Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis

Atopic dermatitis (AD) is a commonly occurring chronic skin disease with high heritability. Apart from filaggrin (*FLG*), the genes influencing atopic dermatitis are largely unknown. We conducted a genome-wide association meta-analysis of 5,606 affected individuals and 20,565 controls from 16 population-based cohorts and then examined the ten most strongly associated new susceptibility loci in an additional 5,419 affected individuals and 19,833 controls from 14 studies. Three SNPs reached genome-wide significance in the discovery and replication cohorts combined, including rs479844 upstream of *OVOL1* (odds ratio (OR) = 0.88, $P = 1.1 \times 10^{-13}$) and rs2164983 near *ACTL9* (OR = 1.16, $P = 7.1 \times 10^{-9}$), both of which are near genes that have been implicated in epidermal proliferation and differentiation, as well as rs2897442 in *KIF3A* within the cytokine cluster at 5q31.1 (OR = 1.11, $P = 3.8 \times 10^{-8}$). We also replicated association with the *FLG* locus and with two recently identified association signals at 11q13.5 (rs7927894; $P = 0.008$) and 20q13.33 (rs6010620; $P = 0.002$). Our results underline the importance of both epidermal barrier function and immune dysregulation in atopic dermatitis pathogenesis.

Atopic dermatitis, or eczema, is one of the most common chronic inflammatory skin diseases, with prevalence rates of up to 20% in children and 3% in adults. It commonly starts during infancy and frequently precedes or co-occurs with food allergy, asthma and rhinitis[1].

Atopic dermatitis has a broad spectrum of clinical manifestations and is characterized by dry skin, intense pruritus and a typical age-related distribution of inflammatory lesions that are frequently superinfected by bacteria and viruses[1]. Profound alterations in skin barrier function and immunologic abnormalities are considered to be key components affecting the development and severity of atopic dermatitis, but the exact cellular and molecular mechanisms remain incompletely understood[1].

There is substantial evidence supporting the idea of a strong genetic component in atopic dermatitis; however, it is not well understood how genetic susceptibility contributes to the development of this condition[2,3]. To date, only null mutations in the *FLG* gene encoding the epidermal structural protein filaggrin have been established as major risk factors[4,5].

The only genome-wide association study (GWAS) of atopic dermatitis in European populations identified a new susceptibility locus at 11q13.5 downstream of *C11orf30* (ref. 6). A second GWAS, recently carried out in a Chinese Han population, identified two new susceptibility loci, one of which (rs6010620 at 20q13.33) also showed evidence for association in a German sample[7]. In a collaborative effort to identify additional risk genes for atopic dermatitis, we conducted a well-powered, two-stage genome-wide association meta-analysis for the EAGLE Consortium.

In the discovery analysis of 5,606 individuals with atopic dermatitis (cases) and 20,565 controls from 16 population-based cohorts of European descent (**Supplementary Tables 1** and **2**), there was little evidence for population stratification at the study level (genomic inflation factor, $\lambda_{GC} \leq 1.08$) or at the meta-analysis level ($\lambda_{GC} = 1.02$), and we detected an excess of association signals beyond those expected by chance (**Supplementary Figs. 1** and **2**).

SNPs from two regions reached genome-wide significance ($P < 5 \times 10^{-8}$) in the discovery meta-analysis (**Fig. 1** and **Supplementary Table 3**): rs7000782 (8q21.13 near *ZBTB10*; OR = 1.14, $P = 1.6 \times 10^{-8}$) and rs9050 (1q21.3 near *TCHH*; OR = 1.33, $P = 1.9 \times 10^{-8}$). Given the proximity of rs9050 to the well-established atopic dermatitis susceptibility gene *FLG*[4,5], we evaluated whether the observed association was due to linkage disequilibrium (LD) with *FLG* mutations. Despite low correlation between rs9050 and the two most prevalent *FLG* mutations in ALSPAC cases ($r^2 = 0.257$ for p.Arg501* (c.1501C>T) and $r^2 = 0.001$
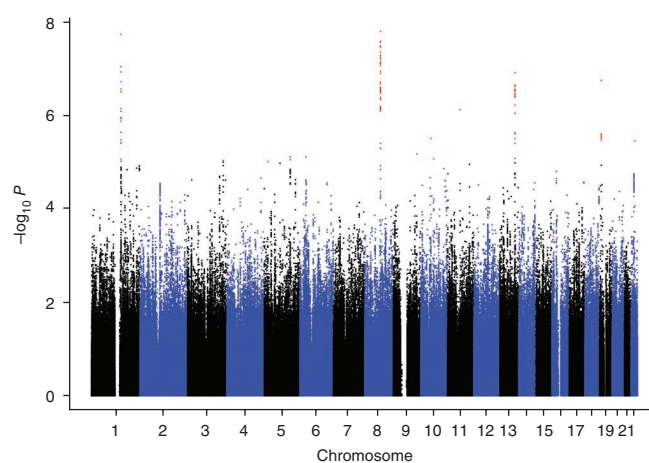

Figure 1 Manhattan plot for the discovery genome-wide association meta-analysis of atopic dermatitis after excluding all SNPs with minor allele frequency (MAF) <1% and r^2 <0.3 or proper info <0.4. $\lambda_{GC} = 1.017$. SNPs with $P < 1 \times 10^{-5}$ are shown in red.

a rs479844(A)

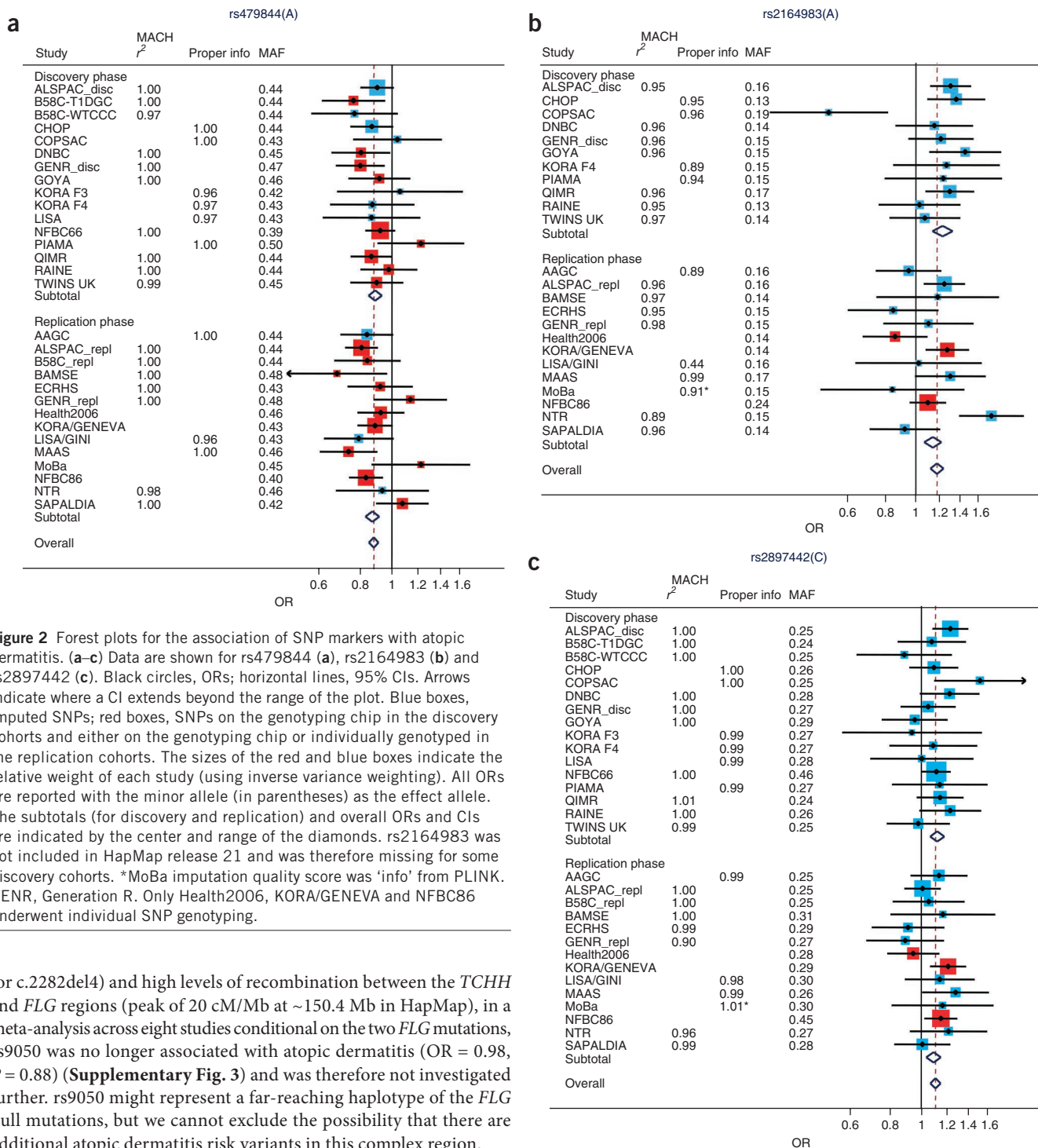**b** rs2164983(A)

c rs2897442(C)

Figure 2 Forest plots for the association of SNP markers with atopic dermatitis. (**a**–**c**) Data are shown for rs479844 (**a**), rs2164983 (**b**) and rs2897442 (**c**). Black circles, ORs; horizontal lines, 95% CIs. Arrows indicate where a CI extends beyond the range of the plot. Blue boxes, imputed SNPs; red boxes, SNPs on the genotyping chip in the discovery cohorts and either on the genotyping chip or individually genotyped in the replication cohorts. The sizes of the red and blue boxes indicate the relative weight of each study (using inverse variance weighting). All ORs are reported with the minor allele (in parentheses) as the effect allele. The subtotals (for discovery and replication) and overall ORs and CIs are indicated by the center and range of the diamonds. rs2164983 was not included in HapMap release 21 and was therefore missing for some discovery cohorts. *MoBa imputation quality score was 'info' from PLINK. GENR, Generation R. Only Health2006, KORA/GENEVA and NFBC86 underwent individual SNP genotyping.

for c.2282del4) and high levels of recombination between the *TCHH* and *FLG* regions (peak of 20 cM/Mb at ~150.4 Mb in HapMap), in a meta-analysis across eight studies conditional on the two *FLG* mutations, rs9050 was no longer associated with atopic dermatitis (OR = 0.98, $P = 0.88$) (**Supplementary Fig. 3**) and was therefore not investigated further. rs9050 might represent a far-reaching haplotype of the *FLG* null mutations, but we cannot exclude the possibility that there are additional atopic dermatitis risk variants in this complex region.

The 11q13.5 locus previously reported to be associated in the only other European GWAS on atopic dermatitis to date[6] was confirmed in our meta-analysis (rs7927894: OR = 1.07, 95% confidence interval (CI)1.02–1.12, $P = 0.008$) (**Supplementary Fig. 4**). Association with the rs6010620 variant, which was reported in a recent Chinese GWAS[7], was similarly confirmed (OR = 1.09, 95% CI 1.03–1.15, $P = 0.002$).

Of the 15 loci reported to be associated with asthma or total serum immunoglobulin E (IgE) levels in a recent GWAS[8], two showed suggestive evidence for association with atopic dermatitis (*IL13*: rs1295686, $P = 0.0008$ and rs20541, $P = 0.0007$; *STAT6*: rs167769, $P = 0.0379$) (**Supplementary Table 4**).

After excluding the rs9050 SNP, we attempted to replicate the remaining 10 most strongly associated loci ($P < 1 \times 10^{-5}$ in the discovery meta-analysis) (**Fig. 2**, **Table 1**, **Supplementary Fig. 5** and **Supplementary Table 3**) in 5,419 cases and 19,833 controls from 14 studies (**Supplementary Tables 1** and **2**). Three of the ten SNPs showed significant association after conservative Bonferroni correction ($P < 0.05/10 = 0.005$) in the replication meta-analysis (with the same direction of effect as in the discovery meta-analysis), including rs479844 near *OVOL1*, rs2164983 near *ACTL9* and rs2897442 in intron 8 of *KIF3A* (**Fig. 2** and **Table 1**). All three SNPs reached

Table 1 Discovery and replication results for loci associated with atopic dermatitis

Chr.	SNP	Position (bp)	Gene	Effect allele	Other allele	Effect allele freq.	Stage	N	OR (95% CI)	P value	P_{het}
11	rs479844	65,308,533	OVOL1	A	G	0.44	I	26,151	0.89 (0.85–0.93)	7.8×10^{-7}	
							II	25,098	0.87 (0.83–0.92)	2.4×10^{-8}	
							I + II	51,249	0.88 (0.85–0.91)	1.1×10^{-13}	0.23
19	rs2164983[a]	8,650,381	ACTL9	A	C	0.15	I	17,403	1.22 (1.13–1.32)	1.8×10^{-7}	
							II	22,996	1.11 (1.04–1.19)	0.002	
							I + II	40,399	1.16 (1.10–1.22)	7.1×10^{-9}	0.004
5	rs2897442	132,076,926	KIF3A	C	T	0.29	I	26,164	1.12 (1.07–1.18)	7.8×10^{-6}	
							II	25,064	1.09 (1.04–1.15)	0.001	
							I + II	51,228	1.11 (1.07–1.15)	3.8×10^{-8}	0.52

Results are for the fixed-effect, inverse variance meta-analysis, with genomic control applied to the individual studies in the discovery meta-analysis. Chr., chromosome; Stage I, discovery screen; stage II, replication; stages I + II, combined analysis; N, number of subjects in each analysis. The heterogeneity P value (P_{het}) for overall heterogeneity between all discovery and replication studies was generated using Cochran's Q test for heterogeneity. All ORs are given with the minor allele representing the effect allele.
[a]rs2164983 was not included in HapMap release 21 and was therefore missing for some discovery cohorts. This SNP showed evidence of heterogeneity (P = 0.004). The random-effects combined (I + II) result for this SNP was OR = 1.14 (95%CI 1.05–1.24), P = 0.001.

genome-wide significance in the combined meta-analysis of the discovery and replication sets: rs479844 with OR = 0.88, P = 1.1 × 10⁻¹³; rs2164983 with OR = 1.16, P = 7.1 × 10⁻⁹; and rs2897442 with OR = 1.11, P = 3.8 × 10⁻⁸. In contrast, rs7000782, which had reached genome-wide significance in the discovery analysis, showed no evidence of association in the replication cohort (P = 0.296). There was no evidence of interaction between the three replicated SNPs (**Supplementary Table 5**).

rs479844 (at 11q13.1) is located <3 kb upstream of OVOL1. The pattern of LD is complex at this locus, but there is a low recombination rate between rs479844 and OVOL1 in Europeans (**Supplementary Fig. 2**). OVOL1 belongs to a highly conserved family of genes involved in regulation of the development and differentiation of epithelial tissues and germ cells[9–11]. The encoded OVOL1 protein functions as a c-Myc repressor in keratinocytes, is activated by the β-catenin–LEF1 complex during epidermal differentiation and is a downstream target of the Wg-Wnt and TGF-β–BMP7–Smad4 developmental signaling pathways[10,12,13]. Apart from their role in the organogenesis of skin and skin appendages[14,15], these pathways are also implicated in postnatal regulation of epidermal proliferation and differentiation[16–18]. Disruption of Ovol1 in mice leads to keratinocyte hyperproliferation, hair shaft abnormalities, kidney cysts and defective spermatogenesis[10,11]. In addition, OVOL1 regulates loricrin (LOR) expression, thereby preventing premature terminal differentiation[10]. Thus, it is reasonable to speculate that variation at this locus might influence epidermal proliferation and/or differentiation, which are known to be disturbed in atopic dermatitis. Analysis of transcript levels of all genes within 500 kb of rs479844 in Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) from 949 ALSPAC individuals revealed a significant association (P = 7 × 10⁻⁵) between rs479844 and the nearby DKFZp761E198 locus, which encodes a hypothetical protein. Likewise, analysis of SNP-transcript pairs in the Multiple Tissue Human Expression Resource (MuTHER) pilot database of skin genome-wide expression quantitative trait loci (eQTL) from 160 samples[19] provided suggestive evidence for an association with DKFZp761E198 in the same direction in one of the twin sets (**Supplementary Fig. 6**). Further investigations are needed to clarify whether the causal variant(s) at this locus exerts its effect by regulating the expression of the DKFZp761E198 transcript.

rs2164983 (at 19p13.2) is located in an intergenic region 70 kb upstream of ADAMTS10 and 18 kb downstream of ACTL9 (encoding a hypothetical protein). ADAMTS proteins are a group of complex, secreted zinc-dependent metalloproteinases, which bind to and cleave extracellular matrix components and are involved in connective tissue

remodeling and extracellular matrix turnover[20,21]. Actin proteins have well-characterized cytoskeletal functions, are important for the maintenance of epithelial morphology and cell migration and have also been implicated in nuclear activities[22–24]. The low recombination rate between rs2164983 and ACTL9 and the recombination peak between this SNP and ADAMTS10 in the HapMap reference set of Utah residents of Northern and Western European descent (CEU) (**Supplementary Fig. 2**) suggests the functional variant may be located within the ACTL9 region. There was no evidence for association between rs2164983 and altered expression of genes within 500 kb of this marker in either the ALSPAC LCL eQTL analysis or the MuTHER skin eQTL data (**Supplementary Fig. 6**).

rs2897442 is located in intron 8 of KIF3A, which encodes a subunit of the kinesin II complex required for the assembly of primary cilia, which are essential for Hedgehog signaling and are implicated in β-catenin–dependent Wnt signaling to induce expression of a variety of genes that influence proliferation and apoptosis[25,26]. Of note, KIF3A is located at 5q31, which is characterized by a complex LD pattern and contains a cluster of cytokine and immune-related genes. This chromosomal location has been linked to several autoimmune and inflammatory diseases, including psoriasis[27,28], Crohn's disease[29,30] and asthma[8,29,31] (**Supplementary Table 4**). In particular, distinct functional IL13 (interleukin 13) variants have been associated with asthma susceptibility[32]. Although rs2897442 is within the KIF3A gene, there is little recombination between this locus and IL4 (encoding interleukin 4). There is a recombination peak between this region and IL13 (**Supplementary Fig. 7a**); however, there also seems to be a secondary association signal in the IL13-RAD50 region, and when making association conditional on rs2897442 in the discovery meta-analysis, the signal in the IL13-RAD50 region is still present, providing evidence of two independent signals (**Supplementary Fig. 7b**). In an attempt to further refine the association at this locus, we analyzed Immunochip data from 1,553 German atopic dermatitis cases and 3,640 population controls, of whom 767 cases and 983 controls were part of the replication stage. The Immunochip is a custom-content Illumina iSelect array that focuses on genes involved in autoimmune disorders and offers an increased resolution at 5q31. In the population tested, the strongest association signal was seen for the rs848 SNP in IL13 (P = 1.93 × 10⁻¹⁰), which is in high LD with the functional IL13 variant rs20541 (r² = 0.979; D′ = 0.995). Additional significantly associated signals were observed for a cluster of tightly linked variants in IL4 (lead SNP rs66913936: P = 2.58 × 10⁻⁸) and KIF3A (rs2897442: P = 8.84 × 10⁻⁷) (**Supplementary Fig. 8** and **Supplementary Tables 6** and **7**). Whereas rs2897442 showed only weak LD with rs848

($r^2 = 0.160$; $D' = 0.483$), it was strongly correlated with rs66913936 ($r^2 = 0.858$; $D' = 0.982$). Likewise, pairwise genotype-conditioned analyses showed that the significant association of rs2897442 with atopic dermatitis was abolished upon conditioning for rs66913936, whereas there an association signal remained after conditioning for rs848 (**Supplementary Tables 6** and **7**). Analysis of the expression levels of all genes within 500 kb of rs2897442 in LCLs derived from ALSPAC individuals revealed a modest association between rs2897442 and *IL13* transcript levels ($P = 2.7 \times 10^{-3}$). No association with transcript levels for any gene within 500 kb of the proxy variant, rs2299009, ($r^2 = 1$) was seen in the MuTHER skin eQTL data (**Supplementary Fig. 6**). However, this result does not exclude the possibilities that this variation may cause a regulatory effect in another tissue or physiological state, that this variant or causative variants in LD may be involved in long-range control of more distant genes[33] or that different functional effects, such as alternative splicing, may be affected.

It is well known that genes that participate in the same pathway tend to be located adjacent to one another in the human genome and are coordinately regulated[34]. Thus, our results and previous findings suggest that there are distinct effects at the 5q31 locus, which might involve loci that are part of a regulatory block in this region. Further efforts, including detailed sequencing and functional exploration, are necessary to fully explore this locus.

The rs2164983, rs1327914 and rs10983837 variants showed evidence of heterogeneity in the meta-analysis ($P < 0.01$). The overall random-effects results for these variants were OR = 1.14 (95% CI 1.05–1.24), $P = 0.001$; OR = 1.06 (95% CI 1.00–1.13), $P = 0.058$; and OR = 1.11 (95% CI 0.98–1.20), $P = 0.155$, respectively. Stratified analysis showed that the effects of rs2164983 and rs1327914 were stronger in the childhood atopic dermatitis cohorts (OR = 1.23, $P = 2.9 \times 10^{-9}$ and OR = 1.12, $P = 2.5 \times 10^{-4}$) than in studies that included atopic dermatitis cases of any age (OR = 1.17, $P = 0.002$ and OR = 1.02, $P = 0.584$; P values for the differences = 0.031 and 0.028, respectively) (**Supplementary Fig. 9**). This stratification did not fully explain the heterogeneity for rs2164983 (in the childhood-only cohorts, the P value for heterogeneity was still <0.01). In the COPSAC cohort, results were noticeably in the opposite direction, and excluding this study gave a heterogeneity P value of 0.069 (OR = 1.17, $P = 8.1 \times 10^{-10}$). However, the COPSAC cases are diagnostically and demographically comparable to those in the other cohorts, and, thus, there is no obvious reason why this cohort should give such a different result. Neither stratification by age of diagnosis nor by whether a physician's diagnosis was required for definition as a case explained the heterogeneity observed for rs10983837. Stratified analyses also indicated a stronger effect of rs2897442 in studies with a more stringent definition of atopic dermatitis (reported physician's diagnosis) (OR = 1.14, $P = 7.0 \times 10^{-9}$) compared to studies in which atopic dermatitis was defined using only self-reported histories of the disease (OR = 1.05, $P = 0.119$) (**Supplementary Fig. 9**). These observations highlight the importance of careful phenotyping and support the claim that atopic dermatitis encompasses distinct disease entities rather than being one illness, as is reflected by the current, relatively broad and inclusive concept of this condition. It is anticipated that the results of molecular studies will enable a more precise classification of atopic dermatitis.

In summary, in this large-scale GWAS of 11,025 atopic dermatitis cases and 40,398 controls, we have identified and replicated two newly identified risk loci for atopic dermatitis near genes that have annotations suggesting roles in epidermal proliferation and differentiation, supporting the importance of abnormalities in skin barrier function in the pathobiology of atopic dermatitis. In addition, we observed an association signal of genome-wide significance from within the cytokine cluster at 5q31.1, which seemed to be composed of two distinct signals, one centered at *IL13-RAD50* and the other at *IL4-KIF3A*, both of which showed moderate association with *IL13* expression. We further observed a signal in the epidermal differentiation complex, representing the *FLG* locus, and replicated the association with the 11q13.5 variant identified in the only other (smaller) published European GWAS of atopic dermatitis to date. Our results are consistent with the hypothesis that atopic dermatitis is caused by both epidermal barrier abnormalities and immunological features. Further studies are needed to identify the causal variants at the associated loci and to understand the mechanisms through which they confer risk for atopic dermatitis.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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ONLINE METHODS

Discovery analysis. For the discovery analysis, we used 5,606 atopic dermatitis cases and 20,565 controls of European descent from 16 population-based cohorts, 10 of which were birth cohorts. Local research ethics committees approved the individual studies, and informed consent was obtained from all participants when necessary (see the **Supplementary Note** for full details of ethics and consent procedures for each study). Additional details on sample recruitment and phenotypes and summary details for each collection are given in the **Supplementary Note** and in **Supplementary Table 1**. Genome-wide genotyping was performed independently in each cohort with the use of various standard genotyping technologies (see **Supplementary Table 2**). Imputation was independently conducted for each study with reference to HapMap release 21 or 22 CEU phased genotypes, and association analysis was performed using logistic regression models based on an expected allelic dosage model for SNPs, adjusting for sex- and ancestry-informative principal components as necessary. SNPs with MAF <1% and poor imputation quality (r^2 <0.3, if using MACH, or proper info <0.4, if using the IMPUTE imputation algorithm) were excluded. After genomic control at the level of the individual studies, we combined association data for ~2.5 million imputed and genotyped autosomal SNPs into an inverse variance fixed-effects additive model meta-analysis. There was little evidence for population stratification at the study level ($\lambda_{GC} \leq 1.08$; **Supplementary Table 2**) or at the meta-analysis level (λ_{GC} = 1.02), and the quantile-quantile plot of the meta-statistic showed a marked excess of detectable association signals well beyond those expected by chance (**Supplementary Fig. 1**).

Replication analysis. For replication, we selected the most strongly associated SNPs from the 10 most strongly associated loci in the discovery meta-analysis (all with $P < 1 \times 10^{-5}$ in stage I; **Table 1**). These SNPs were analyzed using *in silico* data from 11 GWAS sample sets not included in the discovery meta-analysis and *de novo* genotyping data from an additional 3 studies (**Supplementary Tables 1** and **2**), for a maximum possible replication sample size of 5,419 cases and 19,833 controls, all of European descent. Association analyses were again conducted for each study using a logistic regression model with similar covariate adjustments, based on expected allelic dosage for the *in silico* studies and allele counts in the *de novo* genotyping studies, and the results underwent meta-analysis with Stata 11.1 software (Statacorp LP). We applied a threshold of $P < 5 \times 10^{-8}$ for genome-wide significance and tested for overall heterogeneity of the discovery and replication studies using the Cochran's Q statistic.

Immunochip analysis. In this analysis, we evaluated 1,553 German atopic dermatitis cases and 3,640 German population controls. Cases were obtained from German university hospitals (Technical University Munich, as part of the GENEVA study, and University of Kiel). Atopic dermatitis was diagnosed on the basis of a skin examination by experienced dermatologists according to standard criteria, which included the presence of chronic or chronically relapsing pruritic dermatitis with the typical morphology and distribution[6]. Controls were derived from the Kooperative Gesundheitsforschung in der Region Augsburg (KORA) population-based surveys[35] and the previously described population-based Popgen Biobank[36]. Of these samples, 767 of the cases and 983 of the controls were also part of the replication stage. Samples with >10% missing data, individuals from pairs of unexpected duplicates or relatives and individuals with outlier heterozygosities of greater than ±5 s.d. from the mean were excluded. The remaining Immunochip samples were tested for population stratification using the principal-components stratification method, as implemented in EIGENSTRAT[37]. The results of principal-component analysis revealed no evidence for population stratification. SNPs that had >5% missing data, MAF <1% or exact Hardy-Weinberg equilibrium ($P_{controls}$ <1 × 10^{-4}) were excluded. Association P values were calculated using χ^2 tests (degree of freedom (d.f.) = 1) and conditional association was analyzed using logistic regression, with both implemented in PLINK[38], from which we also derived OR values and their respective CIs.

ALSPAC expression analysis. RNA was extracted from LCLs generated from 997 unrelated ALSPAC individuals using an RNeasy extraction kit (Qiagen) and was amplified using the Illumina TotalPrep-96 RNA Amplification kit (Ambion). Expression was evaluated using Illumina HT-12 v3 BeadChip arrays. Each individual sample was run with two replicates. Expression data were normalized by quantile normalization between replicates and then by median normalization across individuals. For 949 ALSPAC individuals, both expression levels and imputed genome-wide SNP data were available (see ALSPAC replication cohort genotyping). For each of the three replicated SNPs that were associated with atopic dermatitis (rs479844, rs2164983 and rs2897442), we used linear regression in Mach2QTL to investigate the association between each SNP and any transcript within 500 kb of this SNP.

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