

The eczema risk variant on chromosome 11q13 (rs7927894) in the population-based ALSPAC cohort: a novel susceptibility factor for asthma and hay fever

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In a genome-wide association study, a common variant on chromosome 11q13.5 (rs7927894[T]) has been identified as a susceptibility locus for eczema. We aimed to analyze the effect of this risk variant on asthma and hay fever and to determine its impact on the general population level in over 9300 individuals of the prospectively evaluated Avon Longitudinal Study of Parents and Children birth cohort. We demonstrate an association of rs7927894[T] with atopic asthma and with hay fever. The largest effect sizes were found in patients with the combined phenotype atopic asthma plus eczema [odds ratio (OR) = 1.50; 95% confidence interval (CI) 1.20–1.88; $P = 3.7 \times 10^{-4}$] and hay fever plus eczema (OR = 1.37; 95% CI 1.15–1.62; $P = 3.8 \times 10^{-4}$). We replicated the effects of rs7927894[T] on eczema-associated asthma and hay fever independently in the German GENUFAD (GEnetic studies in NUclear Families with Atopic Dermatitis) study and show that they are significantly larger than the effect observed in eczema. The estimated population attributable risk fractions for eczema, eczema-associated atopic asthma or hay fever were 9.3, 24.9 and 23.5%, respectively. Finally in eczema, we found a synergistic interaction of rs7927894[T] with filaggrin gene (*FLG*) mutations, which are a major cause of epidermal barrier dysfunction, and replicated the interaction in the German Multicenter Allergy Study birth cohort. The synergistic effect of rs7927894[T] and *FLG* mutations on eczema risk as well as the association of both variants with eczema-associated atopic asthma and hay fever point to an involvement of rs7927894[T] in a functional pathway that is linked to the barrier defect.

INTRODUCTION

Allergic diseases such as eczema, asthma and hay fever are the most common chronic disorders in childhood and a major public health problem in developing and industrialized countries (1). The allergic diseases are closely associated with one another and show strong familial and intraindividual clustering, suggesting common disease etiology. Clinically, however, they differ markedly, affecting different organ systems and showing

distinct epidemiologic characteristics. Eczema is a chronic inflammatory skin disease that usually develops within the first 2 years of life (2), whereas asthma and hay fever affect the airways with typical onset at school age or adulthood (3). An important, but by no means universal, trigger for the development of allergic disorders is the immunoglobulin E (IgE)-mediated hypersensitivity to common environmental allergens (atopy). A substantial subset of children suffer from multiple allergic conditions, facing a lifetime of impaired health.

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Allergic disorders are complex trait diseases, resulting from a combination of multiple interacting genetic and environmental factors rendering the identification of risk factors extremely challenging. Accordingly, only a few genetic risk variants were replicated in independent studies and the majority of allergic risk variants identified so far are disease-specific (4,5). We have recently performed a genome-wide association study (GWAS) on eczema in order to identify novel disease-related genes (6). In that study, a single risk locus on chromosome 11q13.5 (rs7927894[T]) was consistently associated with eczema in all four study groups from Central Europe. The association of this single-nucleotide polymorphism (SNP) with eczema has recently been confirmed in an independent case-control study population from Ireland (7). Although the functional implication of the associated variant has not yet been resolved, rs7927894 seems to play an important role in chronic inflammatory disorders of the epithelium since it has also been identified as a susceptibility factor for Crohn's disease (8), a chronic inflammatory bowel disease with a defect of the intestinal barrier.

We aimed to study the effect of rs7927894 on asthma and hay fever in the population-based Avon Longitudinal Study of Parents and Children (ALSPAC) cohort comprising more than 14 000 children born in 1991 and 1992 in the Avon area, UK (9). This unselected birth cohort from England enabled us to analyze the association of the risk variant with a range of allergic diseases. Moreover, the population-based recruitment allowed us to assess the effect sizes on the population level and to investigate factors potentially modifying the disease risk.

RESULTS

A summary of the ALSPAC study population including the genotyping results and the allergic phenotypes under study is provided in Table 1. In order to assess a potential selection bias, we compared the study population with those individuals who were not included due to lacking genotype data (Supplementary Material, Table S1). We found an over-representation of children with allergic diseases in the study population. Additionally, regarding the included co-factors, the study population was enriched for older mothers, longer gestation periods and higher birth weight, whereas maternal smoking during pregnancy was less frequent. Differences in the distribution of the co-factors between each of the two subgroups were further tested for heterogeneity of the effects on eczema and asthma. For qualitative co-factors, the effects on eczema or asthma were homogenous in both groups and, for quantitative co-factors, the same trend was observed.

Association analyses were performed under an additive model, which best fit our data, confirming the results of the initial GWAS (6). All odds ratios (ORs) reported for the ALSPAC cohort were adjusted for gender, maternal smoking during pregnancy, the presence of older siblings, gestation length, birth weight and maternal age. The unadjusted results were very similar to the adjusted results; we therefore present only the adjusted results.

Table 1. Characterization of the ALSPAC study population (n = 9395)

Phenotype	Number of individuals (%)
Male sex	4849/9395 (51.6%)
Eczema	
Overall	1528/5271 (29.0%)
Atopic	410/3026 (13.5%)
Non-atopic	541/3026 (17.9%)
Asthma	
Overall	763/6569 (11.6%)
Atopic	301/4379 (6.9%)
Non-atopic	227/4379 (5.2%)
Hay fever	817/6796 (12.0%)
Atopy ^a	1105/5480 (20.2%)
Genotype	
CC	3483/9395 (37.1%)
CT	4426/9395 (47.1%)
TT	1486/9395 (15.8%)

^aIgE-mediated hypersensitivity to common allergens (atopy) was determined by SPT to house dust mites, grass and cats.

The effect of rs7927894 on asthma and eczema

We replicated the previously reported association of the T allele of rs7927894 with eczema in this population-based cohort with a similar effect size [OR = 1.17; 95% confidence interval (CI) 1.08–1.28; *P* = 3.0 × 10^{−4}; Table 2, A]. By including data on IgE-mediated hypersensitivity (atopy), we demonstrated that the association of the risk allele was mainly due to individuals with the atopic form of eczema (OR = 1.20; 95% CI 1.03–1.40; *P* = 0.020), whereas only a marginal effect (OR = 1.07; 95% CI 0.93–1.23; *P* = 0.34) was observed in eczema patients with non-atopic eczema.

In the overall study population, the rs7927894 polymorphism did not significantly increase the risk for asthma (Table 2, B). However, after stratification for IgE-mediated hypersensitivity (atopy), we found an association of the risk allele with atopic asthma (OR = 1.25; 95% CI 1.06–1.49, *P* = 9.8 × 10^{−3}). Since asthma is often associated with eczema, we investigated whether the association of rs7927894 with atopic asthma was related to the presence of eczema (Table 2, B). Indeed, we observed an increase in the OR for atopic asthma plus eczema (OR = 1.50; 95% CI 1.20–1.88; *P* = 3.7 × 10^{−4}), whereas there was no association with asthma in the absence of eczema (OR = 0.96; 95% CI 0.61–1.52; *P* = 0.86). To evaluate whether this increased OR merely reflected the association with eczema or represented an effect of rs7927894 on eczema-associated asthma, we focused on the children with eczema and tested for association of rs7927894 with atopic asthma. Interestingly, in this subgroup, rs7927894[T] was significantly associated with atopic asthma (OR = 1.45; 95% CI 1.12–1.86; *P* = 3.9 × 10^{−3}). In order to confirm the effect of rs7927894 on eczema-associated atopic asthma, we studied 761 unrelated children with eczema from the German GENUFAD (Genetic studies in NUClear Families with Atopic Dermatitis) study. Among these children, the risk allele frequency was 49.4% in the presence of asthma and 41.3% in the absence of asthma, thus providing independent replication of the association with atopic asthma (OR = 1.38; 95% CI 1.08–1.78; *P* = 8.2 × 10^{−3}; Table 3).

Table 2. The role of rs7927894 in allergic disease

	Phenotype	Genotype CC	CT	TT	Risk allele frequency (%)	Adjusted ^a OR (95% CI)	P-value
A	Eczema (<i>n</i> = 5271)						
	Yes	520	717	291	42.5	1.17 (1.08–1.28)	3.0×10^{-4}
	No	1458	1713	572	38.2		
	Eczema and atopy (<i>n</i> = 3026)						
	Atopic eczema	136	190	84	43.7	1.20 (1.03–1.40)	0.020
B	Non-atopic eczema	191	263	87	40.4	1.07 (0.93–1.23)	0.34
	Controls	795	946	334	38.9		
	Asthma (<i>n</i> = 6569)						
	Yes	264	365	134	41.5	1.10 (0.99–1.23)	0.089
	No	2171	2752	883	38.9		
	Asthma and atopy (<i>n</i> = 4379)						
	Atopic asthma	91	148	62	45.2	1.25 (1.06–1.49)	9.8×10^{-3}
	Non-atopic asthma	82	110	35	39.6	0.99 (0.81–1.20)	0.89
	Controls	1416	1822	613	39.6		
	Asthma, eczema, and atopy (<i>n</i> = 2420)						
	Atopic asthma and eczema	48	82	44	48.9	1.50 (1.20–1.88)	3.7×10^{-4}
	Atopic asthma, no eczema	16	18	7	39.0	0.96 (0.61–1.52)	0.86
	Eczema, no atopic asthma	189	260	92	41.0	1.07 (0.93–1.23)	0.35
C	Controls	636	764	264	38.8		
	Hay fever (<i>n</i> = 6796)						
	Yes	269	405	143	42.3	1.16 (1.04–1.29)	7.4×10^{-3}
	No	2272	2814	893	38.5		
	Hay fever and eczema (<i>n</i> = 3914)						
	Hay fever and eczema	89	158	63	45.8	1.37 (1.15–1.62)	3.8×10^{-4}
	Hay fever, no eczema	67	81	31	39.9	1.09 (0.87–1.36)	0.44
	Eczema, no hay fever	292	380	138	40.5	1.13 (1.00–1.26)	0.044
D	Controls	1041	1189	385	37.5		
	Atopy ^b (<i>n</i> = 5480)						
	Yes	399	504	202	41.1	1.08 (0.98–1.19)	0.14
	No	1610	2091	674	39.3		

OR, odds ratio; *n*, number of genotyped individuals, for whom the respective phenotype data were available; the control group comprises all individuals, who do not belong to any of the disease groups.

^aORs were adjusted for gender, maternal smoking during pregnancy, the presence of older siblings, gestation length, birth weight and age of the mother.

^bIgE-mediated hypersensitivity to common allergens (atopy) was determined by SPT to house dust mites, grass and cats.

Table 3. The effect of rs7927894 on asthma and hay fever in children with eczema of the GENUFAD study

Phenotype	Genotype CC	CT	TT	Risk allele frequency (%)	OR	95% CI	P-value
Atopic asthma (<i>n</i> = 682)							
Yes	42	80	40	49.4	1.38	1.08–1.78	8.2×10^{-3}
No	166	278	76	41.3			
Hay fever (<i>n</i> = 757)							
Yes	61	133	58	49.4	1.41	1.13–1.74	1.4×10^{-3}
No	168	260	77	41.0			

OR, odds ratio. *n*, number of genotyped individuals, for whom the respective phenotype data were available.

The association of rs7927894 with hay fever

We found an association of the risk allele with hay fever in the overall study population (OR = 1.16; 95% CI 1.04–1.29; $P = 7.4 \times 10^{-3}$; Table 2, C). Stratification of the ALSPAC cohort according to the presence or absence of eczema (Table 2, C) identified a strong association of the risk allele with the combined phenotype hay fever plus eczema (OR = 1.37; 95% CI 1.15–1.62; $P = 3.8 \times 10^{-4}$). In contrast, in the absence of eczema, the association with hay fever was not significant (OR = 1.11; 95% CI 0.89–1.37; $P = 0.36$). We again restricted the analysis to children with eczema and evaluated

rs7927894 as a risk factor for hay fever in this subgroup. We detected a significant association of the risk variant with eczema-associated hay fever (OR = 1.23; 95% CI 1.01–1.49; $P = 0.036$), which was also confirmed independently in the 761 children with eczema of the GENUFAD study (OR = 1.41; 95% CI 1.13–1.74; $P = 1.4 \times 10^{-3}$; Table 3).

The role of rs7927894 in atopy

We then evaluated whether rs7927894 had an effect on IgE-mediated sensitization to common allergens (atopy)

independent of clinical evidence for allergic disease. The sensitization status was determined based on the skin prick test (SPT) reaction to house dust mites, grass and cats, which accounted for 95% of all positive SPT reactions in the ALSPAC cohort (10). Although, in the overall study population, there was a trend toward the same risk allele (Table 2, D), this weak effect obviously reflected the presence of concomitant allergic disorders; in the subgroup of IgE-sensitized individuals without any symptoms of allergic disease ($n = 74$), we did not find an independent effect of rs7927894[T] on atopy when compared with the non-sensitized subgroup ($n = 1283$; adjusted OR = 0.95; 95% CI 0.68–1.35; $P = 0.64$).

Interaction analysis of rs7927894 with FLG mutations

The rs7927894 risk allele is associated with eczema and with concomitant allergic airways disease, like the previously described loss-of-function mutations in the filaggrin gene (*FLG*) (11). We therefore investigated whether an interaction between rs7927894[T] and the two most common *FLG* mutations influenced the eczema risk.

Compared with the children who carried neither genetic risk factor, the presence of rs7927894[T] or of the combined *FLG* mutations increased the eczema risk and children with both risk factors were at highest risk (Table 4). The relative excess risk due to interaction (RERI) of 0.38 ($P_{\text{empirical}} = 0.028$) indicated that the risk conferred by the combination of both risk factors was significantly higher than the sum of the independent effects. In order to confirm the interaction, we genotyped 871 children of the German Multicenter Allergy Study (MAS) birth cohort. In the MAS, the effect sizes for the different risk groups were strikingly similar to the ALSPAC cohort (Fig. 1). In addition, we confirmed the synergistic effect of rs7927894[T] and the combined *FLG* mutations on the eczema risk as indicated by an RERI of 0.81 ($P_{\text{empirical}} = 0.041$; Table 4).

Finally, we tested for interaction between the two genetic risk factors in the development of atopic asthma and eczema-associated asthma. There was no evidence for interaction in ALSPAC (data not shown). However, due to the smaller sample size, the estimated power to observe an interaction of the same effect size as in eczema at a significance level $\alpha = 0.05$ was only 30% for atopic asthma and 22% for eczema plus asthma. Therefore, we cannot exclude such an effect on asthma.

The population-wide risk for allergic diseases attributable to rs7927894

We used the population attributable risk fraction (PARF) to estimate the effect of the risk variant on the population level. The PARF was calculated for those allergic diseases that were significantly associated with rs7927894 (Table 5). Although the effect sizes of the associations with these phenotypes were moderate, the PARFs were high, ranging from 9.3 to 24.9% for eczema alone and in combination with atopic asthma.

Table 4. Interaction between rs7927894 and the FLG mutations in the ALSPAC and MAS

rs7927894[T]	FLG mut	Eczema No. of cases ^a	No. of controls ^a	RR (95% CI)	RERI (P-value)
ALSPAC					
No	No	358	1057		
Yes	No	664	1724	1.10 (0.98–1.23)	
No	Yes	51	74	1.61 (1.28–2.03)	
Yes	Yes	125	111	2.09 (1.80–2.43)	0.38 (0.028)
MAS					
No	No	77	193		
Yes	No	123	266	1.11 (0.87–1.41)	
No	Yes	14	17	1.58 (1.03–2.44)	
Yes	Yes	20	8	2.50 (1.85–3.38)	0.81 (0.041)

RR, risk ratio; the risk ratio indicates the fold risk to develop eczema compared with the children who do not carry any of the risk factors. RERI, relative excess risk due to interaction.

^aThe smaller number of cases and controls compared with Table 1 is due to the individuals with incomplete genotypes, who were excluded from the interaction analysis.

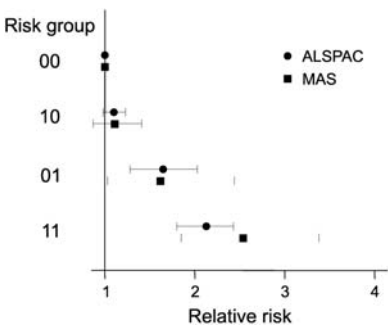


Figure 1. Similarity of the effect sizes of the interacting genetic risk factors in the ALSPAC and MAS. The four risk groups correspond to all combinations of the two risk factors; no risk allele (00), rs7927894[T] and no *FLG* mutation (10), *FLG* mutation and no rs7927894[T] (01), and rs7927894[T] and *FLG* mutation (11). Relative risks of eczema are indicated by circles (ALSPAC) and squares (MAS). Horizontal bars represent the 95% CIs.

Table 5. PARF of rs7927894 in allergic disease

Phenotype	PARF (%)
Eczema	9.3
Atopic eczema	10.5
Atopic asthma	16.7
Atopic asthma and eczema	24.9
Hay fever	11.9
Hay fever and eczema	23.5

DISCUSSION

In a GWAS, we have recently identified a common variant on chromosome 11q13 (rs7927894[T]) to be a risk factor for eczema. The association of this variant with eczema has been confirmed in an independent case–control study population from Ireland (7). Here, we provide novel evidence for the association of rs7927894[T] with atopic asthma and hay fever. Beyond replicating the association of rs7927894[T]

with eczema, we show that the development of eczema is essential for the associated allergic disease phenotypes and that there is a stronger effect of rs7927894[T] on the atopic subtype of eczema. We demonstrate a genetic interaction with filaggrin gene (*FLG*) mutations in two different cohorts with similar effect sizes. Our results in the population-based ALSPAC birth cohort render this variant a robust genetic susceptibility factor for allergic diseases on the population level.

The population-based recruitment enabled us to evaluate the association of rs7927894 with different allergic phenotypes in a general population sample and to select affected and control individuals based on longitudinal, prospectively collected data. Interestingly, rs7927894[T] was not only associated with eczema but also with atopic asthma and hay fever. The highest disease risk was identified for children having the combined allergic phenotypes atopic asthma plus eczema as well as hay fever plus eczema, suggesting an involvement of the risk allele in the development of multiple clinical manifestations of allergic inflammation. The analysis of the combined allergic phenotypes also revealed that there was no disease risk in the absence of eczema, pointing to an essential role of eczema in the allergic airways disease associated with rs7927894. Consequently, the risk variant might influence a disease mechanism leading to the 'atopic march', which refers to a typical sequence of allergic disease manifestations in childhood starting with infantile eczema, and progressing to allergic airways disease at school age (12). Only a fraction of infants with eczema undergo this development. Thus, the identification of genetic risk factors for this unfavorable course is important to elucidate the relationship between the clinical entities, to identify individuals at risk and to design specific preventive measures.

Importantly, we could exclude that the effect on eczema-associated atopic asthma and hay fever only reflected the association with concomitant eczema. The investigation of rs7927894[T] among children with eczema revealed that the variant provided a significant risk for atopic asthma and hay fever in this subgroup. This result was confirmed in an independent study population from Germany, consisting of more than 700 children with eczema. Our results suggest a common mechanism underlying eczema and promoting disease progression to asthma and hay fever in these children.

The association of rs7927894[T] with eczema has recently been confirmed in an independent case-control study from Ireland (7). By including data on IgE-mediated hypersensitivity (atopy), we were able to define the disease phenotype in more detail. Interestingly, the risk variant had a stronger effect on the atopic form of eczema, whereas there was a marginal effect in the absence of atopy. In addition, we could clearly demonstrate that atopy *per se* was not associated with the risk allele, confirming a key role of eczema in the allergic disease phenotypes associated with rs7927894[T] and indicating that atopy is not the primary disease manifestation of the risk variant.

Finally, the population-based ALSPAC cohort enabled us to assess the impact of the risk variant on a general population sample. We calculated a PARF of 9.3% for atopic eczema and of over 20% for the combined allergic phenotypes, indicating a substantial effect on the population level. The PARF of this variant for eczema almost reached that of the *FLG* mutations

which are strong genetic risk factors and for which a PARF of 15% was reported in the same population (13).

A comparison between the study population, which was genotyped for rs7927894, and the non-study group revealed that allergic diseases were over-represented in the study population. This may be attributed to the greater interest of parents who have an affected child to participate in the follow-ups of the study. Provided that the allele frequency in the study population corresponds to that of the general population, a larger disease prevalence in the study population would lead to a conservative estimate of the PARF as well as the relative risk. In a large study on Crohn's disease, an allele frequency of 39.6% has been reported for rs7927894[T] in the UK population (8). In our study population, we detected a very similar risk allele frequency of 39.3%. Hence, the validity of the PARF and the RERI should not be affected.

An association with eczema and with eczema-associated allergic airways disease, as well as a stronger effect on the atopic subtype of eczema but lack of association with atopy, has previously been noted for *FLG* mutations, which are a major cause of the epidermal barrier defect (11,14). The potential involvement of both risk factors, rs7927894 and the filaggrin gene mutations, in the atopic march prompted us to test for interaction. We identified a synergistic interaction of rs7927894[T] with loss-of-function mutations in *FLG*. For carriers of both genetic risk factors, the disease risk was significantly higher than that expected from an additive model. Notably, we confirmed the interaction with very similar effect sizes in an independent population-based birth cohort, the MAS from Germany. This interaction was not detected in a recent case-control study from Ireland (7). Since the size of the synergistic effect found in the ALSPAC and MAS birth cohorts was moderate, this is most likely attributable to a lack of power. The synergistic effect of rs7927894[T] and *FLG* mutations on the eczema risk, as well as the overlap between the association patterns of both variants in eczema-associated allergic airways disease, points to an involvement in a common functional pathway that defines this unfavorable disease subtype. Accordingly, like the *FLG* mutations, rs7927894[T] might be a risk factor for skin barrier dysfunction, resulting in increased systemic exposure to antigen stimuli and, finally, in IgE-mediated hypersensitivity and allergic airways disease. A potential role in the epithelial barrier is further supported by the finding that rs7927894[T] was reported as a risk factor for Crohn's disease (8), which is a chronic inflammatory bowel disease characterized by a defective intestinal barrier.

In conclusion, our study robustly demonstrates the importance of the rs7927894 risk variant for the development of eczema-associated allergic disorders in the general population. We have provided independent confirmation of the association of rs7927894[T] with eczema in a population-based cohort. Moreover, we identified the association of this variant with eczema-associated asthma and hay fever, suggesting a potential role in the atopic march. In eczema, we identified a genetic interaction of rs7927894[T] with the *FLG* mutations, which points to an involvement in a pathway linked to the barrier defect. Further studies of this genomic region are required in order to resolve the functional implication of the risk variant. They might provide novel mechanisms and therapeutic targets which are

common to chronic inflammatory barrier diseases of the skin, the airways and the gastrointestinal tract.

MATERIALS AND METHODS

Study populations

The ALSPAC cohort is a longitudinal, population-based birth cohort consisting of 14 062 children (13 868 children after excluding all multiples but the first-born child) born between April 1, 1991 and December 31, 1992 in Avon, UK. The cohort has previously been described in detail (9,15). Further information is available on the ALSPAC website (<http://www.bristol.ac.uk/alspac/>). The study population comprised all individuals who were genotyped for rs7927894 ($n = 9395$).

To replicate the association of rs7927894 with eczema-associated asthma and hay fever, we studied 761 unrelated German children with eczema from the extended GENUFAD cohort, recruited through at least one child having moderate-to-severe eczema with an age of onset below 2 years. Of those, a total of 682 individuals had information on atopic asthma and 757 on hay fever. The GENUFAD study has previously been described in detail (11).

To replicate the interaction of rs7927894 with filaggrin loss-of-function mutations in another population-based cohort, we investigated the MAS, a German birth cohort of 1314 children born in 1990 and followed until the age of 13 years. Detailed description of allergic phenotypes has been reported (11,16). DNA samples of 871 children were available for genotyping. The institutional review boards of all centers approved the study and written informed consent was obtained from the parents.

Phenotypes

In the ALSPAC cohort, all phenotypes were defined based on questionnaires filled in by the mothers. At 6, 18, 30, 42, 54, 69, 81 and 103 months after birth, mothers were asked whether their child had skin rashes in the joints and creases of the body in the past 12 months. At 81, 91, 103, 128, 157 and 166 months after birth, mothers were asked whether their child had asthma or hay fever in the past 12 months. In addition, mothers were asked whether a doctor had ever diagnosed asthma (at 91, 128 and 166 months after birth) or eczema (at 128 and 166 months after birth). Referring to previous articles on eczema in the ALSPAC cohort (13,17), we defined children with eczema as those with at least two positive reports on flexural dermatitis between 6 and 42 months. For the definition of asthma or hay fever, we required at least three positive reports on the respective disease between 81 and 166 months. The absence of a disease was declared if no positive report was ever given on the respective disease. For 664 children, parents have repeatedly reported flexural dermatitis between 6 and 42 months, but they have also reported a contradicting doctor's diagnosis. Those children were excluded from the study.

SPT for common inhalant and food allergens was performed at age 7 or 8 years as reported previously (10). In that study, it was demonstrated that sensitization to grass, house dust mites (*Dermatophagoides pteronyssinus*) or cats accounted for more

than 95% of all positive SPT reactions in the ALSPAC cohort. Accordingly, individuals were divided into atopic and non-atopic based on a positive (mean wheal diameter ≥ 2 mm) or negative (mean wheal diameter < 2 mm) SPT response, respectively, to grass, house dust mites or cats.

In the GENUFAD study, all subjects had moderate-to-severe eczema diagnosed by a doctor, with an age of onset below 2 years, as described previously (11). Eczema in the MAS cohort was defined by the presence of (i) a reported physician's diagnosis, (ii) a parental report of eczema symptoms or (iii) visible eczema at any follow-up between 3 months and 3 years of age. For the absence of eczema, no positive report on the disease up to the age of 13 years was required.

Genotyping of rs7927894

Genotyping of rs7927894 in the ALSPAC cohort was performed by KBiosciences (Hoddesdon, Herts, UK) using a proprietary, competitive allele-specific PCR system, KASPar (www.kbiosciences.co.uk). In the GENUFAD and MAS samples, rs7927894 was genotyped by TaqMan allelic discrimination (Applied Biosystems, Foster City, CA, USA) as described previously (6). The genotyping success rate was 99.2%, and there was no deviation from the Hardy–Weinberg equilibrium for rs7927894. We refer to SNP rs7927894 as a C to T exchange according to the plus strand of the human genome reference sequence. The T allele corresponds to the risk allele from the previous GWAS (6), in which the complementary A allele was reported. The two most frequent loss-of-function mutations in *FLG*, R501X and 2282del4, were genotyped in the ALSPAC cohort and in the MAS cohort as described previously (11,13). Complete genotypes (rs7927894, R501X and 2282del4) were available in 79.9% of individuals. Comparing the proportions of eczema cases among the individuals with complete and incomplete genotypes indicated no bias (28.8 versus 29.8%, $P = 0.50$).

Statistical analyses

The study population and the non-study group were compared regarding the frequency or median of single risk factors and co-factors. Significance was obtained from the χ^2 test and the Wilcoxon rank-sum statistic, respectively. Heterogeneity of ORs was evaluated by Cochran's Q -test, and equality of regression parameters for quantitative co-factors was tested in a z -test. ORs obtained from unconditional logistic regression were used to analyze the association between rs7927894 and disease phenotypes under study. The core model included the co-factors gender, maternal smoking during pregnancy, the presence of older siblings, gestation length, birth weight and age of the mother. The significance of the logistic model was expressed as the P -value of the likelihood ratio test for the 'full model' (with risk factor and co-factors included) versus the 'null model' (with co-factors only). A two-sided P -value of < 0.05 was considered statistically significant.

To establish whether an interaction between the two risk factors A (rs7927894) and B (*FLG* mutations) existed, the RERI (18) was calculated as $RERI = \text{relative risk (A and B)} - \text{relative risk (A without B)} - \text{relative risk (B without A)}$.

A) + 1. Interaction was defined as departure from the additive model. A RERI >0 or <0 indicates a superadditive or subadditive effect, respectively. We chose (with replacement) 100 000 bootstrap samples from the original sample, each of which was the same size as the original sample, and obtained a significance level. To evaluate the power of RERI, we performed bootstrap resampling of the original data set ($B = 1000$) and calculated $RERI_B$ for each sample with empirical P -values P_B obtained from 1000 permutations of the disease phenotype. For data sets of low sample size and sparse cell counts, we applied the RERI with continuity correction (19).

The PARF, which is defined as the fraction of disease cases in a population which can be prevented by elimination of a given risk factor, was calculated based on the risk ratios as described in Hennekens and Buring (20). Statistical significance was obtained from the χ^2 test. Statistical analyses were performed using the software R.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online

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Conflict of Interest statement. None declared.

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