Multi-ethnic genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis

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Abstract

Genetic association studies have identified 21 loci associated with atopic dermatitis risk predominantly in populations of European ancestry. To identify further susceptibility loci for this common complex skin disease, we performed a meta-analysis of >15 million genetic variants in 21,399 cases and 95,464 controls from populations of European, African, Japanese and Latino ancestry, followed by replication in 32,059 cases and 228,628 controls from 18 studies. We identified 10 novel risk loci, bringing the total number of known atopic dermatitis risk loci to 31 (with novel secondary signals at 4 of these). Notably, the new loci include candidate genes with roles in regulation of innate host defenses and T-cell function, underscoring the important contribution of (auto-)immune mechanisms to atopic dermatitis pathogenesis.

Atopic dermatitis (eczema) is a common inflammatory skin disease affecting 15–30% of children and 5-10% of adults[1]. Its pathogenesis involves skin barrier abnormalities and a T-cell-driven cutaneous inflammation. Atopic dermatitis has significant genetic contributions, with heritability estimates of up to 90%[2] in Europeans. The strongest known risk factors are null mutations of the filaggrin (*FLG*) gene, resulting in epidermal barrier deficiency[3-5]. Genome-wide association (GWA) studies have identified 20 additional loci (10 in Europeans, 8 in Japanese, 2 in Chinese populations), mostly implicated in immune dysregulation[6-12]. Genetic modeling suggests further loci may be identified with well-powered GWAS[13]. We therefore carried out a multi-ethnic meta-analysis of 26 studies comprising 21,399 cases and 95,464 controls imputed to the 1000 Genomes Project Phase 1 reference panel (Supplementary Note 1 & Supplementary Table 1). 15,539,996 variants with ≥1% MAF were analyzed.

A fixed effects meta-analysis of the 22 European studies identified 21 genome-wide significant ($p<5x10^{-8}$) loci (Table 1, Fig 1, Supplementary Figs 1-4), and a multi-ethnic meta-analysis identified an additional 6 loci with log10 Bayes Factor>6.1, 4 of which (10q21.2, 6p21.33, 11p13, 2p13.3) also showed nominal association in the European analysis (Table 1). These 27 loci included all 11 loci previously associated with atopic dermatitis in Europeans and 5 loci originally reported in Japanese. Three Japanese loci (6p21.33, 10q21.2, 2q12.1) were also strongly associated in the European analysis, whereas two (3q13.2, 11p15.4) may represent Japanese-specific signals (Supplementary Figs 1&2), with the European confidence interval ruling out all but very small effects (OR<1.03, Table 1). Furthermore, a locus originally reported in a Chinese GWAS (20q13.33) showed association in Europeans. We identified 11 novel loci for atopic dermatitis. Four (11q24.3, 10p15.1, 8q21.13, 2p25.1) were previously associated with self-reported allergy[14], and another (8q21.13) with

asthma[15]. Two novel variants (5p13.2 and 2p25.1) showed statistically significant evidence of heterogeneity between European and non-European studies (Cochran's Q p~0.01, Supplementary Table 2). Both showed little evidence for association in non-Europeans (particularly Japanese, Supplementary Fig.2). The CIs also overlapped for all variants when comparing pediatric (defined as onset by age 6) with any-age onset studies (Supplementary Fig.3). Within Europeans there was some evidence of heterogeneity in effect sizes between studies amongst known variants (e.g. 11q13.5 I^2=62.9%, p<0.0001; 11p13 I^2=55.6%, p=0.0011) but little evidence amogst novel variants (I^2 range=0-40%, all p>0.02, Supplementary Fig.2). Nevertheless, studies with phenotype definition based on a dermatological exam tended to report larger effect sizes than studies using self-report (Supplementary Fig.4), which is to be expected, assuming a moderate degree of phenotypic misclassification in the latter. The inclusion of studies utilizing self-report is therefore likely to bias estimates of the effect size towards the null, and this should be borne in mind when interpreting the odds ratios from our study. Given the primary aim of GWA studies is the detection of novel loci, the increase in sample size achieved by including these studies is so large that any potential detrimental effect on statistical power is more than outweighed and the expected direction of bias means there is unlikely to be an issue of spurious findings (corroborated by Supplementary Fig.4)."

Seven of the 21 established asthma loci[15-20], 7 of the 10 allergic sensitization loci[21], and 6 of 14 self-reported allergy loci[14] showed association with atopic dermatitis (p<0.05), all with consistent directions of effect, supporting common atopic mechanisms in atopic dermatitis and allergy (Supplementary Table 3). However, several studies used here contribute to multiple GWASs, which may bias this overlap. Nevertheless, a substantial proportion of the loci associated with other atopic conditions appear not to be strongly associated with atopic dermatitis.

Twenty-one of the 27 atopic dermatitis-associated loci have previously been implicated in other immune-mediated traits (Supplementary Table 4), most notably inflammatory bowel disease (IBD) and psoriasis. We therefore compared significant results from GWAS of IBD[22], psoriasis[23], ankylosing spondylitis[24], multiple sclerosis[25], rheumatoid arthritis[26] and type 1 diabetes[27]with results from our present study of atopic dermatitis. Of 163 established IBD risk variants, 39 reached p<0.05 for atopic dermatitis (Supplementary Table 5, 8.1 expected, $p=2.4x10^{-16}$), 35 with the same direction of effect (sign test p<0.0001), consistent with the observational association between the two diseases[28-30]. Of the 36 known psoriasis variants, 15 reached p<0.05 for atopic dermatitis (Supplementary Table 6, 1.8 expected, $p=6x10^{-11}$), 10 with the same direction of effect (sign test p=0.30). However, these conditions rarely clinically co-occur[31] and the most strongly associated genetic variants show opposite directions of effect[32]. Therefore our results, suggesting a more complex genetic relationship, might warrant further investigation. SNPs robustly associated with other auto-immune

diseases were also more likely to be nominally associated with atopic dermatitis than expected by chance, but there was little evidence of any consistency in direction of effect (Supplementary Tables 7–10). These findings did not appear to be affected by contamination by common controls across studies. Analyses performed excluding common cohorts, yielded similar results (data not shown).

Conditional analysis showed evidence for secondary independent signals at 4 known atopic dermatitis loci (2q12.1, 4q27, 11p13, 5q31.1, Supplementary Table 11), one of which (5q31.1) has been previously reported[9]. In the epidermal differentiation complex (1q21.2–3, where *FLG* is located) the signals near *MRPS21* (rs7512552) and *IL6R* (rs12730935 or the known functional mutation rs2228145) were independent from *FLG*, whereas the top signal near *LCE3E* (rs61813875) appears to be partially tagging the R501X *FLG* mutation (r^2=0.49) and showed no significant residual association (P>0.05) after conditioning on the 4 most prevalent *FLG* mutations (Supplementary Tables 12&13).

To identify additional variants of biological relevance not reaching genome-wide significance, we applied gene-set enrichment analysis using Meta-Analysis Gene-set Enrichment of variaNT Associations (MAGENTA)[33] (Supplementary Table 14). A significant enrichment of 22 partially overlapping gene-sets (FDR<=0.01) related to innate immune signaling and T-cell polarization was observed (Supplementary Fig.5).

For replication, we selected the lead SNPs from the 11 novel loci, 9 candidate SNPs from the MAGENTA analysis (with $p<10^{-5}$ mapping to gene-sets with FDR<0.05), and 3 SNPs representing potentially novel secondary signals. These were investigated in 18 studies (32,059 cases and 228,628 controls, Supplementary Table 1). Amongst the European studies, 11 of the 20 novel loci reached a Bonferroni-corrected threshold (α=0.0025) with 1-sided tests in a fixed effects analysis (Table 2). However, one of these showed evidence of heterogeneity (10p15.1, p=0.041) and was not significant in a random effects analysis (p=0.019, Supplementary Table 15). Two of the gene-set selected SNPs reached genome-wide significance in the combined analysis (2q37.1, 12q15). A random effects analysis of all replication cohorts (European and other ethnicities) show broadly consistent results (though only 6 reach genome-wide significance), with no clear population-specific effects (Supplementary Table 16 & Fig.6).

All 3 secondary signals showed significant association in the replication-phase conditional analysis (Supplementary Table 11).

As a preliminary step towards understanding the functional underpinnings of the atopic dermatitis genetic associations, we established a 'credible set' of SNPs (all with strong association) for each

locus as described in the online methods[34]. We reviewed these SNPs' functional annotations in ENCODE Consortium and Roadmap Epigenomics Consortium data, evaluated expression quantitative trait locus (eQTL) effects in MuTHER[35], reviewed evidence of differential expression, and surveyed relevant mouse mutants (see Supplementary Note 2 and Tables 17–21). Regions of DNase hypersensitivity from the ENCODE and Roadmap data[36,37] were strongly enriched for atopic dermatitis association compared to the rest of the genome (Supplementary Fig.7 & Table 22), particularly in immune cells (Th0, Th1, Th17 $p<0.0001$), this enrichment was observed well below the genome-wide significance threshold, indicating the presence of additional undetected risk variants. We observed multiple cis-eQTLs (Bonferroni-corrected $p<7x10^{-4}$) in lymphoblastoid cell lines (LCLs) or skin (Supplementary Tables 17&19). The most significant were two variants from the credible set at 2p13.3, which were strong eQTLs for CD207/langerin in skin (rs4852714 $p=1.23x10^{-10}$, rs6723629 $p=1.67x10^{-10}$, LD with lead SNP $r^2=0.56$,D'=0.96, and $r^2=0.53$,D'=0.93, respectively, 99% posterior probability that atopic dermatitis and eQTL signals colocalize). rs4852714 is also in an open-chromatin region with histone marks indicative of promoter/enhancer activity in LCLs (Supplementary Tables 18,19 & Fig.8). *CD207* encodes an intracellular pattern recognition receptor expressed in subpopulations of dendritic cells, in particular epidermal Langerhans cells (LCs) which play a vital role in the induction of tolerance and direction of adaptive immune responses[38]. CD207 binds to carbohydrates present e.g. on microorganisms and exerts anti-viral/anti-fungal defense mechanisms[39]. Of note, atopic dermatitis is characterized by an increased susceptibility towards skin infection with pathogens such as *Staphylococcus aureus*, herpes simplex virus, and *Malassezia* species[40], and differences in langerin function might contribute to this dysregulated cutaneous immunity.

There is longstanding evidence that skin barrier defects and inappropriate immune responses to environmental antigens[1] contributes to atopic dermatitis. However, evidence for autoimmune mechanisms, in particular in the context of progression to the chronic phase, has only recently emerged[41]. Interestingly, the majority of our novel susceptibility loci harbor candidate genes with functional annotations related to autoimmunity. At 14q13.2, the lead SNP (rs2038255) is intronic to *PPP2R3C* (a protein phosphatase component regulating B-cell maturation and survival), the dysregulation of which has been associated with murine autoimmunity[42] and the signal colocalizes with a strong *KIAA0391* eQTL signal (Supplementary Table 19). The lead 5p13.2 variant (rs10214237) is located 4kb downstream of the gene encoding the alpha-chain of the IL7 receptor (IL7R), which is a key mediator in T-cell-driven autoimmunity and inflammation[43]. Of interest, the credible set contains an *IL7R* missense variant (rs6897932, $p=1.6x10^{-7}$, $r^2=0.94$ with lead SNP), which displays the same effect direction with multiple sclerosis[44,45]. The risk allele leads to an enhanced bioavailability

of IL7[46], which in mice causes severe dermatitis with intense pruritus and high IgE levels, i.e. atopic dermatitis-like features[47]. Likewise, as part of the autosomal-dominant hyper-IgE syndrome, rare dominant negative mutations in the gene encoding *STAT3* (in which our lead 17q21.2 variant is intronic) cause severe dermatitis and high serum IgE levels, as well as recurrent *S.aureus* skin infections, which may be driven by impaired Th17 cell differentiation and effector function[48,49]. *STAT3* might thus represent an example for risk gene/pathway shared between a complex trait and a related Mendelian condition[50,51], harboring highly penetrant severe effect rare mutations and common milder effect variants. At 8q21.13, the closest candidate gene is *ZBTB10* encoding a zinc finger protein, which is a putative repressor of the Sp1, Sp3 and Sp4 transcription factors[52]. Variants in moderate LD (r^2>0.7) with the lead variant for atopic dermatitis were previously associated with self-reported allergy[14] and a combined asthma and hay fever phenotype[53]. However, although not excluding *ZBTB10* as the causal gene, the credible SNP set comprises a 47kb interval on the other side of a recombination peak (60cM/Mb). The variant most likely to be regulatory amongst this set, deletion rs5892724 (r^2=0.82 with lead SNP), is located in open chromatin in several cell types including CD4+ helper T-cells, and affects a STAT3 binding site[49,54]. At 11q24.3 the most plausible candidate gene is *ETS1*, which encodes a transcription factor with a range of immune functions including Th17 and B-cell differentiation and function; ETS1-deficient mice display autoimmune features[55]. ETS1 appears to be additionally involved in keratinocyte differentiation and formation of the cornified envelope[56]. Additional variants identified through the gene-set approach implicate genes with cytokine signaling functions (*INPP5D*, *TRAF3*, *SOCS3* and a cytokine cluster on 12q15).

In conclusion, we have identified 10 new loci robustly associated with atopic dermatitis in Europeans (6 of which also reach genome-wide significance in random effects analysis across studies of all ethnicities), bringing the total number of susceptibility loci to 31 (24 in Europeans), with evidence of secondary signals at 4 of these. Altogether, in the subset of European studies with clinically defined cases, previously established and newly identified variants explain approximately 12.3% and 2.6% of the variance in liability, respectively (Supplementary Table 23). All novel susceptibility loci are related to (auto-)immune regulation, in particular innate signaling and T-cell activation and specification, and there appears to be a substantial genetic overlap with other inflammatory and autoimmune diseases. Whilst not detracting from the importance of maintaining the skin barrier in the prevention and treatment of atopic dermatitis, our findings lend support to new therapeutic approaches targeted at immune modulation[57].

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AAGC provided results for the discovery analysis.

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552 **Figure Legend**

553 **Figure 1. Atopic dermatitis GWAS meta-analysis results.** (A) Manhattan plot of European fixed
554 effects meta-analysis. (B) Manhattan plot of the multi-ethnic MANTRA meta-analysis of all studies.
555 Arrows mark variants not associated in the European-only analysis. (C) QQ plot of the European
556 analysis - lambda=1.054.