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Longitudinal analysis of the effect of water hardness on atopic eczema: evidence for gene–environment interaction*

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Summary

Background Several studies have identified an association between water hardness and atopic eczema (AE); however, there is a paucity of longitudinal data in early life.

Objectives To examine whether water hardness is associated with an increased risk of AE and skin barrier dysfunction in infants and to assess effect modification by filaggrin (FLG) loss-of-function variants.

Methods We performed a longitudinal analysis of data from infants in the Enquiring About Tolerance (EAT) study, who were enrolled at 3 months and followed up until 36 months of age.

Results Of 1303 infants enrolled in the EAT study, 91·3% (n = 1189) attended the final clinic visit and 94·0% (n = 1225) of participants' families completed the 36-month questionnaire. In total, 761 (58·4%) developed AE by 36 months. There was no overall association between exposure to harder (> 257 mg L^{-1} $CaCO_3$) vs. softer (≤ 257 mg L^{-1} $CaCO_3$) water: adjusted hazard ratio (HR) 1·07, 95% confidence interval (CI) 0·92–1·24. However, there was an increased incidence of AE in infants with *FLG* mutations exposed to hard water (adjusted HR 2·72, 95% CI 2·03–3·66), and statistically significant interactions between hard water plus FLG and both risk of AE (HR 1·80, 95% CI 1·17–2·78) and transepidermal water loss (0·0081 g m^{-2} h^{-1} per mg L^{-1} $CaCO_3$, 95% CI 0·00028–0·016).

Conclusions There is evidence of an interaction between water hardness and *FLG* mutations in the development of infantile AE.

What's already known about this topic?

- Several cross-sectional studies have found an association between domestic water hardness exposure and atopic eczema (AE) risk.
- Loss-of-function mutations in the skin barrier gene filaggrin (*FLG*) are the strongest genetic risk factor for AE.

What does this study add?

- There was no overall association between AE risk and exposure to harder vs. softer domestic water in a large, well-phenotyped cohort of infants living in England and Wales followed up at 3–36 months of age.

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Conflicts of interest
None to declare.

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- However, infants with at least one *FLG* loss-of-function mutation exposed to harder water have a threefold increased risk of developing AE up to age 36 months compared with infants with wild-type *FLG* exposed to softer water.

Atopic eczema (AE) is a common inflammatory skin condition affecting around 20% of children, which carries a significant impact on quality of life.[1] Loss-of-function (LOF) mutations in the skin barrier gene filaggrin (*FLG*) are important risk factors for the development of AE.[2] However, as genetic risk factors do not fully explain the observed risk of AE, environmental factors are likely to play an important additional role, including antibiotic exposure, water hardness and pet ownership.[3] For instance, it has previously been shown that interactions between genetic and environmental exposures, such as cat ownership, in early life in those with *FLG* LOF mutations may potentiate the effect of such environmental exposures in those with genetically weakened skin barriers.[4]

Hard domestic water is the result of dissolved minerals from the percolation of water through rock in the environment. The key minerals that constitute hardness are $CaCO_3$ and $MgCO_3$. When domestic water is hard to very hard (>250 mg L^{-1} $CaCO_3$) this can lead to limescale build-up in heating systems and the formation of soap scum (calcium stearate) on the skin, clothes and bedding. Anecdotally, patients report that their skin feels drier or their AE gets worse if they move from a soft- to a hard-water area.

In 1998, a cross-sectional study showed an increased risk of AE in primary-school children living in a hard- vs. a soft-water area.[5] Two further cross-sectional studies among schoolchildren in Japan and Spain confirmed this association.[6,7] More recently, a Danish birth cohort found a 5% increase in the prevalence of eczema within the first 18 months of life for each five-unit increase in domestic water hardness (range 6·60–35·90 German degrees of hardness, 118–641 mg L^{-1} $CaCO_3$).[8,9]

Several mechanisms have been proposed for the way in which hard water may lead to AE development. Increased deposition of detergents such as sodium lauryl sulfate (SLS) on the skin, altered calcium signalling in the epidermis, and a rise in skin-surface pH, with a resulting increase in protease activity, could all have detrimental effects on skin barrier function. Such hypotheses are supported by experimental work that examined the combined effect of water hardness and SLS on skin irritation in 83 people with or without AE and with or without *FLG* LOF mutations. This study showed skin barrier impairment (raised transepidermal water loss, TEWL) and raised skin-surface pH with higher $CaCO_3$ exposure, particularly in those with an *FLG* mutation and a past history of AE. Changes in both TEWL and objectively measured skin erythema correlated with increased deposition of SLS in skin washed with hard vs. softened water.[10] Importantly, chlorine exposure did not increase skin inflammation, TEWL or SLS deposition.

In a hairless mouse model, low extracellular concentrations of calcium ions in the upper epidermis led to exocytosis of lamellar bodies, required for skin barrier repair, independently of skin barrier disruption.[11] An experimental pilot study of 11 dogs with pruritus reported an interaction between shampoo and hard water. A protective effect was seen on skin barrier function when using shampoo with ultrapure soft water (< 1 mg L^{-1} $CaCO_3$) compared with shampoo and tap water (158 mg L^{-1} $CaCO_3$).[12]

Our group has previously shown in a cross-sectional analysis from the Enquiring About Tolerance (EAT) study among 3-month old infants from England and Wales that combined exposure to hard water and high chlorine was associated with a 61% increase in the odds of AE on skin examination [adjusted odds ratio 1·61, 95% confidence interval (CI) 1·09–2·38].[13] These results also suggested an interaction between hard water and *FLG* LOF mutations, not only for AE but also for skin barrier dysfunction measured by TEWL, although formal interaction testing showed results that were not statistically significant. The present study extends the analysis beyond 3 months of age to test longitudinally the hypothesis that early-life exposure to hard water is associated with AE and skin barrier dysfunction in infants with and without *FLG* LOF mutations.

Patients and methods

A secondary analysis of data on infants aged 3–36 months enrolled in the EAT study was performed. The EAT study was a randomized controlled trial comparing early vs. standard introduction of allergenic foods in 1303 generally well, breastfed infants born at term (≥ 37 weeks).[14] Infants were recruited at 3 months of age from the general population across England and Wales. The aim of the EAT study was to determine whether early introduction of common dietary allergens would prevent food allergies. The sample size was determined by the intervention component in the EAT study.

Primary outcome

Two definitions of AE were used for the primary analyses: parent-reported, doctor-diagnosed AE and visible AE on skin examination. Visible AE was determined at the 3-, 12- and 36-month visits using a U.K. diagnostic criteria-based photographic protocol adapted for infants.[15] Parents were asked about new-onset AE, including whether this diagnosis was confirmed by a doctor, through online questionnaires at monthly intervals from 3 to 12 months, then every 3 months thereafter. A combined end point of visible AE and parent-reported AE was used for the survival analysis. Time of onset of AE prior to 3 months was imputed using a combination of parent-reported, doctor-diagnosed AE, with time of first

topical steroid use. AE severity was determined by the Scoring Atopic Dermatitis (SCORAD) index.[16]

Secondary outcomes

A secondary outcome was TEWL as a measure of skin barrier function. TEWL was measured at 3 and 12 months with the Biox Aquaflux AF200 closed condenser-chamber device (Biox Systems Ltd, London, U.K.) on unaffected skin of the volar aspect of the forearm.[17] Parents were advised not to use any skincare products on the infant's arms for the preceding 24 h. Measurements were performed in our environmentally controlled clinical research facility (ambient temperature 20 ± 2°C, relative room humidity 32–50%) after at least 20 min of acclimatization. In all children the mean of three separate TEWL measurements was calculated.

Water hardness exposure and covariates

Data on domestic water $CaCO_3$ concentrations in mg L^{-1} were obtained from local water supply companies for each participant's household based on postcode at the time of study recruitment. Data were collected on covariates, including sex, ethnicity, home location, maternal age, socioeconomic status (maternal age at leaving full-time education), ownership of a water softener, family history of AE and other allergic diseases, frequency of bathing, and use of topical moisturizers and bathing products, through parental questionnaires. Data on indices of multiple deprivation, a measure of socioeconomic position, were obtained from official statistics based on the postcode of residence.

Filaggrin genotyping

Venous blood samples were screened for the six most common *FLG* mutations using TaqMan allelic discrimination assays (mutations R501X, 2282del4, R2447X, S3247X; ABI 7900 HT; Applied Biosystems, Foster City, CA, U.S.A.) or by sizing of fluorescent polymerase chain reaction products on an Applied Biosystems 3130 DNA sequencer (mutations 3673delC and 3702delG). These six mutations detect 99% of *FLG* mutation carriers in the U.K. population.

Statistical analysis

As in our previous cross-sectional analysis of the effect of water hardness at 3 months of age in the EAT study,[14] water hardness exposure was dichotomized based on the median value of $CaCO_3$ across the whole EAT cohort. Our a priori hypothesis was that the risk of AE with hard water would be increased in those with *FLG* LOF mutations. We therefore planned to test for an interaction between water hardness and *FLG*, even in the absence of a statistically significant main effect of water hardness. *FLG* status was modelled using a two-level dominant genetic model whereby infants were assigned as having an *FLG* LOF mutation if they were heterozygous or homozygous for the null allele in at least one of the two single-nucleotide polymorphisms.

Children were retained in the analysis from 3 months until the first of development of AE, dropout or 36 months of age. We did not create a combined water hardness–chlorine variable, as was done in our cross-sectional analysis at 3 months, as our own subsequent mechanistic work in patients with and without AE and *FLG* LOF mutations showed no additional increase in skin barrier disruption secondarily to chlorine exposure.[10] SCORAD was categorized as mild (1–15) or moderate-to-severe (> 15).

The relationships between covariates and AE were explored using Kaplan–Meier plots and univariate Cox regression. Multivariable adjustment was made for likely confounders: home location (rural or urban), ethnicity (white or nonwhite), indices of multiple deprivation (deciles) and water softener present (yes or no). The effect of adding in the study randomization group as a covariate was examined. Analyses were conducted using Stata, version 15 (StataCorp, College Station, TX, U.S.A.).

An exact partial-likelihood method was used to handle tied failures. Interactions among selected variables in the main effects model were examined based on plotting the ratios of hazard ratios using *fintplot*.[18] Likelihood ratio tests were used to compare model fit with and without addition of the selected interaction terms. The proportional hazards assumption for the overall model was tested using *stphtest*. A cutoff of ≥ 15 g m^{-2} h^{-1} was used to define 'high' TEWL, based on the upper quartile of value of TEWL in EAT participants at enrolment without visible AE, and consistently with our previous publications.[13,19] The relationship between water hardness and TEWL was analysed using a generalized estimating equation with an equal-correlation model and conventionally derived variance estimator for standard error. All estimates are reported with 95% CIs. The population attributable fraction (PAF) was calculated as PAF (%) = p(HR − 1) / [(p(HR − 1) + 1] × 100%, where p is the prevalence of the risk factor and HR is the hazard ratio of the disease risk in the exposed over the nonexposed.

Results

Of 1303 infants enrolled in the EAT study, 91·3% (n = 1189) attended the final clinic visit, and 94·0% (n = 1225) of participants' families completed the 36-month questionnaire. Water hardness data based on $CaCO_3$ levels were available for all participants. Water hardness values ranged from 3 mg L^{-1} to 490 mg L^{-1}, with a median of 257 mg L^{-1} (Fig. 1). The distribution of water hardness values was negatively skewed (Fig. S1; see Supporting Information). *FLG* genotype was available for 1206 participants (92·6%). Exposure to hard water was independent of *FLG* mutation status ($\chi^2_{1\ d.f.} P = 0 \cdot 84$) Infants living in hard-water areas were, at enrolment, more likely to be of nonwhite ethnicity and more likely to use a moisturizer and have a water softener installed, and were less likely to use bubble bath or have pets (Table 1). In total, 1204 participants

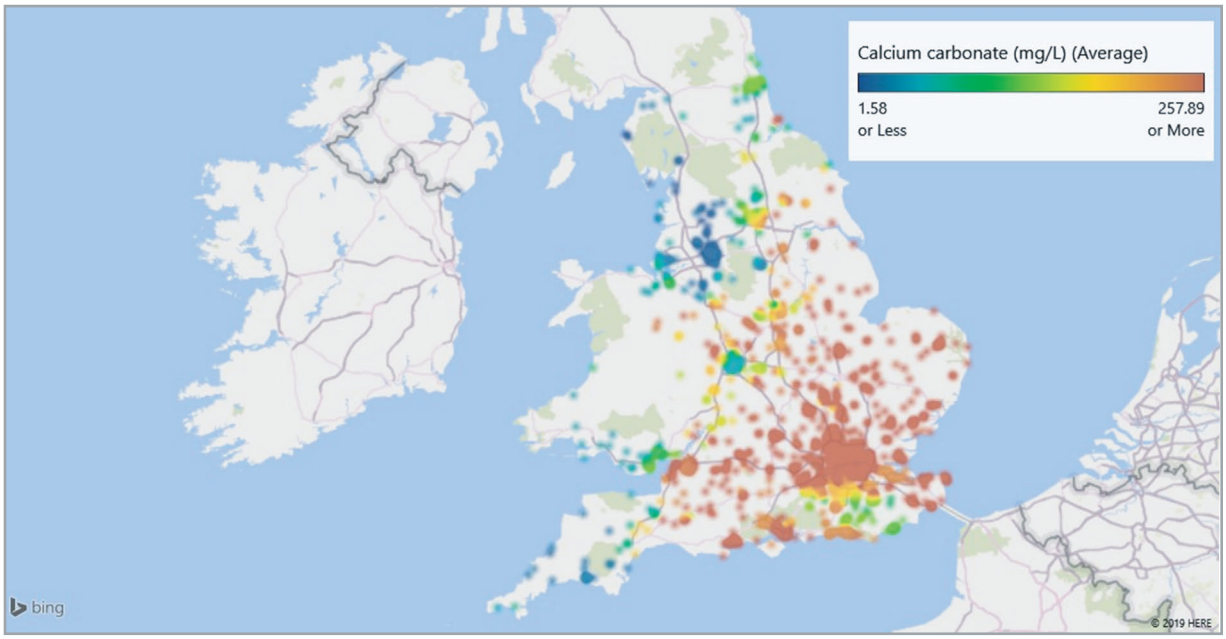

Fig 1. Heatmap of England and Wales showing average calcium carbonate levels (mg L^{-1}) based on participants' postcodes at enrolment.

were included in this analysis, and their demographic characteristics were broadly similar to those of thee full EAT cohort (Table S1; see Supporting Information).

Visible atopic eczema with domestic hard-water exposure

At 3 months, 183 infants (27·6%) exposed to harder water had visible AE, compared with 134 (21·0%) exposed to softer water (P = 0·005) (Table 2). By 36 months, this difference had attenuated (P = 0·69) (Table 2). Moderate-to-severe AE (SCORAD > 15) was more common in harder-water areas than in softer-water areas (6·6% vs. 4·4%, P = 0·02) at 3 months; however, this relationship was not present at 12 or 36 months.

Parent-reported atopic eczema risk with domestic hard-water exposure

Overall, 761 infants (58·4%) developed parent-reported AE by 36 months of age. There was no significant difference in the risk of parent-reported AE between infants exposed to harder vs. softer water (log-rank P = 0·20) (Fig. S2; see Supporting Information). There was no association between $CaCO_3$ exposure (per mg L^{-1}) and the risk of parent-reported AE [hazard ratio (HR) 1·00, 95% CI 1·00–1·00; P = 0·62], and this was unchanged after adjustment for confounders (HR 1·00, 95% CI 1·00–1·00; P = 0·33). When water hardness was dichotomized on the median value of $CaCO_3$ in the cohort, there was a small, nonstatistically significant increased risk of parent-reported AE with exposure to above vs. below median water hardness levels (HR 1·10, 95% CI 0·95–1·28; P = 0·20), even after adjustment for confounders (HR 1·07, 95% CI 0·92–1·24; P = 0·39). This risk equates to a PAF of 3·4% overall with water hardness exposure. There was evidence of violation of the proportionality assumption, based on water hardness and ethnicity. Stratification by ethnicity improved proportionality and the effect estimates were unchanged (P = 0·39).

Effect of *FLG* loss-of-function mutation status on atopic eczema risk

In total, 141 of 1206 infants (11·7%) carried a *FLG* LOF mutation and, of those, 102 (72·3%) developed visible AE on skin examination, compared with 413 of 988 (41·8%) with wild-type *FLG*. The risk of parent-reported AE was also significantly increased in those with an *FLG* LOF mutation: HR 2·04, 95% CI 1·64–2·53; P < 0·001 (Fig. S3; see Supporting Information).

Effect of *FLG* loss-of-function mutation status on risk of atopic eczema with domestic hard-water exposure

Stratifying by *FLG* LOF mutation status, there was an increase in the cumulative prevalence of visible AE with increasing water hardness exposure across the various timepoints (log-rank P < 0·001) (Figs 2, 3). In the adjusted multivariable model there was a higher risk of AE in those with *FLG* LOF mutations exposed to harder vs. softer water (HR 2·72, 95% CI 2·03–3·66; Table S2; see Supporting Information), with a statistically significant multiplicative interaction term (HR 1·80, 95% CI 1·17–2·78; P-value for interaction = 0·008), which improved the fit of the model. This risk equates to a PAF of 23·2% in those with *FLG* and hard-water coexposure. There were no significant interactions between water hardness, *FLG* LOF mutations and other key variables (Fig. S4; see Supporting Information).

Table 1 Population demographics by exposure to below or above the median $CaCO_3$ concentrations (257 mg L^{-1}) at enrolment into the Enquiring About Tolerance (EAT) study (n = 1303)

	$CaCO_3$ below median, n = 639	$CaCO_3$ above median, n = 664	P-value
Sex male	316 (49·5)	337 (50·8)	0·64
Age at enrolment (years), mean ± SD	3·40 ± 0·23	3·38 ± 0·23	0·16
Ethnicity white	562 (87·9)	542 (81·6)	0·002
Home location urban	479 (75·1)	527 (79·5)	0·06
Maternal education (age at completion in years)			
≤ 16	35 (5·5)	39 (5·9)	0·93
17–18	86 (13·5)	86 (13·0)	
≥ 19	517 (81·0)	539 (81·2)	
Index of multiple deprivation (deciles)			
1 – most deprived	34 (5·3)	17 (2·6)	0·20
2	47 (7·4)	44 (6·6)	
3	56 (8·8)	63 (9·5)	
4	56 (8·8)	63 (9·5)	
5	64 (10·0)	78 (11·8)	
6	68 (10·6)	87 (13·1)	
7	64 (10·0)	75 (11·3)	
8	84 (13·1)	79 (11·9)	
9	89 (13·9)	75 (11·3)	
10 – least deprived	77 (12·1)	82 (12·4)	
EAT study randomization: assigned to intervention	318 (49·8)	334 (50·3)	0·85
Family atopy status			
Maternal			
Atopy[a]	404 (63·3)	410 (61·8)	0·58
Atopic eczema	228 (35·7)	221 (33·3)	0·36
Paternal			
Atopy[a]	349 (54·7)	342 (51·6)	0·26
Atopic eczema	124 (19·4)	136 (20·5)	0·63
Parental			
Atopy[a]	524 (82·1)	542 (81·7)	0·86
Skincare and bathing			
Water softener present at home	12 (1·9)	54 (8·1)	< 0·001
Bathing ≥ 5 times per week	259 (43·0)	245 (39·4)	0·21
Moisturizer use ≥ 5 times per week	180 (29·9)	234 (37·6)	0·004
Bubble bath used	215 (35·7)	173 (27·8)	0·003
Bath emollient used	104 (17·2)	128 (20·6)	0·14
Shampoo used	202 (33·5)	190 (30·5)	0·27
Soap used	50 (8·3)	60 (9·6)	0·41
FLG loss-of-function mutation	68 (11·7)	75 (12)	0·84
Antibiotic exposure	122 (19·1)	123 (18·5)	0·79
Pet ownership	316 (49·5)	238 (35·9)	< 0·001
Any household members smoking	87 (13·6)	81 (12·2)	0·45
Vaginal delivery	479 (74·7)	496 (74·7)	0·91

The data are presented as n (%) unless stated otherwise. P-values for heterogeneity were calculated using χ^2-statistics. [a]Asthma, atopic eczema or hay fever.

Transepidermal water loss and domestic hard-water exposure

TEWL was measured in 1300 infants (99·8%) at 3 months, and 1103 infants (84·7%) at 12 months. Median TEWL levels increased overall by 0·94 g m^{-2} h^{-1} (IQR −1·63 to 3·66) between 3 and 12 months. Of those with measured TEWL, increased TEWL (≥ 15 g m^{-2} h^{-1}) was observed in 32·3% at 3 months and 38·4% at 12 months. Table S3 (see Supporting Information) shows the effect of hard water on TEWL stratified by *FLG* LOF mutation status. In children with visible AE without *FLG* LOF mutations, a slightly higher proportion had high TEWL in those exposed to harder vs. softer water at 3 months (11·9% vs. 9·1%, $\chi^2_{1\,d.f.} = 1 \cdot 9$, P > 0·05), and this difference was greater in those with *FLG* mutations (46·7% vs. 23·5%, $\chi^2_{1\,d.f.} = 5 \cdot 4$, P = 0·02). The differences did not persist at 12 months (8·6% vs. 9·4% and 23·1% vs. 25·0%, respectively, P > 0·05 for both). In those without visible AE, no significant differences were observed in TEWL, at either 3 or 12 months, between those exposed

Table 2 Point and cumulative prevalence of visible atopic eczema at 3, 12 and 36 months, stratified by water hardness exposure

Visit age, outcome	$CaCO_3$ below median[a] n = 639	$CaCO_3$ above median[a] n = 664	$CaCO_3$ at quartile 1 n = 327	$CaCO_3$ at quartile 2 n = 329	$CaCO_3$ at quartile 3 n = 325	$CaCO_3$ at quartile 4 n = 322
3 months						
AE	134 (21·0)	183 (27·6) P = 0·005	71 (21·7)	71 (21·6)	95 (29·3)	80 (24·8) P = 0·07
AE severity						
Mild	106 (16·6)	140 (21·1)	54 (16·5)	60 (18·2)	71 (21·8)	61 (18·9)
Moderate–severe	28 (4·4)	44 (6·6) P = 0·02	17 (5·2)	11 (3·3)	25 (7·7)	19 (5·9) P = 0·12
Raised TEWL	196 (30·7)	224 (33·8) P = 0·23	98 (30·0)	104 (31·7)	114 (35·1)	104 (32·5) P = 0·57
12 months						
AE	144 (25·2)	156 (26·9) P = 0·52	79 (26·7)	71 (24·6)	73 (25·6)	77 (27·4) P = 0·88
AE severity						
Mild	115 (20·1)	122 (21·0)	63 (21·3)	58 (20·1)	59 (20·7)	57 (20·3)
Moderate–severe	28 (4·9)	31 (5·3) P = 0·87	16 (5·4)	12 (4·2)	12 (4·2)	19 (6·8) P = 0·83
Cumulative AE	222 (46·9)	251 (53·1) P = 0·24	117 (35·8)	115 (35·0)	124 (38·3)	117 (36·3) P = 0·84
Raised TEWL	203 (37·2)	221 (39·7) P = 0·39	107 (37·7)	101 (36·7)	115 (42·0)	101 (37·4) P = 0·58
36 months						
AE	124 (21·2)	132 (22·1) P = 0·69	67 (22·1)	62 (20·9)	68 (23·6)	59 (20·1) P = 0·75
AE severity						
Mild	74 (12·6)	94 (15·6)	39 (12·8)	39 (13·1)	50 (17·2)	40 (13·5)
Moderate–severe	45 (7·7)	36 (6·0) P = 0·20	24 (7·9)	21 (7·1)	15 (5·2)	21 (7·1) P = 0·83
Cumulative AE	265 (47·9)	288 (52·1) P = 0·47	136 (41·6)	139 (42·2)	149 (46·0)	129 (40·1) P = 0·47

Data are presented as n (%). P-values for heterogeneity were calculated using χ^2-statistics. AE, atopic eczema; TEWL, transepidermal water loss. [a]Median value is 257 mg L^{-1}.

to harder vs. softer water, irrespectively of *FLG* LOF mutation status.

There was no statistically significant relationship between water hardness and TEWL as a continuous outcome between 3 and 12 months: 0·00061 g m^{-2} h^{-1} per mg L^{-1} $CaCO_3$ (95% CI −0·0018–0·0030; P = 0·62). Within strata of *FLG*, there were also no significant correlations between $CaCO_3$ at 12 months and TEWL at 12 months (Fig. S5; see Supporting Information). However, after adjustment for confounders and inclusion of an interaction term in the model between water hardness and *FLG*, there was a small statistically significant increase in TEWL of 0·0081 g m^{-2} h^{-1} per mg L^{-1} in association with higher $CaCO_3$ levels (95% CI 0·00028–0·016; P-value for interaction = 0·042; Fig. S6; see Supporting Information).

Discussion

In this longitudinal analysis of the EAT study we found that infants with at least one *FLG* LOF mutation exposed to harder water have a threefold increased risk of developing AE compared with infants with wild-type *FLG* exposed to softer water. This risk equates to a PAF of 3% overall with water hardness exposure and 23·2% in those with *FLG* and hard-water coexposure. Combined exposure to hard water and *FLG* mutations was also associated with a slight increase in skin barrier dysfunction, as measured by TEWL, between 3 and 12 months of age. These results support the growing body of evidence for the multifactorial aetiology of AE, and provide a plausible insight into how a commonly encountered exposure, hard water, might interact with a genetically weakened skin barrier in early life to lead to further deterioration in skin barrier function, loss of epidermal water and the initiation of eczematous skin inflammation.[20]

This was a hypothesis-driven analysis of a large, well-characterized cohort of children, with comprehensive assessment of known confounders. Our findings are likely to be representative of the population in England and Wales as the study population was drawn from the general population. The measurement of *FLG* LOF mutation status and TEWL in the infants along with detailed phenotyping is a further strength. As in any observational study, there is the possibility of bias. The

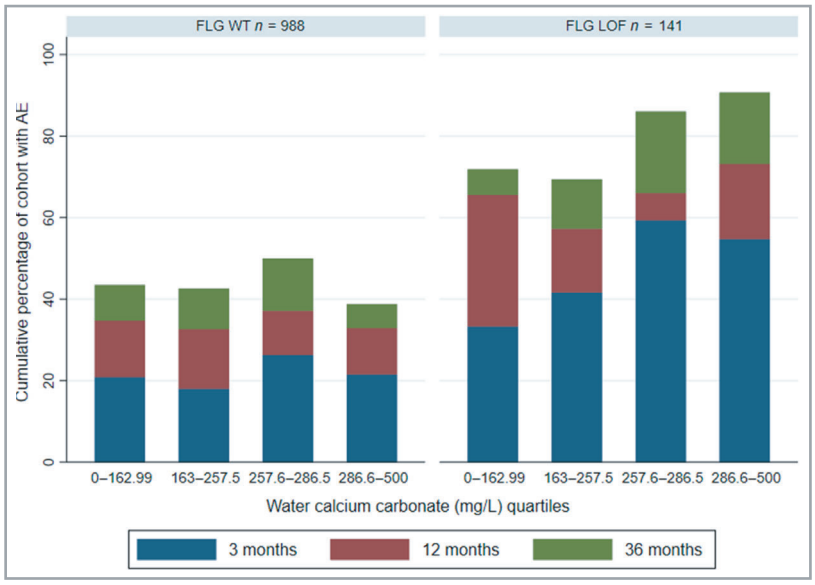

Fig 2. Cumulative prevalence of visible atopic eczema (AE) at 3, 12 and 36 months stratified by water hardness exposure in infants with and without filaggrin (FLG) loss-of-function mutations. LOF, loss-of-function; WT, wild-type.

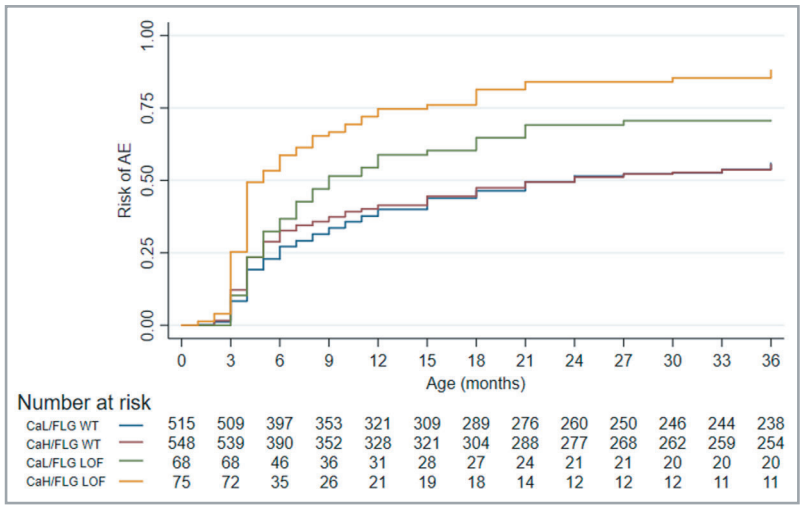

Fig 3. Kaplan–Meier plot of parent-reported atopic eczema (AE) risk stratified by filaggrin (FLG) status; log-rank $P < 0{\cdot}001$. CaL, calcium carbonate below median; CaH, calcium carbonate above median; WT, wild-type; LOF, loss of function.

calculated estimates represent the true causal effect of water hardness on AE risk, assuming no model misspecification and no unmeasured confounding. The model diagnostics suggest good model specification. There may also be misclassification of the exposure, as water hardness was measured only at baseline, and we did not formally capture whether participants moved. However, the moves that we were aware of occurred locally, and this effect would not be expected to happen in a differential way and should therefore not lead to biased estimates.

A high proportion (58%) of infants developed parent-reported, doctor-diagnosed AE by 36 months of age, raising the possibility of over-reporting of the outcome. However, a sensitivity analysis using only investigator-assessed visible AE as the outcome, rather than the composite outcome of parent-reported, doctor-diagnosed AE, yielded similar risk estimates (adjusted HR 2·57, 95% CI 1·91–3·46). As the dataset was from a randomized trial, we examined the effect of including the randomization group in the model, which did not appreciably alter the risk estimates.

The lack of a longitudinal statistically significant association between water hardness and AE overall is in keeping with the results of the Spanish birth cohort study by Font-Ribera *et al.*,[21] which showed no relationship between water hardness and AE at 14 months and 4 years of age. The overall PAF of 3% in our EAT study cohort is consistent with the value of

2% reported recently by Engebretsen *et al.*[9] in their analysis of a Danish cohort, but the additional contribution of *FLG* LOF mutations was not examined in the Danish population. The significant interaction between *FLG* LOF mutations and water hardness we found longitudinally is consistent with the trend towards an association observed in our previous cross-sectional analysis.

The heterogeneity of observational study results may reflect differences in study design; the ranges of water hardness exposure, population age and ethnicity; and definitions of AE. It may also reflect the changing impact of hard water on AE risk over time, seen with maturation of the skin of the growing child. For instance, the 90th percentile of $CaCO_3$ in the study of Miyake *et al.* from Japan was 76 mg L^{-1}.[7] This would be considered soft water in the U.K. context.

The observed effect of hard water on skin barrier function is consistent with our own mechanistic work, using washing experiments in adults with and without *FLG* LOF mutation, which demonstrated that hard water leads to an increase in skin barrier dysfunction (raised TEWL and erythema), partly mediated by an increase in SLS deposition on the skin.[10] The relationship between water hardness and TEWL is stronger at 3 months than at 12 months of age, suggesting that the deleterious effects of hard water on skin barrier function might lessen over time with stratum corneum maturation.

Based on our longitudinal population-based analysis of a carefully phenotyped U.K. population, domestic hard water is an important risk factor for the development of AE in infants aged 3–36 months who have a *FLG* LOF mutation. An interventional study is underway to examine whether installing water softeners in the homes of high-risk babies who live in hard-water areas reduces their risk of developing skin barrier impairment and AE (Clinicaltrials.gov: NCT03270566).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Histogram showing distribution of $CaCO_3$ concentrations based on postcode at enrolment.

Fig S2. Kaplan–Meier plot of parent-reported atopic eczema risk with exposure to harder (> 257 mg L^{-1} $CaCO_3$) vs. softer water (≤ 257 mg L^{-1} $CaCO_3$).

Fig S3. Gene-only effect of *FLG* loss-of-function mutations on risk of atopic eczema.

Fig S4. Forest plot summarizing interactions of key variables with water hardness in relation to parent-reported atopic eczema risk.

Fig S5. Scatterplots of transepidermal water loss at 12 months of age in infants with and without atopic eczema, stratified by filaggrin mutation status.

Fig S6. Modelled marginal effect of water $CaCO_3$ level and *FLG* mutation inheritance on predicted transepidermal water loss from 3 to 12 months of age.

Table S1 Comparison of baseline characteristics between the full dataset and the analytical dataset.

Table S2 Crude and adjusted hazard ratios for parent-reported atopic eczema stratified by water hardness exposure (high/low) and *FLG* mutation status (yes/no).

Table S3 Influence of water hardness (high/low) on transepidermal water loss and atopic eczema prevalence by *FLG* mutation status.