

Genome-wide association study identifies two new susceptibility loci for atopic dermatitis in the Chinese Han population

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Atopic dermatitis is a chronic, relapsing form of inflammatory skin disorder that is affected by genetic and environmental factors. We performed a genome-wide association study of atopic dermatitis in a Chinese Han population using 1,012 affected individuals (cases) and 1,362 controls followed by a replication study in an additional 3,624 cases and 12,197 controls of Chinese Han ethnicity, as well as 1,806 cases and 3,256 controls from Germany. We identified previously undescribed susceptibility loci at 5q22.1 (*TMEM232* and *SLC25A46*, rs7701890, $P_{\text{combined}} = 3.15 \times 10^{-9}$, odds ratio (OR) = 1.24) and 20q13.33 (*TNFRSF6B* and *ZGPAT*, rs6010620, $P_{\text{combined}} = 3.0 \times 10^{-8}$, OR = 1.17) and replicated another previously reported locus at 1q21.3 (*FLG*, rs3126085, $P_{\text{combined}} = 5.90 \times 10^{-12}$, OR = 0.82) in the Chinese sample. The 20q13.33 locus also showed evidence for association in the German sample (rs6010620, $P = 2.87 \times 10^{-5}$, OR = 1.25). Our study identifies new genetic susceptibility factors and suggests previously unidentified biological pathways in atopic dermatitis.

Atopic dermatitis is a common complex skin disease that is characterized by chronically relapsing skin inflammation, which often occurs in association with other atopic manifestations, such as allergic rhinitis and asthma. The clinical phenotype of atopic dermatitis is complex, and it has a prevalence of 1.4% to 22.3% worldwide¹. It has been suggested that genetic factors have an important role in the development of atopic dermatitis². Linkage and candidate gene association studies have identified several genetic loci that predispose carriers to atopic dermatitis, such as *FLG* (ref. 3). In particular, one recent genome-wide association study (GWAS) of atopic dermatitis in European populations has identified an atopic dermatitis susceptibility gene, *C11orf30* (ref. 4), that was also implicated in genetic susceptibility to Crohn's disease⁵. However, these genetic factors do not fully explain the genetic risk to atopic dermatitis, suggesting that additional genetic factors remain to be discovered. Furthermore, genetic heterogeneity of disease susceptibility variants between ethnic groups is common in complex diseases^{3,6}. To search for more susceptibility genes for atopic dermatitis, we carried out a GWAS of atopic dermatitis in 1,012 cases and 1,362 controls from southern China (Online Methods), followed

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Table 1 Sample summary information in the GWAS and replication studies

Analyses	Population	Cases				Controls		
		Sample size	Mean age (s.d.)	Mean age of onset	Male/female (%)	Sample size	Mean age (s.d.)	Male/female
GWAS	Southern Chinese	1,012	5.01 (7.63)	1.37 (4.68)	58.1/41.9	1,362	33.10 (13.60)	55.4/44.6
Replication 1	Northern Chinese	1,119	7.06 (9.81)	2.46 (6.98)	58.5/41.5	1,479	29.79 (13.14)	48.2/51.8
Replication 2	Southern Chinese	2,505	4.90 (6.64)	1.22 (3.88)	60.8/39.2	10,718	27.22 (12.45)	53.1/46.9
Replication 3	German	1,806	22.29 (15.70)	No data	43.9/56.1	3,256	45.78 (15.21)	47.2/52.8
Total		6,442	5.44 (7.78) ^a	1.55 (4.99) ^a	59.7/40.3 ^a	16,815	28.08 (12.79) ^a	52.8/47.2 ^a

^aSample summary information in the Chinese population.

by a replication study on an additional 3,624 cases and 12,197 controls of Chinese Han ethnicity, as well as 1,806 cases and 3,256 controls from Germany (Table 1).

Our GWAS dataset was comprised of 620,901 SNPs and copy number variant (CNV) probes. After quality control (Online Methods), we used the genotype data of 491,905 autosomal SNPs in 1,012 cases (with a mean age of 5.01 years) and 1,362 controls for association testing. Population structure analysis indicated minimal overall inflation of the genome-wide statistical results because of population stratification ($\lambda_{GC} = 1.049$; Fig. 1 and Supplementary Figs. 1, 2). No SNP exceeded genome-wide significance for association ($P < 5 \times 10^{-8}$).

To validate the results of the GWAS analysis, we selected 67 SNPs for replication in two independent samples of Chinese Han ethnicity (replication 1: 1,119 cases with a mean age of 7.06 years and 1,203 controls; replication 2: 2,505 cases with a mean age of 4.90 years and 6,762 controls; Supplementary Table 1). These SNPs included 44 SNPs with $P < 1 \times 10^{-4}$ in the genome-wide discovery analysis of 1,012 cases with atopic dermatitis and 1,362 healthy controls and 12 SNPs that showed stronger association evidence after including the additional 3,144 'pseudo-controls' in the genome-wide discovery analysis. The pseudo-controls were genotyped in a series of GWAS in the Chinese Han population for psoriasis⁷, vitiligo⁸, systemic lupus erythematosus (SLE)⁹ and leprosy¹⁰ (Online Methods). In addition, the replication analysis included 11 SNPs that were located within or close to seven susceptibility or candidate genes with confirmed or suggestive association with atopic diseases (atopic dermatitis and/or asthma) in European and Asian populations. These genes were reported by either GWAS or more than two independent candidate gene studies, and we selected those SNPs within the respective genes that showed the smallest P values in our initial GWAS analysis (Online Methods).

After quality control (Online Methods), 64 of the 67 selected SNPs for replication remained for further association analysis. We first performed the association analysis in the two independent Chinese replication samples separately, and then carried out a joint analysis in the combined GWAS and replication samples of Chinese Han ethnicity. The replication analysis revealed three SNPs in two loci that showed association with atopic dermatitis at genome-wide significance ($P_{combined} < 5 \times 10^{-8}$): rs3126085 and rs11204971 at 1q21.3 ($P_{combined} = 5.75 \times 10^{-12}$, OR = 0.83 and $P_{combined} = 5.02 \times 10^{-9}$, OR = 0.86, respectively) and rs7701890 at 5q22.1 ($P_{combined} = 4.33 \times 10^{-8}$, OR = 1.23; Supplementary Table 1).

To increase the sample size and therefore the statistical power of the replication analysis, we genotyped the most promising 19 SNPs (of the 67 selected SNPs) that showed supportive association evidence in the initial validation study in an additional 276 controls for the replication 1 sample and 3,956 controls for the replication 2 sample. Eighteen SNPs passed quality control (Online Methods) and were used for association analysis. Of these 18 SNPs, the associations at 7 SNPs in 3 loci (1q21.3, 5q22.1 and 20q13.33) reached genome-wide significance: rs3126085 and rs11204971 at 1q21.3 ($P_{combined} = 5.90 \times 10^{-12}$,

OR = 0.82 and $P_{combined} = 1.20 \times 10^{-10}$, OR = 0.85, respectively); rs7701890, rs10067777, rs13360927 and rs13361382 at 5q22.1 ($P_{combined} = 3.15 \times 10^{-9}$, OR = 1.24, $P_{combined} = 1.20 \times 10^{-8}$, OR = 1.24, $P_{combined} = 2.45 \times 10^{-8}$, OR = 1.22 and $P_{combined} = 4.02 \times 10^{-8}$, OR = 1.22, respectively); and rs6010620 at 20q13.33 ($P_{combined} = 3.0 \times 10^{-8}$, OR = 1.17; Table 2 and Supplementary Table 2). The association at rs3126085 and rs11204971 at 1q21.3 showed low heterogeneity ($I^2 = 39.4\%$ and $I^2 = 28.0\%$, respectively) among the GWAS and two replication samples (Supplementary Table 3). Consequently, the analysis of the combined GWAS and replication 1 and 2 samples under a random effects model yielded less significant associations at these two SNPs ($P_{combined} = 5.90 \times 10^{-12}$, OR = 0.82 and $P_{combined} = 1.20 \times 10^{-10}$, OR = 0.85, respectively) than the combined analysis under a fixed effects model ($P_{combined} = 2.34 \times 10^{-13}$, OR = 0.83 and $P_{combined} = 3.70 \times 10^{-10}$, OR = 0.85, respectively), but the associations remained significant at the genome-wide level. In addition, one promising SNP (rs2393903 at 10q21.2) failed to be genotyped in the expanded control samples, which showed a genome-wide association barely below significance ($P_{combined} = 1.05 \times 10^{-7}$, OR = 1.15; Table 2 and Supplementary Table 2). Further studies using larger sample sizes are needed to verify its significance for atopic dermatitis susceptibility. The detailed association results of the 19 SNPs are shown in Supplementary Table 2.

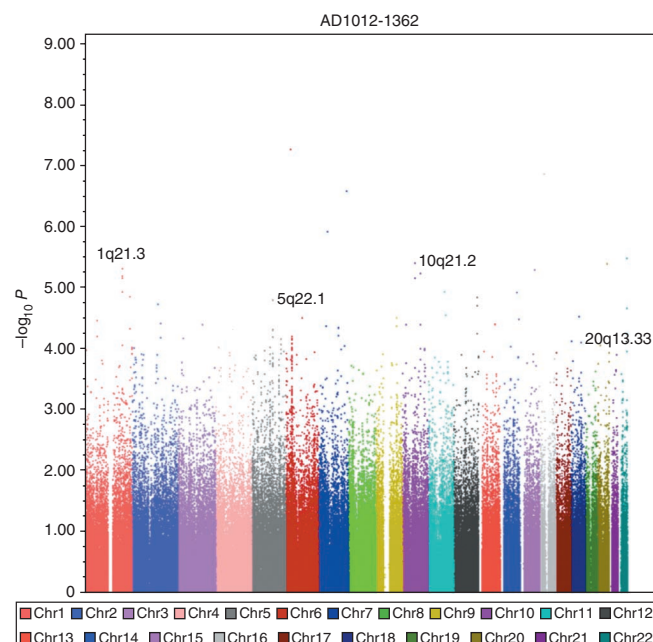


Figure 1 Genome-wide association results from the initial GWAS analysis. The genome-wide P values of the Cochran-Armitage trend test from 491,905 polymorphic SNPs in 1,012 atopic dermatitis cases and 1,362 controls of Chinese Han ancestry are presented. The chromosomal distribution of all the P values ($-\log_{10} P$) is shown.

Table 2 Association evidence for four SNPs at four loci in GWAS, replication and combined studies of Chinese Han

SNP		rs3126085	rs7701890	rs2393903	rs6010620
Chromosome		1q21.3	5q22.1	10q21.2	20q13.33
Gene		<i>FLG</i>	<i>TMEM232/SLC25A46</i>	<i>ZNF365</i>	<i>TNFRSF6B/ZGPAT</i>
Level of evidence		Confirmed locus	New locus	Suggestive locus	New locus
Minor allele		G	G	G	G
GWAS (1,012 cases, 1,362 controls)	Case	0.3898	0.1657	0.5094 ^d	0.3177
	Control	0.4273	0.1241	0.4431	0.2680
	OR	0.86	1.40	1.31	1.27
	<i>P</i> ^a	9.43×10^{-3}	4.87×10^{-5}	6.03×10^{-6}	1.87×10^{-4}
Replication 1 (1,119 cases, 1,479 controls)	Case	0.4087	0.1243	0.5252 ^d	0.2814
	Control	0.4430	0.1120	0.4885	0.2598
	OR	0.87	1.13	1.16	1.12
	<i>P</i> ^a	1.51×10^{-2}	1.79×10^{-1}	1.44×10^{-2}	8.42×10^{-2}
Replication 2 (2,505 cases, 10,718 controls)	Case	0.3690	0.1597	0.4773	0.2904
	Control	0.4230	0.1354	0.4541	0.2643
	OR	0.80	1.21	1.10	1.14
	<i>P</i> ^a	1.27×10^{-11}	1.08×10^{-5}	6.16×10^{-3}	1.86×10^{-4}
Combined (4,636 cases, 13,559 controls)	Case	0.3835	0.1526	0.4960	0.2943
	Control	0.4249	0.1310	0.4562	0.2629
	OR	0.82	1.24	1.15	1.17
	<i>P</i> ^a	5.90×10^{-12b}	3.15×10^{-9}	1.05×10^{-7c}	3.00×10^{-8}
	P-HWE	0.9397	0.3790	0.8876	0.2219
	Call rate	0.96	0.97	0.96	0.99

^aTwo-tailed *P* values from the Cochran-Armitage trend test. ^bThe *P* value was conducted by random effects model ($R^2 > 25\%$). ^cCombined *P* value of rs2393903 was based on 4,636 cases and 9,327 controls. ^dMajor allele frequency of rs2393903 in GWAS and replication 1.

To further assess the impact of the three new loci (5q22.1, 10q21.2 and 20q13.33) on genetic risk of atopic dermatitis in diverse populations, we analyzed the six SNPs of the three loci in one additional cohort from Germany (replication 3: 1,806 cases and 3,256 controls). Notably, the G allele of rs6010620 at 20q13.33 showed a consistent risk effect in both the Chinese Han ($P = 3.0 \times 10^{-8}$, OR = 1.17) and German ($P = 2.87 \times 10^{-5}$, OR = 1.25) samples (Table 3). We found no evidence for genetic interaction in the Chinese samples when testing pairwise interactions between the lead SNPs rs3126085 at 1q21.3, rs7701890 and rs2416257 at 5q22.1, rs2393903 at 10q21.2, rs7936562 at 11q13.5 and rs6010620 at 20q13.33 ($P_{\text{all}} > 0.1$, data not shown).

To uncover the susceptibility genes implicated in each newly discovered locus, we investigated the patterns of recombination and linkage disequilibrium (LD) around the risk-associated SNPs (Fig. 2). At 5q22.1, *TMEM232* and *SLC25A46* seem to be plausible candidate genes (Fig. 2). Four significant SNPs (rs10067777, rs7701890, rs13360927 and rs13361382) in *TMEM232* were in strong LD with each other (all pairwise $r^2 > 0.82$ in the HapMap CHB data, all pairwise $r^2 > 0.84$ in our GWAS sample set). Conditional logistic regression analysis showed that after we controlled for the genetic effect of the top SNP (rs7701890) in the locus, the associations at the other three SNPs disappeared (the smallest $P_{\text{condition}} = 0.19$), indicating that the associations at the four SNPs were not independent of each other. Little is known about the function of the annotated genes in the 5q22.1 locus. *TMEM232* encodes a transmembrane protein 232 belonging to the functional class of tetraspan transmembrane proteins, whereas *SLC25A46* belongs to the solute carrier 25 (SLC25) family. This family encodes mitochondrial carrier proteins, which could shuttle metabolites across the inner mitochondrial membrane¹¹. Further fine mapping and functional studies are needed to identify and characterize the causal gene(s) at this locus.

At 20q13.33, there were eight genes—*PRIC285*, *GMEB2*, *STMN3*, *RTKL1*, *TNFRSF6B*, *ARFRP1*, *ZGPAT* and *LIME1*—within a single

200-kb LD block around rs6010620 (Fig. 2). Expression quantitative trait loci (eQTL) analysis using the Sanger Institute Genevar database (see URLs) suggested that the risk-associated SNP rs6010620 might be associated with *TNFRSF6B* and *ZGPAT* expression ($P = 0.024$ and $P = 0.039$, respectively; Supplementary Table 4), although the associations are suggestive and need to be validated. *TNFRSF6B* has an important role in adaptive immune responses; it acts on T-cell^{12,13}, dendritic cell^{14,15} and macrophage responses¹⁶. Individuals with atopic dermatitis show increased mRNA expression of serum *TNFRSF6B* as compared to healthy individuals and control subjects with non-atopic inflammatory disease¹⁷. *ZGPAT* encodes a zinc finger CCCH domain-containing protein that is involved in the epidermal growth factor receptor (*EGFR*) pathway¹⁸. *EGFR* is over-expressed in lesional skin of individuals with atopic dermatitis, and blocking *EGFR* induces dysregulated chemokine expression in keratinocytes and leads to enhanced skin inflammation¹⁹. These results indicate that *TNFRSF6B* and *ZGPAT* are plausible candidate gene(s) for atopic dermatitis. However, further fine mapping and functional studies will be required to identify true susceptibility genes at 20q13.33.

At 10q21.2, the association signal refers to a single gene (*ZNF365*; Fig. 2). *ZNF365* encodes the zinc finger protein 365. Disruption of normal *ZNF365* function causes mitotic failure, possibly because of centrosome defects or incomplete cytokinesis²⁰. *ZNF365* has also been associated with Crohn's disease⁵. Thus, our results strengthen the hypothesis of joint barrier disease genes.

In addition to the previously unidentified susceptibility loci above, we also analyzed eleven SNPs within or close to seven previously reported susceptibility or candidate genes (*FLG* (ref. 3), *C11orf30* (ref. 4), *WDR36* (ref. 21), *ADRB2* (ref. 22), *IL12B* (refs. 23,24), *CMA1* (refs. 25,26) and *CTNNA3* (ref. 27)) for atopic disease (atopic dermatitis and/or asthma) identified by previous studies. Our study provided strong evidence for *FLG* (rs3126085, $P_{\text{combined}} = 5.90 \times 10^{-12}$, OR = 0.82; Fig. 2) and suggestive evidence for *C11orf30* (rs7936562,

Table 3 Association evidence for eight SNPs at four loci in the German population

			Replication 3 (1,806 cases, 3,256 controls)				
SNP	Chromosome	Gene	Minor allele	MAF ^a		OR	<i>P</i> ^b
				Case	Control		
rs3126085	1q21.3	<i>FLG</i>	A	0.1367	0.1437	0.99	8.84 × 10 ^{−1c}
rs11204971	1q21.3	<i>FLG</i>	G	0.1355	0.1435	0.99	9.73 × 10 ^{−1c}
rs7701890	5q22.1	<i>TMEM232/</i> <i>SLC25A46</i>	G	0.1003	0.1027	0.94	3.35 × 10 ^{−1}
rs10067777	5q22.1	<i>TMEM232/</i> <i>SLC25A46</i>	G	0.0749	0.0780	0.96	5.91 × 10 ^{−1}
rs13360927	5q22.1	<i>TMEM232/</i> <i>SLC25A46</i>	G	0.0801	0.0790	1.05	5.00 × 10 ^{−1}
rs13361382	5q22.1	<i>TMEM232/</i> <i>SLC25A46</i>	A	0.0793	0.0788	1.05	5.15 × 10 ^{−1}
rs2393903	10q21.2	<i>ZNF365</i>	A	0.3937	0.3962	0.98	6.32 × 10 ^{−1}
rs6010620 ^d	20q13.33	<i>TNFRSF6B/ZGPAT</i>	A	0.1886	0.2282	0.80	2.87 × 10 ^{−5}

^aMAF, minor allele frequency. ^bTwo-tailed P values from the Cochran-Armitage trend test. ^cImputed P value. ^dThe G allele of rs6010620 showed a consistent risk effect between the Chinese Han and the German populations but the minor allele is A in the German population.

$P_{\text{combined}} = 2.98 \times 10^{-4}$, OR = 0.91) and *WDR36* (rs2416257, $P_{\text{combined}} = 9.80 \times 10^{-3}$, OR = 1.14) being associated with atopic dermatitis in the Chinese Han population. In contrast, our study showed no evidence that *ADRB2*, *IL12B*, *CMA1* or *CTNNA3* were associated with atopic dermatitis ($P_{\text{smallest}} = 1.22 \times 10^{-2}$; **Supplementary Table 5**).

FLG is an established susceptibility gene for atopic dermatitis with various mutations in diverse populations^{3,28}. *FLG* encodes a key protein for the maintenance of the skin barrier function²⁹, which is disrupted in atopic dermatitis³⁰. In the Chinese population, c.3321delA is

the most common mutation of *FLG* (ref. 31). Therefore, we sequenced the c.3321delA mutation in 1,080 Chinese atopic dermatitis cases and 892 controls from our GWAS sample. We observed LD between the major Chinese *FLG* mutation c.3321delA and our lead SNP rs3126085 ($D' = 0.976$, $r^2 = 0.029$; **Supplementary Table 6**). The c.3321delA mutation showed stronger association (OR = 3.37, $P = 6.86 \times 10^{-12}$) than rs3126085 (OR = 0.83, $P = 5.19 \times 10^{-3}$; **Supplementary Table 6**). Furthermore, controlling for the genetic effect of the c.3321delA mutation by using conditional logistic regression analysis also abolished

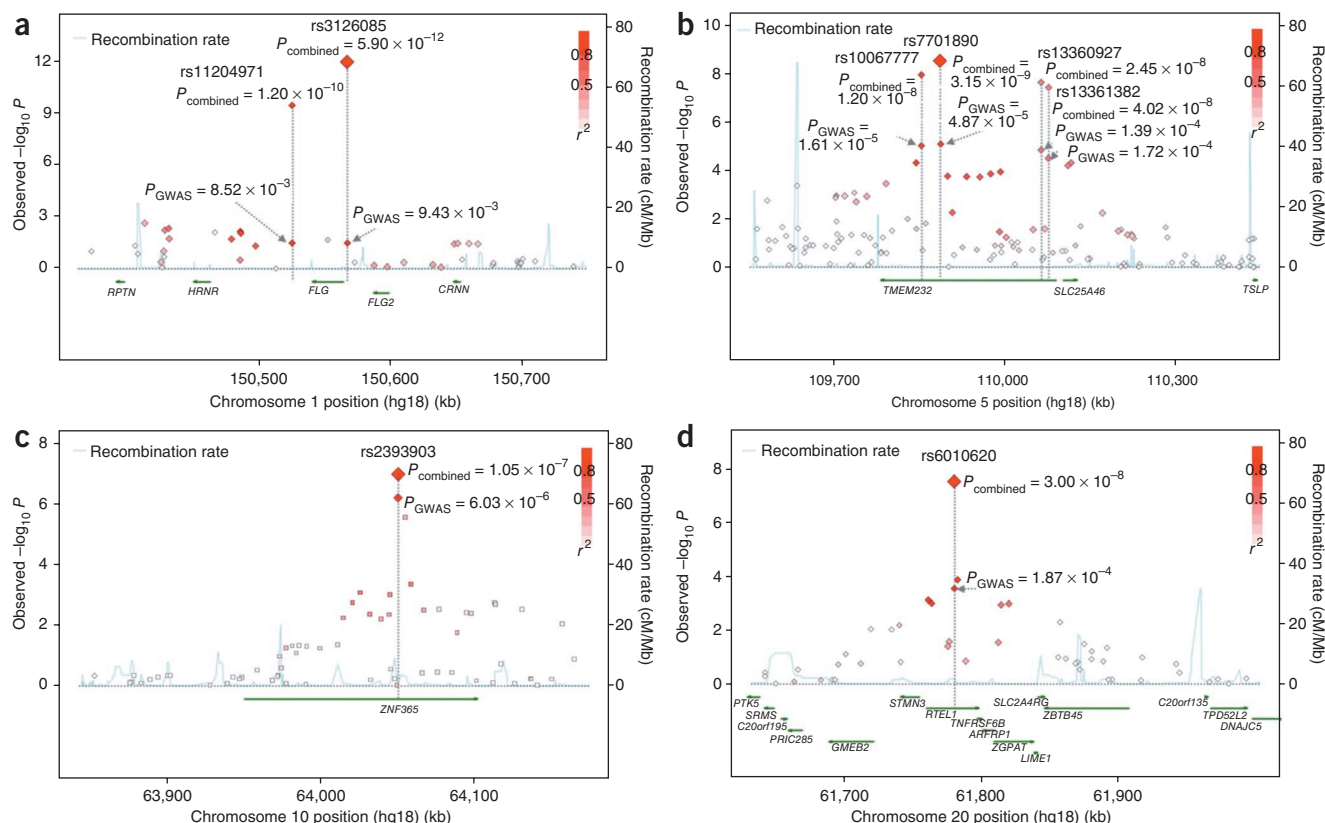


Figure 2 Association scatter plots for four atopic dermatitis susceptibility loci. We plotted the P values of SNPs (shown as $-\log_{10}$ values in y axis from the genome-wide single-marker association analysis using the Cochran-Armitage trend test) against their map positions (x axis). The color of each SNP spot reflects its r^2 with the top SNP (large red diamond) within each association locus, changing from red to white. Estimated recombination rates (based on the combined CHB and JPT samples from the HapMap project) are plotted in light blue. Gene annotations were adapted from the UCSC Genome Browser (see URLs). (a) 1q21.3; (b) 5q22.1; (c) 10q21.2; (d) 20q13.33.

the association at rs3126085 ($P = 0.16$; **Supplementary Table 6**). These indicate that the rs3126085[A] allele is correlated with the c.3321delA mutation and that the association at rs3126085 is mainly driven by the c.3321delA mutation. Likewise, the association of rs6661961 in a recent European GWAS of atopic dermatitis was shown to be caused by common European *FLG* mutations⁴. These two SNPs—rs6661961 and rs3126085—are only weakly correlated with each other in both European ($D' = 0.472$, $r^2 = 0.052$ in CEU) and Chinese ($D' = 0.532$, $r^2 = 0.198$ in CHB) populations. Consistently, the imputation-based association analysis did not show an association of rs3126085 with atopic dermatitis in the German population ($P = 0.884$, power 73%), nor an association of rs6661961 in the Chinese sample ($P = 0.248$, power 81%). Our study therefore indicates that these two SNPs probably mirror population-specific *FLG* mutations, but comprehensive fine-mapping of *FLG* in different populations is needed to prove allelic heterogeneity.

Larger sample sizes using more dense genetic markers will also help to elucidate the basis for potential ethnicity-related disparities in atopic dermatitis association for the other six previously reported loci (*C11orf30*, *WDR36*, *ADRB2*, *IL12B*, *CMA1* and *CTNNA3*; **Supplementary Table 5**) and the two newly identified loci (5q22.1 and 10q21.2; **Table 3**) between different populations, which will provide a better understanding on the genetic basis of atopic dermatitis.

Our GWAS on atopic dermatitis in the Chinese Han population has identified two previously unknown genetic susceptibility loci and suggested new biological pathways in atopic dermatitis. Further studies in additional and ethnically diverse cohorts are needed to increase our understanding of the complex genetic architecture of atopic dermatitis and to gain better insights into its proposed genetic heterogeneity.

URLs. Sanger Institute Genevar database, <ftp://ftp.sanger.ac.uk/pub/genevar/>; UCSC Genome Browser, <http://genome.ucsc.edu/>.

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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AUTHOR CONTRIBUTIONS

X.-J.Z. conceived of this study and obtained financial support. X.-J.Z., Z.-R.Y., S.Y., L.-D.S., F.-L.X., Y. Li and W.-M.Z. participated in the design and were responsible for sample selection, genotyping and project management. H.Z., A.F., H.S., R.F.-H., S.W., M.S., Q.L., X.-L.C., Y.-F.G., Ming L., Y.-H.Z., J.-P.T., H.W., X.-Y.Z., X.-Y. Liu, L.M., A.-P.J., X.-H.G., C.-X.T., S.-P.S., Z.-Y.L., X.-L.Z., J.C., X.-Y. Luo, X.D., W.S., C.-P.S., X.-B.F., X.-P.H., J.-Q.H., Y.W., Y.-X.B., S.-M.H., Y.-M.L., K.-J.Z., D.-Y.H., Min L., X.Z., B.-R.G., Y. Liu, S.-M.Z., X.-Y.Y., Y.-Q.R., Y.C., M.G., P.-G.W., H.L., S.-X.L., C.-J.Y., G.-S.L., Z.-X.W., H.-Y.T. and X.F. conducted sample selection and data management, undertook recruitment, collected phenotype data, undertook related data handling and calculation, managed recruitment and obtained biological samples. F.-S.Z., G.C., X.-D.Z. and P.L. performed genotyping analysis. J.-J.L., H.-Y.T., X.-F.T. and X.-B.Z. undertook data processing, statistical analysis and bioinformatics investigations. All the authors contributed to the final paper, with X.-J.Z., Z.-R.Y., S.Y., L.-D.S., F.-L.X., Y. Li and W.-M.Z. having key roles.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects. Four independent study groups were evaluated. All samples from initial GWAS, replication 1 and replication 2 were Chinese Han, obtained from doctors through collaboration with multiple hospitals in China. Cases and controls for the GWAS in the initial stage included 1,012 atopic dermatitis cases and 1,362 controls from southern China; replication 1 consisted of 1,119 atopic dermatitis cases and 1,479 controls from northern China and replication 2 consisted of 2,505 atopic dermatitis cases and 10,718 controls from southern China. All the cases with atopic dermatitis were diagnosed according to standard criteria in the presence of chronic or chronically relapsing pruritic dermatitis with the typical morphology and distribution³². Clinical information was collected from the affected individuals through a full clinical checkup by physician specialists. Additional demographic information was collected from cases and controls through a structured questionnaire. All controls were clinically assessed to be without atopic dermatitis, other atopic diseases, systemic disorders or family history of atopic diseases (including first-, second- and third-degree relatives). All participants provided written informed consent. The study was approved by the Institutional Ethical Committee of each hospital and was conducted according to the Declaration of Helsinki principles. Replication 3 (1,806 cases and 3,256 controls) from Germany was provided by A. Franke. All cases were obtained from German university hospitals (University of Kiel and Technical University Munich), and the German control individuals were derived from the PopGen Biobank³³.

Genotyping and quality controls in the GWAS. The genome-wide genotyping analysis was conducted using Illumina Human610-Quad BeadChips at State Key Laboratory of Dermatology, Ministry of Science and Technology, Hefei, Anhui, China. Genotyping was performed as described⁷ according to the Infinium HD protocol from Illumina. Briefly, 200 ng of genomic DNA from each sample was whole-genome amplified, fragmented, precipitated and resuspended in the appropriate hybridization buffer. Denatured samples were hybridized on prepared Illumina Human610-Quad BeadChips. After hybridization, the BeadChip oligonucleotides were extended by a single labeled base, which was detected by fluorescence imaging with an Illumina Bead Array Reader. Normalized bead intensity data obtained for each sample were loaded into the Illumina BeadStudio 3.2 software, which converted the fluorescence intensities into SNP genotypes. The clustering of genotypes was carried out with the GenCall software version 6.2.0.4, which assigns a quality score to each locus and an individual genotype confidence score that is based on the distance of a genotype from the center of the nearest cluster. Our GWAS dataset was comprised of 620,901 SNPs and CNV probes. All SNPs on the X, Y and mitochondrial chromosomes as well as CNV-related SNPs were excluded. SNPs were also excluded if they showed a call rate <90% in cases or controls, a MAF <1% in the population or significant deviation from Hardy-Weinberg equilibrium (HWE) in the controls ($P \leq 10^{-7}$). In the GWAS stage, 1,039 atopic dermatitis cases and 1,409 controls were genotyped initially using the Illumina Human610-Quad BeadChip array. Forty samples with genotyping call rate less than 98% were removed from analysis (19 cases and 21 controls). We then examined potential genetic relatedness based on pairwise identity by state for all the successfully genotyped samples using PLINK 1.07 software. In total, 17 samples (6 cases and 11 controls) were removed because of sample duplications and relatedness. In addition, 2 cases and 15 controls were removed for outliers in principal components analysis (PCA) by using the software package EIGENSTRAT³⁴. The original script from EIGENSTRAT was modified to extract the principal components for plotting (**Supplementary Fig. 2**). After quality control, the genotype data of 491,905 autosomal SNPs in 1,012 cases and 1,362 controls were used for the GWAS analysis.

SNP selection for replication. We selected 44 SNPs with $P < 1 \times 10^{-4}$ based on the 1,012 atopic dermatitis cases and 1,362 healthy controls and further selected an additional 12 SNPs with improvement of association evidence in the genome-wide analysis of 1,012 cases and 4,506 controls (1,362 healthy controls and the additional 3,144 pseudo-controls). All the 3,144 pseudo-controls (taking other diseases as controls) were genotyped using the same Illumina Human610-Quad BeadChips in a series of GWAS of various diseases in the Chinese Han population, including psoriasis, leprosy, vitiligo and SLE. The improvement of association evidence by the inclusion of the 3,144 samples

as controls was used as one of the criteria for selecting SNPs for replication analysis; however, because these additional controls had diseases other than atopic dermatitis, these 3,144 samples were not included in the association analysis of the replication study or in the final joint association analysis of the combined samples. All the association results of the current study are free of the influence of these 3,144 immunity-related disease subjects on association. Additional criteria for SNP selection were high MAF (MAF > 0.05 both in cases and controls) and P value of HWE ($P \geq 10^{-7}$ in controls). We also selected 11 SNPs located in or close to seven susceptibility or candidate genes (*FLG*, *C11orf30*, *ADRB2*, *WDR36*, *IL12B*, *CMA1* and *CTNNA3*) with confirmed or suggestive association evidence for atopic diseases in terms of atopic dermatitis and asthma in the European and Asian populations. These genes were reported by either previous GWAS or more than two independent candidate gene studies, and the SNPs in the appropriate gene showed the smallest P values in the initial GWAS. In total, 67 SNPs were selected for the replication (replication 1: 1,119 cases and 1,203 controls; replication 2: 2,505 cases and 6,762 controls; **Supplementary Table 1**). The 19 most promising of the 67 SNPs were further genotyped by adding additional 276 controls in replication 1 and 3,956 controls in replication 2 (**Supplementary Table 2**). The six most significant SNPs of the 67 SNPs were further genotyped in replication 3 (1,806 cases and 3,256 controls) from Germany (**Table 3**).

Genotyping and quality controls in replication studies. Genotyping analyses of replications 1 and 2 were conducted by using the Sequenom MassArray system at State Key Laboratory of Dermatology, Ministry of Science and Technology, Hefei, Anhui, China. Approximately 15 ng of genomic DNA was used to genotype each sample. Locus-specific PCR and detection primers were designed using the MassARRAY Assay Design 3.0 software (Sequenom). Following the manufacturer's instructions, the DNA samples were amplified by multiplex PCR reactions, and the PCR products were then used for locus-specific single-base extension reactions. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using MALDI-TOF mass spectrometry. The mass spectrograms were analyzed by the MassARRAY Typer software (Sequenom). To evaluate the quality of the genotype data for the replication analysis, 100 randomly selected samples from the GWAS stage were re-genotyped in the replication study using the Sequenom system. The concordance rate between the genotypes from the Illumina and the Sequenom analyses was >99%. Genotyping of replication 3 was done using TaqMan assays (Applied Biosystems) at the Christian-Albrechts University Kiel in Kiel, Germany. Quality control was performed in each dataset separately using PLINK version 1.07. *FLG* c.3321delA mutation sequencing in our GWAS sample was performed by Sanger sequencing using ABI 3730xl DNA Analyzer (Applied Biosystems) at Beijing Genomics Institute. In each case-control cohort (replications 1–3), we excluded SNPs with a call rate <90%, low MAF (MAF < 0.05) or deviation from HWE ($P < 0.01$) in the controls. After quality control, 64 SNPs were left for further analysis in replication 1, and 18 of the 19 SNPs passed the quality control and were left for final analysis in replication 2. All six SNPs passed quality control in replication 3 and were left for downstream analysis.

Statistical analysis. In the GWAS stage, we examined potential genetic relatedness based on pairwise identity by state for all of the successfully genotyped samples using PLINK 1.07 software. For all the first-, second- and third-degree pairs detected, the subject of each pair with a lower call rate was removed from further analysis. PCA was performed for our 2,374 GWAS samples (1,012 cases and 1,362 controls), and the results were combined with those for 206 reference HapMap samples separately (**Supplementary Fig. 2**). We used the quantile-quantile plots to evaluate the overall significance of the genome-wide association results and the potential effect of population stratification. We further calculated the genomic control inflation factor and found it to be minimal ($\lambda_{GC} = 1.049$), suggesting minimal inflation of the genome-wide association results from population stratification. The extent of heterogeneity was assessed by using the I^2 index³⁵ (**Supplementary Table 3**). The I^2 index describes the proportion of the total variability that is caused by heterogeneity. In general, $I^2 < 25\%$ is considered as no heterogeneity, $I^2 = 25\text{--}50\%$ is considered as low heterogeneity, $I^2 = 50\text{--}75\%$ is considered as moderate heterogeneity and $I^2 > 75\%$ is considered as strong heterogeneity. Therefore, we set the threshold of I^2 to be 25% in our

analysis. GWAS and replication analyses were performed using the Cochran-Armitage trend test. The joint analysis of all combined samples of Chinese Han was conducted either by random effects model³⁵ ($I^2 > 25\%$) or using Cochran-Mantel-Haenszel stratification analysis³⁶ ($I^2 < 25\%$). The calculation of the P value in the GWAS, replication 1, replication 2, replication 3 and the combined association analysis was done using two-tailed tests. We used multiple logistic regression analysis to test the independence of multiple associations at 5q22.1. Haplotype analyses were performed using Haploview v4.2 to generate haplotype frequencies and calculate the significance of the associations³⁷. Recombination plots of each newly discovered susceptibility locus were generated using the information from the HapMap project (CHB and JPT samples).

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