

Signatures of positive selection are enriched in genome-wide associated allergy alleles

To the Editor,

The prevalence of allergy has rapidly increased over the last few decades and generally associated with industrialization. Epidemiological data and limited evidence from genetic studies suggest that the prevalence of allergy in modern settings is likely to increase due

to positive selective pressures.^{1,2} In this respect, we systematically screened for the signatures of positive selection at allergy-associated loci from four large meta-analysis of GWAS (Table S1), to determine the potential evolutionary factors contributing to persistence of allergic disorders.

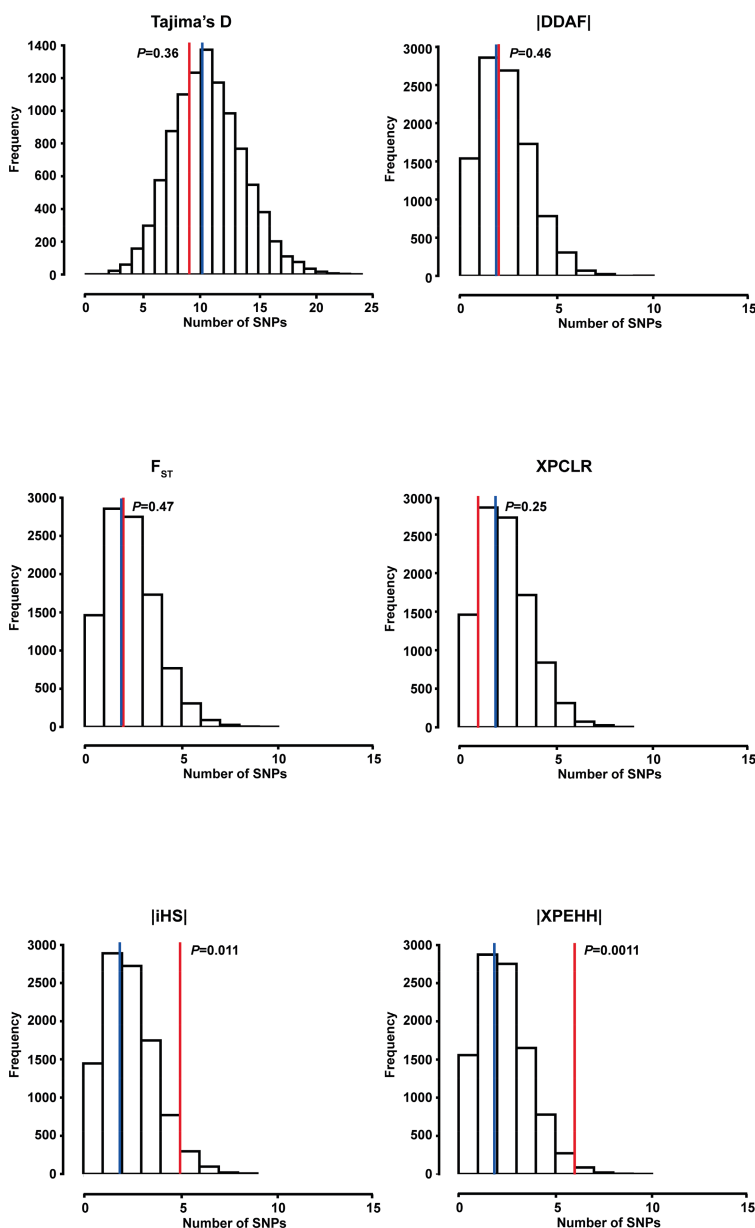


FIGURE 1 Enrichment of allergy-associated SNPs in signatures of positive selection across 26 populations from 1000 Genomes Project. Each histogram represents the distribution of 100 sets of randomly matched negative control SNPs with values of six statistical metrics above the 99th percentile of an empirical distribution, sampled 10,000 times at random (Tajima's $D < -1.004$; $|DDAF| > 0.5239$; $F_{ST} > 0.4156$; $XPCLR > 651.8505$; $|iHS| > 1.7716$; $|XPEHH| > 1.0526$). The blue line represents the mean of empirical null distribution. The red line represents the observed number of allergy-associated SNPs with values of six statistical metrics above the 99th percentile of the empirical distribution

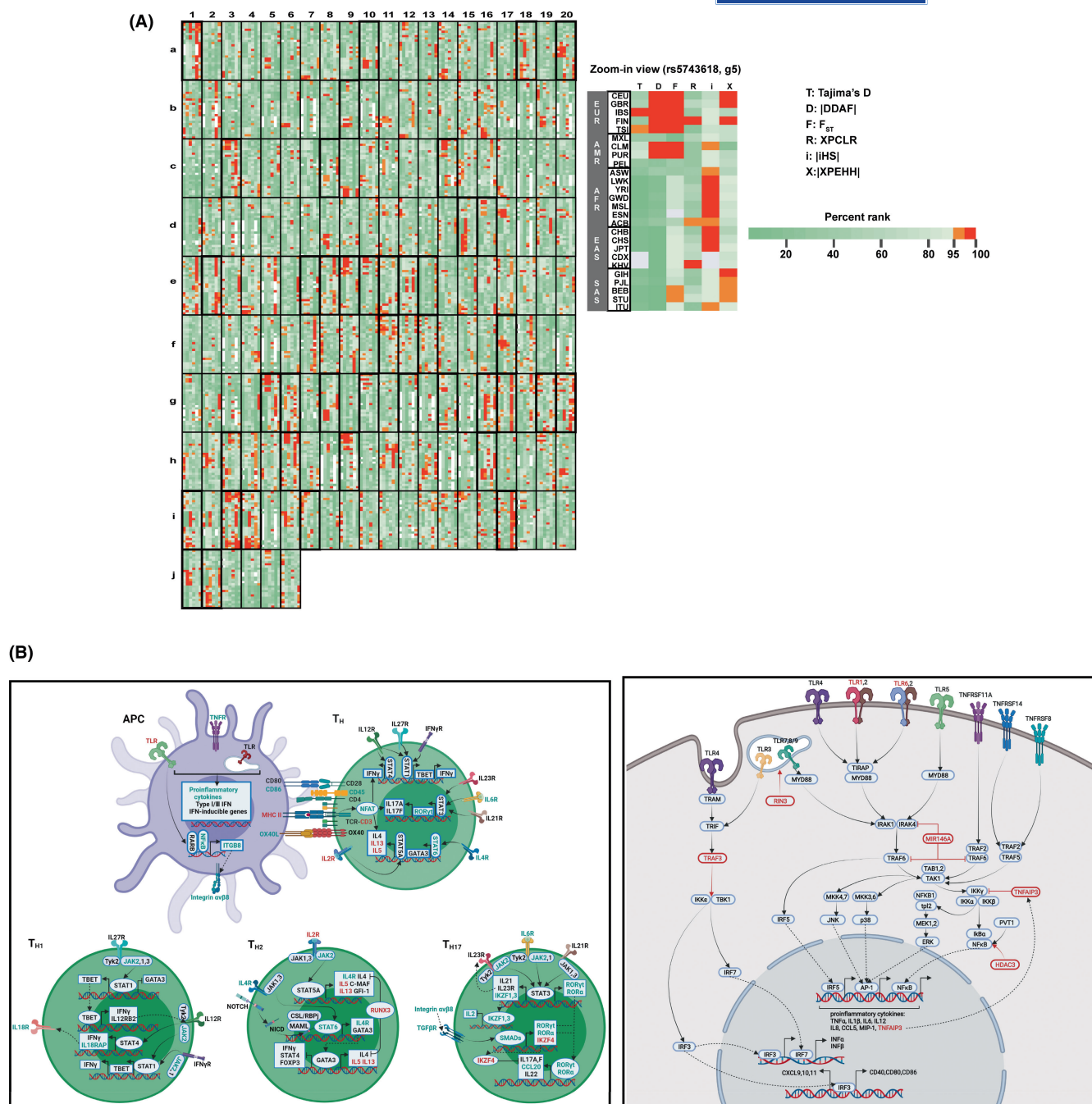


FIGURE 2 Positive selection signals for allergy-associated SNPs and loci. (A) Heat maps of positive selection signals for allergy-associated SNPs. Each box represents an individual SNP, with the zoom view of box g5 providing an example of the percent rank of six statistical metrics of positive selection (horizontal axis) in genomic background distribution determined across 26 individual populations (vertical scale) for SNP rs5743618. Positively selected SNPs are displayed in coarse boxes, and other SNPs are displayed in thin boxes. The intensity of color shows high to low percent rank as indicated in the scale bar. Combination of vertical letter (a to j) and horizontal number (1 to 20) was encoded for each SNP, as shown in Table S1-S3. (B) Comparison of biological processes of GO and KEGG pathways implicated in plausible target genes of allergy with (red bold) and without (green bold) evidence of positive selection. Left: Differentiation of T_H1 , T_H2 , and T_H17 cells. Right: TLR signaling in antigen presenting cells. (Figures were created with BioRender.com)

Genetic data across 26 populations were extracted from 1000 Genomes Project to investigate signatures of positive selection, based on six statistical metrics evaluating reduction in genetic diversity (Tajima's D), high-frequency derived alleles (DDAF), differences between populations (F_{ST} and XPCLR) and long haplotypes

(iHS and XPEHH) at 186 allergy-associated variants, 100 sets of randomly matched negative control single nucleotide polymorphisms (SNPs), and a positive control set of SNPs with positively selected peaks (Tables S1-S3). Three supervised machine learning models, including extreme gradient boosting (XGBoost), adaptive

boosting (AdaBoost), and random forest (RF), were employed to predict the individual SNPs experiencing positive selection (Figure S1). The ethics approval and subjects' informed consent had previously been obtained in the original research. Enrichment analysis of the biological processes from gene ontology (GO) terms over-represented and KEGG pathway for plausible target genes harboring positively selected alleles or not were conducted with Metascape.

In the empirical distribution of negative control SNP sets sampled 10,000 times at random from 26 populations or non-mixed 20 populations, allergy-associated SNPs were significantly enriched in the recent selected sweeps indexed both by two haplotype-based tests $|iHS|$ ($p = 0.011$ and 0.019) and $|XPEHH|$ ($p = 0.0011$ and 0.015) (Figures 1, S2). Separate analysis for each superpopulation demonstrated that allergy-associated SNPs exhibited multiple signatures in several positive terms in European superpopulation, indexed by $|DDAF|$ ($p = 0.012$), F_{ST} ($p < 0.0001$), $XPCLR$ ($p = 0.055$), and $|iHS|$ ($p = 0.043$) (Figure S3). The combination of the six statistical metrics indicated that global signatures of positive selection were more likely to be enriched at allergy-associated alleles in all 26 populations and in 20 non-admixed populations ($p = 0.005$ and 0.007), with significant evidence of global positive selection was detected in EUR superpopulation ($p = 1.39E-07$). Using XGBoost model with the best performance, a total of 42 out of 186 allergy-associated SNPs were identified to be subjected to positive selection (Figure 2A), of which 27 SNPs showed cis-regulatory effects on gene expression and two SNPs were coding variants which impact protein functions (Table S4). Plausible target genes harboring positively selected alleles were observed to be enriched in biological processes related to innate immune response and host defense, especially in pathogen-related responses (Figures 2B, S4).

Variants in functional regions of genome are frequently enriched for signatures of positive selection associated with complex diseases assessed by GWAS.³ In accordance with previous studies,^{4,5} the present study has shown that positive selection favored the genetic variants associated with susceptibility to allergy, and a majority of these variants showed functional effects. These findings not only reinforce the link between evidence of selective pressure and variants with measurable effects on inflammatory phenotypes, but also provide a noteworthy list of positively selected variants, which can be prioritized for investigation in future functional studies.

The primary and strong drivers for natural selections have been proposed to be pathogens. Several plausible candidate genes with evidence of positive selection show remarkable enrichment of innate immune responses and host defense-related pathways against pathogens, suggesting that positive selection may play a role in maintaining the genetic association between allergy and infection. However, the past pathogenic pressures that explain persistence of present-day inflammatory disorders remain open to question. The influence of directional selection should be interpreted with caution as some variants have opposite effects on allergy and other inflammatory disorders,⁶ possibly explained by the difference underlying the causal variant in region and pleiotropy. In addition,

the complexity of gene-environment interactions should also be noted.

In conclusion, this study indicates that variants associated with allergy are significantly enriched for signatures of positive selection, supporting an important role for evolutionary perspective in susceptibility to chronic inflammatory disorders.

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CONFLICT OF INTEREST

All authors declare no financial or commercial conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Soluble ST2 enhances IL-33-induced neutrophilic and pro-type 2 inflammation in the lungs

To the Editor,

Soluble suppression of tumorigenicity 2 (sST2) is a decoy receptor for interleukin 33 (IL-33). We previously reported that high serum levels of sST2 predict unfavorable outcomes in patients with pneumonia¹ or asthma,² although its function in the lungs remains poorly understood. We hypothesized that sST2 modifies IL-33-induced neutrophilic inflammation in the lungs. We measured levels of sST2, IL-33, and pro-neutrophilic or pro-Th17 chemokines in the sputum of patients with asthma, chronic obstructive pulmonary disease (COPD), or asthma-COPD overlap (ACO), and administered a recombinant soluble form of ST2 (ST2-Fc) and/or IL-33 to mice.

Both sST2 and IL-33 were high in patients with ACO, whereas only sST2 was high in patients with asthma and only IL-33 was high in patients with COPD (Figure 1A,B; Table S1). There was no correlation between sST2 and IL-33 levels (Figure 1C). IL-33 levels were positively correlated with chemokine (CXC motif) ligand 8 (CXCL8) and chemokine (CC motif) ligand 20 (CCL20) but were not correlated with CXCL1 levels (Figure 1D). We expected sST2 to negatively regulate IL-33-induced pro-neutrophilic inflammation, but this was not the case (Figure 1E). Conversely, elevated levels of both sST2 and IL-33 (characteristic of ACO patients) were associated with elevation of CXCL1 (as reported previously³) and CCL20 levels (Figure 1F). Cell counts were not conducted at the time of collection, so they could not be analyzed. These results do not support the hypothesis that sST2 works as a decoy receptor for IL-33 in human airways.

Based on the clinical data, we suspected that sST2 may be a carrier protein. To test this idea, we administered mice with mouse (m) IL-33 and/or mST2-Fc at 2.5 and 25 µg protein, respectively (molar ratio, 1:2.8), because the concentration of sST2 in the sputum of patients with ACO was approximately 10 times that of IL-33 (medians of 927 and 74.8 pg/m, respectively; Table S1). Surprisingly, mST2-Fc augmented the mIL-33-induced influx of neutrophils and dendritic cells into the alveolar spaces, as well as bronchial inflammation and secretion of CXCL1, matrix metalloproteinase 9 (MMP9), dsDNA, and CCL17 into the airspaces (Figure 2A-C). Also, mIL-33 promoted an influx of eosinophils (Figure 2A) and secretion of IL-5 (data not shown) into the airspaces, but ST2-Fc did not augment this process. Furthermore, we detected ST2-Fc-IL-33 complexes and enhanced recovery of mIL-33 and mST2-Fc in BAL fluid (Figure 2D). Finally, we confirmed that ST2-Fc-IL-33 complex formation did occur between mST2-Fc and mIL-33 in vitro (Figure 2E). Therefore, mST2-Fc augments mIL-33-induced airway neutrophilia and pro-type 2 inflammation via complex formation.

We thus demonstrated that ST2-Fc enhances IL-33 activity in vivo. Similar forms of augmentation, caused by several types of cytokines and their corresponding anti-cytokine monoclonal antibodies, have been reported. For instance, anti-IL-4 and anti-granulocyte-colony stimulating factor (G-CSF) monoclonal antibodies enhance IL-4 and G-CSF, respectively, in mice, via the formation of immune complexes in the circulation system.⁴ These findings support our discovery that ST2-Fc augments IL-33 activity.