Letter to the Editor

A genome-wide association study reveals 2 new susceptibility loci for atopic dermatitis

To the Editor:

Association studies have identified a total of 23 European and Asian genetic susceptibility loci for atopic dermatitis (AD), although these explain only a small fraction of the estimated total heritability.[1,2]

To identify further risk loci for AD, we analyzed an imputed data set of more than 1.6 million genetic markers from 924 unrelated German tertiary care cases and 5506 population-based control subjects, followed by an additional replication study in a further 1383 AD cases and 1728 control subjects (see the Methods section and Table E1 in this article's Online Repository at www.jacionline.org). Cases used for the screen had also been genotyped on Affymetrix 500K/5.0 platforms (Affymetrix, Santa Clara, Calif) and used for a previous genome-wide association study (GWAS).[3]

Genome-wide single nucleotide polymorphism (SNP) genotyping of patients was performed by an Affymetrix service facility (South San Francisco, Calif) using the Affymetrix Genome-Wide Human SNP Array 6.0 (1000k), according to the manufacturer's protocols. SNP genotype imputation was carried out with MACH version 1.10.16 and HapMap II CEU phased haplotypes release 22 (http://hapmap.ncbi.nlm.nih.gov/downloads/phasing/2007-08_rel22/phased/) as the reference data set to increase marker density. After stringent quality control filtering (see the Methods section in this article's Online Repository), the initial GWAS sample set consisted of 870 cases and 5293 control subjects with 1,623,390 markers. Genetic heterogeneity was found to be moderate, with an estimated genomic inflation factor λ1000 value of 1.076, and the quantile-quantile plot showed substantial deviation from the expected distribution of genome-wide *P* values in the tail of the distribution (see Fig E1 in this article's Online Repository at www.jacionline.org).

After visual inspection of the regional plots of 812 SNPs showing a disease association with *P* values of 10^{-3} or less by means of clumping (clump command with default settings in PLINK: $P1 \leq 10^{-3}$, $P2 \leq .05$, $r^2 \geq 0.8$, kb = 250), 98 SNPs were carried forward to replication genotyping using ligation-based SNPlex or TaqMan (Applied Biosystems, Foster City, Calif) technology. After quality control (see the Methods section in this article's Online Repository), 1383 AD cases, 1728 control subjects, and 69 SNPs remained for association analysis (complete results are presented in Table E2 in this article's Online Repository at www.jacionline.org). Ten of these SNPs showed a nominally significant association with *P* values of less than .05 in the replication cohort. In the combined analysis (GWAS and replication data sets) 5 loci achieved *P* values of less than 5×10^{-7} (Table I and see Fig E2 in this article's Online Repository at www.jacionline.org for regional association plots). A marker within the filaggrin *(FLG)* locus achieved genome-wide significance in the combined analysis (rs12144049, $P_{comb} = 1.02 \times 10^{-16}$). After excluding carriers of common *FLG* mutations (R501X, 2282del4, R2447X, and S3247X), this loci no longer showed a significant association. Furthermore, an SNP located within the *RAD50/IL13/KIF3A* locus at 5q31.1 showed strong association (rs3091307, $P_{comb} = 4.18 \times 10^{-7}$), which tags previously reported susceptibility markers in the *RAD50/KIF3A* locus: rs2040704 ($r^2 = 0.68$),[4] rs3798135 ($r^2 = 0.76$), and rs2240032 ($r^2 = 0.76$).[5] In contrast, it is in low linkage disequilibrium ($r^2 < 0.36$) with the *IL13* risk variants rs848[1] and rs20541,[2] supporting the notion of several independent association signals in this region.[1]

Twelve further susceptibility loci reported previously in European population GWASs were replicated successfully ($P_{GWAS} \leq 5 \times 10^{-2}$). Only 1 reported locus, *ACTL9*, failed in the screen ($P_{GWAS} = 5.23 \times 10^{-2}$). Regarding susceptibility loci reported exclusively in GWASs of Asian populations, 5 loci (*GLB1*, *CCDC80*, *ZNF365*, *OR10A/3/NLRP10*, and *CYP24A1/PFDN4)* showed evidence for association in Europeans for the first time. Thus 21 of 23 reported AD risk loci could be confirmed. Detailed results of all AD susceptibility loci previously reported in GWASs from population with European and Asian ancestry are provided in Table II.[1-3,6-8]

Two novel loci not previously implicated in AD reached classical genome-wide significance ($P_{comb} < 5 \times 10^{-8}$) in the combined analysis: xin actin-binding repeat-containing protein 2 *(XIRP2)*, also known as *CYMA3*, at chromosome 2q24.3, with the lead variant rs6720763 ($P_{comb} = 4.37 \times 10^{-8}$; odds ratio, 1.29) located in intron 2, and doublesex and mab-3-related transcription factor-like family A1 *(DMRTA1)* at chromosome 9p21.3, with the lead variant rs10738626 ($P_{comb} = 1.45 \times 10^{-8}$; odds ratio, 0.81) located 73 kb upstream.

Thus far, very little is known about the function of *XIRP2* and *DMRTA1*. For *XIRP2*, a cross-linking function between skeletal muscle and F-actin[9] and an involvement as an interactor in the cohesion-*RAD21* interactome has been reported,[10] and *DRMTA1* has primarily been implicated in sex differentiation.[11]

In silico bioinformatic analysis of all SNPs in full linkage disequilibrium ($r^2 = 1$ based on 1000G data) with the lead variants (see the supplementary text section and Tables E3a, E3b, E4a, and E4b in this article's Online Repository at www.jacionline.org) indicated that the *XIRP2* lead variant rs6720763 is located within a potentially regulatory region because it is positioned within a DNaseI-hypersensitive site, transcription factors have been observed to bind to that region, alterations in transcription factor binding are predicted, and the DNA sequence is highly conserved. In an expression quantitative trait locus analysis with the MuTHER study data,[12] none of the investigated *DMRTA1* and *XIRP2* SNPs showed expression quantitative trait locus evidence on both loci.

PCR analysis in a panel of 13 different human tissues and cell types and immunohistochemistry in healthy epidermis and lesional AD epidermis showed RNA expression in skin and keratinocytes and immunoreactivity in lesional and healthy epidermis for both *XIRP2* and *DMRTA1*. Furthermore, both genes showed high mRNA expression in skeletal muscle, spleen, and testis tissue, and in addition, *DMRTA1* was expressed in the pancreas, lung, kidney, brain, small intestine, colon, and thymus (see the Results section and Figs E3-E5 in this article's Online Repository at www.jacionline.org). Electrophoretic mobility shift assays with Cy5-labeled oligonucleotides using nuclear extracts derived from human HaCaT keratinocytes and competition shifts with different amounts of unlabeled probes (see the Results section in this article's Online Repository) showed allele-specific protein-DNA interactions for both identified risk SNPs (Fig 1 and see Fig E6 in this article's Online Repository at www.jacionline.org);

TABLE I. SNPs with a *P* value of 5×10^{-7} or less in the combined analysis

					GWAS				Replication				Combined	
					Cases: 870, control subjects: 5293				Cases: 1383, control subjects: 1728				Cases: 2253, control subjects: 7021	
dbSNP ID	Chromosome	Position	A1, A2	Locus	AF_ca	AF_co	P_{GWAS}	OR (95% CI)	AF_ca	AF_co	P_{Repl}	OR (95% OR)	P_{comb}	OR
rs12144049	1	150707534	C, T	*EDC*	0.369	0.279	1.87×10^{-8}	1.47 (1.31-1.64)	0.346	0.286	5.89×10^{-7}	1.33 (1.19-1.48)	1.02×10^{-16}	1.39
rs6720763	2	167700532	C, T	*XIRP2* (intron)	0.221	0.18	5.03×10^{-5}	1.31 (1.15-1.48)	0.211	0.174	2.65×10^{-4}	1.27 (1.12-1.44)	4.37×10^{-8}	1.29
rs3091307	5	132017035	A, G	*RAD50 /IL13/ KIF3A*	0.276	0.228	5.42×10^{-5}	1.3 (1.14-1.47)	0.283	0.247	1.69×10^{-3}	1.2 (1.07-1.35)	4.18×10^{-7}	1.24
rs10738626	9	22363457	C, T	*DMRTA1*	0.443	0.494	1.96×10^{-6}	0.77 (0.69-0.86)	0.474	0.517	7.95×10^{-4}	0.84 (0.76-0.93)	1.45×10^{-8}	0.81
rs1665050	15	57080897	A, G	*RNF111* (intron)	0.299	0.252	7.12×10^{-5}	1.27 (1.13-1.43)	0.296	0.255	3.48×10^{-4}	1.23 (1.1-1.38)	9.65×10^{-8}	1.25

SNPs are ranked by chromosomal position (National Center for Biotechnology Information build 36 [hg18]). ORs and 95% CIs for allele A1 are shown.
A1, Minor allele; *A2*, major allele; *AF_ca/AF_co*, allele frequencies in cases/control subjects; *OR*, odds ratio; $P_{GWAS}/P_{Repl}/P_{comb}$, *P* values in GWAS/replication/combined analysis.

TABLE II. Replication results of 23 established AD loci from GWASs

Locus	Reported gene(s)	Reported SNP(s)	References	Top SNP in region	Position	EA/RA	OR	*P* value
Loci identified through GWASs in European populations								
1q21.3	Tags *FLG* signal	rs3126085	1, 3, 6	rs12144049	152440176	C/T	1.47 (1.31-1.64)	1.87×10^{-8}
4q27	*IL2/IL21*	rs17389644	1	rs17454584	123497697	A/G	0.83 (0.77-0.89)	1.60×10^{-4}
5q31.1	*RAD50/IL13/IL4/KIF3A*	rs1295686, rs2897442, rs848	1, 7, 8	rs3091307	131995843	G/A	1.3 (1.14-1.47)	5.42×10^{-5}
6p21.33	*HLA-C/HLA-B/MICA*	rs9368677, rs2251396	2, 8	rs3021366	31324100	A/C	0.48 (0.33-0.78)	2.36×10^{-5}
6p21.33	*BAT1*	rs2844509	2	rs3130048	31510924	C/T	0.75 (0.68-0.82)	4.88×10^{-5}
6p21.33	*C6orf48*	rs9368699	2	rs9368699	31802541	C/T	0.51 (0.32-0.83)	2.55×10^{-4}
6p21.33	*TNXB/CREBL1*	rs12153855	2	rs12153855	32074804	C/T	0.77 (0.68-0.86)	5.55×10^{-3}
11p12	*PRR5L*	rs12295535	1	rs7945962	36344202	A/G	0.79 (0.74-0.84)	2.16×10^{-5}
11q13.1	*OVOL1*	rs479844	7, 8	rs479844	65551710	T/C	1.20 (1.25-1.14)	5.22×10^{-4}
11q13.5	*C11orf30/LRRC32*	rs7927894, rs11236809, rs7110818	1, 3, 8	rs2155219	76281593	T/G	0.81 (0.76-0.86)	1.74×10^{-4}
16p13.13	*CLEC16A/DEXI*	rs9923856, rs2041733	1, 8	rs2041733	11223454	C/G	1.23 (1.18-1.28)	1.31×10^{-4}
17q21.32-33	*ZNF652*	rs16948048	1	rs7209400	47440466	C/T	0.85 (0.80-0.90)	3.32×10^{-3}
19p13.2	*ACTL9*	rs2164983	7	rs12611036	8789381	A/G	1.12 (1.08-1.18)	5.23×10^{-2}
Loci identified through GWASs in Asian populations								
2q12.1	*IL18R1/IL18RAP/SLC9A4*	rs13015714, rs759382	1, 8	rs13015714	102945378	T/G	1.23 (1.17-1.29)	6.55×10^{-4}
3p22.3	*GLB1*	rs7613051	8	rs13091893	33065339	G/C	1.13 (1.07-1.19)	3.43×10^{-2}
3q13.2	*CCDC80*	rs12634229	8	rs2129844	112376308	T/C	1.52 (1.37-1.67)	2.93×10^{-3}
5q22.1	*TMEM232/SLC25A46*	rs7701890	6	rs11241070	109858821	G/A	1.17 (1.08-1.26)	6.47×10^{-2}
6p21.32	*GPSM3*	rs176095	8	rs6941112	32158319	A,G	1.31 (1.25-1.37)	1.66×10^{-5}
7p22.2	*CARD11*	rs4722404	8	rs11983024	3081727	C/G	0.82 (0.76-0.88)	1.75×10^{-3}
10q21.2	*ZNF365*	rs10995251	8	rs7919747	64380336	T/C	0.71 (0.62-0.8)	3.28×10^{-4}
11p15.4	*OR10A3/NLRP10*	rs878860	8	rs10769866	7968359	G/C	0.88 (0.82-0.94)	1.89×10^{-2}
20q13.2	*CYP24A1/PFDN4*	rs16999165	8	rs6013912	52807221	C/T	0.80 (0.74-0.86)	8.61×10^{-5}
20q13.33	*TNFRSF6B*	rs909341	1, 6	rs16984240	62328742	T/C	0.79 (0.71-0.87)	6.83×10^{-3}

Previously reported genes and SNPs (dbSNP ID) are sorted by chromosomal position (locus). The top associated SNP from imputed GWAS data for each locus (±250 kb) is displayed.
EA/RA, Effect/reference allele; *OR*, odds ratio.

however, further studies are needed to substantiate the effects on expression and causality.

In summary, through analysis of imputed SNP data in 870 German cases and 5293 German control subjects, followed by replication in 1383 additional German cases and 1728 control subjects, we could confirm 14 of the previously established 15 European AD susceptibility loci and, for the first time, replicate 5 of 8 loci thus far only reported in Asians and identified 2 new susceptibility loci with genome-wide significance in the combined analysis at chromosomes 2q24.3 *(XIRP2)* and 9p21.3 *(DMRTA1)*. We provided preliminary functional evidence for a regulatory potential of the lead variants at these 2 novel loci, but further fine mapping and functional analyses will be needed to clarify whether the lead SNP is the true causal variant and to further elucidate its functional role. The 2 novel loci increase the explained heritability for AD in Europeans from 19.2% to 21.5% (see Table E5 in this article's Online Repository at www.jacionline.org).

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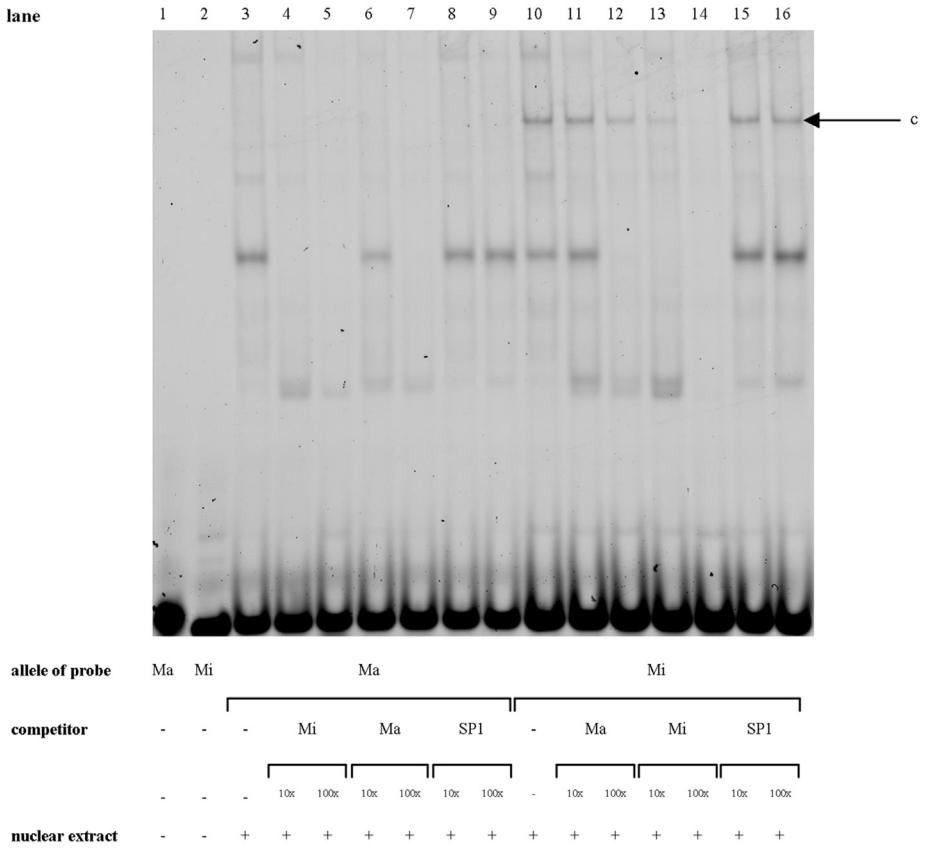

FIG 1. Allele-specific protein-DNA interactions of rs6720763 *(XIRP2)*. Only the probe containing the minor (*Mi;* risk) allele shows allele-specific protein binding (*lane 10, arrow c*) compared with the major *(Ma)* allele *(lane 3)*. Unlabeled minor allele probes efficiently competed with labeled minor allele probes (*lanes 13* and *14*) in contrast to the major allele (*lanes 11* and *12*) or SP1 (*lanes 15* and *16*).

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