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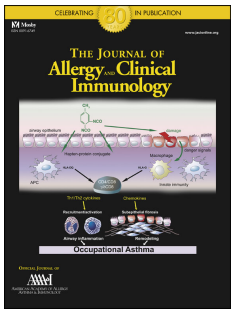

The Association between Domestic Water Hardness, Chlorine and Atopic Dermatitis Risk in Early Life: A Population-Based Cross-Sectional Study

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Title: The Association between Domestic Water Hardness, Chlorine and Atopic Dermatitis Risk in Early Life: A Population-Based Cross-Sectional Study

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ABSTRACT

Background: Domestic water hardness and chlorine have been suggested as important risk factors for atopic dermatitis (AD).

Objective: To examine the link between domestic water calcium carbonate and chlorine concentrations, skin barrier dysfunction (raised TEWL) and AD in infancy.

Methods: We recruited 1303 three month old infants from the general population and gathered data on domestic water calcium carbonate ($CaCO_3$ mg/L) and chlorine (Cl_2 mg/L) concentrations from local water suppliers. At enrolment, infants were examined for AD and screened for filaggrin (*FLG*) skin barrier gene mutation status. Transepidermal water loss (TEWL) was measured on unaffected forearm skin.

Results: $CaCO_3$ and chlorine levels were strongly correlated. A hybrid variable of above and below median levels of $CaCO_3$ and total chlorine was constructed: a baseline group of low $CaCO_3$/low total chlorine (CaL/ClL), high $CaCO_3$/low total chlorine (CaH/ClL), low $CaCO_3$/high total chlorine (CaL/ClH) and high $CaCO_3$/high total chlorine (CaH/ClH). Visible AD was more common in all three groups versus the baseline group: CaH/ClL adjusted OR (AOR) 1.87 (95%CI 1.25-2.80, p=0.002), CaL/ClH AOR 1.46 (95%CI 0.97-2.21, p=0.07) and CaH/ClH AOR 1.61 (95%CI 1.09-2.38, p=0.02). The effect estimates were greater in children carrying filaggrin mutations but formal interaction testing between water quality groups and filaggrin status was not statistically significant.

Conclusions: High domestic water $CaCO_3$ levels are associated with an increased risk of AD in infancy. The influence of elevated total chlorine levels remains uncertain. An intervention trial is required to see whether installation of a domestic device to lower $CaCO_3$ levels around the time of birth can reduce this risk.

Clinical Implications

Domestic water hardness is an important risk factor for AD development and skin barrier dysfunction already during the first three months of life, especially in genetically predisposed infants.

Capsule Summary

In a cohort recruited from the general population, visible AD was more common in three month old infants exposed to domestic water with raised levels of calcium carbonate.

Keywords: Filaggrin, eczema, atopic dermatitis, transepidermal water loss, water hardness

Abbreviations:

AD – atopic dermatitis

CI – confidence interval

FLG – Filaggrin

OR – odds ratio

AOR – adjusted odds ratio

SCORAD - Scoring Atopic Dermatitis Index

TEWL – transepidermal water loss

Introduction

Atopic dermatitis (AD: syn. 'atopic eczema', 'childhood eczema') is the commonest inflammatory skin disease and affects around 20% of children in the UK.[1] Skin barrier impairment and dry skin are hallmarks of AD and likely to be important triggers of eczematous skin inflammation in early life, partly through genetic predisposition, in particular inheritance of filaggrin (*FLG*) skin barrier gene mutations. We have previously shown that carriage of *FLG* skin barrier mutations is associated with an increase in transepidermal water loss (TEWL) and xerosis already by three months of life, even in unaffected children.[2] In addition to *FLG* mutation inheritance, there are a number of potential environmental exposures that may contribute to the breakdown of the skin barrier in early life, including domestic water hardness ($CaCO_3$) and chlorine concentration.[1] Rain water is naturally low in $CaCO_3$ but it collects minerals, such as calcium, as it percolates through rock. The local geology therefore has a major impact on the hardness of the water supply. In the UK, domestic water tends to be harder in the south compared to the north. Chlorine is universally added to tap water and is a potential skin irritant.[3]

Ecological studies in the UK, Spain and Japan have shown consistent positive associations between domestic water hardness and AD risk among schoolchildren.[4-6] However, the link between domestic water hardness and AD has not been studied in early infancy, when around 50% of all AD cases manifest clinically for the first time,[7] and furthermore, *FLG* mutation inheritance and skin barrier impairment (raised TEWL) have not been considered in this context either.

Although an observer blind parallel-group randomized controlled trial with conventional ion-exchange water softeners among 6 month to 16 year old UK children with moderate to severe AD did not show a beneficial effect on disease severity,[8] it is still possible that high

104 domestic water $CaCO_3$ or chlorine levels are involved in the initiation of eczematous skin
105 inflammation. We therefore studied the association between $CaCO_3$ and chlorine
106 concentrations as well as *FLG* skin barrier gene mutation inheritance, skin barrier function
107 (TEWL), AD risk and severity among three month old infants.
108

Methods

This was a cross-sectional study among 1303 three-month old infants recruited from the general population in England and Wales between October 2009 and April 2012 (www.eatstudy.co.uk). The sample size was determined by the intervention component of the EAT Study.[9] All children were generally well, exclusively breastfed and born at term (≥37 weeks gestation). Following written parental consent, children were examined for AD, using a UK diagnostic criteria-based photographic protocol, adapted for infants.[10] AD severity was determined by the Scoring Atopic Dermatitis (SCORAD) index.[11] TEWL was measured with the Biox Aquaflux® AF200 closed condenser chamber device on the unaffected skin of the volar aspect of the forearm.[12] Participants' parents were advised not to use any skin care products on the infant's arms for the preceding 24 hours. Measurements were performed in our environmentally controlled Clinical Research Facility (ambient temperature 20 $^{+}/_{-}$ 2^{0}C, relative room humidity 32-50%), after at least 20 minutes of acclimatization. Measurements were not taken if the child was visibly distressed or crying. In all children we calculated the mean of three separate TEWL measurements. Venous blood samples were screened for the six commonest *FLG* mutations using TaqMan allelic discrimination assays (mutations R501X, 2282del4, R2447X, S3247X; Applied Biosystems, ABI 7900 HT, Foster City, California) or by sizing of fluorescent PCR products on an Applied Biosystems 3130 DNA sequencer (mutations 3673delC, and 3702delG). These six mutations detect 99% of *FLG* mutation carriers in the UK population. Data on domestic water calcium carbonate and free and total chlorine levels in mg per litre (mg/L) were gathered from local UK water suppliers for each participant's household based on post code at time of study recruitment. We also collected information on potential confounders, including sex, ethnicity, home location, maternal age, socio-economic status (maternal age at leaving full-time education), ownership of a water

softener, family history of AD and other allergic diseases, frequency of bathing, and the use of topical moisturisers and bathing products via parental questionnaires.

Statistical analysis

Water content data was available for all participants for $CaCO_3$, but local water companies were only able to provide total and free chlorine values for 1287 and 809 participants respectively. $CaCO_3$ levels were strongly correlated with both total chlorine and free chlorine levels (Figure 1). Furthermore, total chlorine and free chlorine levels were highly correlated (Figure 2). To avoid incorporating strongly correlated variables in the models and given that significantly more participants had total chlorine data, a hybrid variable of above and below median levels of $CaCO_3$ and total chlorine was constructed: a baseline group of low $CaCO_3$/low total chlorine (CaL/ClL), high $CaCO_3$/low total chlorine (CaH/ClL), low $CaCO_3$/high total chlorine (CaL/ClH) and high $CaCO_3$/high total chlorine (CaH/ClH). A univariate analysis was undertaken, investigating the association between this variable and the potential confounding factors. Two principle outcomes were investigated: visible AD at enrolment and raised TEWL. Raised TEWL was defined as ≥15 g/m^2h, based on the upper quartile value of TEWL in participants without visible AD at enrolment (15.00 g/m^2h) as used in our previous publications.[2,13] Logistic regression models for the two principle outcomes were created with two levels of adjustment. The first incorporated factors found to be significantly associated with the outcomes in the univariate analysis. The second also included moisturizer and bubble bath use. Filaggrin mutation inheritance was also included in the models. Water softeners were installed in the homes of a small number of participants (66 families, 5.1% of the cohort). The analysis presented in this paper was undertaken including water softener ownership as a potential confounding variable. The argument for this was

157 that conventional water softeners remove calcium carbonate but have no effect on the
158 chlorine content of the water. However, to ensure that this did not introduce a bias in the
159 analysis, the effect of excluding EAT participants with water softeners was explored by
160 undertaking the same analyses, removing these infants, and the effect estimates were not
161 significantly different. Formal statistical tests for interaction between filaggrin status and the
162 hybrid $CaCO_3$/Cl variable were undertaken. Stata 10.1 (StataCorp, Texas) was used for the
163 analyses.
164

Results

24.3% (317/1302) of all participating infants had AD at 3 months confirmed by skin examination, mostly mild with a median SCORAD of 7.5 (range 3.5-75.0). TEWL levels ranged from 6.5-82.1 g/m^{2}h, with a median of 12.8 g/m^{2}h, and inter-quartile range (IQR) of 10.8-16.1 g/m^{2}h. Raised TEWL (≥15 g/m^{2}h) was present in 32% of participants.

$CaCO_3$ levels ranged from 3-490 mg/L, with a median of 257 mg/L, and inter-quartile range (IQR) of 162-286 mg/l. For total chlorine the range was 0.04-1.06 mg/L, median 0.37 mg/L and IQR of 0.26-0.49 mg/L. The geographical distribution of the principle exposure variables in England and Wales is mapped in Figure 3.

Water $CaCO_3$/Cl content were significantly associated with ethnicity (non-white participants less likely to live in low/low areas) and home location (with urban areas more likely to have a high/high content). Maternal age was associated with water $CaCO_3$/Cl content, with mothers being significantly older in both high $CaCO_3$ groups. Water softener use was most common in the high $CaCO_3$/low Cl group. Water $CaCO_3$/Cl content was not associated with a family history of AD or allergic diseases (Table I).

With regard to the skin care variables, there was a strong association with moisturizer use (highest in the high/high group) and the use of bubble bath (highest in the low/low group and lowest in the high/high group) (Table I).

AD risk and domestic water calcium carbonate and chlorine concentration

For the outcome visible AD at the enrolment visit, the condition was more common in all three groups, compared with the baseline low/low group: CaL/ClL 18.7% (OR 1.00 -

baseline), CaH/ClL 27.9% (OR 1.68, 95%CI 1.16-2.44, p=0.006), CaL/ClH 23.1% (OR 1.31, 95%CI 0.89-1.93, p=0.17) and CaH/ClH 27.6% (OR 1.66, 95%CI 1.16-2.38, p=0.006). In Table II: Model 2, the effect of adjustment for filaggrin status, sex, ethnicity, maternal age, water softener presence and home location enhanced the effect estimates for CaH/ClL AOR 1.87 (95%CI 1.25-2.80, p=0.002) and CaL/ClH AOR 1.46 (95%CI 0.97-2.21, p=0.07), but not for CaH/ClH AOR 1.61 (95%CI 1.09-2.38, p=0.02). We also explored the effect of additionally including moisturizer and bubble bath usage as confounders in our model given the associations found in univariate analysis, and the risk estimates for CaH/ClL and CaL/ClH remained stable (AOR 1.74 (95% CI 1.13-2.68, p=0.01) and AOR 1.39 (0.90-2.17) p=0.14), but there was attenuation in the CaH/ClH estimate (AOR 1.26 (95% CI 0.83-1.92) p=0.28). However, the validity of including these two variables is questionable because of their strong correlation with AD, and this is reviewed further in the discussion.

Transepidermal water loss and domestic water calcium carbonate and chlorine concentration

Table III shows the results of the same analysis using raised TEWL (≥15 g/m^{2}h) as the outcome. Effect estimates for the three water content groups were greater than 1.00, both in the crude and adjusted models, approaching statistical significance for the CaH/ClH group.

Exploring the potential interaction with filaggrin mutation inheritance

There was a very strong association between filaggrin mutation carriage and visible AD (AOR 3.84, 95%CI 2.64-5.59, p<0.0005) and raised TEWL (AOR 3.59, 95%CI 2.48-5.19, p<0.0005).

Furthermore, when we explored whether there was an interaction effect of filaggrin status on the relationship between water content group and visible AD, the effect estimates for the interaction terms were greater than 1.00 for the high calcium carbonate groups (CaH/ClL AOR 2.10 (95%CI 0.74-5.99, p=0.17), CaL/ClH AOR 0.83 (95%CI 0.27-2.60, p=0.75) and CaH/ClH AOR 1.32 (95%CI 0.49-3.55, p=0.59) but missed conventional statistical significance.

However, in contrast to AD, for raised TEWL the interaction terms were more consistently elevated for both raised calcium carbonate groups, suggesting an association between raised TEWL and specifically raised $CaCO_3$ levels but only amongst infants carrying a filaggrin mutation: CaH/ClL AOR 2.13 (95%CI 0.77-5.91, p=0.15), CaL/ClH AOR 0.55 (95%CI 0.18-1.65, p=0.29) and CaH/ClH AOR 2.22 (95%CI 0.83-5.93, p=0.11).

This finding was explored in more detail for $CaCO_3$ alone in Figure 4, where the $CaCO_3$ level is plotted against mean TEWL amongst children with and without filaggrin mutation. As with the previous analysis, TEWL and $CaCO_3$ were positively associated, but only amongst the filaggrin mutation carrying infants.

Infants were divided into four categories depending on their AD status and their raised TEWL status. Within each water $CaCO_3$/Cl group, the relative distribution of infants for these four categories is given in the columns in Table IV, stratified by filaggrin status. For example, the data presented in the first column demonstrates that of the 266 infants (without a filaggrin mutation) living in low $CaCO_3$, low total Cl areas, 67% had neither AD or raised TEWL, 17% had raised TEWL only (but no AD), 8% had AD only (but no raised TEWL) and 8% had both raised TEWL and AD.

Figures 5A & 5B present the data from Table IV in graphical form. In Figure 5a it can be seen that AD is more common in the three water quality groups compared with the baseline

group in participants without and with a filaggrin mutation. In contrast, there is no obvious variation between $CaCO_3$/Cl groups in the proportion with raised TEWL but not AD (orange bars). However, infants with AD (navy bar) in Figure 5A can be split into children with AD *and* raised TEWL (navy with orange border) and those with AD but normal TEWL (navy) (Figure 5B). Amongst children with raised TEWL (orange and navy with orange border combined), the proportion with AD appears higher in the raised $CaCO_3$ groups (percentages indicated in the figure), an effect apparent in children with and without filaggrin mutations, but of greater magnitude in the former. AD severity (SCORAD) was not influenced by water hardness and chlorine concentration.

Discussion

Infants exposed to above average levels of water hardness had a statistically significantly increased risk of having visible eczema at three months of age, whether this was accompanied by high or low total chlorine levels, compared to those living in low $CaCO_3$ water areas. There was the suggestion that inheritance of a *FLG* skin barrier gene mutation enhanced this effect, although the statistical test for interaction was not significant.

Exposure to high total chlorine levels alone was also associated with increased visible eczema at three months (46% higher) but the results missed statistical significance.

Similar patterns were seen for the associations between water hardness groups and elevated TEWL but the effect estimates were more attenuated and not statistically significant.

In addition, there was some evidence to suggest that raised $CaCO_3$ levels influenced the phenotypical expression of AD amongst those with raised TEWL levels both in children with and without filaggrin mutations.

To the best of our knowledge, this is the first study on the association between domestic water calcium carbonate, chlorine concentrations and AD risk among infants. Our findings are likely to be representative of the population in England and Wales because the study population was drawn from a wide geographical area, covering a broad spectrum of calcium carbonate concentrations, wider for instance than in the Lancet publication by Nally et al., which recruited primary and secondary schoolchildren from across Nottinghamshire.[4] A further strength of our study is that all children were physically examined, rather than relying on a questionnaire diagnosis alone, which was the case in all other studies on this topic. We were also able to assess the effect on skin barrier function (TEWL) and potential effect modification through *FLG* mutation inheritance.

The role of a broad range of confounders was explored. Ethnicity was associated with water content with more non-white participants living in high $CaCO_3$/high total chlorine areas of the UK. These areas predominate in the south east of England and particularly London, and London is the most ethnically diverse area in the UK, with the highest proportion of minority ethnic groups and the lowest proportion of the white ethnic group at 59.8 per cent.[14] Furthermore, non-white EAT participants lived significantly closer to London on average than white participants (data not shown). Non-white ethnicity was strongly associated with risk of atopic dermatitis, a relationship for which there is an extensive literature.[15] Non-white ethnicity was also strongly associated with raised TEWL, as has been reported previously.[16]

Whilst the inclusion of variables such as sex, ethnicity, maternal age and home location (rural versus city) would seem to be non-contentious, much more open to debate was the decision as to whether to include variables relating to skin care. The concern is that bathing frequency and usage of bathing products as well as skin moisturisation practice are all strongly influenced by a skin condition, in particular the presence of AD. Thus in a cross sectional study such as this, even though the infants were very young at assessment, bathing skin care practice could have already changed because of the emergence of AD or dry skin, potentially resulting in reverse causality. While we did not directly measure $CaCO_3$ and chlorine concentrations in individual participant's households, UK post codes contain on average only 12 addresses with an inherent precision of around 100m.[17] It is therefore likely that the data we received from commercial domestic water suppliers closely matched the actual domestic water hardness and chlorine levels of individual households.

Our findings are in keeping with the other studies conducted among schoolchildren in the UK, Japan, and Spain,[4-6] suggesting that the association is real. Assuming a direct causal relationship between domestic water hardness and AD risk, it may be that calcium carbonate has a direct

289 detrimental effect on skin barrier integrity, contributing to skin dryness and the development of
290 eczematous skin inflammation. Alternatively, another environmental factor directly related to
291 water hardness, such as alkalinity, may be responsible. The higher the domestic water $CaCO_3$
292 concentration, the higher its alkalinity, and the higher the pH on the skin. An increase in pH on and
293 in the stratum corneum leads to enhanced protease activity, which in turn accelerates the
294 breakdown of corneodesmosomes and reduces lipid lamellae synthesis, all contributing to skin
295 barrier breakdown.[18] This hypothesis is further supported by our finding that the association
296 between water hardness and TEWL risk is more enhanced (albeit not achieving statistical
297 significance) among children who carry a *FLG* mutation skin barrier gene mutation. Our analyses
298 suggested that the effect was not conferred by a differential usage of more protease-containing
299 soaps and shampoos in high water hardness or high chlorine areas.
300 Interactions between $CaCO_3$ and chlorine levels, other chemical water constituents, the skin
301 microflora and stratum corneum may also play a role, and this warrants further research.
302 Unfortunately, UK water companies stopped routinely measuring magnesium levels in 2003, and
303 we were therefore not able to account for this in our analyses.
304 It is interesting to note that the profilaggrin polypeptide encoded by the *FLG* gene possesses a
305 calcium binding domain of unknown function, which is cleaved off when the proprotein is
306 proteolytically processed into functional filaggrin during the biogenesis of the stratum corneum.[19]
307 Moreover, there is a calcium gradient within the living cell layers of the epidermis, whereby
308 increasing calcium concentration is involved in regulating expression of late-differentiation
309 proteins such as filaggrin and in triggering the terminal differentiation process that leads to skin
310 barrier formation.[20] For example, knockout of the skin's calcium sensing receptor leads to failure
311 of epidermal differentiation both *in vitro* and *in vivo*.[21] Although it is not known how
312 environmental sources of calcium influence the physiology of skin barrier formation, in light of the

essential role of this mineral in the process of epidermal differentiation, it is tempting to speculate that the effects we observed may act by perturbation of this mechanism.

Other findings of a potential effect of chlorine are consistent with McNally et al. who reported a correlation between the concentration of chlorine in domestic tap water (comparing the lowest to highest categories of chlorine concentration) and the 1-year prevalence (AOR 1.33, 95% CI 1.04-1.7) and lifetime prevalence of AD (AOR 1.23, 95% CI 1.00-1.52) in children aged 6-11 before the adjustment of potential confounders, but not afterwards.[4] Miyake et al. reported a correlation between high chlorine concentration (<19.8 mg/l compared to >28.0 mg/l) and the lifetime prevalence of AD in children aged 6-12 only, after adjustment for potential confounders (AOR 1.06, 95% CI 1.03-1.10).[5] Interestingly, in this study the chlorine levels were much higher than in the UK, and there was also a strong positive linear trend between the concentration of chlorine and water hardness (Pearson's coefficient 0.57, $p = 0.0001$), whereas we observed a negative trend.

Chlorine is added to domestic water across the UK, leading to ubiquitous exposure and a narrow range of concentrations across the study population, making it more difficult to determine epidemiological effects. We also did not have information on children's exposure to swimming pools, which contain much higher chlorine levels than domestic water and could have an additional detrimental effect on skin barrier function and AD risk. The fact that the high chlorine/low $CaCO_3$ areas had an elevated risk of AD might contribute to explaining why the SWET study was unsuccessful.[8] This used ion-exchange water softeners which use a synthetic polystyrene resin to remove calcium and magnesium ions from household water, replacing them with sodium ions, thus eliminating the hardness. Ion-exchange water softeners have little impact on chlorine levels, however, which requires a charcoal based filter system for complete removal.

In conclusion, domestic water $CaCO_3$ content is an important risk factor for AD development and possibly skin barrier dysfunction during the first three months of life, potentially more in genetically predisposed infants. Whether chlorine also contributes to these issues remains uncertain. We are in the preparation phase of an intervention trial to assess whether installation of a water softening device in high risk children around the time of birth is able to attenuate this risk and whether any additional benefit may be accrued by also reducing chlorine levels.

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Members of the EAT Study Team include:

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Figure 1: Relationship between water total and free chlorine and calcium carbonate content

Figure 2. Relationship between total chlorine and free chlorine levels

Figure 3. Geographical distribution of high/low calcium carbonate and total chlorine levels for all EAT study participants. Each dot represents a participating child's home location.

Figure 4. Relationship between TEWL at 3 months of age and water hardness by filaggrin status for those with and without AD

Figures 5 The influence of water content on TEWL and AD prevalence by filaggrin status. In panel A the navy bars represent those with AD (Categories 1 & 2 combined in Table IV). The orange bars represent the infants with raised TEWL but no AD (Category 3 in Table IV). In panel B the same data as panel A is shown but the AD category is divided into those with raised TEWL (Category 2 in Table IV - navy with orange border) and those with normal TEWL (Category 1 in Table IV - navy). In each column amongst those with raised TEWL (Category 3 in Table IV - orange & Category 2 in Table IV - navy with orange border), the percentage with AD (Category 2 in Table IV - navy with orange border) is given.

Table I: Population demographic by exposure to above and below median water hardness and total chlorine concentrations

		Low $CaCO_3$ Low total Cl N=343	High $CaCO_3$ Low total Cl N=305	Low $CaCO_3$ High total Cl N=294	High $CaCO_3$ High total Cl N=345	p value
Demography						
Sex	Male	182 (53.1)	156 (51.2)	132 (44.9)	172 (49.9)	0.21
	Female	161 (46.9)	149 (48.9)	162 (55.1)	173 (50.1)	
Ethnicity	White	306 (89.2)	260 (85.3)	254 (86.4)	268 (77.7)	**<0.0005**
	Non-White	37 (10.8)	45 (14.8)	40 (13.6)	77 (22.3)	
Home location	Urban	252 (73.5)	220 (72.1)	226 (77.1)	297 (86.3)	**<0.0005**
	Rural	91 (26.5)	85 (27.9)	67 (22.9)	47 (13.7)	
Maternal education (age at completion)	≤16	20 (5.8)	23 (7.5)	15 (5.1)	15 (4.4)	0.51
	17-18	47 (13.7)	45 (14.8)	38 (13.0)	40 (11.6)	
	>18	276 (80.5)	237 (77.7)	240 (81.9)	290 (84.1)	
Family history						
Maternal age (in years)	19-32	158 (46.1)	112 (36.7)	140 (47.8)	135 (39.1)	**0.01**
	33-46	185 (53.9)	193 (63.3)	153 (52.2)	210 (60.9)	
Siblings		214 (62.4)	186 (61.0)	180 (61.2)	222 (64.4)	0.80
Skin variables at 3 months						
Filaggrin mutation		43 (13.9)	34 (12.0)	24 (8.8)	40 (12.2)	0.30
Visible AD		64 (18.7)	85 (27.9)	68 (23.1)	95 (27.6)	**0.02**
SCORAD - infants with AD (median)		7.2	7.5	7.1	9.4	NS
Family atopy status						
Maternal						
AD		126 (36.7)	105 (34.4)	101 (34.5)	113 (32.9)	0.76
Maternal atopy (E, A or HF)		223 (65.0)	195 (63.9)	180 (61.4)	207 (60.2)	0.55
Paternal						
AD		67 (19.5)	69 (22.6)	56 (19.1)	64 (18.6)	0.59
Paternal atopy (E, A or HF)		181 (52.8)	164 (53.8)	166 (56.7)	168 (48.8)	0.26
Parental						
Parental atopy (E, A or HF)		281 (81.9)	257 (84.3)	241 (82.3)	273 (79.4)	0.45
Skin care						

Water softener present in home		7 (2.2)	32 (11.1)	4 (1.4)	17 (5.2)	**<0.0005**
Frequency of bathing	Never or 1/week	55 (16.7)	45 (15.9)	42 (15.4)	52 (16.0)	0.52
	2-4 times/week	129 (39.2)	117 (41.2)	117 (43.0)	156 (48.0)	
	5-6 times/week	38 (11.6)	36 (12.7)	32 (11.8)	26 (8.0)	
	Daily or more	107 (32.5)	86 (30.3)	81 (29.8)	91 (28.0)	
Use of moisturiser	Never or 1/week	154 (46.8)	143 (50.4)	144 (52.9)	124 (38.2)	**0.003**
	2-4 times/week	73 (22.2)	51 (18.0)	51 (18.8)	60 (18.5)	
	5-6 times/week	21 (6.4)	15 (5.3)	15 (5.3)	18 (5.5)	
	Daily or more	81 (24.6)	75 (26.4)	75 (26.4)	123 (37.9)	
Bath products used		264 (80.2)	221 (77.8)	216 (79.4)	260 (80.0)	0.89
Bubble bath used		131 (39.8)	89 (31.3)	84 (30.9)	81 (24.9)	**0.001**
Soap used in bath		31 (9.4)	24 (8.5)	19 (7.0)	36 (11.1)	0.29
Bath emollient used		58 (17.6)	47 (16.6)	45 (16.5)	77 (23.7)	0.33
Shampoo used		112 (34.0)	90 (31.7)	90 (33.1)	96 (29.5)	0.68

Table II: Crude and adjusted odds ratios (95% CI) of visible AD at 3 months

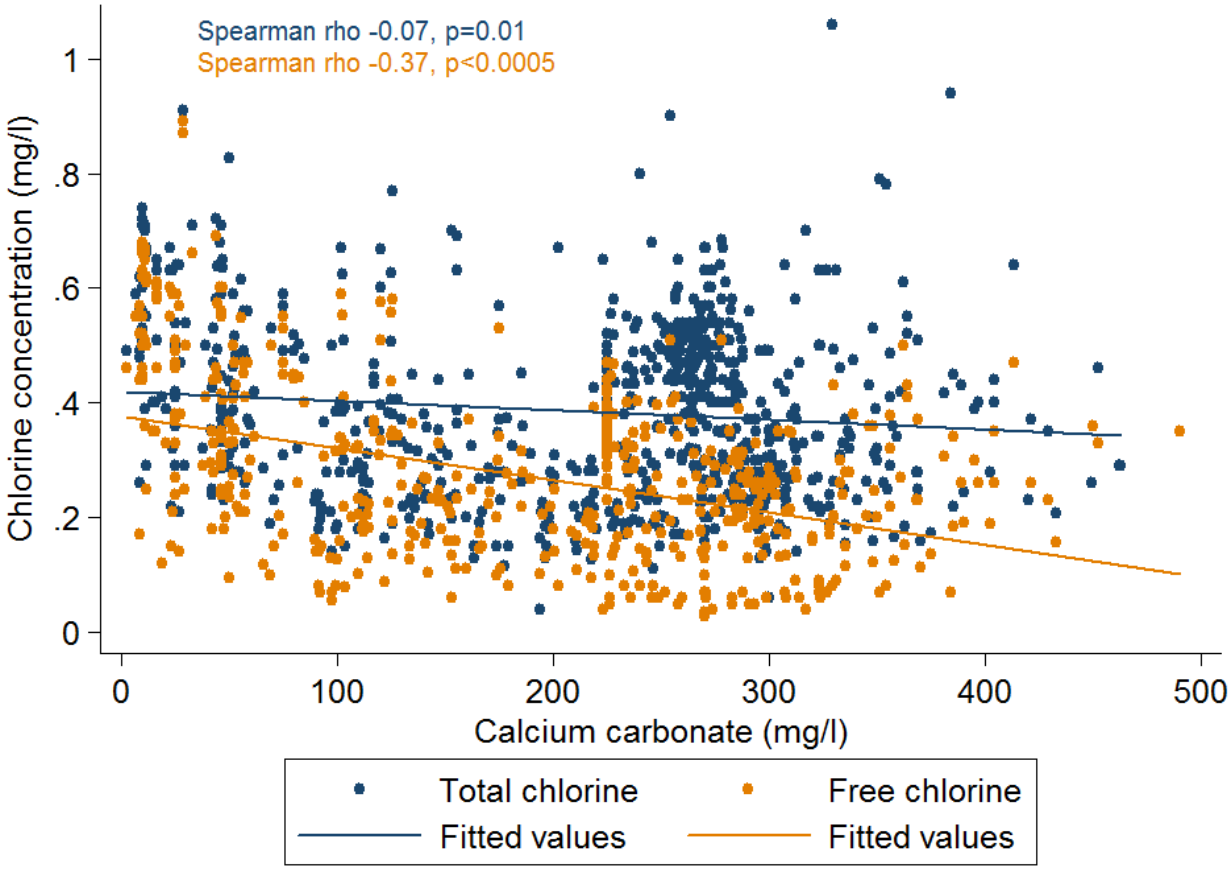
	Model 1 (crude)		Model 2 (adjusted)	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Water content				
Low $CaCO_3$/Low total Cl	1.0 (Baseline)	-	1.0 (Baseline)	-
High $CaCO_3$/Low total Cl	1.68 (1.16-2.44)	**0.006**	1.87 (1.25-2.80)	**0.002**
Low $CaCO_3$/High total Cl	1.31 (0.89-1.93)	0.17	1.46 (0.97-2.21)	**0.07**
High $CaCO_3$/High total Cl	1.66 (1.16-2.38)	**0.006**	1.61 (1.09-2.38)	**0.02**
Filaggrin (mutation present)			3.84 (2.64-5.59)	**<0.0005**
Sex (female)			0.78 (0.59-1.03)	**0.08**
Ethnicity (non-white)			2.12 (1.49-3.02)	**<0.0005**
Maternal age (≥33 years)			1.24 (0.94-1.64)	0.13
Water softener (present)			0.70 (0.35-1.39)	0.31
Home location (rural)			1.06 (0.76-1.49)	0.72

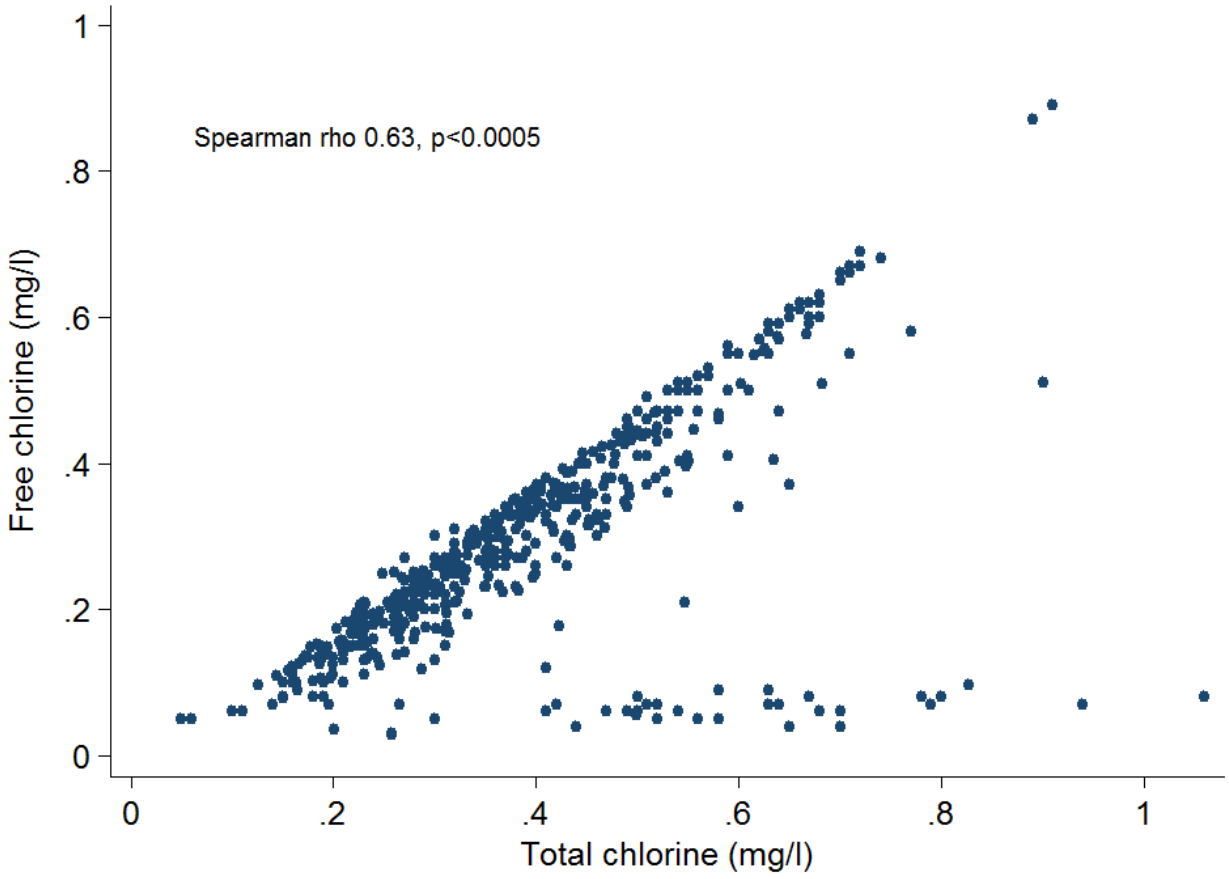
Table III: Crude and adjusted odds ratios (95% CI) for raised TEWL (≥15 g/m^{2}h) at 3 months

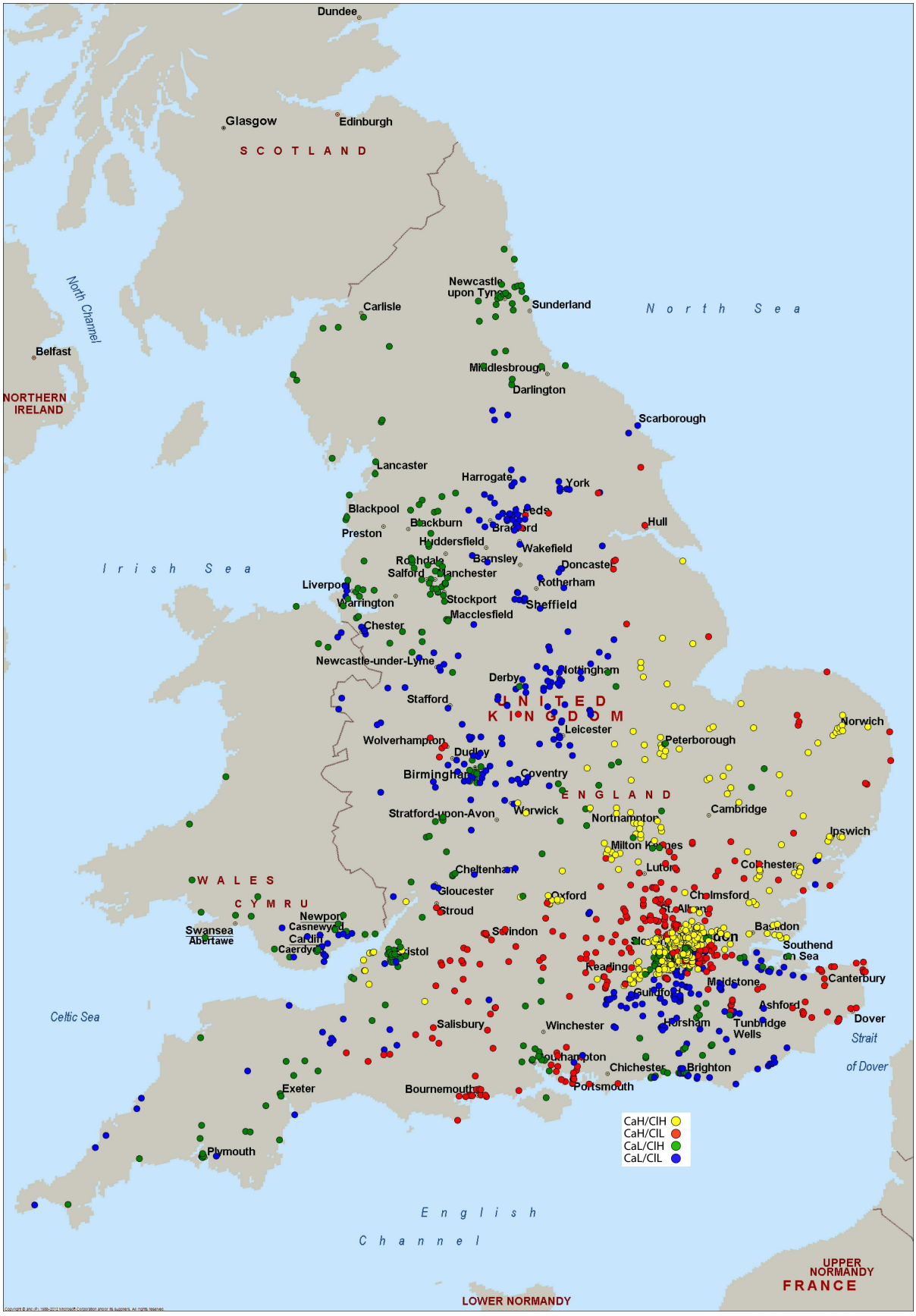
	Model 1 (crude)		Model 2 (adjusted)	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Water content				
Low $CaCO_3$/Low total Cl	1.0 (Baseline)	-	1.0 (Baseline)	-
High $CaCO_3$/Low total Cl	1.11 (0.79-1.55)	0.54	1.22 (0.84-1.77)	0.29
Low $CaCO_3$/High total Cl	1.13 (0.81-1.59)	0.47	1.25 (0.87-1.81)	0.23
High $CaCO_3$/High total Cl	1.33 (0.96-1.83)	0.088	1.35 (0.95-1.81)	0.09
Filaggrin (mutation present)			3.59 (2.48-5.19)	**<0.0005**
Sex (female)			0.68 (0.53-0.88)	**0.003**
Ethnicity (non-white)			2.02 (1.44-2.82)	**<0.0005**
Maternal age (≥33 years)			0.87 (0.67-1.21)	0.28
Water softener (present)			0.50 (0.25-1.00)	0.05
Home location (rural)			0.84 (0.61-1.16)	0.29

Table IV. The influence of water quality on TEWL and AD prevalence, by filaggrin status

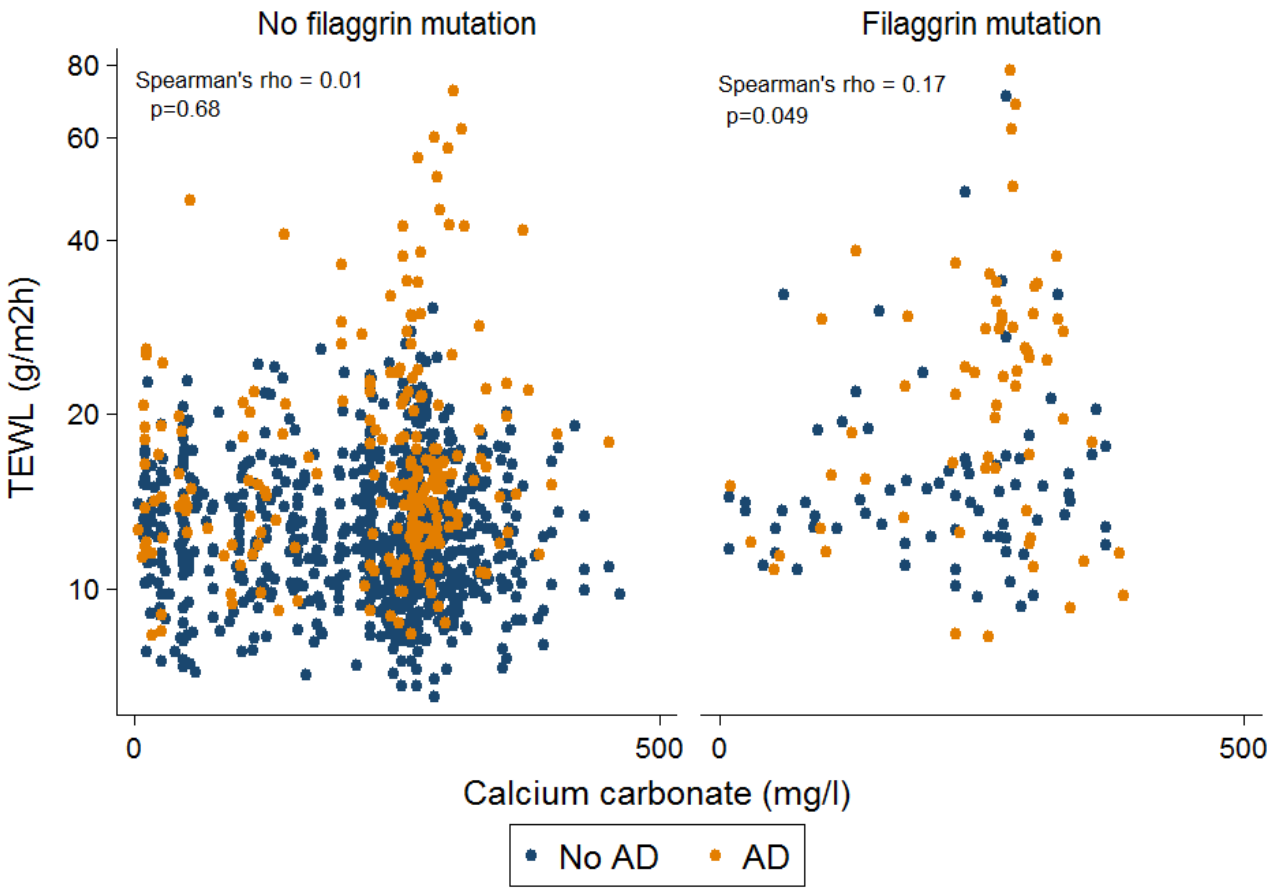
Category	AD	Raised TEWL	No filaggrin mutation				Filaggrin mutation			
			Low $CaCO_3$ Low total Cl	High $CaCO_3$ Low total Cl	Low $CaCO_3$ High total Cl	High $CaCO_3$ High total Cl	Low $CaCO_3$ Low total Cl	High $CaCO_3$ Low total Cl	Low $CaCO_3$ High total Cl	High $CaCO_3$ High total Cl
(1)	**Yes**	No	21 (8%)	32 (13%)	29 (12%)	34 (12%)	5 (12%)	5 (15%)	4 (17%)	3 (8%)
(2)	**Yes**	**Yes**	22 (8%)	29 (12%)	25 (10%)	35 (12%)	10 (23%)	17 (50%)	5 (21%)	18 (45%)
(3)	No	**Yes**	46 (17%)	36 (15%)	51 (21%)	54 (19%)	10 (23%)	5 (15%)	4 (17%)	9 (23%)
(4)	No	No	177 (67%)	150 (61%)	142 (57%)	163 (57%)	18 (42%)	7 (21%)	11 (46%)	10 (25%)
Total			**266**	**247**	**247**	**286**	**43**	**34**	**24**	**40**


Spearman rho -0.07, p=0.01
Spearman rho -0.37, p<0.0005
Chlorine concentration (mg/l)
0
.2
.4
.6
.8
1
0
100
200
300
400
500
Calcium carbonate (mg/l)
Total chlorine
Fitted values
Free chlorine
Fitted values





Calcium carbonate and total chlorine level categories – CaH = high calcium carbonate, ClH = high chlorine, CaL = low calcium carbonate, ClL = low chlorine



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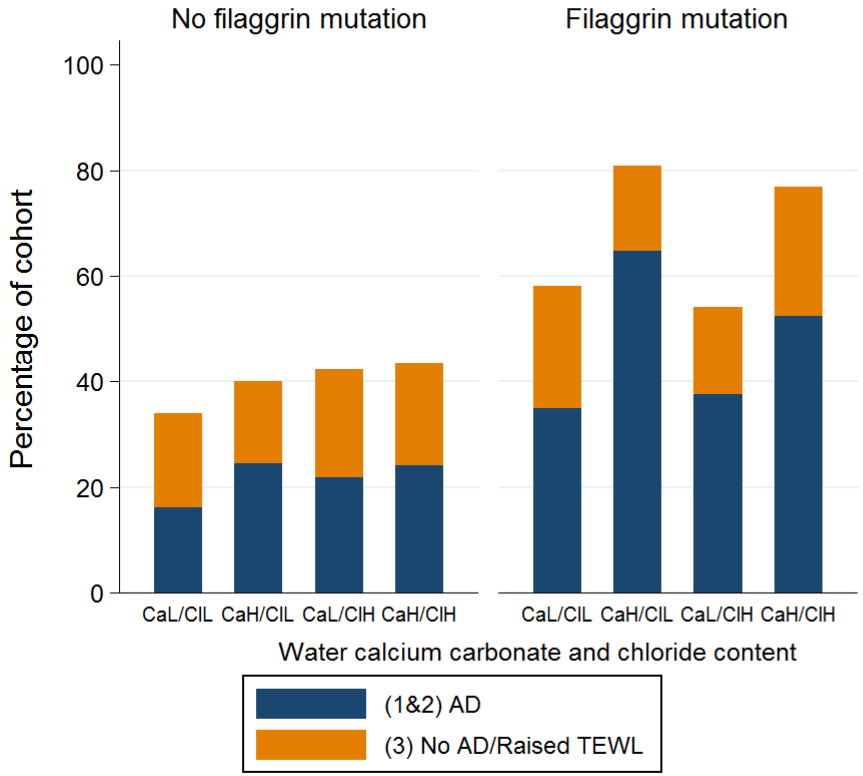

B