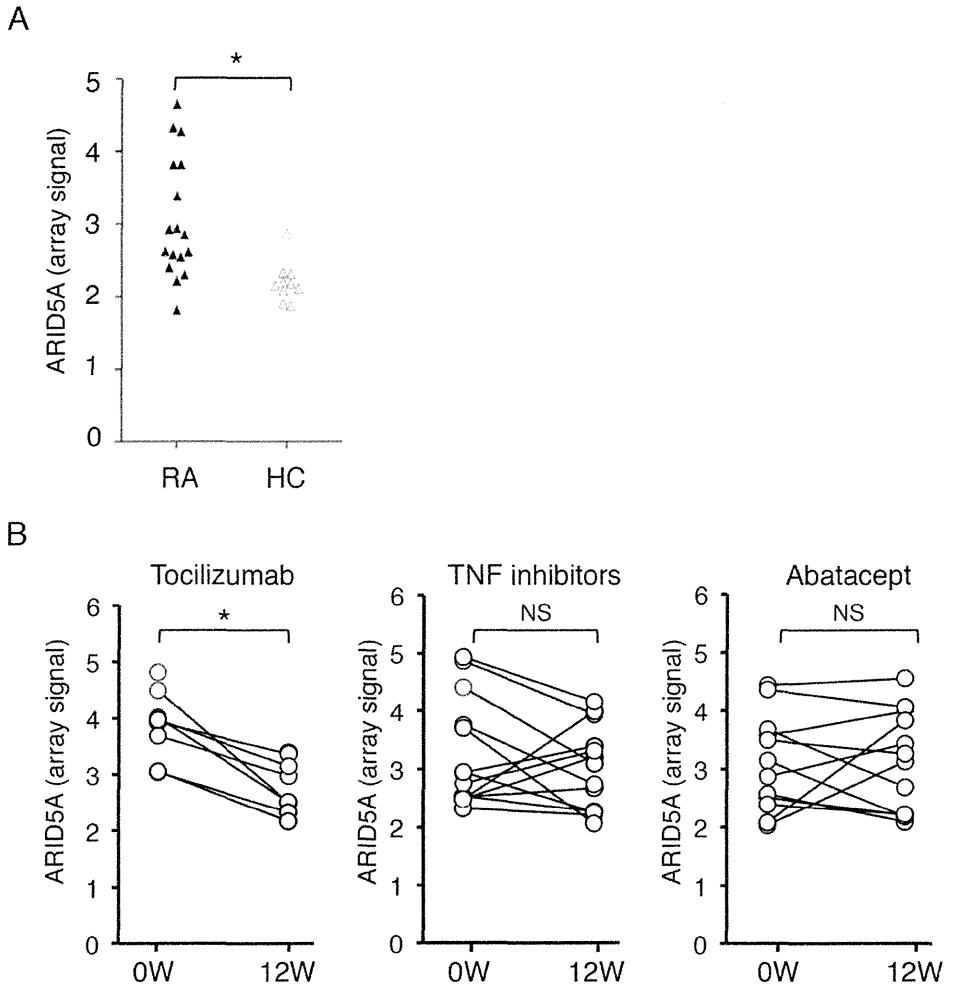

Figure 1. The expression of ARID5A in CD4+ T cells from RA patients is decreased by Tocilizumab therapy
(A) CD4+ T cells from untreated RA patients (n = 17) and those from healthy controls (HC) (n = 10) were subjected to DNA microarray analysis. Shown are array signals of ARID5A. *p<0.01. (B) CD4+ T cells were isolated from RA patients who showed good clinical responses to the treatment with Tocilizumab (n = 8), TNF inhibitors (n = 13), or Abatacept (n = 12) at just before and 12 weeks after the treatment. Samples were subjected to DNA microarray analysis and array signals of ARID5A before and after the treatment were compared in each treatment group. *p<0.01. NS = not significant.
77x80mm (300 x 300 DPI)

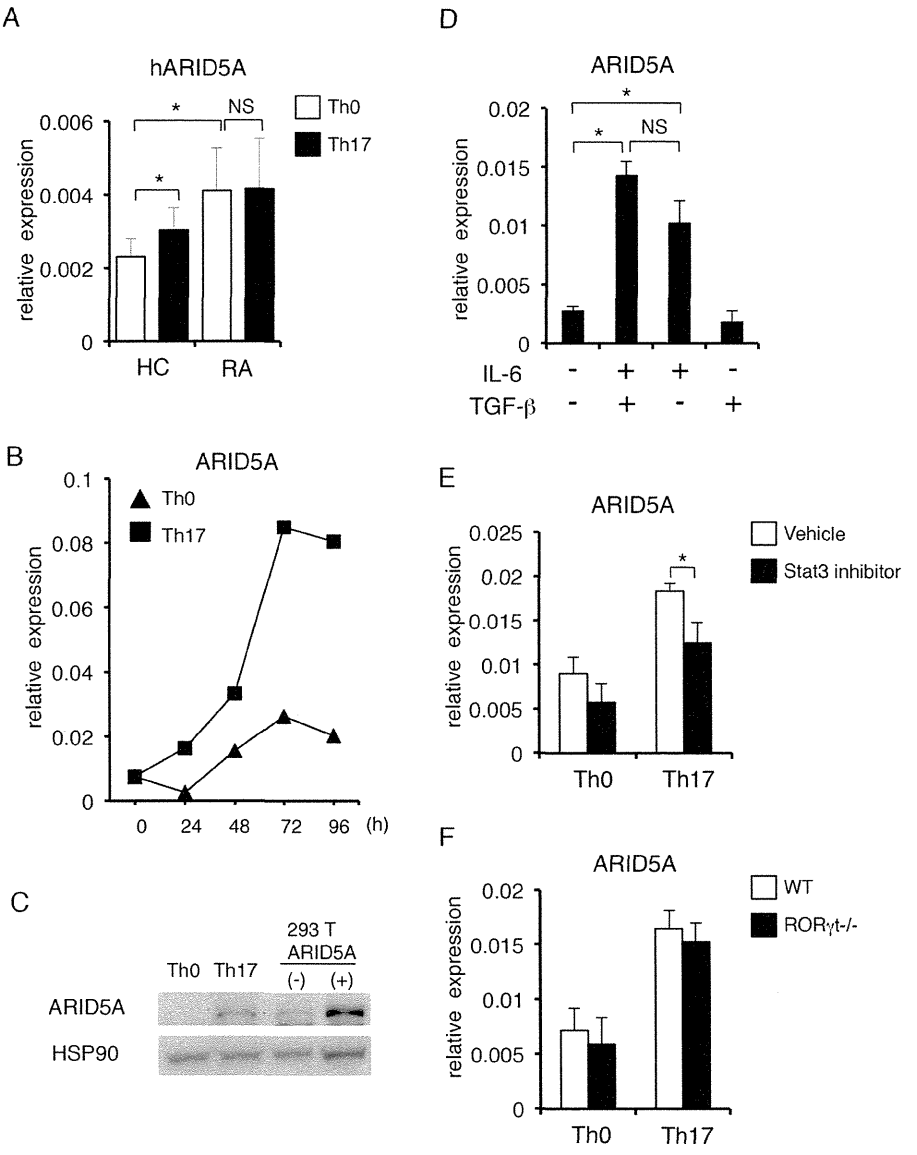

Figure 2. IL-6-Stat3 signaling induces ARID5A expression in human and murine Th17 cells
(A) Memory CD4+ T cells from healthy controls (n=6) or untreated RA patients (n=6) were stimulated with anti-CD3/CD28 in Th0 or Th17-polarizing conditions and the expression of ARID5A was measured by qPCR. *p<0.05. (B) Murine naïve CD4+ T cells were stimulated in Th0 or Th17-polarizing conditions for indicated time periods and the expression of ARID5A was measured by qPCR. Data are representative of three independent experiments. (C) Naïve CD4+ T cells were stimulated with anti-CD3/CD28 in Th0 or Th17-polarizing conditions and were subjected to immunoblotting with anti-ARID5A antibody. As controls, lysates of 293T cells that were transfected with pcDNA3-ARID5A or empty pcDNA3 were used. (D-F) (D) Naïve CD4+ T cells were stimulated with anti-CD3/CD28 in the presence or absence of IL-6 and/or TGF-β. (E) Naïve CD4+ T cells were stimulated with anti-CD3/CD28 in Th0 or Th17-polarizing conditions in the presence or absence of Stat3 inhibitor VI. (F) Naïve CD4+ T cells from RORγt-deficient mice or littermate wild-type (WT) mice were stimulated with anti-CD3/CD28 in Th0 or Th17-polarizing conditions. ARID5A expression was measured by qPCR. Data are means ± SD from three experiments. *p<0.05.

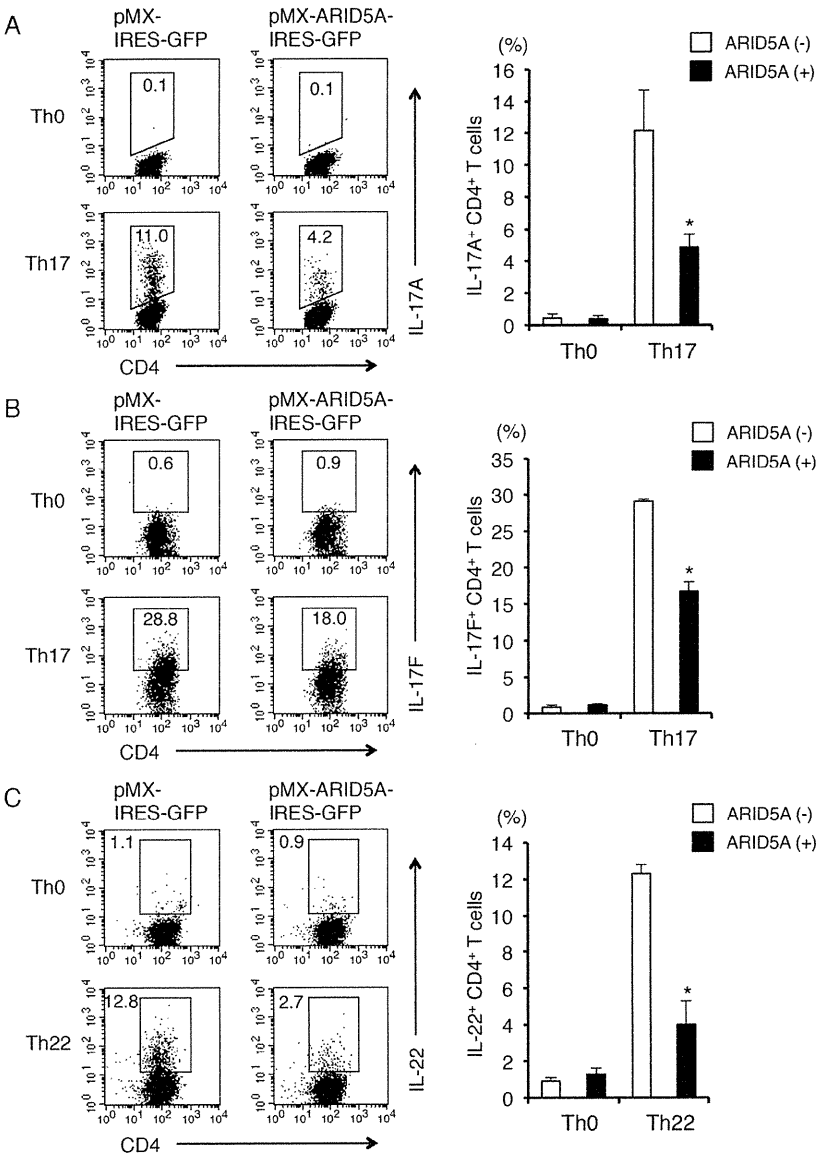

Figure 3. ARID5A inhibits Th17 cell differentiation
(A-C) Murine naïve CD4+ T cells were stimulated with anti-CD3/CD28 in Th0 conditions for 24 h and then cells were infected with a retrovirus of pMX-ARID5A-IRES-GFP or pMX-IRES-GFP (as a control) in Th0, Th17-, or Th22-polarizing conditions. Three days later, cells were re-stimulated with PMA plus ionomycin for 5 h and intracellular cytokine profiles of IL-17A (A), IL-17F (B), and IL-22 (C) in GFP+ CD4+ T cells were evaluated by FACS analysis. Left panels show representative data of cytokine profiles in CD4+ T cells, and right panels show the frequency of IL-17A, IL-17F, or IL-22-producing CD4+ T cells. Data are means ± SD from four experiments. *, significantly different from the mean value of the corresponding control response, $p<0.05$.
114x159mm (300 x 300 DPI)

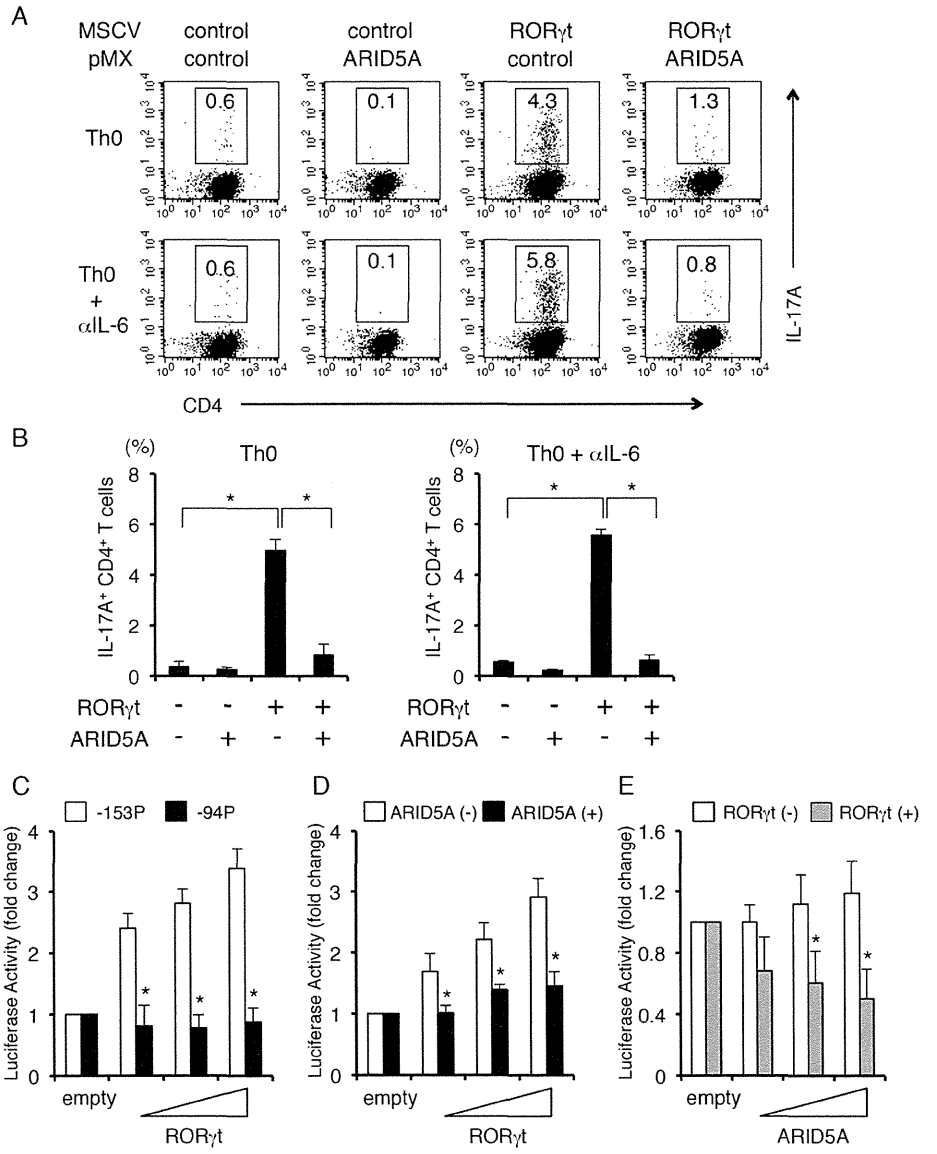

Figure 4. ARID5A inhibits RORγt-induced Th17 cell differentiation

(A-B) Murine naïve CD4+ T cells were stimulated with anti-CD3/CD28 in Th0 conditions for 24 h and then doubly infected with retroviruses of pMX-based virus (pMX-ARID5A-IRES-GFP or pMX-IRES-GFP) and MSCV-based virus (MSCV-myc-RORγt-IRES-Thy1.1 or MSCV-IRES-Thy1.1). Twenty-four hours later, cells were stimulated with anti-CD3/CD28 for 3 days in the presence or absence of anti-IL-6 antibody. Cells were re-stimulated with PMA+ionomycin for 5 h and the expression of IL-17A in doubly-infected CD4+ T cells (GFP+ Thy1.1+ CD4+ cells) was evaluated. Shown are representative FACS profiles (A) and means ± SD of the frequency of IL-17A-producing CD4+ T cells (B). n = 4 experiments. *p<0.01. (C) EL4 cells were transfected with -153 mIL17p-Luc or -94 mIL17p-Luc in the presence of MSCV-myc-RORγt-IRES-Thy1.1 (0.25, 0.5, or 1 μg) or empty MSCV-myc-IRES-Thy1.1. (D) EL4 cells were transfected with -153 mIL17p-Luc in the presence of pcDNA3-ARID5A or empty pcDNA3 and various amounts (0.25, 0.5, or 1 μg) of MSCV-myc-RORγt-IRES-Thy1.1. (E) EL4 cells were transfected with -153 mIL17p-Luc in the presence of MSCV-myc-RORγt-IRES-Thy1.1 or empty MSCV-myc-IRES-Thy1.1 and various amounts (0.25, 0.5, or 1 μg) of

pcDNA3-ARID5A. Data are means ± SD. n = 4 experiments. *, p<0.05.
113x142mm (300 x 300 DPI)

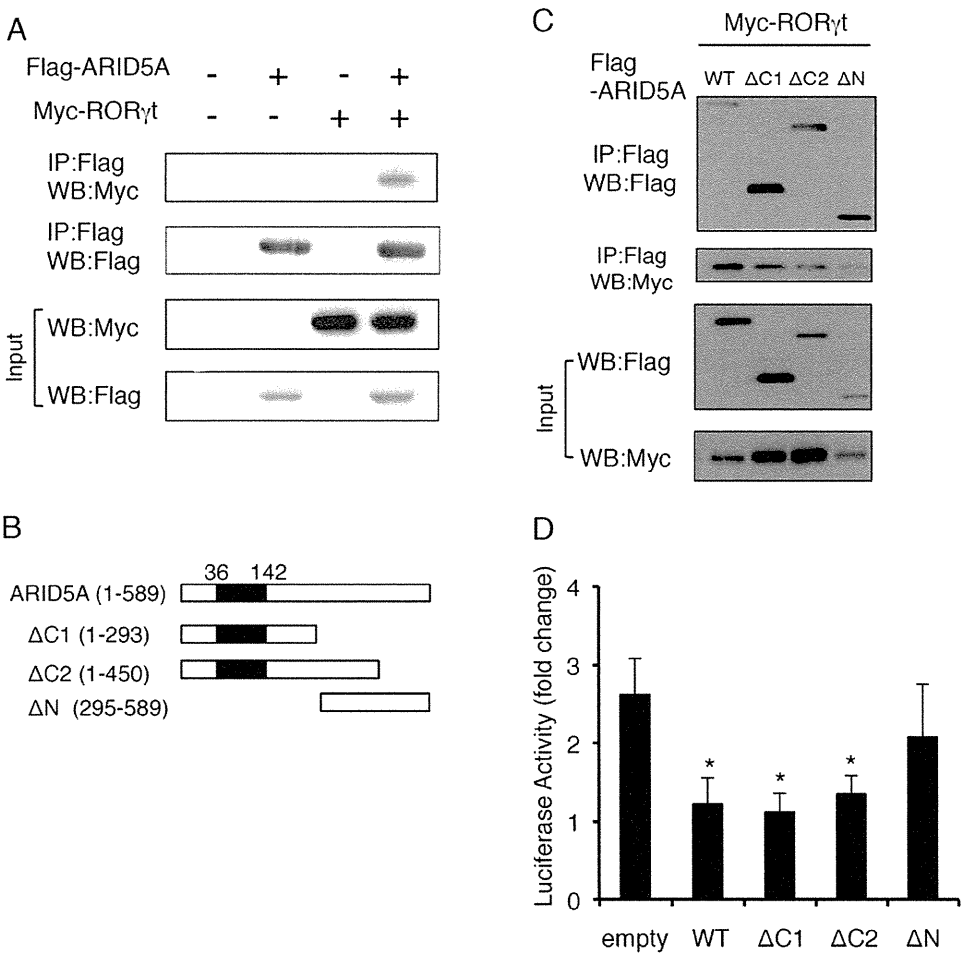

Figure 5. ARID5A physically associates with RORγt and inhibits its function
(A) 293T cells were co-transfected with Flag-tagged ARID5A (pcDNA3-Flag-ARID5A) and Myc-tagged RORγt (MSCV-myc-RORγt-IRES-Thy1.1). Whole cell lysate was immunoprecipitated with anti-Flag mAb and blotted with anti-Myc mAb. Shown are representative of 4 independent experiments. (B) A schematic diagram of truncated mutants of ARID5A. ΔC1 and ΔC2 indicate N-terminal amino acid 1-293 and 1-450 of ARID5A, respectively. ΔN indicates C-terminal amino acid 295-589 of ARID5A. (C) 293T cells were transfected with Flag-tagged wild-type (WT) or mutant ARID5A and Myc-tagged RORγt. Co-immunoprecipitation assay was performed as described in A. (D) EL4 cells were transfected with -153 mIL17p-Luc in the presence of MSCV-myc-RORγt-IRES-Thy1.1 (1 μg) and empty pcDNA3, pcDNA3 ARID5A (WT), or its truncated mutants. Twenty-four hours after the transfection, the luciferase activity of reporter constructs was determined by dual luciferase assay. Data are means ± SD of fold induction of luciferase activity relative to MSCV-myc-IRES-Thy1.1-transfected cells. n = 4 experiments. *, significantly different from the mean value of the empty pcDNA3-transfected cells, $p<0.05$.
80x83mm (300 x 300 DPI)

nature genetics

High-density genotyping study identifies four new susceptibility loci for atopic dermatitis

Atopic dermatitis is a common inflammatory skin disease with a strong heritable component. Pathogenetic models consider keratinocyte differentiation defects and immune alterations as scaffolds[1], and recent data indicate a role for autoreactivity in at least a subgroup of patients[2]. *FLG* (encoding filaggrin) has been identified as a major locus causing skin barrier deficiency[3]. To better define risk variants and identify additional susceptibility loci, we densely genotyped 2,425 German individuals with atopic dermatitis (cases) and 5,449 controls using the Immunochip array followed by replication in 7,196 cases and 15,480 controls from Germany, Ireland, Japan and China. We identified four new susceptibility loci for atopic dermatitis and replicated previous associations. This brings the number of atopic dermatitis risk loci reported in individuals of European ancestry to 11. We estimate that these susceptibility loci together account for 14.4% of the heritability for atopic dermatitis.

Genome-wide association studies (GWAS) have shown remarkable overlap across immune-mediated diseases[4]. Two European GWAS on atopic dermatitis established four susceptibility loci (*C11orf30*, *OVOL1*, *ACTL9* and *RAD50-IL13-KIF3A*) in addition to *FLG*[5,6]. At *C11orf30*, the same allele also confers risk to asthma[7] and Crohn's disease[8]. For *RAD50-IL13*, locus agonistic effects were observed for asthma[9], and locus antagonistic effects were observed for psoriasis[10]. Two further loci were reported in a Chinese GWAS (*TNFRSF6B-ZGPAT* and *TMEM232-SLC25A46*)[11]. All loci were confirmed in a recent Japanese GWAS, which additionally reported eight new loci (*IL1RL1-IL18R1-IL18RAP*, *MHC*, *OR10A3-NLRP10*, *GLB1*, *CCDC80*, *CARD11*, *ZNF365* and *CYP24A1-PFDN4*)[12]. However, the causal variants at all loci except *FLG* are unknown. To better define susceptibility variants and evaluate loci implicated in other immune-mediated diseases, we genotyped 2,425 German cases with atopic dermatitis and 5,449 German population controls (**Supplementary Table 1a**) using the Immunochip array[13] followed by replication in four independent collections (**Supplementary Table 1b–d**).

After quality control, 128,830 SNPs with a minor allele frequency >1% were available for analysis (Online Methods). The initial comparison of the case-control frequencies yielded 131 and 663 SNPs within 5 and 33 genomic loci with $P_{\text{Immunochip}} < 5 \times 10^{-8}$ and $P_{\text{Immunochip}} < 10^{-4}$, respectively (**Fig. 1**). Of the five atopic dermatitis loci previously reported in European ancestry populations, three reached conservative genome-wide significance (GWS, defined as $P < 5 \times 10^{-8}$; $P_{1q21.3} = 4.51 \times 10^{-20}$, $P_{5q31.1} = 1.99 \times 10^{-14}$ and $P_{11q13.5} = 5.22 \times 10^{-11}$) (**Table 1**). For all three of these loci, we observed stronger association signals as compared to those of previously reported SNPs[5,6] (**Supplementary Table 2**), for which Immunochip data were used to refine the 5q31.1 locus[6]. Variant rs72702813 at 1q21.3 is located 5,275 bases upstream of *LCE3A* (encoding late cornified envelope gene 3A), a member of the *LCE3* group, which contains a psoriasis risk-associated deletion (*LCE3C_LCE3B*-del)[14] and encodes proteins that are involved in barrier repair with differential expression in atopic dermatitis[15].

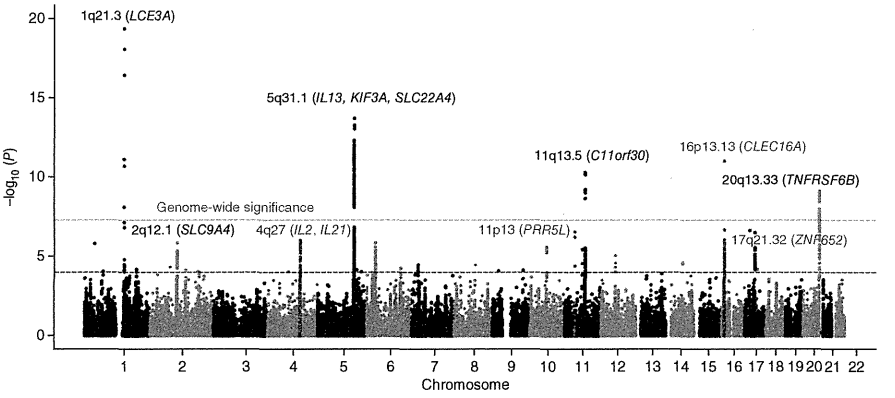

Figure 1 Manhattan plot of the Immunochip association statistics highlighting atopic dermatitis susceptibility loci. The red horizontal line indicates a genome-wide significance threshold of $P = 5 \times 10^{-8}$, and the black horizontal line indicates the threshold for follow-up genotyping of the most strongly associated SNPs ($n = 34$) with $P_{\text{Immunochip}} < 10^{-4}$ from each associated locus in an independent case-control collection (**Supplementary Table 1b**). SNPs within five known and four newly associated loci (depicted in blue) (**Table 1**) reached the GWS threshold for association with atopic dermatitis in the combined analysis of the Immunochip discovery and replication stages (**Supplementary Table 1a–c**).

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Received 6 October 2012; accepted 25 April 2013; published online 2 June 2013; doi:10.1038/ng.2642

Table 1 Susceptibility loci associated with atopic dermatitis in Europeans at GWS

Chr.	Association boundaries (kb)	dbSNP ID	A1	A2	AF_{cases}	$AF_{controls}$	Key genes (*n* additional genes in the locus)	Discovery Immunochip (2,425/5,449)[a]		Replication (1,951/4,599)[a]		Immunochip + replication (4,376/10,048)[a]		Associations to other traits
								P	OR (95% CI)	*P*	OR (95% CI)	$P_{combined}$	OR (95% CI)	
Previously reported atopic dermatitis susceptibility loci[b] meeting genome-wide significance														
1q21.3	150803–151051	rs72702813	T	G	0.08147	0.04056	*LCE3A* (15)	4.51×10^{-20}	1.91 (1.66–2.19)	2.22×10^{-16}	2.46 (1.98–3.04)	1.49×10^{-33}	2.06 (1.83–2.31)	PS
2q12.1	102225–102619	rs759382	C	A	0.2866	0.2454	*SLC9A4* (5)	1.40×10^{-6}	1.21 (1.12–1.30)	9.69×10^{-6}	1.23 (1.12–1.35)	6.01×10^{-11}	1.22 (1.15–1.29)	AS, CD, CeliD, EC, WBCT
5q31.1	131812–132167	rs848	T	G	0.2679	0.2067	*IL13* (8)	1.99×10^{-14}	1.36 (1.25–1.47)	2.33×10^{-15}	1.47 (1.34–1.62)	8.22×10^{-28}	1.40 (1.32–1.49)	AS[c], CD, IgE[c], PS[c], HL[c], PIC, FIB, EC, CRP
11q13.5	75724–76017	rs7110818	A	G	0.4992	0.4392	*C11orf30* (1)	5.22×10^{-11}	1.25 (1.17–1.34)	7.20×10^{-7}	1.35 (1.20–1.53)	3.33×10^{-16}	1.28 (1.21–1.36)	AS, CD, ALRH, IgEgs, UC
20q13.33	61678–61872	rs909341	A	G	0.1809	0.226	*TNFRSF6B* (8)	7.73×10^{-10}	0.76 (0.70–0.83)	1.90×10^{-7}	0.77 (0.70–0.85)	7.77×10^{-16}	0.77 (0.72–0.82)	CD, U[c], polBD, GL
New atopic dermatitis susceptibility loci meeting genome-wide significance														
4q27	123204–123784	rs17389644	A	G	0.2774	0.2396	*IL2-IL21* (2)	1.16×10^{-6}	1.21 (1.12–1.30)	0.0026	1.16 (1.05–1.28)	1.39×10^{-8}	1.19 (1.12–1.26)	RA, CeliD, UC, T1D, T1DA, IgEgs, PSNP, AA
11p13	36355–36438	rs12295535	A	G	0.04045	0.02376	*PRR5L*	2.71×10^{-7}	1.63 (1.35–1.96)	4.32×10^{-7}	1.75 (1.41–2.17)	7.96×10^{-13}	1.68 (1.46–1.93)	–
16p13.13	10930–11218	rs2041733	A	G	0.4903	0.4528	*CLEC16A-DEXI*	1.00×10^{-11}	1.26 (1.18–1.35)	3.07×10^{-5}	1.18 (1.09–1.28)	3.44×10^{-15}	1.23 (1.17–1.29)	MS, T1D, T1DA, PBC, IgA, AA, ALRH
17q21.32	44641–44875	rs16948048	G	A	0.4252	0.3848	*ZNF652* (5)	6.46×10^{-5}	1.15 (1.07–1.23)	8.45×10^{-6}	1.20 (1.11–1.30)	2.92×10^{-9}	1.17 (1.11–1.23)	H

[a]Number of cases/number of controls. [b]We replicated the association at the 20q13.33 (*TNFRSF6B*) and 2q12.1 (*SLC4A*) loci, previously reported in Chinese and Japanese populations, respectively, with GWS in Europeans. Two other known atopic dermatitis loci in Europeans from previous GWAS (*OVOL1* and *ACTL9*) are sparsely covered on the Immunochip (see the main text and **Supplementary Fig. 1**). [c]A known risk SNP for the listed trait is in high LD ($r^2 > 0.9$) with the atopic dermatitis hit SNP (Online Methods). The *P* values and ORs were calculated with respect to the minor allele. Chr., chromosome of the marker; genomic positions were retrieved from NCBI dbSNP build v130 (genome build hg18); A1, minor allele (corresponds to the risk allele, except for rs909341, which is protective); A2, major allele; AF, allele frequency of A1 estimated from Immunochip and replication; key gene(s), candidate gene(s) in the region; AA, alopecia areata; ALRH, allergic rhinitis; AS, asthma; CD, Crohn's disease; CeliD, celiac disease; CRP, C-reactive protein levels; EC, eosinophil counts; FIB, fibrinogen; GL, glioma; H, height; HL, Hodgkin's lymphoma; IgA, immunoglobulin A; IgE, total IgE levels; IgEgs, IgE grass sensitization; MS, multiple sclerosis; PBC, primary biliary cirrhosis; PIC, platelet counts; polBD, pediatric-onset inflammatory bowel disease; PS, psoriasis; PSNP, progressive supranuclear palsy; RA, rheumatoid arthritis; T1D, type 1 diabetes; T1DA, type 1 diabetes autoantibodies; UC, ulcerative colitis; WBCT, white blood cell types.

Analysis of linkage disequilibrium (LD) patterns showed that rs72702813 does not tag the psoriasis deletion ($D' = 1.0$, $r^2 = 0.09$ with proxy SNP rs4112788 (ref. 14)) but is in moderate LD ($D' = 0.63$, $r^2 = 0.38$) with the known *FLG* mutation c.2282del4 (**Supplementary Table 3**). After conditioning on *FLG* mutations (p.Arg501X, c.2282del4, p.Arg2447X and p.Ser3247X), rs72702813 no longer showed association ($P_{cond} = 0.94$, odds ratio (OR) = 0.99, 95% confidence interval (CI) 0.77–1.28).

Another locus at *CLEC16A* (16p13.13), which was not previously known to be associated with atopic dermatitis, attained GWS ($P_{rs2041733} = 1.00 \times 10^{-11}$, OR = 1.26, 95% CI 1.18–1.35) (**Table 1**). For the two remaining established loci, we observed a significant signal for *OVOL1* (11q13.1) ($P_{rs11820062} = 3.60 \times 10^{-6}$) but not *ACTL9* (19p13.2) ($P_{rs2967682} = 0.18$) (**Supplementary Fig. 1**), which is sparsely covered on the Immunochip ($r^2 = 0.15$ between rs2967682 and the lead SNP rs2164983 from a previous GWAS[6]). Furthermore, we replicated the association at the *TNFRSF6B* (20q13.33) locus ($P_{rs909341} = 7.73 \times 10^{-10}$, OR = 0.76, 95% CI 0.70–0.83), which was previously reported in a Chinese population, with GWS in Europeans. This gene encodes a soluble decoy receptor (DcR3) that acts as an immunomodulator (for example, in support of T helper type 2 (T_H2) cell polarization, which is a hallmark feature of atopic dermatitis)[16]. DcR3 is overexpressed in inflamed epithelia, and increased serum concentrations of this protein have been reported in autoimmune and inflammatory diseases[17,18]. In line with this, we observed a slight overexpression in serum from patients with atopic dermatitis ($P_{Fisher} = 0.00049$; **Supplementary Fig. 2**). However, immunohistochemistry showed strong epidermal staining with no clear differences between atopic dermatitis lesional and healthy skin (**Supplementary Fig. 3**). No proxy SNPs ($r^2 > 0.5$) were available for the 5q22.1 locus identified in the Chinese population (*TMEM232-SLC25A46*). For the recently reported loci in a Japanese population, we observed significant associations at 2q12.1 (*IL1RL1-IL18R1-IL18RAP*, $P_{rs13015714} = 2.81 \times 10^{-5}$, OR = 1.18, 95% CI 1.09–1.27), 6p21.3 (*GPSM3*, $P_{rs176095} = 2.53 \times 10^{-5}$, OR = 0.83, 95% CI 0.76–0.90) and 7p22 (*CARD11*, $P_{rs6978200} = 2.34 \times 10^{-3}$, OR = 1.12, 95% CI 1.04–1.20, $r^2 = 0.58$ with the reported SNP rs4722404). We found no association for 3p21.33 (*GLB1*, $P_{rs35480293} = 0.80$, $r^2 = 0.92$ with the reported SNP rs6780220) or 10q21.2 (*ZNF365*, $P_{rs10995251} = 0.08$). The 3q13.2 (*CCDC80*) and 20q13 (*CYP24A1-PFDN4*) loci have more limited coverage on the Immunochip array.

To identify additional susceptibility loci, we analyzed the most strongly associated SNPs ($n = 34$) with $P_{Immunochip} < 10^{-4}$ after the clumping procedure from each associated locus in an independent set of 794 German cases and 3,338 controls (**Supplementary Table 1b**). We further genotyped SNPs replicated at the 0.05 significance level ($n = 15$; **Supplementary Table 4**) in 1,157 Irish childhood cases and 1,261 controls (**Supplementary Table 1c**). In a meta-analysis ($P_{Immunochip+Repl}$) of the discovery ($P_{Immunochip}$) and replication (P_{Repl}) stages (**Supplementary Table 1a–c**), SNPs within six distinct regions met the GWS threshold (**Table 1**). Again, we observed association at 16p13.13 for SNP rs2041733 in *CLEC16A* ($P_{Immunochip+Repl} = 3.44 \times 10^{-15}$, $OR_{Immunochip+Repl} = 1.23$, 95% CI 1.17–1.29). *CLEC16A* encodes a sugar-binding, C-type lectin expressed on B lymphocytes, natural killer cells and dendritic cells that is functionally active through an immunoreceptor tyrosine-based activation motif (ITAM)[19]. Several SNPs in *CLEC16A* have been associated with immune-mediated diseases such as multiple sclerosis, type 1 diabetes[20,21] and alopecia areata[22], a frequent comorbidity of atopic dermatitis.

We found a significant association at 11p13 for rs12295535 in *PRR5L* ($P_{Immunochip+Repl} = 7.96 \times 10^{-13}$, $OR_{Immunochip+Repl} = 1.68$, 95% CI 1.46–1.93), which encodes a protein that promotes apoptosis[23].

Table 2 Susceptibility loci associated with atopic dermatitis in Japanese and Chinese replication case-control studies

Chr.	Association boundaries (kb)	dbSNP ID	A1	A2	Key genes (*n* additional genes in the locus)	Immunochip + Replication Europeans (4,376/10,048)[a] *P*	Immunochip + Replication Europeans (4,376/10,048)[a] OR (95% CI)	Replication Japan (2,397/7,937)[a] *P*	Replication Japan (2,397/7,937)[a] OR (95% CI)	Replication China (2,848/2,944)[a] *P*	Replication China (2,848/2,944)[a] OR (95% CI)
Previously reported atopic dermatitis susceptibility loci[b]											
1q21.3	150803–151051	rs72702813	T	G	*LCE3A* (15)	1.49×10^{-33}	2.06 (1.83–2.31)	–	–	0.3261	1 (0–∞)
2q12.1	102225–102619	rs759382	C	A	*SLC9A4* (5)	6.01×10^{-11}	1.22 (1.15–1.29)	1.36×10^{-9}	1.22 (1.15–1.30)	0.4540	0.97 (0.90–1.05)
5q31.1	131812–132167	rs848	T	G	*IL13* (8)	8.22×10^{-28}	1.40 (1.32–1.49)	5.14×10^{-10}	1.24 (1.16–1.33)	1.28×10^{-6}	1.21 (1.12–1.31)
11q13.5	75724–76017	rs7110818	A	G	*C11orf30* (1)	3.33×10^{-16}	1.28 (1.21–1.36)	6.34×10^{-6}	1.16 (1.09–1.24)	0.0320	1.08 (1.01–1.17)
20q13.33	61678–61872	rs909341	A	G	*TNFRSF6B* (8)	7.77×10^{-16}	0.77 (0.72–0.82)	7.74×10^{-4}	0.83 (0.84–0.95)	1.52×10^{-7}	0.82 (0.76–0.88)
New atopic dermatitis susceptibility loci											
4q27	123204–123784	rs17389644	A	G	*IL2-IL21 (2)*	1.39×10^{-8}	1.19 (1.12–1.26)	0.2492	1.06 (0.96–1.18)	0.1599	1.08 (0.97–1.21)
11p13	36355–36438	rs12295535	A	G	*PRR5L*	7.96×10^{-13}	1.68 (1.46–1.93)	0.0074	1.31 (1.08–1.60)	0.1588	1.13 (0.95–1.34)
16p13.13	10930–11218	rs2041733	A	G	*CLEC16A-DEXI*	3.44×10^{-15}	1.23 (1.17–1.29)	0.0063	1.09 (1.03–1.18)	1.23×10^{-4}	1.18 (1.08–1.28)
17q21.32	44641–44875	rs16948048	G	A	*ZNF652* (5)	2.92×10^{-9}	1.17 (1.11–1.23)	1.87×10^{-5}	1.22 (1.12–1.34)	0.04224	1.10 (1.00–1.20)

[a]Number of cases/number of controls. [b]We replicated the association at the 20q13.33 (*TNFRSF6B*) and 2q12.1 (*SLC4A*) loci, previously reported in Chinese and Japanese populations, respectively, with GWS in Europeans. Two other known atopic dermatitis loci in Europeans from previous GWAS (*OVOL1* and *ACTL9*) are sparsely covered on the Immunochip (see the main text and **Supplementary Fig. 1**). Chr., chromosome of the marker; genomic positions were retrieved from NCBI dbSNP build v130 (genome build hg18); A1, minor allele; A2, major allele; key gene(s), candidate gene(s) in the region. *P* values and ORs were calculated with respect to the minor allele. rs72702813 failed replication genotyping in the Japanese study due to technical reasons.

At 2q12.1, the associated SNP (rs759382, $P_{\text{Immunochip+Repl}} = 6.01 \times 10^{-11}$, $\text{OR}_{\text{Immunochip+Repl}} = 1.22$, 95% CI 1.15–1.29) maps to a 400-kb LD block encompassing four genes (*IL1RL1*, *IL18R1*, *IL18RAP* and *SLC9A4*). *IL1RL1* encodes a receptor for IL-33, which promotes T_H2 cell responses[24], and the products of *IL18RAP* and *IL18R1* form the receptor for IL-18, which has multiple immunologic functions, including the induction of T_H1 cell responses. Various SNPs in *IL1RL1*, *IL18R1* and *IL18RAP* are associated with asthma and related traits, and the effect has been attributed to *IL1RL1* (refs. 9,25,26). In addition, variants in *IL18R1* and *IL18RAP* have been associated with Crohn's disease[27] and celiac disease[28]. Stepwise conditional regression identified evidence for three independent signals (rs759382 in *SLC9A4*; rs3771180 in *IL1RL1*, which was previously implicated in asthma[29]; and rs10185897 in *IL1RL1-IL18R1*) with $P < 5 \times 10^{-4}$ and showed that the recently reported variant rs13015714 (ref. 12) tags rs759382 (**Supplementary Table 5**).

We found additional significant associations for rs16948048 at 17q21.32 (*ZNF652*, $P_{\text{Immunochip+Repl}} = 2.92 \times 10^{-9}$, $\text{OR}_{\text{Immunochip+Repl}} = 1.17$, 95% CI 1.11–1.23) and rs17389644 at 4q27 (*IL2-IL21*, $P_{\text{Immunochip+Repl}} = 1.39 \times 10^{-8}$, $\text{OR}_{\text{Immunochip+Repl}} = 1.19$, 95% CI 1.12–1.26). *ZNF652* encodes a transcriptional repressor that is implicated in epithelial cancers[30]. IL-2 has pleiotropic immunoregulatory functions, in particular control of the proliferation and survival of regulatory T cells[31]. The *IL2* locus is tightly linked with *IL21*, and variants in *IL2*, its high-affinity receptor *IL2RA* and *IL21* have been associated with multiple immune-mediated diseases. None of the SNPs chosen from the major histocompatibility complex replicated. Regional association plots of the nine atopic dermatitis susceptibility loci with GWS in Europeans are shown in **Supplementary Figure 4**. The four newly associated loci collectively increase the explained heritability from 9% to 14.4% (**Supplementary Table 6**).

To further determine the impact of the new susceptibility loci identified in Europeans on atopic dermatitis risk in diverse populations, we tested them for association in 2,397 adult Japanese cases and 7,937 controls from a recent GWAS[12] and 2,848 adult Chinese cases and 2,944 controls (**Supplementary Table 1d**). In the Japanese study population, all new loci except *IL2-IL21* passed the Bonferroni-corrected significance threshold ($P < 0.05/6 = 0.008$) for replication, and in the Chinese study population, two loci (*CLEC16A* and *TNFRSF6B*) passed this threshold (**Table 2** and **Supplementary Table 4**). Thus, *CLEC16A* and *TNFRSF6B* seem to be relevant to atopic dermatitis in both these European and Asian populations, whereas results for the other loci might reflect phenotypic and ancestry differences between the studies.

Because atopic dermatitis is often coexpressed with asthma, to enhance the interpretation of our findings, we analyzed Immunochip data from an independent set of 733 German cases with asthma[32] and 2,503 controls for atopic dermatitis, asthma, atopic dermatitis without asthma and asthma without atopic dermatitis (Online Methods). We found that all of the newly identified susceptibility loci associated primarily with atopic dermatitis (**Supplementary Table 7**).

For the nine loci associated at GWS (**Table 1**), we identified seven coding SNPs highly correlated ($r^2 > 0.9$ in 1000 Genomes Project European samples) with the lead SNPs (**Supplementary Table 8**). However, these nonsynonymous SNPs are predicted *in silico* to have a nondamaging effect on protein products.

Analysis of whole-blood samples from 740 German control individuals identified evidence for correlation between the expression of *IL1RL1*, *ARAP3*, *MAP3K11* and *STMN3* and SNP alleles in high LD ($r^2 > 0.95$) with the most strongly associated SNPs listed in **Table 1** (**Supplementary Table 9**). Examination of expression levels in skin biopsies from 64 healthy controls run on HU133 Plus 2.0 arrays[33] yielded no evidence for *cis*-regulatory effects (**Supplementary Table 10**). However, a regulatory effect in another physiological state (for example, atopic dermatitis) cannot be ruled out.

We next looked for statistical interactions (allelic-by-allelic epistasis) between lead SNPs of each locus shown in **Table 1** (**Supplementary Table 11**). One SNP pair (rs848 (*IL13*) and rs2041733 (*CLEC16A*)) showed evidence for interaction ($P = 5.41 \times 10^{-4}$) after Bonferroni correction ($P < 0.05/36 = 1.39 \times 10^{-3}$). rs848 is in tight LD with the functional *IL13* variant rs20541 ($r^2 = 0.979$), which affects the activation of the signal transducer and activator of transcription 6 (STAT6) signaling pathway[34]. CLEC16A is thought to act through its ITAM, the ligation of which modulates JAK-STAT signaling[35]. Thus, the observed interactions reflect potential functional links, which need further investigation.

In summary, our dense genotyping approach using the Immunochip array identified four new atopic dermatitis risk loci in Europeans (**Table 1**), adding 5.4% to the estimate of explained atopic dermatitis heritability and bringing the total to 14.4% heritability explained by currently reported susceptibility loci. Our results expand the catalog of genetic loci implicated in atopic dermatitis and provide evidence for a substantial contribution of loci shared with other immune-mediated diseases.

URL. PopGen biobank, http://www.popgen.de.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

ACKNOWLEDGMENTS

We thank all individuals with atopic dermatitis, their families, control individuals and clinicians for their participation. We thank T. Wesse, T. Henke, S.S. Sabet, S. Greve, I. Urbach, G. Patone, C. Flachmeier, S. Kolberg and G. Born for expert technical help. This study was supported by the Deutsche Forschungsgemeinschaft (DFG) grant FR 2821/2-1, the German Ministry of Education and Research (BMBF) through the National Genome Research Network (NGFN; 01GS 0818 and 01GS 0812 to Y.-A.L.) and the PopGen biobank. S.W. is supported by a Heisenberg fellowship of the DFG (WE 2678/7-1). The project received infrastructure support through the DFG Clusters of Excellence 'Inflammation at Interfaces'. A.D.I. is supported by the National Children's Research Centre, Dublin. A.D.I. and I.M. are supported by the Wellcome Trust (reference 090066/B/09/Z and 092530/Z/10/Z).

S. Brand is supported by DFG grant BR1912/6-1 and the Else-Kröner-Fresenius-Stiftung (stipend 2010_EKES.32). We acknowledge US National Institutes of Health grant 1R01CA141743-01 (R.H.D., principal investigator) and the University of Pittsburgh Genomics and Proteomics Core Laboratories for the Immunochip genotyping services. This work was supported by Wellcome Trust Programme and Bioresources grants (090066/B/09/Z and 092530/Z/10/Z) to W.H.I.M. and A.D.I. and a Wellcome Trust Strategic Award (098439/Z/12/Z) to W.H.I.M. Parts of this study were funded by the General Program of National Natural Science Foundation of China (31171224), the Program for New Century Excellent Talents in University (NCET-11-0889), the Science and Technological Foundation of Anhui Province for Outstanding Youth (1108085J10) and a pre-project of State Key Basic Research Program 973 of China (2012CB722404).

AUTHOR CONTRIBUTIONS

S.W., A.F., Y.-A.L. and D.E. designed the experiments. E.R., J.E.-G., A.M., I.M., N.H., H.S., U.M.-H., M.H., M. Kubo, W.H.I.M. and N.N. performed wet lab experiments. D.E., H.B. and S.M. analyzed the data. S.W., Y.-A.L., N.N., L.M., R.F.-H. and T.W. provided German case samples. H.S. helped providing case samples. M.T., A.T., Y.N., T.H. and M. Kubo provided Japanese replication data. A.D.I., S. Brown, M.A.M. and C.M.F. provided Irish replication data. L.S., X. Zuo, S.Y. and X. Zhang provided Chinese replication data. B.O.B. provided German control samples from the Echinococcus Multilocularis and Internal Diseases in Leutkirch (EMIL) study. P.H. and M.M.N. provided German control samples. S. Brand, J.G. and C.B. provided German control samples, which were genotyped at the University of Pittsburgh Genomics and Proteomics Core Laboratories (R.H.D., principal investigator). J.W. and T.I. provided German control samples. M. Kabesch provided Immunochip data from the cases with asthma from the Multicenter Asthma Genetics in Childhood (MAGIC) and International Study of Asthma and Allergies in Childhood (ISAAC) studies. H.P., K.H., T.I., C.H., L.C.T., P.S. and J.T.E. contributed and analyzed expression data. S.W., A.F., S.S., N.H. and Y.-A.L. supervised the experiments. D.E., H.B., S.W. and A.F. wrote the paper. All authors reviewed, edited and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Study subjects.

Immunochip data. All German cases with atopic dermatitis (**Supplementary Table 1a**) were recruited from tertiary dermatology and pediatrics clinics based at four centers (Technische Universität Munich, as part of the Gene-Environment Association (GENEVA) study, the University of Kiel, the University of Bonn and University Children's Hospital, Charité Universitätsmedizin Berlin, as part of the Genetic Studies in Nuclear Families with Atopic Dermatitis (GENUFAD) study). Atopic dermatitis was diagnosed on the basis of a skin examination performed by experienced dermatologists and pediatricians according to standard criteria, which included the presence of chronic or chronically relapsing pruritic dermatitis with the typical morphology and distribution[36]. A total of 2,461 German controls were obtained from the PopGen biorepository[37]. A total of 1,545 cases with atopic dermatitis and all 2,461 controls from PopGen were genotyped at the Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, and 880 cases with atopic dermatitis were genotyped at the Max-Delbrück-Centrum (MDC) for Molecular Medicine (Berlin-Buch, Germany). Nine-hundred seventy-nine German controls were selected as part of an independent population-based sample from the general population living in the region of Augsburg (Cooperative Heath Research in the Region of Augsburg (KORA)), southern Germany[38], and were genotyped at the Helmholtz Center in Munich. Three hundred and two control individuals were of south German ancestry and were part of the control population from Munich recruited from the Bavarian Red Cross; 208 control individuals were recruited from the Charité-Universitätsmedizin Berlin. These samples were genotyped at the University of Pittsburgh Genomics and Proteomics Core Laboratories (R.H.D., principal investigator). The Bonn controls (n = 1,499) were recruited from the population-based epidemiological Heinz Nixdorf Recall study[39] and genotyped at the Life and Brain Center at the University Clinic in Bonn. Of the cases with atopic dermatitis used for the screen with available phenotype information, 33.1% and 31.0% (**Supplementary Table 1a**) suffered from comorbid asthma.

For *in silico* analysis of the selected SNPs for asthma, Immunochip data from 733 German cases with asthma from the MAGICS and German ISAAC studies[9], as well as 2,503 controls from PopGen, were used.

Replication data. For follow-up genotyping (**Supplementary Table 1b**), we used 794 cases recruited at tertiary dermatology clinics in Munich, Bonn, Kiel and Hannover (Technische Universität Munich, as part of the GENEVA study, the University of Bonn, the University of Kiel and Medizinische Hochschule of Hannover). A total of 2,412 German control individuals were selected as part of the EMIL study, an independent population-based sample from the general population living in Leukirch, southern Germany[40]. A total of 926 German control individuals were selected as part of an independent population-based sample from the general population living in the region of Augsburg (KORA), southern Germany[38], and were genotyped at the Helmholtz Center in Munich. The Irish case-control collection consisted of 1,157 unrelated children of self-reported Irish ancestry with moderate-to-severe atopic dermatitis recruited from the tertiary referral pediatric dermatology clinic based at Our Lady's Children's Hospital, Dublin (**Supplementary Table 1c**). A total of 1,261 unselected control samples were obtained from the population-based Trinity Biobank Control samples.

For further replication, a total of 2,397 Japanese cases and 7,937 Japanese controls were analyzed (**Supplementary Table 1d**). Cases were recruited from several medical institutes, including Fukujuji Hospital, Iizuka Hospital, Juntendo University, Hospital Iwate Medical University School of Medicine, National Hospital Organization Osaka National Hospital, Nihon University, Nippon Medical School, Shiga University of Medical Science, Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokushukai Hospital and Tokyo Metropolitan Geriatric Hospital[41]. Controls included 6,018 cases with one of five diseases (cerebral aneurysm, esophageal cancer, endometrial cancer, chronic obstructive pulmonary disease or glaucoma) who did not have atopic dermatitis or bronchial asthma in BioBank Japan, 1,018 healthy volunteers from members of the Rotary Club of Osaka-Midosuji District 2660 Rotary International in Japan and 901 healthy subjects from the PharmaSNP Consortium. A total of 2,848 Chinese Han case samples with atopic dermatitis and 2,944 Chinese Han control samples (**Supplementary Table 1d**) were provided by S.Y. and X. Zhang.

Written, informed consent was obtained from all study participants, and the institutional ethical review committees of the participating centers approved all protocols.

Immunochip genotyping. DNA samples were genotyped using the Immunochip, which is an Illumina iSelect HD custom genotyping array. The Immunochip is a BeadChip developed for highly multiplexed SNP genotyping of complex DNA. Data were analyzed using Illumina's GenomeStudio Genotyping Module. The NCBI build 36 (hg18) map was used (Illumina manifest file Immuno_BeadChip_11419691_B.bpm), and normalized probe intensities were extracted for all samples that passed standard laboratory quality-control thresholds.

Immunochip genotype calling and quality control. Genotype calling was performed with the GenomeStudio GenTrain 2.0 algorithm (Illumina's GenomeStudio data analysis software) and the custom generated cluster file of Trynka *et al.* (based on an initial clustering of 2,000 UK samples and subsequent manual readjustment of cluster positions)[13].

SNPs that had >5% missing data, a minor allele frequency <1% or deviated from Hardy-Weinberg equilibrium (exact $P < 10^{-4}$ in controls) per sample study were excluded using PLINK software version 1.07 (ref. 42). Sample quality control measures included sample call rate, overall heterozygosity, relatedness testing and other metrics (**Supplementary Figs. 5–7**). The remaining 2,425 cases with atopic dermatitis and 5,449 controls were tested for population stratification using the principal components stratification method as implemented in EIGENSTRAT[43]. Principal component analysis revealed no population stratification in the remaining samples; no population outliers were detected. A total of 128,830 polymorphic SNPs were available for analysis. A quantile-quantile plot of the full association analysis showed a marked excess of significant associations in the tail of the distribution (**Supplementary Fig. 8a**), which was due primarily to hundreds of highly significant association signals from a few associated (fine-mapped) regions. A quantile-quantile plot using 2,714 'null' SNPs not associated with autoimmune disease (bipolar disease–associated SNPs)[13] is shown as negative control, and the inflation factor inferred from this showed only modest inflation (λ = 1.01; **Supplementary Fig. 8b**).

Replication genotyping. For replication genotyping, we selected the most strongly associated SNP (n = 34) with $P < 10^{-4}$ from each associated locus by means of PLINK's clumping procedure (using default settings: $P_1 < 0.0001$, $P_2 < 0.01$, $r^2 \geq 0.5$, kb = 250) representing 23 loci (see also **Supplementary Table 4**). Follow-up replication genotyping in the German study population was carried out using our Sequenom iPlex platform from Sequenom and TaqMan technology from Applied Biosystems. Replication typing in the Irish case-control collection was done using TaqMan technology from Applied Biosystems. Quality control was done for each country population separately. Individuals with >8% missing data were removed. SNPs that had >3% missing data or deviated from Hardy-Weinberg equilibrium (exact $P < 0.01$ in controls) per sample population were excluded. P values for allele-based tests of phenotypic association for each single replication population were calculated using R 2.14.2 (ref. 44). PLINK's meta-analysis function was used to obtain P values for the replication data set (P_{Repl}) (**Supplementary Table 1b,c**) and the combined discovery-replication data set ($P_{Immunochip+Repl}$) (**Supplementary Table 1a–c**). We used the commonly accepted threshold of $P = 5 \times 10^{-8}$ for joint P values to define statistical significance.

The Japanese replication set was typed using multiplex PCR–based Invader assay (Third Wave Technologies). Genotyping in the Chinese replication cohort was carried out using Sequenom technology.

Annotation of association boundaries. LD regions (association boundaries) around focal SNPs were defined by extending in both directions a distance of 0.1 centimorgans (cM) or until another SNP with $P < 10^{-5}$ was reached, in which case the process was repeated from this SNP. For each locus, candidate genes within regions are listed in columns labeled 'key gene(s)' in **Tables 1** and **2** and are listed in more detail in **Supplementary Table 4**.

Annotation of associations to other phenotypes. Overlaps with other phenotypes were annotated with the National Human Genome Research Institute

(NHGRI) GWAS catalog[45] (www.genome.gov/gwastudies, accessed December 19, 2012). All known associations with $P < 5 \times 10^{-8}$ to any disease or primary phenotype were included. For each atopic dermatitis susceptibility locus with association boundaries defined in **Table 1**, we annotated all phenotypes that had at least one associated SNP within the region. We also checked whether the hit SNP in the NHGRI GWAS catalog was the same as, or in high LD with ($r^2 > 0.9$), the atopic dermatitis hit SNP.

Stepwise conditional logistic regression and joint analysis. Multiple associated SNPs were selected through a stepwise selection procedure using GCTA (Genome-wide Complex Trait Analysis)[46] using SNP markers at 2q12.1 and a threshold P value of 5×10^{-4} to declare evidence for independently associated SNPs (–massoc-p 5e-4) (**Supplementary Table 5a**).

Expression quantitative trait loci look up. We analyzed gene expression data measured previously in whole blood (fasting conditions) and skin specimens. For analysis of whole blood, we used Illumina Human HT-12 v3 Expression BeadChip data from 740 adult individuals of the German population-based KORA (Cooperative Heath Research in the Region of Augsburg) F4 study performed in 2006–2008 (ref. 47) (**Supplementary Table 9**). For analysis of skin, we used Affymetrix HU133 Plus 2.0 arrays data from 57 healthy individuals[33] (**Supplementary Table 10**).

Statistical interaction analysis. To look for interactions between associated loci, we considered all distinct pairs ($n = 36$) of the nine lead SNPs listed in **Table 1** (see **Supplementary Table 11**).

Immunohistochemistry. Immunohistochemical staining of paraffin-embedded tissue of eight biopsies taken from lesions of patients with atopic dermatitis compared to eight healthy sex- and age-matched control persons was done by using monoclonal mouse anti-DcR3 (see **Supplementary Fig. 3**).

Serum measurements. Analysis of DcR3 serum concentrations was done using the DuoSet ELISA development system from R&D Systems (Wiesbaden, Germany) according to the manufacturer's instructions.

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