











Four childhood atopic dermatitis subtypes identified from trajectory and severity of disease and internally validated in a large UK birth cohort

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Summary

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Funding sources

See Appendix 1 for full details.

Conflicts of interest

A.C. reports personal fees from Novartis, Philips, Sanofi, Stallergenes Greer and Thermo Fisher Scientific, outside the submitted work. K.A. reports consultancy for Target RWE and institutional grant funding from Pfizer, outside the submitted work.

A.D.I. has received consultancy fees from AbbVie, Arena Pharmaceuticals, Benevolent AI, Chugai, Dermavant, Eli Lilly, Genentech, LEO Pharma, Menlo Therapeutics, Novartis, Pfizer, Regeneron, Sanofi and UCB. All others report no conflicts of interest.

K.A. and S.M.L. are joint senior authors.

DOI 10.1111/bjd.19885

Background Atopic dermatitis (AD) disease activity and severity is highly variable during childhood. Early attempts to identify subtypes based on disease trajectory have assessed AD presence over time without incorporating severity.

Objectives To identify childhood AD subtypes from symptom severity and trajectories, and determine associations with genetic risk factors, comorbidities and demographic and environmental variables.

Methods We split data from children in the Avon Longitudinal Study of Parents and Children birth cohort into development and validation sets. To identify subtypes, we ran latent class analyses in the development set on AD symptom reports up to age 14 years. We regressed identified subtypes on nongenetic variables in mutually adjusted, multiply imputed (genetic: unadjusted, complete case) multinomial regression analyses. We repeated analyses in the validation set and report confirmed results.

Results There were 11 866 children who contributed to analyses. We identified one Unaffected/Rare class (66% of children) and four AD subtypes: Severe–Frequent (4%), Moderate–Frequent (7%), Moderate–Declining (11%) and Mild–Intermittent (12%). Symptom patterns within the first two subtypes appeared more homogeneous than the last two. Filaggrin (*FLG*) null mutations, an AD polygenic risk score (PRS), being female, parental AD and comorbid asthma were associated with higher risk for some or all subtypes; *FLG*, AD-PRS and asthma associations were stronger along a subtype gradient arranged by increasing severity and frequency; *FLG* and AD-PRS further differentiated some phenotypes from each other.

Conclusions Considering severity and AD trajectories leads to four well-defined and recognizable subtypes. The differential associations of risk factors among and between subtypes is novel and requires further research.

What's already known about this topic?

- Atopic dermatitis (AD) is a heterogeneous condition in terms of both disease activity and severity.
- Childhood AD phenotypes in previous studies have focused only on disease activity.

What does this study add?

- We incorporate disease severity over time to derive four clinically recognizable AD phenotypes using data-driven methods.
- Disease severity improved over time in all phenotypes (even in those with high probability of activity in late childhood and adolescence).
- Several established risk factors, including genetic associates, were associated with our proposed phenotypes, with most factors more strongly associated with phenotypes reporting the worst symptoms. Fewer factors differentiated between more Frequent and Declining/Intermittent phenotypes.

Atopic dermatitis (AD) (eczema) is characterized by considerable heterogeneity including clinical morphology, disease severity, age at presentation, disease course and comorbidities. Many attempts have been made to address this heterogeneity by identifying AD subtypes, which may improve diagnosis, more accurately estimate clinical prognoses, inform management or better predict treatment efficacy or effectiveness.¹ In older literature AD is largely subtyped into allergic and nonallergic forms; recent evidence suggests that this dichotomization is not clinically useful² because IgE levels or sensitization status alone do not predict symptom resolution or treatment response.³ Recent efforts using longitudinal cohorts have attempted to identify AD subtypes from trajectories of childhood disease activity^{4–6} or patterns of atopic disease overall.^{7–10} However, none of these categorizations account for disease severity, which is the primary predictor of quality of life, sleep disruption, comorbidities including mental health outcomes, and need for systemic treatments.^{11,12} Identifying factors early in the disease course that could differentiate between mild but persistent cases and severe but resolving cases is important both for understanding aetiology and outcomes and for informing patient prognoses and treatment options.

In this paper, we aim to identify AD subtypes based on trajectories of flexural dermatitis in children related to both presence and severity of disease, and quantify their relationships with known and suspected AD risk factors. We note that subtypes based on disease trajectories over time are not the same as directly observed phenotypes.¹³ However, as the term ‘phenotypes’ is commonly used to describe AD subtypes, we use this nomenclature in this paper.

Methods

Study population

We used data from the Avon Longitudinal Study of Parents and Children (ALSPAC).^{14,15} Pregnant women resident in Avon, UK with expected dates of delivery between 1 April 1991 and 31 December 1992 were invited to take part and 14 541 pregnancies were enrolled initially. Of these, there were 14 676 fetuses, resulting in 14 062 live births and 13 988 children who were alive at 1 year of age, 96% of

whom are of white ethnicity. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool.¹⁶

Atopic dermatitis phenotype determination

AD presence was collected from questionnaires at 11 ages between 6 and 166 months (13.8 years). Mothers reported whether their children experienced flexural dermatitis (FD) via the International Study of Asthma and Allergies in Childhood (ISAAC)-validated question:¹⁷ ‘Has your child had an itchy, dry skin rash in the joints and creases of his body (e.g. behind the knees, elbows, under the arms) in the past year?’ Participants who responded ‘yes’ were followed up with an assessment of self-rated severity, ‘How bad was this?’ with options: ‘no problem’, ‘mild’, ‘quite bad’ and ‘very bad’. From these two responses we derived a five-category severity variable. Participants who did not complete the questionnaire or did not answer these questions were coded as ‘missing’.

Other variables

Family history of atopic diseases, environmental exposures and social class were taken from parental questionnaires during pregnancy. Breastfeeding, comorbidities and siblings were taken from questionnaires at various ages (for definitions see Methods S1 in the Supporting Information).

Genetic polymorphisms

DNA was obtained from blood samples using standard extraction techniques.¹⁸ In children with genome-wide genotyping data, we created an AD polygenic risk score (PRS) using the 25 single-nucleotide polymorphisms (SNPs) previously identified as associated with AD ($P < 5 \times 10^{-8}$),¹⁹ where dosage genotypes were extracted and weighted by the (risk-increasing) genome-wide association study beta coefficient, summed and standardized to 1 SD.

In children with Filaggrin (*FLG*) loss-of-function genotyping data, null mutations in any of R2447X, S3247X, 2282del4 or

R501X were coded as FLG-present, and FLG-absent was coded otherwise.

Statistical methods

We randomly split the ALSPAC cohort into two equally sized groups, one (development cohort) for identifying AD phenotypes and associated characteristics, and the other (validation cohort) for internally validating the identified phenotypes. In both groups we excluded individuals with fewer than two of the 11 requested FD reports.

Phenotype identification

We identified phenotypes in the development group using latent class analysis (LCA). FD variables were modelled as joint categorical outcomes in children with and without FD symptoms to allow the LCA to identify a negative control phenotype without AD in addition to AD phenotypes.

We ran models with three to eight classes and compared various model fit statistics (see Methods S1), estimated class sizes and clinical interpretation (by clinicians with AD management expertise) to determine the best classification. From the best fitting model, we estimated individuals' posterior class probabilities and identified their most likely phenotype as the class with the highest probability. We drew heatmaps for each phenotype showing individuals' symptom severity across ages, with a dendrogram to visualize common patterns.

Association of variables with phenotypes

We explored three methods²⁰ to account for phenotype uncertainty (LCA class probabilities) in regression models (Figure S1; see Supporting Information). We chose the method that ignores uncertainty and treats phenotypes as known quantities, and report 99% confidence intervals (CIs) to correct for this method's potential for overprecision.

We regressed sex, family history of AD, asthma, hay fever, parental education, pet cats and dogs, maternal smoking, breastfeeding (ever and duration), comorbid asthma and older siblings in the household on phenotypes in unadjusted and mutually adjusted multinomial regressions. These factors have been previously associated with AD.²¹ In the mutually adjusted model, we excluded 'ever breastfed' to avoid collinearity with breastfeeding duration and additionally adjusted for FLG null mutations to investigate independence of family history risk factors. We estimated crude genetic risk by regressing FLG, SNPs and the PRS on AD phenotypes in unadjusted multinomial regressions, treating SNP and PRS effects as linear.

Phenotype validation

To assess internal validity, we ran the final LCA model in the validation cohort and visually compared resulting phenotypes with those from the development cohort. We repeated the multinomial regressions and qualitatively compared results with development cohort results.

Missing data handling

We assumed missing FD reports were at random given observed reports and estimated the LCA using full information maximum likelihood (FIML). We checked the solution for sensitivity to departures from this assumption (see Methods S1). We assumed missing nongenetic regression variables were at random given observed variables, social class and comorbid hay fever, and used the multivariate imputation by the chained equations procedure²² to impute them. We ran regression models on 10 imputed datasets and combined results using Rubin's rules.²³ We performed complete case genetic analyses, assuming genetic data missingness was completely at random.

Software

LCAs were run in MPlus v8.2 (Muthén & Muthén, Los Angeles, CA, USA) and all other analyses in Stata v16.0 (StataCorp, College Station, TX, USA) and R v3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical approval

Ethical approval for this study was obtained from the ALSPAC Ethics and Law Committee (B2510) and the London School of Hygiene and Tropical Medicine Institutional Review Board (EPNCZK46).

Results

Sample characteristics

There were 13 972 children born as singletons or twins who were eligible for inclusion. We randomly allocated half ($n = 6986$) to each of the development and validation cohorts. Within each cohort, 85% had data on FD on two or more questionnaires (development: 5927; validation: 5939) and were included in the analyses. In the development and validation cohorts, respectively, just under half were female (48%/49%); 7.7%/7.4% carried a FLG null mutation and 28%/29%, 19%/18% and 43%/43% had at least one parent with a history of AD, asthma and hay fever, respectively. All characteristics were balanced between cohorts (Table 1). Reports of FD activity decreased over time. Generally, the activity peaked at 18 months while the lowest activity was observed at age 166 months, similar to the corresponding response rates (92%/91% at 18 months and 57%/56% at 166 months) (Table S1; see Supporting Information).

Atopic dermatitis phenotypes from infancy to adolescence

From data in the development cohort, we determined the optimal number of phenotypes from LCA models to be five, based on the Bayesian information criterion (Results S1 and Figure S2; see Supporting Information) and clinical interpretability (Figure S3; see Supporting Information). Children

Table 1 ALSPAC study characteristics

	Development cohort (N = 5927), n (%)	Validation cohort (N = 5939), n (%)
Sex		
Male	3099 (52)	3031 (51)
Female	2828 (48)	2908 (49)
Social class ^a		
I	693 (12)	677 (11)
II	2031 (34)	1999 (34)
III(n)	1203 (20)	1219 (21)
III(m)	387 (6.5)	414 (7)
IV	113 (1.9)	125 (2.1)
V	18 (0.3)	7 (0.1)
Missing	1482 (25)	1498 (25)
At least one parent with university degree		
No	4393 (74)	4420 (74)
Yes	1288 (22)	1238 (21)
Missing	246 (4.1)	281 (4.7)
Parental AD		
No	2563 (43)	2557 (43)
Yes	1678 (28)	1700 (29)
Missing	1686 (28)	1682 (28)
Parental asthma		
No	2967 (50)	3041 (51)
Yes	1123 (19)	1059 (18)
Missing	1837 (31)	1839 (31)
Parental hay fever		
No	1826 (31)	1812 (31)
Yes	2534 (43)	2549 (43)
Missing	1567 (26)	1578 (27)
Any older siblings		
No	433 (7.3)	454 (7.6)
Yes	2105 (35)	1962 (33)
Missing	3389 (57)	3523 (59)
Pet cat during pregnancy		
No	4011 (68)	3965 (67)
Yes	1723 (29)	1778 (30)
Missing	193 (3.2)	196 (3.3)
Pet dog during pregnancy		
No	4353 (73)	4358 (73)
Yes	1381 (23)	1385 (23)
Missing	193 (3.2)	196 (3.3)
Mum smoked during first 3 months of pregnancy		
No	4458 (75)	4408 (74)
Yes	1307 (22)	1361 (23)
Missing	162 (2.7)	170 (2.9)
Breastfeeding		
Never	1409 (24)	1349 (23)
< 3 months	1214 (20)	1240 (21)
3–5 months	855 (14)	908 (15)
6 months +	1857 (31)	1802 (30)
missing	592 (10)	640 (11)
Asthma by 7.5 years		
No	3242 (55)	3188 (54)
Yes	816 (14)	829 (14)
Missing	1869 (31)	1922 (32)
Hay fever by 10.5 years		
No	2877 (48)	2856 (48)
Yes	784 (13)	732 (12)
Missing	2266 (38)	2351 (40)

(continued)

Table 1 (continued)

	Development cohort (N = 5927), n (%)	Validation cohort (N = 5939), n (%)
FLG null mutation		
No	3985 (67)	3891 (66)
Yes	456 (7.7)	441 (7.4)
Missing	1486 (25)	1607 (27)
Polygenic risk score (standardized)		
Median (IQR)	−0.13 (−0.78 to 0.63)	−0.10 (−0.74 to 0.62)
Missing	1971 (33)	2113 (36)

^aI, professional occupations; II, managerial and technical occupations; III(n), skilled occupations–nonmanual; III(m), skilled occupations–manual; IV, partly skilled occupations; V, unskilled occupations. AD, atopic dermatitis; ALSPAC, Avon Longitudinal Study of Parents and Children; IQR, interquartile range.

were generally clearly classified into phenotypes, with high probability of being in their most likely phenotype (mean 74–90%) and low probabilities of being in the other four (mean 0.1–10%) (Figure S4 and Table S2; see Supporting Information). There was no evidence the LCA solution was sensitive to missing data patterns and it was consistent across the development and validation cohorts (Figures S5, S6 and Tables S2, S3; see Supporting Information).

Based on reported severity and duration of FD, we labelled the classes: Unaffected/Rare, to which 66% of children were assigned with highest probability, Severe–Frequent AD (4%), Moderate–Frequent AD (7%), Moderate–Declining AD (11%) and Mild–Intermittent AD (12%). Children in a particular phenotype did not always report the same symptoms as each other, but heatmaps in children with complete data showed similar patterns with more consistency among individuals in each Frequent phenotype than among individuals in the Declining and Intermittent phenotypes (Figure S7; see Supporting Information).

Figure 1 illustrates the distributions of FD severity at each age and how they changed over time for each phenotype. Children in the Severe–Frequent phenotype almost always reported FD and rarely characterized it as ‘no problem’. For example, at 6 months of age, FD was present and rated ‘very bad’ or ‘quite bad’ in 29% of the group, rising to 49% at 18 months, although heterogeneity was present within this and other subtypes. Children in the Moderate–Frequent phenotype also almost always reported FD, although they usually rated it less severely than children with Severe–Frequent AD and occasionally rated it as ‘no problem’. Children with Moderate–Declining AD reported symptoms similar to those with Moderate–Frequent AD up to age 2.5 years, but after this age, their likelihood of FD declined. Children with Mild–Intermittent AD more often reported absence of FD than presence, and when present usually rated it ‘mild’ or ‘no problem’. Up to 9% of the Unaffected/Rare group reported FD before age

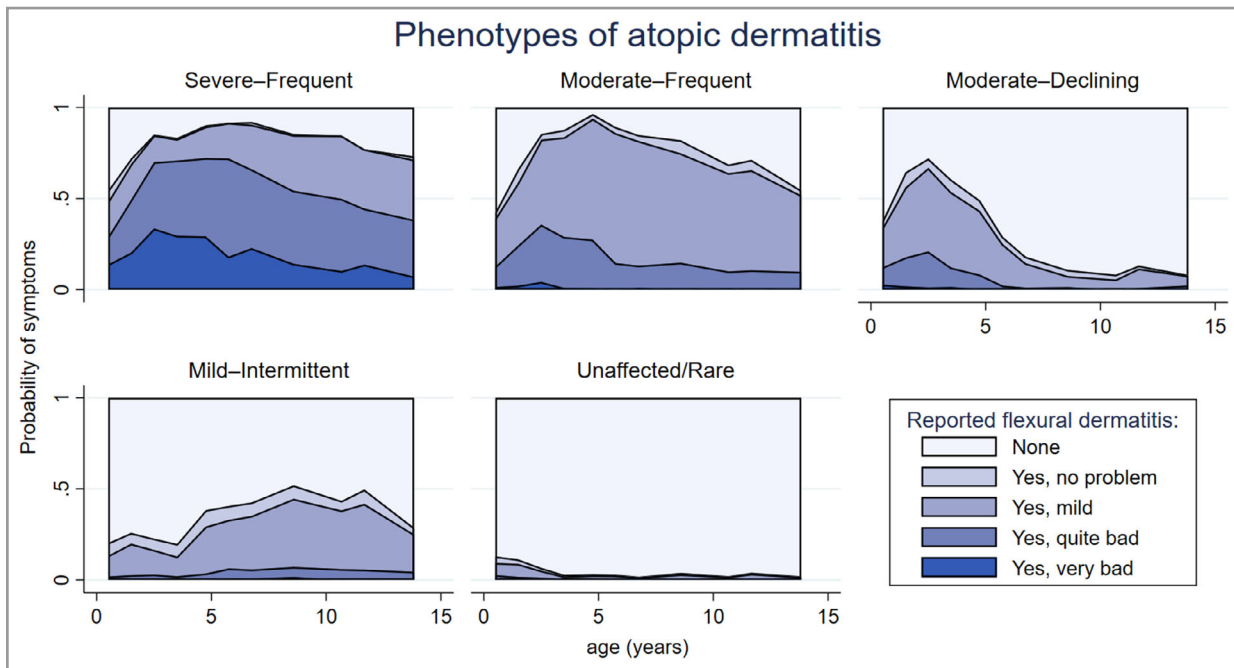


Figure 1 Atopic dermatitis (AD) phenotypes. Five phenotypes of AD (including an Unaffected/Rare phenotype without AD) identified by latent class analysis on 5927 ALSPAC children whose mothers responded to at least two questionnaires between the ages of 6 months and 14 years. Probabilities of flexural dermatitis and its severity are shown by age for children assigned to each phenotype (missing reports not indicated): Severe-Frequent, $n = 230$ (3.9%); Moderate-Frequent, $n = 408$ (6.9%); Moderate-Declining, $n = 676$ (11%); Mild-Intermittent, $n = 684$ (12%); Unaffected/Rare, $n = 3929$ (66%).

2.5 years, mostly 'mild', but rarely reported it ($\leq 3\%$ of the time) thereafter. Proportions of individuals with missing symptoms are shown in Figure S8 (see Supporting Information).

The distribution of children among phenotypes in the validation cohort was similar to the distribution in the development cohort, except that relatively fewer children were assigned to the Mild-Intermittent phenotype (8% vs. 12%) and more to Moderate-Frequent (9% vs. 7%) and Moderate-Declining (13% vs. 11%) phenotypes.

Variables associated with phenotypes

Table 2 shows the distributions of clinical, demographic and genetic variables used in the regression analyses by most likely phenotype.

We used the Unaffected/Rare phenotype as the reference group in multiple imputation analyses (Table S4; see Supporting Information). The unadjusted and adjusted estimates were similar, except for parental asthma, hay fever and education: each unadjusted estimate showed a higher risk for all but Mild-Intermittent AD, which attenuated after adjustment. The adjusted estimates in the development and validation cohorts were also similar (Figure 2).

After adjusting for the other variables and FLG, we found that comorbid asthma, parental AD and sex were associated with AD phenotype (all $P < 0.0001$). Children with asthma were three times more likely to be in the Severe-Frequent

group [relative risk ratio (RRR) 3.19, 99% CI (2.02–5.06)], twice as likely to be Moderate-Frequent [RRR 2.07 (1.45–2.97)], and 1.43 (1.03–1.99) and 1.44 (1.07–1.94) times as likely to be Moderate-Declining and Mild-Intermittent, respectively. There was strong evidence ($P = 0.0001$) for this differential association with each phenotype. Children with a family history of AD had twice the risk of Severe-Frequent [RRR 1.88 (1.23–2.87)] or Moderate-Frequent [RRR 1.95 (1.43–2.66)] disease, 1.66 (1.30–2.12) times the risk of Moderate-Declining disease and 1.40 (1.08–1.81) times the risk of Mild-Intermittent disease, but there was no evidence that these associations were truly differential ($P = 0.0987$). Girls were more likely than boys to have Mild-Intermittent AD [RRR 1.67 (1.35–2.08)]. There was no evidence that the other variables listed in Table 2 were independently associated with AD phenotypes.

The patterns of associations above were replicated in the validation cohort with similar estimates and CIs, except that differential parental AD RRRs were detectable across phenotypes ($P = 0.0039$). Additionally, in the validation cohort there was borderline evidence ($P = 0.0063$) that maternal smoking was associated with phenotypes after adjusting for other variables; there was similar evidence ($P = 0.0135$) for the same association in the development cohort. Children whose mothers smoked were less likely to have Moderate-Frequent AD in the development cohort and less likely to have Moderate-Declining AD in the validation cohort; the other associations were inconclusive.

Table 2 ALSPAC development cohort characteristics across each 'most likely' atopic dermatitis phenotype^a

	Severe–Frequent	Moderate–Frequent	Moderate–Declining	Mild–Intermittent	Unaffected/Rare
Phenotype, N (%)	230 (3.9)	408 (6.9)	676 (11)	684 (12)	3929 (66)
Sex					
Male	115 (3.7)	202 (6.5)	366 (12)	287 (9.3)	2129 (69)
Female	115 (4.1)	206 (7.3)	310 (11)	397 (14)	1800 (64)
At least one parent with university degree					
No	158 (3.6)	286 (6.5)	480 (11)	502 (11)	2967 (68)
Yes	63 (4.9)	113 (8.8)	170 (13)	164 (13)	778 (60)
Missing	9 (3.7)	9 (3.7)	26 (11)	18 (7.3)	184 (75)
Parental atopic dermatitis					
No	73 (2.8)	138 (5.4)	278 (11)	296 (12)	1778 (69)
Yes	95 (5.7)	170 (10)	249 (15)	218 (13)	946 (56)
Missing	62 (3.7)	100 (5.9)	149 (8.8)	170 (10)	1205 (71)
Parental asthma					
No	99 (3.3)	192 (6.5)	344 (12)	364 (12)	1968 (66)
Yes	61 (5.4)	99 (8.8)	155 (14)	124 (11)	684 (61)
Missing	70 (3.8)	117 (6.4)	177 (9.6)	196 (11)	1277 (70)
Parental hay fever					
No	56 (3.1)	106 (5.8)	198 (11)	232 (13)	1234 (68)
Yes	122 (4.8)	207 (8.2)	325 (13)	287 (11)	1593 (63)
Missing	52 (3.3)	95 (6.1)	153 (9.8)	165 (11)	1102 (70)
Any older siblings					
No	17 (3.9)	38 (8.8)	62 (14)	49 (11)	267 (62)
Yes	86 (4.1)	168 (8)	248 (12)	285 (14)	1318 (63)
Missing	127 (3.7)	202 (6)	366 (11)	350 (10)	2344 (69)
Pet cat during pregnancy					
No	156 (3.9)	277 (6.9)	453 (11)	466 (12)	2659 (66)
Yes	67 (3.9)	123 (7.1)	205 (12)	205 (12)	1123 (65)
Missing	7 (3.6)	8 (4.1)	18 (9.3)	13 (6.7)	147 (76)
Pet dog during pregnancy					
No	168 (3.9)	327 (7.5)	505 (12)	524 (12)	2829 (65)
Yes	55 (4)	73 (5.3)	153 (11)	147 (11)	953 (69)
Missing	7 (3.6)	8 (4.1)	18 (9.3)	13 (6.7)	147 (76)
Mum smoked during first 3 months of pregnancy					
No	164 (3.7)	337 (7.6)	517 (12)	535 (12)	2905 (65)
Yes	59 (4.5)	63 (4.8)	146 (11)	131 (10)	908 (69)
Missing	7 (4.3)	8 (4.9)	13 (8)	18 (11)	116 (72)
Breastfeeding					
Never	49 (3.5)	81 (5.7)	139 (9.9)	160 (11)	980 (70)
< 3 months	40 (3.3)	76 (6.3)	160 (13)	128 (11)	810 (67)
3–5 months	27 (3.2)	64 (7.5)	98 (11)	109 (13)	557 (65)
6 months+	86 (4.6)	157 (8.5)	231 (12)	238 (13)	1145 (62)
Missing	28 (4.7)	30 (5.1)	48 (8.1)	49 (8.3)	437 (74)
Asthma by 7.5 years					
No	96 (3)	211 (6.5)	360 (11)	417 (13)	2158 (67)
Yes	62 (7.6)	90 (11)	106 (13)	119 (15)	439 (54)
Missing	72 (3.9)	107 (5.7)	210 (11)	148 (7.9)	1332 (71)
FLG null mutation					
No	121 (3)	261 (6.5)	461 (12)	481 (12)	2661 (67)
Yes	55 (12)	55 (12)	66 (14)	58 (13)	222 (49)
Missing	54 (3.6)	92 (6.2)	149 (10)	145 (9.8)	1046 (70)
Polygenic risk score (standardized)					
Median (IQR)	0.39 (–0.38 to 1.47)	0.18 (–0.73 to 0.98)	–0.03 (–0.74 to 0.76)	–0.02 (–0.73 to 0.64)	–0.22 (–0.82 to 0.54)
Missing ^b	73 (32)	125 (31)	200 (30)	189 (28)	1384 (35)

^aAll values are n (%) unless otherwise noted. ^bMissing polygenic risk scores are tabulated within phenotype, while missingness in other variables is tabulated across phenotypes. ALSPAC, Avon Longitudinal Study of Parents and Children; IQR, interquartile range.

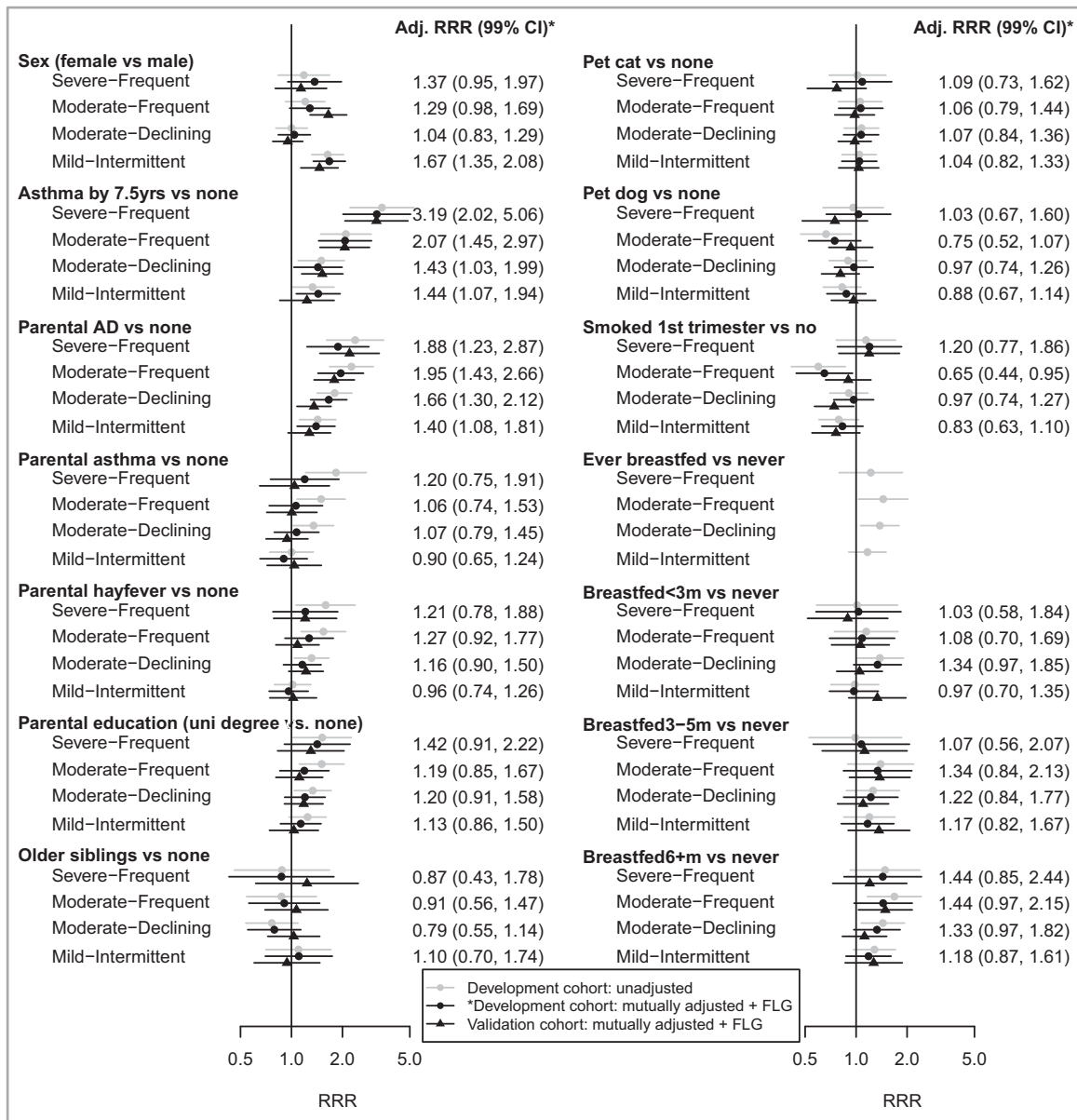


Figure 2 Associations of demographic and clinical characteristics with atopic dermatitis phenotypes. Unadjusted and mutually adjusted phenotype associations in the development cohort (grey and black circles) and adjusted associations in the validation cohort (black triangles). All adjusted associations include additional adjustment for FLG null mutations. Stated effects and confidence intervals are for adjusted associations in the development cohort. AD, atopic dermatitis; Adj, adjusted; RRR, relative risk ratio; CI, confidence interval.

Genetic phenotype associates

Of children included in this study, 8773 (74%) had FLG genotype data and 7782 (66%) had available genome-wide genotype data.

FLG loss-of-function mutations

FLG was strongly associated with AD phenotypes ($P = 4.26 \times 10^{-32}$), with strong evidence of higher risks for more severe and frequently active phenotypes ($P = 1.23 \times 10^{-10}$). Children with FLG mutations were more

than four times more likely to have Severe-Frequent AD [RRR 4.32 (3.07–6.07)], more than twice as likely to have Moderate-Frequent AD [RRR 2.41 (1.81–3.22)], 1.80 (1.39–2.34) times more likely to have Moderate-Declining AD and 1.52 (1.13–2.04) times more likely to have Mild-Intermittent AD than children without FLG mutations (Table 3).

Standardized genetic score

The PRS was strongly associated with AD phenotypes ($P = 1.22 \times 10^{-28}$), with strong evidence of higher risks for more severe and frequently active phenotypes

($P = 8.60 \times 10^{-11}$). A score increase of 1 SD was associated with a relative risk increase of: 1.67 (1.45–1.93) for Severe–Frequent, 1.37 (1.23–1.52) for Moderate–Frequent, 1.17 (1.07–1.27) for Moderate–Declining and 1.13 (1.02–1.24) for Mild–Intermittent AD. Removing rs11205006 from the score, which tags the *FLG* locus, and the remainder of the chromosome 1 SNPs made little difference to these associations (Table S5; see Supporting Information).

Single-nucleotide polymorphisms

Eight SNPs on chromosomes 1, 6, 11, 14 and 19 were associated with AD phenotypes ($P = 8.63 \times 10^{-10}$ to 1.19×10^{-2} ; Table 3). Of these, five were associated with Severe–Frequent AD (RRR range 1.31–2.99), six with Moderate–Frequent (RRR range 1.17–2.07) and one with Moderate–Declining

(RRR 1.96). There was no evidence that any single SNP was associated with Mild–Intermittent AD.

Association differences between atopic dermatitis phenotypes

We explored whether any of the variables we investigated could distinguish between the four AD phenotypes. In six post-hoc Bonferroni-corrected pairwise comparisons from the multinomial regression models, we found evidence that the relative risk associated with asthma, sex, *FLG* null mutations and the AD-PRS differed among some phenotype pairs (Table S6; see Supporting Information). In particular, the AD-PRS and *FLG* were able to discriminate between four of six pairs showing a significantly higher relative risk in the more severe and/or frequently affected phenotypes. The remaining comparisons were

Table 3 Associations between genetic profiles and atopic dermatitis phenotypes in ALSPAC children

	Chr	Nearest gene	Global P-value ^b	Severe–Frequent, ^a N = 305	Moderate–Frequent, ^a N = 634	Moderate–Declining, ^a N = 1008	Mild–Intermittent, ^a N = 845	Equality P-value ^c
				RRR (99% CI)	RRR (99% CI)	RRR (99% CI)	RRR (99% CI)	
FLG null mutation			4.26E-32	4.32 (3.07–6.07)	2.41 (1.81–3.22)	1.80 (1.39–2.34)	1.52 (1.13–2.04)	1.23E-10
Polygenic risk score			1.22E-28	1.67 (1.45–1.93)	1.37 (1.23–1.52)	1.17 (1.07–1.27)	1.13 (1.02–1.24)	8.60E-11
Individual SNP associations								
rs11205006_a	1	LCE5A, FLG-ASI	9.94E-07	1.55 (1.24–1.93)	1.22 (1.03–1.44)	1.11 (0.97–1.28)	1.10 (0.95–1.28)	3.20E-03
rs2228145_c	1	IL6R	3.69E-04	1.31 (1.06–1.63)	1.15 (0.99–1.34)	0.95 (0.83–1.08)	0.93 (0.81–1.07)	1.49E-04
rs61813875_g	1	LCE3E, CRCT1	8.63E-10	2.99 (1.79–4.98)	2.07 (1.34–3.19)	1.96 (1.36–2.84)	1.33 (0.84–2.09)	9.82E-03
rs1057258_c	2	INPP5D	5.81E-02	1.21 (0.90–1.63)	0.99 (0.81–1.21)	0.97 (0.82–1.14)	0.86 (0.72–1.01)	4.81E-02
rs112111458_a	2	CD207	6.68E-02	1.18 (0.85–1.65)	1.00 (0.80–1.25)	1.17 (0.96–1.42)	0.91 (0.75–1.10)	4.42E-02
rs13015714_g	2	IL18R1	5.40E-02	1.07 (0.83–1.38)	1.23 (1.03–1.47)	1.06 (0.92–1.24)	1.02 (0.87–1.20)	1.74E-01
rs17389644_a	4	IL21	9.94E-02	1.17 (0.91–1.51)	1.07 (0.89–1.29)	1.15 (0.99–1.33)	1.02 (0.87–1.21)	4.36E-01
rs10214237_t	5	IL7R	3.01E-01	0.96 (0.75–1.21)	1.06 (0.89–1.26)	1.01 (0.88–1.17)	1.13 (0.96–1.32)	3.66E-01
rs2897442_c	5	KIF3A	4.78E-02	1.29 (1.02–1.63)	1.11 (0.93–1.32)	0.99 (0.86–1.15)	0.99 (0.85–1.16)	4.02E-02
rs12153855_t	6	TNXB	8.75E-03	1.04 (0.72–1.50)	1.42 (1.06–1.91)	1.07 (0.86–1.33)	1.22 (0.96–1.56)	1.32E-01
rs6473227_c	8	ZBTB10	2.49E-01	1.12 (0.90–1.40)	1.09 (0.93–1.28)	1.07 (0.94–1.22)	1.06 (0.93–1.22)	9.47E-01
rs10995251_c	10	ZNF365	5.82E-01	0.99 (0.79–1.23)	0.99 (0.84–1.16)	1.08 (0.95–1.24)	1.03 (0.89–1.18)	5.87E-01
rs12295535_t	11	PRR5L	8.22E-03	1.55 (0.92–2.61)	1.21 (0.80–1.84)	1.37 (0.99–1.91)	0.78 (0.50–1.22)	1.83E-02
rs479844_g	11	OVOL1	1.17E-05	1.51 (1.21–1.90)	1.17 (1.00–1.37)	1.06 (0.93–1.21)	1.06 (0.92–1.22)	1.34E-03
rs7127307_t	11	ETS1	5.28E-01	1.05 (0.84–1.30)	1.07 (0.92–1.25)	1.00 (0.88–1.13)	1.07 (0.94–1.23)	6.39E-01
rs7927894_t	11	C11orf30	2.48E-02	1.21 (0.98–1.50)	1.09 (0.94–1.28)	0.96 (0.84–1.09)	1.10 (0.96–1.26)	3.74E-02
rs2227483_t	12	IL22	9.80E-01	1.03 (0.83–1.29)	1.01 (0.86–1.18)	1.00 (0.88–1.14)	1.03 (0.90–1.18)	9.75E-01
rs2143950_t	14	PPP2R3C	1.19E-02	1.24 (0.95–1.62)	1.26 (1.04–1.53)	1.01 (0.86–1.20)	1.06 (0.89–1.27)	5.74E-02
rs7146581_c	14	TRAF3	1.83E-01	1.09 (0.84–1.42)	1.16 (0.96–1.40)	1.09 (0.94–1.27)	1.04 (0.88–1.22)	6.78E-01
rs2041733_t	16	CLEC16A	4.41E-01	0.98 (0.79–1.22)	1.12 (0.96–1.30)	1.00 (0.88–1.14)	1.03 (0.90–1.18)	4.20E-01
rs11657987_t	17	PGS1	9.36E-02	1.22 (0.99–1.51)	1.09 (0.94–1.27)	1.02 (0.90–1.15)	1.06 (0.92–1.21)	2.44E-01
rs16948048_g	17	ZNF652	7.69E-02	1.18 (0.95–1.46)	0.92 (0.79–1.08)	0.98 (0.86–1.11)	0.92 (0.80–1.06)	5.50E-02
rs17881320_t	17	STAT3	2.25E-01	1.30 (0.91–1.86)	1.12 (0.85–1.47)	1.13 (0.90–1.41)	1.09 (0.86–1.39)	7.37E-01
rs2164983_a	19	ACTL9	1.26E-05	1.34 (1.02–1.77)	1.40 (1.15–1.70)	1.16 (0.98–1.37)	1.18 (0.99–1.42)	1.49E-01
rs6010620_g	20	RTEL1	9.39E-02	1.14 (0.88–1.47)	1.17 (0.97–1.41)	1.05 (0.90–1.21)	1.11 (0.94–1.30)	6.05E-01

^aCompared with the Unaffected/Rare population, n = 4990; ^bNull hypothesis: RRR1 = RRR2 = RRR3 = RRR4 = 0; ^cNull hypothesis: RRR1 = RRR2 = RRR3 = RRR4. ALSPAC, Avon Longitudinal Study of Parents and Children; *FLG*, Filaggrin gene; SNP, single-nucleotide polymorphism; Chr, chromosome; RRR, relative risk ratio; CI, confidence interval.

inconclusive, generally producing CIs consistent with important effects in both directions.

Discussion

We identified four phenotypes of AD based on disease severity and trajectories during childhood: Severe–Frequent, Moderate–Frequent, Moderate–Declining and Mild–Intermittent disease. Healthcare providers and patients might be reassured that in all subgroups, the probability of reporting ‘quite bad’ or ‘very bad’ symptoms declined with age (with evidence of within-group heterogeneity). Associations with some established risk factors (FLG null mutations, AD-PRS, family history of AD) and comorbidities (asthma) were present in all phenotypes with evidence of a gradient of association from more severe to less severe phenotypes. AD-PRS and FLG mutations were further able to differentiate many of our phenotypes from each other, highlighting the need for increased understanding of drivers of disease severity/activity throughout life in people with AD, including the role of gene–environment interactions.

Our findings add novel information about patterns of disease severity over time, and are consistent with prior cross-sectional studies on disease severity and longitudinal studies focused on disease activity alone. While disease activity (i.e. the presence of any symptoms at a particular age) and disease severity (i.e. the impact of those symptoms) are sometimes correlated, they are separate concepts, and it is important to differentiate them.²⁴ For example, a small patch of mild eczema that persists for years may be quite different from extensive severe disease that resolves quickly.

Paternoster *et al.*⁵ conducted a previous study in ALSPAC with external validation,²⁵ Hu *et al.*⁶ examined a multi-ethnic population up to age 10 years, and Roduit *et al.*⁴ examined a European population up to age 6 years. All reported phenotypes described onset timing and persistence during childhood, and between them there were some commonly described phenotypes (early-persistent, early-transient, late-onset). Hu *et al.*⁶ concluded that established risk factors have little capacity to differentiate eczema phenotypes. By additionally incorporating severity, the most clinically relevant aspect of AD, we have been able to show that genetic factors, in particular, have the potential to differentiate between phenotypes.

Our study has several strengths. We used a well-characterized, population-based cohort; hence, our results are generalizable to similar settings and populations. Our large sample ($n = 11\,866$) offered precision, and our phenotypes were internally reproducible, lending confidence to our findings. We applied robust statistical methods to minimize imprecision and bias in our regression estimates.

Our study also has limitations. We used a measure of self-reported AD, which has not been directly validated in ALSPAC, but has been shown to be a reasonable predictor in other contexts, including validation by physical exam in the UK²⁶ and physician assessment in a multicentre US study.²⁷ Missing data could have introduced bias into our estimates, although we tried to minimize this risk with FIML estimation and multiple

imputation techniques. There is uncertainty in phenotype assignment that could affect regression results; however, we compared several methods of accounting for it and found little difference in our results, suggesting insensitivity to this issue. Finally, there are few data sources recording AD severity, and we were unable to find a large cohort with similarly characterized children to externally validate our findings, although we did attempt this using a small cohort (Figure S9; see Supporting Information).

The LCA method involves probabilistic classification into subgroups and individual disease trajectories can vary within subgroups. For example, on average, active disease becomes less likely over time in the Moderate–Declining group, but an individual classified into this phenotype may still have active FD in late childhood. Future work may consider how early aggressive treatment for severe AD might modify the natural history.²⁸

Our study adds important information about patterns of AD severity over time. The identified phenotypes were stable across sensitivity analyses and had strong genetic associations, providing support for using them in future work towards a better aetiological understanding of AD, including the role of environmental risk factors throughout the life course, and towards better clinical trial design. Additional research is underway to determine the utility of our phenotypes for prognosis and to inform priorities for early intervention.

Acknowledgments

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. We also thank Drs Sadia Haider and Toshinori Nakamura at the National Heart & Lung Institute, Imperial College London, for their valuable input into the design of this research.

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Appendix 1 Funding sources

This article presents independent research supported by a Wellcome Trust Senior Research Fellowship in Clinical Science to S.M.L. (205039/Z/16/Z) and a National Institute of Arthritis and Musculoskeletal and Skin Diseases Career Development Award to K.A. (K23AR073915). This work was also supported by British Skin Foundation Innovative grant (8066) and Health Data Research UK (LOND1), which is funded by the UK Medical Research Council, Engineering and Physical Sciences Research Council, Economic and Social Research Council, Department of Health and Social Care (England), Chief Scientist Office of the Scottish Government Health and Social Care Directorates, Health and Social Care Research and Development Division (Welsh Government), Public Health Agency (Northern Ireland), British Heart Foundation and Wellcome Trust. It was also supported by the European Research Council under the European Union’s Seventh Framework Programme (FP7/2007–2013) / ERC grant agreement no 668954. The UK Medical Research Council and Wellcome Trust (grant ref.: 217065/Z/19/Z) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and A.R.M. will serve as guarantor for the contents of this paper.

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funders.

Supporting Information

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

Methods S1 Clinical, demographic and genetic variables; Statistical methods; Missing data.

Results S1 Phenotypes; internal validation; external validation.

Table S1 Distribution of flexural dermatitis in Avon Longitudinal Study of Parents and Children (ALSPAC) children at 11 timepoints.

Table S2 Average latent class analysis (LCA) classification probabilities by most likely phenotype.

Table S3 Individual phenotype classifications from latent class analysis (LCA) using full cohorts compared with LCA using complete cases.

Table S4 Associations between atopic dermatitis risk factors and phenotypes in the Avon Longitudinal Study of Parents and Children (ALSPAC) development and validation cohorts.

Table S5 Sensitivity of polygenic risk score to removal of chromosome 1 single-nucleotide polymorphisms (SNPs).

Table S6 Pairwise comparisons of atopic dermatitis risk factors between four atopic dermatitis phenotypes.

Figure S1 Comparison of statistical analysis methods.

Figure S2 Latent class analysis model fit statistics.

Figure S3 Clinical interpretations of phenotypes from six latent class analysis models.

Figure S4 Phenotype classification probabilities.

Figure S5 Sensitivity checks on phenotype derivation.

Figure S6 Phenotype assignment across 10 imputed datasets.

Figure S7 Heatmap of flexural dermatitis reports for individuals with complete cases ($n = 2083$).

Figure S8 Atopic dermatitis phenotypes showing missing data.

Figure S9 Atopic dermatitis phenotypes derived from MAAS.