Functional and clinical translation of asthma and allergy associated genetic variants in IL33 and IL1RL1

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Chapter 5

Phenotypic and functional translation of *IL1RL1* locus polymorphisms in lung tissue and asthmatic airway epithelium

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Abstract

The *IL1RL1* (ST2) gene locus is robustly associated with asthma; however, the contribution of single nucleotide polymorphisms (SNPs) in this locus to specific asthma subtypes and the functional mechanisms underlying these associations remain to be defined. We tested for association between *IL1RL1* region SNPs and characteristics of asthma as defined by clinical and immunological measures and addressed functional effects of these genetic variants in lung tissue and airway epithelium. Utilizing 4 independent cohorts (Lifelines, Dutch Asthma GWAS [DAG], Genetics of Asthma Severity and Phenotypes [GASP], and Manchester Asthma and Allergy Study [MAAS]) and resequencing data, we identified three key signals associated with asthma features. Investigations in lung tissue and primary bronchial epithelial cells identified context-dependent relationships between the signals and *IL1RL1* mRNA and soluble protein expression. This was also observed for asthma-associated IL1RL1 nonsynonymous coding TIR domain SNPs, representing the fourth studied *IL1RL1* signal. Bronchial epithelial cell cultures from asthma patients, exposed to exacerbation-relevant stimulations, revealed modulatory effects for all 4 signals on *IL1RL1* mRNA and/or protein expression, suggesting SNP-environment interactions. The *IL1RL1* TIR signaling domain haplotype affected IL-33–driven NF-κB signaling, while not interfering with TLR signaling. In summary, we identify that *IL1RL1* genetic signals potentially contribute to severe and eosinophilic phenotypes in asthma, as well as provide initial mechanistic insight, including genetic regulation of IL1RL1 isoform expression and receptor signaling.

Keywords

Cell Biology, Genetics, Asthma, Genetic variation, Molecular genetics

Introduction

Asthma is a chronic airway disorder characterized by inflammation and widespread variable airflow obstruction that is often reversible, either spontaneously or with treatment (197). Over the years, a significant genetic component to asthma has been identified, and today, over 130 single nucleotide polymorphisms (SNPs) have been reported to be associated with asthma and allergic disease in genome-wide association studies (GWAS) (1,54,198). One of the most replicated asthma-associated genetic signals is the chromosome 2q12 locus, containing the IL-1 receptor like 1 (*IL1RL1*), IL18R1, and IL-18 receptor accessory protein (*IL18RAP*) genes (21-23,54). IL1RL1 is predominantly expressed as 2 major splice variants, one of which contains the transmembrane domain encoding the membrane bound receptor (ST2L, IL1RL1-b) that facilitates signal transduction through a Toll–IL-1 receptor (TIR) domain by interacting with/binding to IL-1 receptor accessory protein (IL1RAP). This IL-33 receptor is expressed on a number of different cell types relevant to asthma, including inflammatory cells such as T-lymphocytes, innate lymphoid cells, basophils, eosinophils, and mast cells, as well as structural cells such as fibroblasts, endothelial cells, and epithelial cells (48,111). The other main splice variant encodes the soluble form of the receptor (sST2, IL1RL1-a), which has been detected in both bronchoalveolar lavage fluid and serum in asthma patients. This splice variant is hypothesized to act as a decoy receptor for its ligand, dampening IL-33 activity (199,200).

The presence of multiple polymorphisms in the *IL1RL1* locus that independently contribute to asthma risk complicates the interpretation of the association signal with the disease (1,26). Because asthma is known to be a multifactorial and heterogeneous disease (197), we hypothesize that different SNPs within the *IL1RL1* locus drive different subtypes or components of asthma via independent and overlapping functional effects. Disease-associated SNPs may exert their functional effects by changing the protein sequence and/or by affecting levels of gene transcription (expression quantitative trait locus; eQTL). Whereas some SNPs affect gene expression under constitutive conditions (constitutive eQTL), it has recently been shown that the effect of a SNP on gene transcription is sometimes observed only in a specific context, such as diseased conditions (inducible eQTL) (201). We hypothesize that the genetic heterogeneity of the *IL1RL1* locus may be partly due to inducible eQTLs that affect gene transcription in asthma patients but not in healthy controls.

In this study, we set out to extend the association of the *IL1RL1* region polymorphisms with asthma diagnosis and to define the relative contribution of SNPs spanning the association signal to characteristics of asthma defined by clinical and immunological measures. To investigate these hypotheses, we used a step-wise study approach ultimately prioritizing selected association signals for functional characterization (Figure 1). Following detection of known common variation in the locus, we identified coding and noncoding variation through resequencing of the *IL1RL1* locus in 2 European populations of asthma patients in order to provide improved understanding of the genetic variation in the *IL1RL1* region. We subsequently related these SNPs to different asthma subtypes in order to identify key

priority SNPs for functional investigation. We tested the presence of specific eQTLs in the lung and bronchial epithelium, with a focus on *IL1RL1* regulation, and we assessed their role in regulating epithelial IL1RL1 expression after stimulation with known asthma factors implicated in disease exacerbation, such as human rhinovirus 16 (RV-16), a known modulator of IL-33 expression (202); European house dust mite (HDM) extract; and in an artificially IL-33 rich environment. Finally, we performed reductionist functional studies to address the effect of coding SNPs in *IL1RL1* on IL-33–induced signal transduction. In the same system, we investigated the effect of IL1RL1 coding SNPs on TLR-2 and -4 signaling, both of which have previously been linked to IL1RL1-TLR crosstalk in the context of tolerance (56,88).

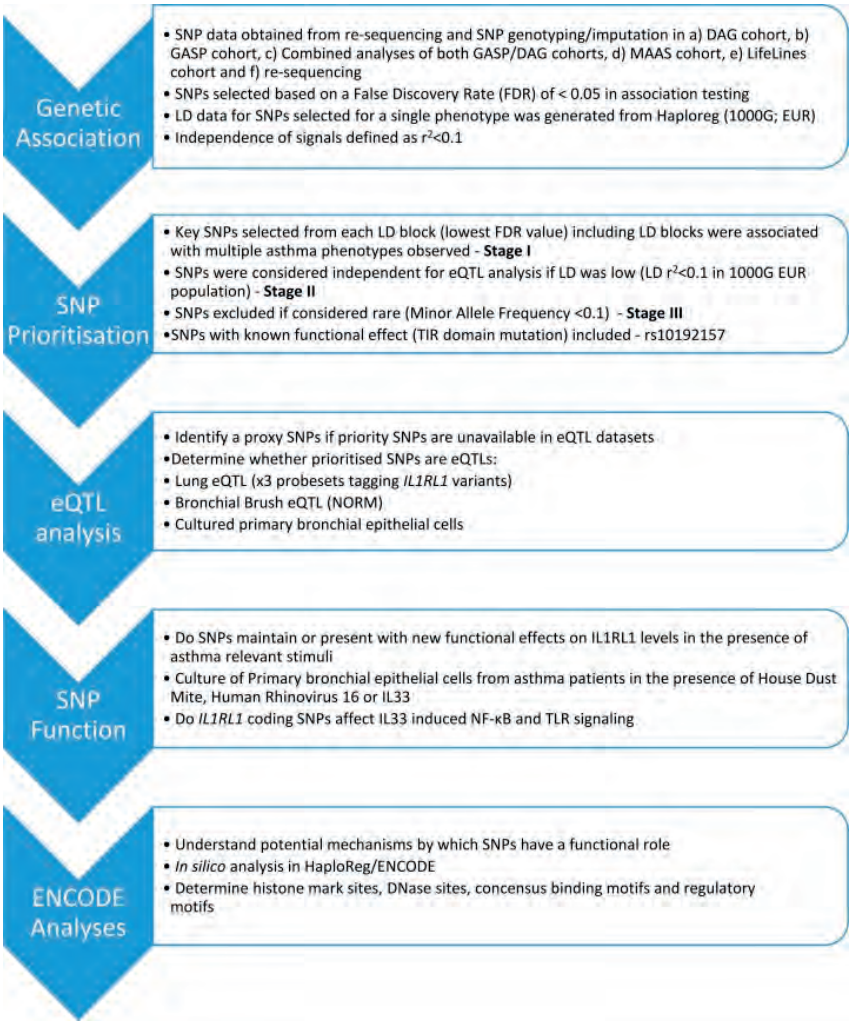


Figure 1: Flow diagram of different stages of investigation carried out in this study.
DAG, Dutch Asthma GWAS; ENCODE, Encyclopaedia of DNA Elements; GASP, Genetics of Asthma Severity and Phenotypes; LD, linkage disequilibrium; MAAS, Manchester Asthma and Allergy Study; NORM, Study to Obtain Normal Values of Inflammatory Variables From Healthy Subjects; SNP, single nucleotide polymorphism.

Material/Methods

Supplemental Methods are available online and below this chapter.

Selection of genetic region and *IL1RL1* SNPs

Selection of region.

For the phenotypic analyses, we selected SNPs with a MAF ≥ 0.01 located in the genomic region 400 kb up- and downstream the IL1RL1 gene (chr2: 102,527,961–103,368,497), which encompasses all of the previously described asthma signals, as well as several additional genes (Supplemental Figure 1). There were 3148 and 3048 SNPs with SNPTEST infoscore > 0.3 present in the GASP and DAG cohorts and 2760 SNPs with PLINK infoscore > 0.7 in Lifelines. Annotated SNP location and function was determined with the use of HaploReg v4.1 (188). All genetic data were annotated relative to assembly GRCh37/hg19. In the MAAS analyses, 2206 SNPs were available in the region for association testing.

Selection of SNPs for cell-based analysis.

SNPs of interest were selected using the following criteria: (a) significant association with asthma subtypes in our genetic association analysis (FDR < 0.05), (b) a MAF over 10% to facilitate subsequent in vitro analysis, and (c) independence based on r2 < 0.1 (in the 1000 genomes CEU population; (186)). SNP rs10192157 was also selected due to its nature as a functional SNP within IL1RL1. Levels of linkage disequilibrium were identified for SNPs at each stage of prioritization utilizing the online software LDlink (203,204) (Supplemental Figures 9 and 10). From this, SNPs were prioritized based on LD (LD r2 < 0.1; Figure 2) and selected for further study (Table 1). A tagging SNP was chosen from each key haplotype block/signal of interest after the selection process ultimately gave 4 SNPs.

ENCODE.

We used data collected by ENCODE (54, 55) to identify potential functional significance of the associated SNPs within HaploReg v4.1 (188,205). The data set was last accessed on the April 29, 2019, at 11:30 a.m.

IL1RL1 TIR domain recombinant experiments

To examine differences in NF-κB signaling between TIR domain haplotypes after IL-33 stimulation in the presence and absence of anti–IL-33 or anti-IL1RL1, we used Kruskal-Wallis test followed by Bonferroni post hoc test. A P < 0.05 value was considered significant. In the TLR experiments, we tested 2 potential functional effects of the IL1RL1-b exon 11 haplotypes based on literature. First, we tested whether the 2 haplotypes of IL1RL1-b showed a differential suppressive effect on TLR2 and TLR4 signaling, as previously reported for IL1RL1-b. IL1RL1-b exon 11 risk and protective haplotypes were overexpressed in HEK-Blue cells sensitive to either TLR2 stimulation with Pam3Cys or TLR4 stimulation with LPS at concentrations of 0, 0.1, 1.0, and 10 ng/mL.

Table 1

SNP	POSITION	GENE	C/NC*	MAF	PHENOTYPE	COHORT	EFFECT	FDR
rs12474258† (Signal A)	2:102816695	IL1RL2	T/C	0.4 (C)	Asthma Eosinophilic Asthma	Lifelines	+ +	0.0399 0.0358
rs4142132† (Signal B)	2:102937482	IL1RL1	A/G	0.49 (A)	FEV_1	GASP+DAG	-	0.0291
rs72825929 (Signal C)	2:103087086	3` IL18RAP	A/G	0.10 (G)	Severe Asthma	NGS	+	0.0351

Summary of 3 selected variants identified in our association analyses and selected for stratification of functional work. MAF, Minor allele frequency; LD, linkage disequilibrium. *C = coded allele, NC = Non-coded allele, † rs12474258 shows limited LD (r2=0.1) with previously reported asthma associated SNP rs13431828 (C) (5, 71, 72), while rs4142132 shows LD (r2=0.54) with the previously reported asthma associated SNP rs1420101 (A) (29, 70)

Table 2

Signals	*Alleles C/NC*	*Risk Allele*	*Lung eQTL proxy*	*Alleles C/NC*	*Risk Allele*	*MAF (EUR)*	*R^2*	*Cultured Epithelial eQTL proxy*	*Alleles C/NC*	*Risk Allele*	*MAF (EUR)*	*R^2*
Signal A rs12474258	T/C	T	None	-	-	-	-	rs995514	T/C	T	0.40 (C)	0.325†
Signal B rs4142132	A/G	A	rs1420088	C/T	C	0.49 (C)	1.00	None	-	-	-	-
Signal C rs72825929	A/G	A	rs11690532	C/T	C	0.17 (T)	0.536	rs17027258	A/G	A	0.10 (G)	0.311‡
Signal D rs10192157	C/T	C	None	-	-	-	-	None	-	-	-	-

Proxies for the four investigated signals used for the functional analyses presented in this manuscript. Proxies were utilised for the three SNPs associated with asthma related phenotypes in the whole lung and/or the epithelial eQTL datasets due to the lack of genotyping of the original tagging SNP in these datasets. Selected proxies were SNPs presenting with the highest r2 value within the dataset. The fourth signal (D) is the TIR homology domain variant selected for follow-up. †Although presenting with a moderately low r2 value, rs995514 was considered an appropriate proxy due to its stronger LD (r2=0.45, EUR) with rs3917243, another SNP within the association region that identified rs12474258. ‡Although presenting with a moderately low r2 value this SNP was also identified (FDR<0.05) as being associated with severe asthma in the re-sequencing dataset. MAF=Minor Allele Frequency
*proxies utilised in the bronchial epithelial brush eQTL have not been included in this table as no association was reported in this eQTL dataset

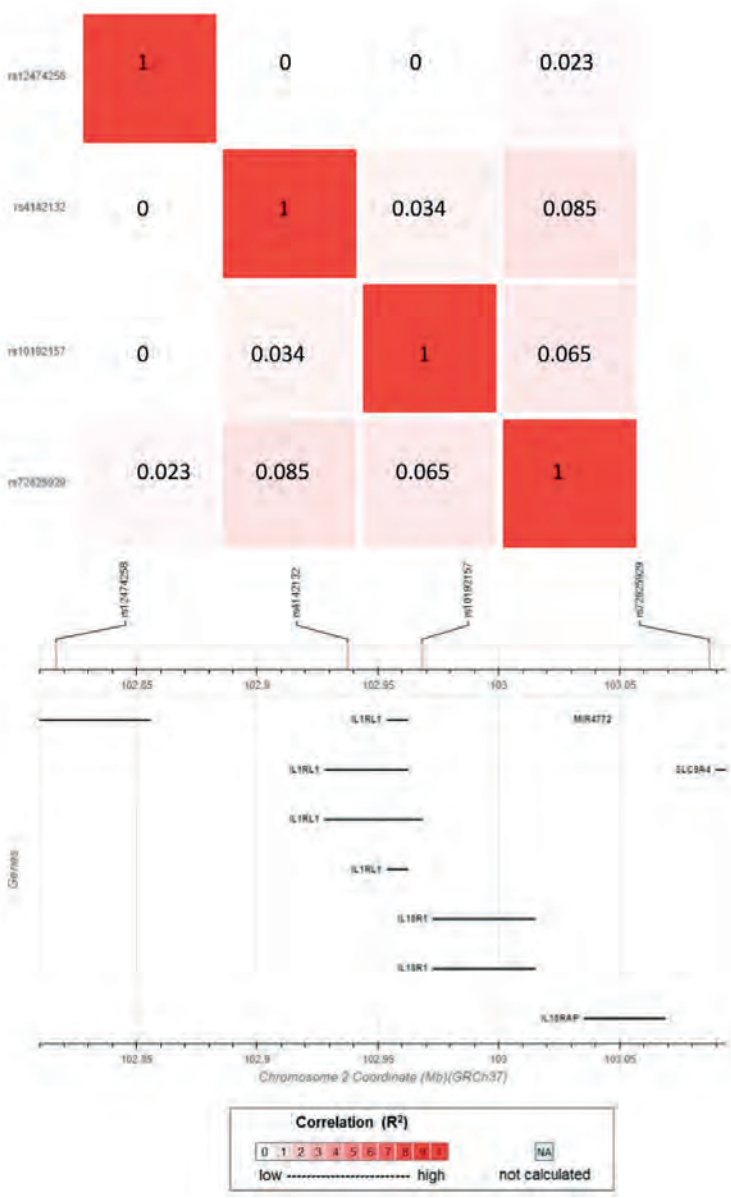


Figure 2

Linkage disequilibrium map of the 4 IL1RL1 variants identified in Stages 1–3 and selected as SNPs for functional study.

Figure identifies the level of linkage disequilibrium between signals identified based on r2 values. Image generated using the EUR population of the Phase I cohort of the 1000 genomes study via the LDmatrix tab of the online software tool LDlink 3.6, available at https://ldlink-nci-nih-gov.proxy-ub.rug.nl/

Statistics

We have included details of statistical tests and criteria used in each analysis in the online supplement; however, the following tests were used: (a) genotype-phenotype association testing (SNPTEST v2.5β or PLINK v1.90b6.7 with a FDR < 0.05 considered statistically significant), (b) resequencing in cases-controls association testing (Syzygy, P < 0.05 corrected for multiple testing [Bonferroni] was considered significant), (c) lung and bronchial biopsy eQTL (SNPtest v2.5β additive genetic model; for the 4 selected signals a P < 0.002 [Bonferroni correction] was considered significant), and (d) cultured cell eQTL, 2-tailed Mann-Whitney U or Kruskal-Wallis with Dunn's correction for multiple testing; P < 0.05 was considered significant. *IL1RL1* TIR domain recombinant experiments; Kruskal-Wallis with Dunn's correction for multiple testing; P < 0.05 was considered significant.

Study approval

The DAG and NORM cohort were approved by the Medical Ethics Committee of the UMCG. For Lifelines, all participants signed an informed consent form before they received an invitation for the physical examination. The Lifelines Cohort Study is conducted according to the principles of the Declaration of Helsinki and in accordance with the UMCG research code. The Lifelines study was approved by the medical ethical committee of the UMCG. The Lung eQTL study was approved by the ethics committees of the Institut universitaire de cardiologie et de pneumologie de Québec and the UBC-Providence Health Care Research Institute Ethics Board for Laval and UBC, respectively. The study protocol was consistent with the Research Code of the UMCG and Dutch national ethical and professional guidelines. In the AHBEC data set, brushes were collected under ethics REC 08/H0406/189 (University of Leicester) and REC 08/H0407/1 (University of Nottingham). All participants in this study provided informed consent. GASP is a multicenter study under ethics GM129901; however, it also includes samples collected under local ethics from Nottingham (recruited 1990-2015), Belfast (recruited 2008-2009), Birmingham (2005-2014), Manchester (recruited 2008-2014), Southampton (recruited 2003-2014), Glasgow (recruited 2002-2014), and Leicester (recruited 2004-2015). All studies had appropriate local ethics approval.

Results

Demographics.

For details of all cohorts used in this study, see Supplemental Methods (supplemental material available online with this article.)

Resequencing of the IL1RL1 region.

Resequencing of the chromosome 2 region containing *IL1RL1* in 200 pooled severe asthma patient DNA samples (Genetics of Asthma Severity and Phenotypes [GASP]) and 200 pooled nonasthmatic, nonallergic subject DNA samples (Nottingham Gedling Cohort) identified a

total of 4107 variants, of which 1899 were designated as valid variant calls (Supplemental Table 1). Case/control analysis for severe asthma using sequencing allele counts identified 8 variants of interest in severe asthma through meeting criteria of FDR < 0.05, with 3 variants surviving quality control (Supplemental Table 2).
Exon resequencing of the *IL1RL1* gene in an additional 95 asthma patients (Dutch Asthma GWAS [DAG]), carried out to increase our pool of sequenced asthma patients, identified a total of 56 variants covering the gene's distal and proximal promoter, introns, and exons (Supplemental Table 3).

Identification of genetic variants associated with subphenotypes of asthma.
Considering the genomic region 400 kb up- and downstream of IL1RL1 (GRCh37 chr2: 102,527,961–103,368,497) that encompasses all known genetic signals associated with asthma (Supplemental Figure 1), we identified association between 3 SNPs and severe asthma (resequencing analyses; Supplemental Table 2), 130 SNPs and asthma (Lifelines cohort; Supplemental Table 4), 316 SNPs and blood eosinophil levels in a general population (Lifelines; Supplemental Table 5), 4 SNPs and atopy (DAG/GASP; Supplemental Table 6), and 3 SNPs and lung function (forced expiratory volume in the first second [FEV1], DAG/GASP; Supplemental Table 7). We did not observe significant associations with lung function (FEV1 and FEV1/forced vital capacity [FVC]) in Lifelines and Manchester Asthma and Allergy Study (MAAS), nor with blood eosinophils, childhood onset asthma, total IgE levels, or lung function ratio (FEV1/FVC) in GASP/DAG.
We selected 4 signals of association for further functional study by considering (a) significant associations (FDR < 0.05) with asthma subtypes in our genetic association analysis, (b) a minor allele frequency > 0.1 to facilitate subsequent in vitro analysis, (c) independence based on r2 < 0.1 in the 1000 genomes EUR population (186), and (d) SNPs that were known to have a functional effect on IL1RL1 receptor signaling (Figure 1). A detailed description of the SNP selection procedure can be found in the Supplemental Methods. The 3 tagging SNPs for the associated signals presented in this manuscript (Signal A: rs12474258, minor allele frequency [MAF]: 0.40, Asthma (T) OR: 1.20, FDR: 0.049, blood eosinophils (T) β: 0.03, FDR: 0.017; Signal B: rs4142132, MAF = 0.49, FEV1 (A) β: –0.07, FDR: 0.029; and Signal C: rs72825929, MAF = 0.10, Severe Asthma (A) $\chi 2$ statistic: 16.4, FDR: 0.035) span the IL1RL1 region (Figure 2) and demonstrate association with severe, eosinophilic asthma subtypes (Table 1). For convenience, we will refer in this paper to the signals tagged by these 3 SNPs as Signal A, Signal B, and Signal C. We also included the previously reported IL1RL1 TIR homology domain coding region variant tagged by rs10192157 (12), referred to as Signal D, for downstream analysis due to its structural changes (Ala433Thr/Gln501Arg/Thr549Ile/Leu551Ser) known to affect/modulate IL1RL1 receptor signaling (26,206).

Linkage disequilibrium between selected signals and known asthma signals.
We identified that, of our 3 selected signals (discounting Signal D, [rs10192157], which was selected as a functional variant based on literature), in the 1000 Genomes EUR population,

only Signal B (rs4142132) is in linkage disequilibrium (LD) with a previously reported asthma association signal (LD r2 > 0.5; Supplemental Figure 2). Signal B includes reported asthma SNPs (rs11685480 [r2 = 1 with rs4142132], rs12479210 [r2 = 0.54], and rs1420101 [r2 = 0.52]). Our remaining selected signals (rs12474258 and rs72825929) show low LD (r2 = 0.1), with SNPs previously associated with asthma diagnosis. For a full visualization of the LD patterns to our 3 selected SNPs, refer to Supplemental Figure 2.

Association testing of previously reported asthma signals with asthma subphenotypes in our cohorts.

A literature search identified 42 studies highlighting 19 reported SNPs associated with asthma (Supplemental Table 8). Based on r2 < 0.1, these associations represent a single, independent signal. We were able to replicate association with asthma diagnosis in the Lifelines cohort for 2 known asthma-associated SNPs rs13431828 (OR: 1.36, FDR: 0.040) and rs10173081 (OR: 1.36, FDR: 0.040) (21,23,25), which represent the same genetic signal (r2 = 1, 1000 Genomes EUR population). The reported asthma risk alleles for rs13431828 (C) and rs10173081 (C) are consistent with the rs12474258 risk allele (C) identified in the current study (i.e., our signal shows the same direction of effect). We did not observe any additional association with these 19 reported SNPs to any of the phenotypes tested in any cohort (FDR < 0.05). Investigation to see if the 19 asthma SNPs also associate to blood eosinophil levels in both our cohorts and the literature; we identified a degree of overlap, confirming the association of Signal A (with which the overlapping SNPs are associated) with asthma and blood eosinophil levels (Supplemental Table 8).

Selected signals act as eQTLs for membrane and soluble IL1RL1 encoding transcripts in lung tissue.

To assess functional consequences of the selected signals, we first performed an eQTL analysis in lung tissue utilizing array data with *IL1RL1* isoform–specific probes. We find that 3 of the 4 signals (Signals B, C, and D tagged by rs142008 [proxy for rs4142132], rs11690532 [proxy for rs72825929], and rs10192157, respectively; Table 2) act as eQTLs for IL1RL1 in whole lung tissue (Table 3). The FEV1 risk allele for Signal B (proxy rs1420088 [C] allele) was shown to be associated with attenuated levels of *IL1RL1* mRNA of isoforms encoding both the soluble (IL1RL1-a) and transmembrane (IL1RL1-b) protein. The asthma risk allele for Signal C (proxy rs11690532 [C]), however, was associated with elevated IL1RL1 mRNA expression (of both transcripts in a combined and independent assay). The asthma risk allele for Signal D (rs10192157 [C]) was associated with lower expression of the transcript encoding the soluble isoform but did not show association with the transcript encoding the transmembrane protein. In summary, these data show that 3 of our prioritized signals are eQTLs for IL1RL1 in lung tissue; however, the 2 asthma-associated risk alleles have opposite directions of effect on IL1RL1 mRNA expression in lung tissue. Interestingly, Signal A — associated with asthma and blood eosinophils — did not demonstrate any IL1RL1 eQTL association in lung tissue. The strongest effect estimate was observed with the IL1RL1

isoform encoding the soluble protein, showing a 10-fold increase over the transmembrane isoform in the presence of the respective risk alleles of Signals B and C (rs1420088 and rs11690532; Table 3).

Association between IL1RL1 SNPs and baseline IL1RL1 expression in cultured bronchial epithelial cells from asthma patients.

To determine the effect of the 4 signals on IL1RL1 expression in cultured bronchial epithelial cells (BECs), we examined the effect of SNPs on baseline expression of *IL1RL1* mRNA isoforms and soluble IL1RL1 (IL1RL1-a) protein in human BECs (HBECs) isolated from asthma patient donors and cultured in vitro (Figure 3 and Supplemental Figures 3 and 4). We observed that Signal B, tagged by the *IL1RL1* intronic variant rs4142132, had an effect on the mRNA levels of the transcripts encoding soluble and membrane IL1RL1 isoforms in HBECs cultured in vitro. The presence of the risk (A) allele associated with a lower FEV1 resulted in lower levels of both IL1RL1 mRNA isoforms ($P < 0.05$) (Figure 3, A and B), an effect mirrored in the whole lung tissue. These results were confirmed at the protein level, where levels of soluble IL1RL1 in HBEC supernatants were lower in carriers of the (A) allele ($P < 0.01$) (Figure 3E).

The allele associated with severe asthma at Signal C (rs17027258 [A]) was associated with elevated IL1RL1 mRNA levels of the transcripts encoding transmembrane IL1RL1 but not with those encoding the soluble isoform in HBECs ($P < 0.05$) (Figure 3, C and D), in agreement with the direction of effect observed in lung tissue. At the protein level, the risk allele (A) was associated with elevated soluble IL1RL1 levels in cellular supernatants (Figure 3G).

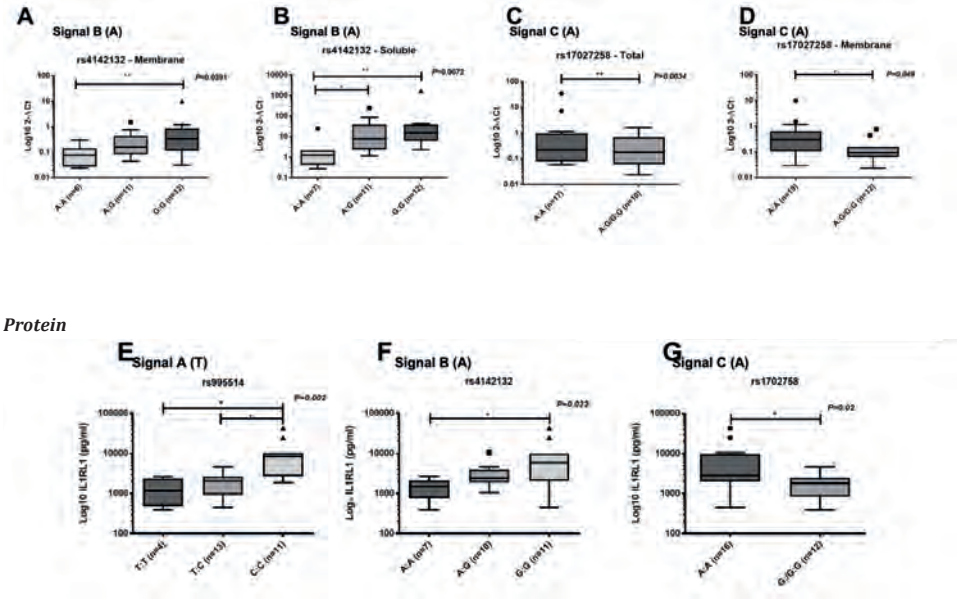
In HBECs, as opposed to no effect in whole lung tissue, the asthma risk (T) allele for Signal A (tagged by rs995514) also associated with elevated blood eosinophil levels, resulted in a lower levels of soluble IL1RL1 protein, but had no effect on mRNA isoforms (Figure 3F and Supplemental Figures 3 and 4). For Signal D (rs10192157), no effect was observed on either *IL1RL1* mRNA or protein levels (Supplemental Figures 3 and 4).

Table 3

				Soluble and membrane *IL1RL1* X100148210_TGI_at			Soluble *IL1RL1* X100148162_TGI_at			Soluble *IL1RL1* X100312840_TGI_at			Membrane *IL1RL1* X100302151_TGI_at		
Signal/SNP	**C/NC***	**Risk Allele**	**MAF**	**BETA**	**SE**	***P***	**BETA**	**SE**	***P***	**BETA**	**SE**	***P***	**BETA**	**SE**	***P***
Signal A rs12474258	C/T	T	0.36 (C)	-0.01	0.05	8.36E-01	-0.01	0.04	7.38E-01	-0.03	0.04	4.58E-01	0.01	0.01	2.47E-01
Signal B rs1420088†	C/T	C	0.44 (C)	**0.96**	**0.04**	**4.88E-137**	**0.78**	**0.03**	**8.87E-148**	**0.74**	**0.03**	**6.09E-150**	**0.08**	**0.01**	**8.89E-13**
Signal C rs11690532††	C/T	C	0.17 (T)	**0.55**	**0.06**	**2.03E-23**	**0.41**	**0.04**	**1.73E-20**	**0.38**	**0.04**	**1.75E-20**	**0.045**	**0.01**	**1.17E-03**
Signal D rs10192157	T/C	C	0.40 (T)	**0.20**	**0.05**	**5.62E-05**	**0.13**	**0.04**	**1.11E-03**	**0.15**	**0.04**	**3.55E-05**	0.004	0.01	7.49E-01

Expression quantitative trait loci (eQTL) analysis for signals (tagging SNPS or proxies) in a dataset for human lung tissue. These analyses identified eQTLs for SNPs rs1420088 and rs11690532 with both membrane and soluble IL1RL1, with the coded alleles (associated with decline in lung function [FEV1] and protective for asthma, respectively) associated with elevations in gene expression in the whole lung. The structural variant rs10192157 is only an eQTL for the soluble isoform of IL1RL1 with the asthma risk allele associated with lower levels of soluble IL1RL1 *C = coded allele, NC = Non-coded allele, †rs1420088 was used as a proxy for rs4142132 *r2=1.0, EUR), ††rs11690532 was used as a proxy for rs72825929 (r2=0.54, EUR), MAF=Minor Allele Frequency

Figure 3



Baseline* IL1RL1 *mRNA and soluble IL1RL1 protein levels are driven by SNPs in cultured human bronchial epithelial cells. *In cultured HBECs lower level of* IL1RL1 *soluble and transmembrane mRNA can be observed in carriers of the risk allele (A) for lower lung function (FEV_1) for Signal B (rs4142132; Panels A & B;* P*<0.05). Increased levels of total and transmembrane* IL1RL1 *mRNA was observed for carriers of the asthma risk allele (A) in Signal C (rs17027258, proxy for rs72825929; Panels C & D;* P*<0.05). Changes in mRNA levels were reflected in soluble IL1RL1 protein levels in matched cellular supernatants (Panels F & G;* P*<0.05). In Signal A, carriers of the asthma risk/elevated blood eosinophil levels allele of SNP rs995514 (proxy for rs12474258) (T) presented with lower levels of IL1RL1 soluble protein (Panel E;* P*=0.002), however this was not observed at the RNA level (see Supplemental Figure 3).*

Effect of asthma-relevant stimuli on IL1RL1 expression.
Next, we considered the possibility that disease state and/or relevant microenvironmental triggers may regulate *IL1RL1* expression in a SNP-dependent fashion. Therefore, we investigated the effect of asthma-relevant stimulations of cultured HBECs obtained from asthma subjects on IL1RL1 expression in carriers and noncarriers of phenotype-associated alleles (i.e., inducible eQTLs). Prior to stratification, we observed an increase in soluble IL1RL1 protein levels in cell supernatants following stimulation with HDM for 24 hours ($P < 0.01$); however, no change was observed with either RV-16 or with IL-33 stimulation ($P > 0.05$) (Figure 4, A and B). Conversely, stimulation with HDM reduced membrane IL1RL1 mRNA levels 3.5-fold (Figure 4C, $P < 0.05$), while RV-16 stimulation reduced soluble IL1RL1 mRNA expression 4.5-fold (Figure 4D, $P < 0.05$). No alterations in IL1RL1 mRNA were observed in response to IL-33; however, a response to IL-33 was confirmed using IL-8 mRNA levels as an outcome (Figure 4E).

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IL1RL1 variation has an impact on IL1RL1 regulation in response to asthma-relevant stimuli.
Stratification based on our 4 selected signals identified that HDM-driven effects on IL1RL1 expression were genotype dependent, with effects observed for all 4 selected SNPs (Figure 5 A–D, Supplemental Figures 5 and 6).

In Signal A (tagged by rs995514), there was a modest increase in the level of soluble IL1RL1 protein (1.63-fold) in the presence of the asthma protective allele (C) (Figure 5A). In Signal C (tagged by rs17027258), presence of the severe asthma risk allele (A) identified modest elevation in IL1RL1 soluble protein expression after HDM stimulation (1.5-fold; $P < 0.01$) (Figure 5B). The largest response to HDM was observed in the functionally relevant TIR domain haplotype (Signal D [tagged by rs10192157]). Here, the presence of the protective allele (T) was associated with a 2-fold increase in soluble IL1RL1 protein after HDM stimulation (Figure 5C). No effect was observed on IL-33 stimulation (Supplemental Figure 5).

At the mRNA level, no apparent inducible eQTL effects were observed for Signals A, C, and D, in contrast to the observed effects at the protein level (Supplemental Figure 6). However, in Signal B, total IL1RL1 mRNA expression was reduced (31.73-fold) in response to HDM but only in cells carrying the allele (G) associated with higher lung function (FEV1) (Signal B, Figure 5D, $P < 0.05$). However, it is important to note that the baseline levels of total IL1RL1 mRNA in GG carriers were significantly higher than that for both AA and AG genotype carriers, in keeping with the findings reported above (Figure 3, A and B).

Table 4: Summary table identifying functional effects of selected signals on IL1RL1 expression and activity.

Signal/SNP	Phenotypic risk allele/trait	Location	Effect allele in functional studies	Recombinant Cell NF-Kb activity	Total *IL1RL1* expression (lung)	Membrane *IL1RL1* expression (lung)	Soluble *IL1RL1* expression (lung)	Total *IL1RL1* expression (HBEC)	Membrane *IL1RL1* expression (HBEC)	Soluble *IL1RL1* expression (HBEC)
Signal A rs12474258	T Asthma Eosinophilic asthma	*IL1RL2* (intronic)	T	ND	-	-	-	-	-	Decrease† Decrease (HDM)†
Signal B rs4142132	A Lower Lung Function (FEV_1)	*IL1RL1* (Intronic)	A	ND	Increase	Increase	Increase	-	Increase	Decrease Decrease† Increase (HDM)‡
Signal C rs72825929	A Severe Asthma	2.7kb 5` of *SLC9A4*	A	ND	Increase	Increase	Increase	Increase	Increase	Increase† Increase (HDM)†
Signal D rs10192157	C Asthma (Literature)	*IL1RL1* (Missense)	C	Increase	Decrease	-	Decrease	-	-	Decrease (HDM)†

Table identifies changes in either lung or cultured epithelial mRNA expression, except were annotated as follows: †identifies additional effect of variation on soluble IL1RL1 protein expression in HBEC supernatants; ‡effect driven by alterations in baseline expression with no difference between genotypes following stimulation

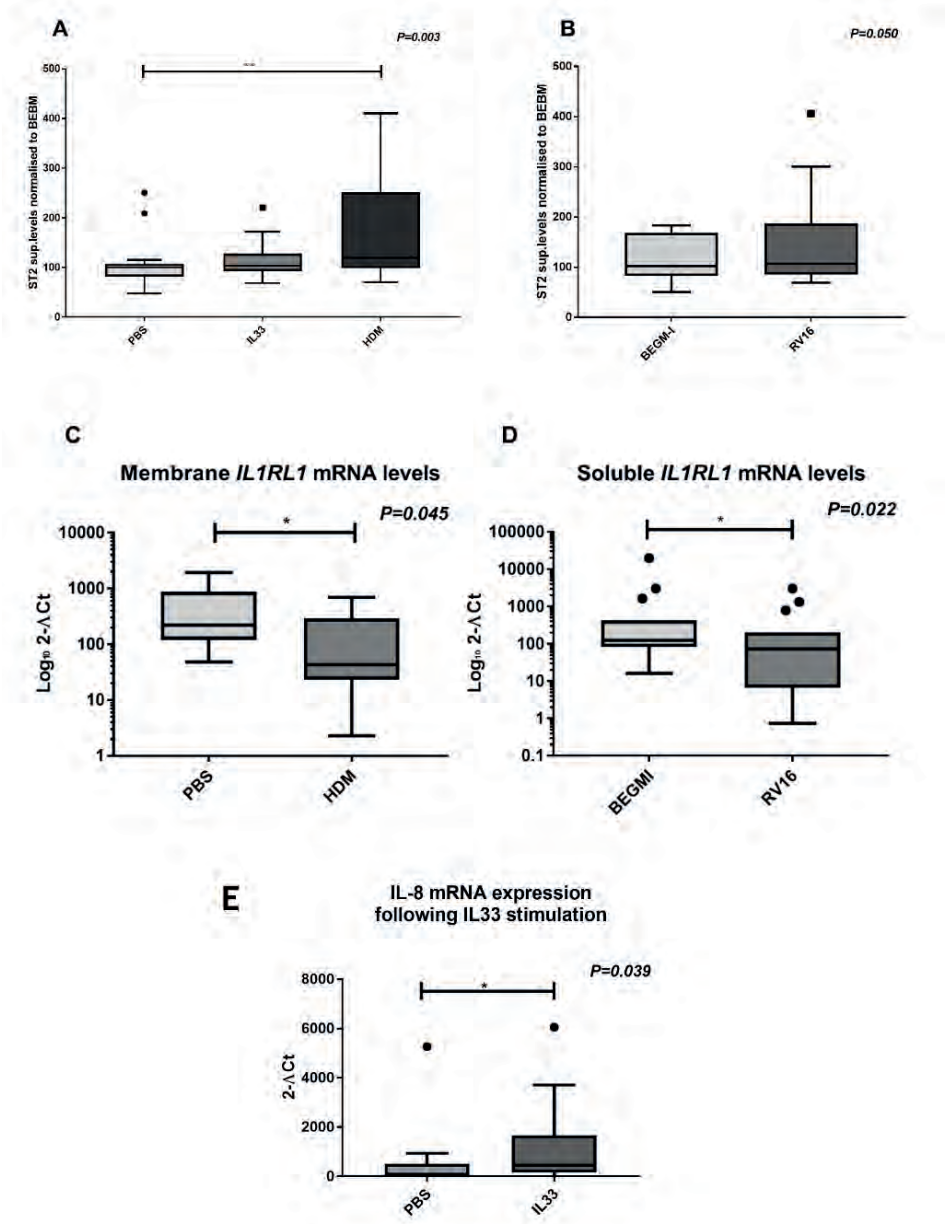

Figure 4: Soluble IL1RL1 Protein levels in supernatant

Asthma relevant microenvironments modulate *IL1RL1* mRNA levels and soluble IL1RL1 protein levels in bronchial epithelial cells isolated from asthma patients and cultured in vitro. Stimulation of cells with 50µg/ml House Dust Mite (HDM) for 24 hours resulted in increased release of soluble IL1RL1 into the cellular supernatant (Panel A, *P*=0.003, n=18). RV-16 (MOI:1) stimulation for 24 hours did not significantly influence IL1RL1 protein release in the cell supernatants (Panel B, P=0.05, n=18). HDM stimulation resulted in a 3.5-fold reduction of membrane IL1RL1 mRNA (Panel C, P=0.045, n=15), while stimulation with RV16 (MOI:1) for 24 hours reduced soluble IL1RL1 mRNA levels 4.4-fold (Panel D, P=0.022, n=15). IL-33 stimulation did not alter IL1RL1 protein or mRNA levels, however did induce IL8 mRNA demonstrating cell activation (Panel E, P=0.039, n=18).

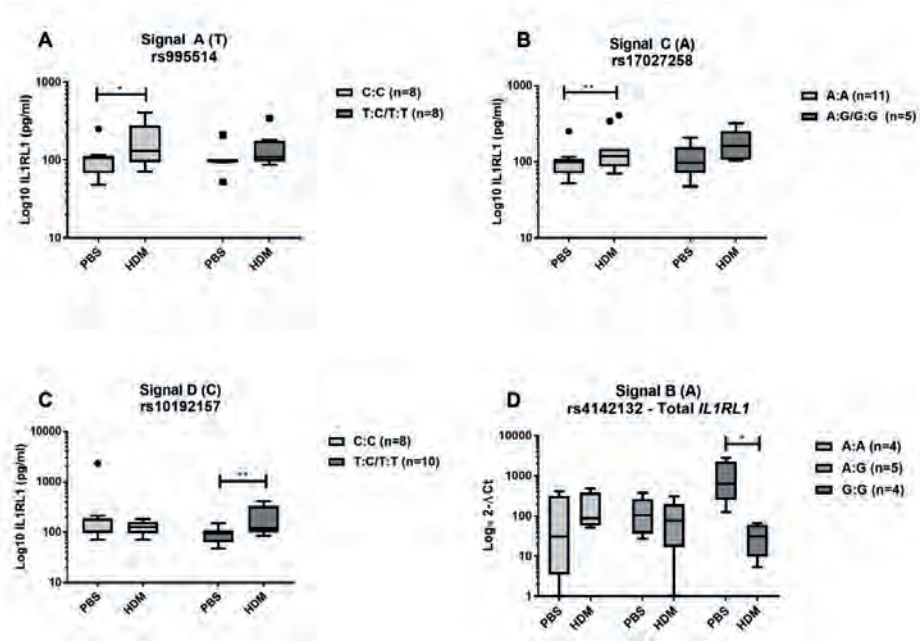

Figure 5: SNPs regulate IL1RL1 mRNA and protein expression levels in response to asthma relevant micro-environments.
Increased release of IL1RL1 protein in response to HDM was present in three of our four selected signals; Signal A (rs995514; proxy for rs12474258) for the protective allele for asthma and elevated blood eosinophils (C) (P<0.05), Signal C (rs17027258; proxy for rs72825929) risk allele for severe asthma (A) (P<0.01) and Signal D (rs10192157) for the protective allele for asthma (T) (P<0.05) (Panels A-C respectively). Decreased levels of total *IL1RL1* mRNA in response to HDM is present only in Signal C (rs4142132) for carriers of the allele protective for reductions in lung function (FEV_1) (G) (Panel D, P<0.05).

IL1RL1 coding region variants associated with asthma influence signaling.
We next tested functional effects of the membrane IL1RL1 TIR domain haplotype that are tagged by Signal D. These haplotypes encode IL1RL1 proteins that present with a 4–amino acid change in the intracellular TIR signaling domain (Ala433Thr/Gln501Arg/Thr549Ile/Leu551Ser) (26). The potential functional effects include the following: (a) IL1RL1-b coding region variants determine the magnitude of signaling response downstream of IL-33 and (b) IL1RL1 haplotypes determine the antiinflammatory effects of anti–IL-33 and anti-IL1RL1 monoclonal antibodies (206). A reductionist recombinant cell line model with a fixed genetic background was used to facilitate these analyses. HEK-Blue-SEAP cells transfected with empty vector or 1 of the 2 IL1RL1 mRNAs encoding the alternative TIR domain IL1RL1 proteins, which demonstrated the same capacity to signal via NF-κB following TNF-α stimulation (Figure 6A). Escalating doses of recombinant IL-33 were able to induce NF-κB signaling, in a dose dependent manner, in the 2 cells lines containing the IL1RL1 protein (Figure 6B). Cells carrying the asthma risk haplotype (Ala433/Gln501/Thr549/Leu551) tagged by the Signal D SNP rs10192157 (CC) demonstrated a 2.9-fold induction at the

highest dose of IL-33 (50 ng/mL) in this cell system, which was significantly higher than the modest activity observed for the asthma protective TIR domain haplotype protein (Thr433/Arg501/Ile549/Ser551) (1.3-fold) (Figure 6B). Additionally, cells carrying the asthma risk haplotype and stimulated with 50 ng/mL IL-33 were more amenable to the antiinflammatory effects of blocking either IL1RL1 or IL-33 using monoclonal antibodies compared with the alternative haplotype, where blocking antibodies had a minimal effect on reducing NF-κB signaling (Figure 6, C and D).

IL1RL1 coding region variants associated with asthma do not modify TLR signaling.
IL1RL1 signaling is thought to modify TLR-2 and -4 activation (87,88); therefore, we hypothesized that the IL1RL1 TIR domain haplotype variation may influence this relationship. For these experiments, we used ILR1L1 overexpression vectors in transfected cells whose functionality was confirmed by the capacity to induce ERK1/2 activation after IL-33 exposure (Supplemental Figure 7). We did not observe an effect of overexpression of the 2 different IL1RL1 exon 11 haplotypes on the sensitivity of HEK-Blue cells to TLR2-induced NF-κB activity after stimulation using a dilution series of Pam3Cys (Supplemental Figure 8A). We also did not observe an IL1RL1 exon 11 haplotype–driven effect on TLR4-induced NF-κB activity in HEK-Blue cells after stimulation with a dilution series of LPS (Supplemental Figure 8B). These studies show that, while the IL1RL1 exon 11 haplotype regulates sensitivity to IL-33 (Figure 6), the proposed regulatory function of IL1RL1-b on TLR2 or TLR4 signaling (87,88) could not be confirmed (Supplemental Figure 8).

Bioinformatic analyses of prioritized signals using ENCODE.
We investigated each signal including SNPs in LD defined by r2 > 0.8 for potential functional effects using the Encyclopaedia of DNA Elements (ENCODE) resource via HaploReg (Supplemental Table 9). Two of the investigated LD blocks tagged by Signals A and B (rs12474258 and rs4142132) were found to have multiple SNPs positioned in enhancer histone mark sites, with DNase hypersensitivity sites also identified within the Signal B region (rs4142132), a region where protein binding consensus sequences exist and affect regulatory motifs. Motifs changed in Signals A and B included cell type–specific transcription factors such as GATA-2, active in mast cells and basophils (145,207) and positively regulating IL1RL1 expression (28,189), and FOXA1/2, active in HBECs (208). Similarly, the functional SNP rs10192157 (Signal D) altered Gfi1 transcription factors linked to type 2 inflammation and a regulator of IL1RL1 expression (209). There was also generic activation–induced transcription factors such as Fos/Jun (AP-1), NF-κB, and cEBP/p300 found to be bound to these motifs. This highlights potential functional effects for 3 of our 4 selected signals of association on both cell type–specific and ubiquitous regulation of IL1RL1 expression (Supplemental Table 9). No SNPs were identified to be in LD for Signal C (rs72825929), and no enhancer histone mark sites, DNase hypersensitivity, or protein motif binding were identified for this SNP. However, rs72825929 modifies the Sox (sex-determining region Y [Sry] box–containing) family of transcriptional factors, which have been shown to be involved

in lung organogenesis, with Sox2 in particular playing a crucial role in the proliferation and differentiation of respiratory epithelial, trachea, airway branching, and Clara cells (210).

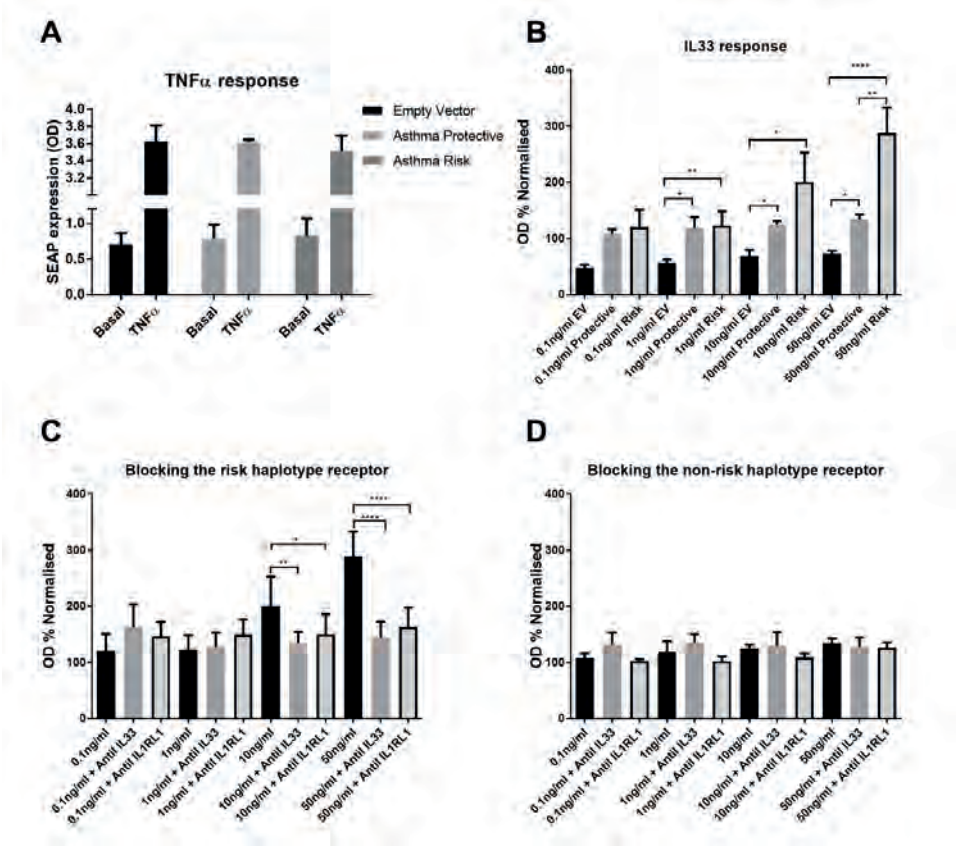

Figure 6: Functional analyses of the IL1RL1 TIR risk haplotype in an in vitro reductionist model identifies an exagerated response to IL-33 that is more amenable to anti-IL-33/IL1RL1 treatment. Transient transfection of HEK-NF-KB-SEAP reporter cells with *IL1RL1* containing the two TIR domain polymorphism haplotypes provides a platform to identify differential NF-KB signalling. Cells transfected with empty vector, *IL1RL1* containing the asthma risk haplotype (Ala433/Gln501/Thr549/Leu551) or IL1RL1 containing the protective haplotype (Thr433/Arg501/Ile549/Ser551) have the same capacity to signal via the NF-KB pathways in response to 10ng/ml TNF-α (Panel A). The presence of the IL1RL1 receptor carrying the asthma risk haplotype identified a 2-fold and 3-fold increase in signalling on stimulation with 10ng/ml and 50ng/ml of human recombinant IL-33 respectively, whereas an attenuated response was observed in the protective haplotype (Panel B). The response induced by 50ng/ml IL-33 in the risk haplotype was amenable to blocking using either 10μg/ml anti-IL-33 or anti-IL1RL1 leading to an anti-inflammatory effect (Panel C). Whereas effect of blocking IL-33 induced inflammation by anti-IL-33 or anti-IL1RL1 was minimal in carriers of the protective TIR domain haplotype (Panel D). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. N=3 for all experiments.

Discussion

Main findings.

We set out to extend our understanding of the *IL1RL1* locus in asthma, one of the most reproducible association signals identified to date, with a particular focus to the contribution of the *IL1RL1* gene. We provide insight into the nature of the genetic association with clinical and immunological features of asthma and the mechanistic underpinnings of these associations with respect to IL1RL1 expression and activity. In this multiple-cohort study, we extend a priori evidence that genetic variation in the IL1RL1 region is important for asthma susceptibility and blood eosinophil counts. In particular, we identify that a functional haplotype consisting of several polymorphisms encoding 4 amino acid changes (increased asthma risk, Ala433/Gln501/Thr549/Leu551) and present in ~50% of the European population enhanced NF-κB activity after IL-33 stimulation. This has potentially important implications for targeted asthma therapies, where expression of the risk haplotype could influence therapeutic efficacy. Functional analyses demonstrated that 3 of our 4 independent signals of association, Signals B, C, and D — tagged by SNPS rs4142132, rs72825929, and rs10192157 — are eQTLs for *IL1RL1* in lung tissue. These regulatory roles were retained in cultured primary HBECs at both the mRNA and protein levels. Interestingly, although not an eQTL in the whole lung data set, Signal A (rs12474258) was an eQTL in cultured BECs for soluble IL1RL1 protein. Thus, our data suggest cell type specificity of eQTL for *IL1RL1*, suggesting that specific subtypes of asthma may be driven by different cell types in different patients. Therefore, different asthma subtypes may be compartment specific, where specific cell types such as lung structural, inflammatory, and isolated immature basal epithelial cells may predominate.

Interestingly, there was a lack of eQTL effects observed in bronchial biopsies and bronchial brushes taken from controls without respiratory diseases, while we did observe eQTLs in cultured primary HBECs of asthma patients, compatible with the context dependency of eQTLs. We therefore investigated whether the selected SNPs act in such a context-dependent manner (i.e., inducible eQTLs), through functional studies. We identified that all of our signals of interest were able to modulate response to HDM, a common allergen associated with asthma and allergies, on IL1RL1 expression in HBECs. These data provide a potential link between IL1RL1 genetic variants and *IL1RL1* regulation of IL-33 inflammation in HDM-induced type 2 immune responses in the lung, where the IL-33/IL1RL1 axis has been shown to be involved (211,212). Finally, we also investigated IL1RL1 coding region variation and established that cells carrying the *IL1RL1* TIR domain asthma risk haplotype (Signal D) presented with an exaggerated inflammatory response to IL-33 that is more amenable to the antiinflammatory effects of either anti–IL-33 or anti-IL1RL1 monoclonal antibodies. This has implications for the targeting of the IL-33/IL1RL1 axis inhibitors to a subset of patients of a specific genotype likely to gain the greatest clinical benefit and is highly relevant, with multiple pharmaceutical companies developing anti–IL-33/IL1RL1 approaches for the treatment of asthma.

Genetic associations.

The locus on chromosome 2q12, which includes the *IL1RL1* gene, has shown a significant replicated association with asthma (15,22,23,67,161) or asthma-relevant traits such as childhood asthma (19,28,213), childhood asthma with exacerbation (19), severe asthma (57), asthma with hay fever (20), type 2 inflammation in asthma (211,212), and blood eosinophil counts (15). Sentinel SNPs identified in these association studies span the 2q12 region, covering multiple genes, with evidence based on linkage disequilibrium pointing to independent association signals (25).

In order to characterize further the contribution of genetic variants to features of asthma, we carried out association testing across the Lifelines/GASP/DAG/MAAS cohorts. This identified that chromosome 2q12 region variation is associated with blood eosinophil numbers (Signal A) and lung function (FEV1) (Signal B). We also investigated SNPs previously associated with asthma or asthma-related traits in the region, with 2 of these SNPs, rs13431828 and rs10173081, being associated with asthma diagnosis in our Lifelines analysis (Supplemental Table 2). We also confirmed phenotypic overlap between asthma and blood eosinophil counts in Signal A by investigating 19 SNPs, previously associated with asthma, in this region in LD with Signal A. Significant overlap between these SNPs and SNPs associated with eosinophilia counts in our cohorts and in the literature was observed (Supplemental Table 8). To complement the investigation of the genetic architecture of the locus, we completed next-generation sequencing analyses and identified 4107 variants spanning the region, of which 3 met criteria for association with severe asthma in case/control analyses (FDR < 0.05) and survived quality control, including sentinel variant rs72825929 (A/G [5′ to SLC9A4]), Signal C. Although limited by small population numbers and pooled analysis, additional support for the association of Signal C with asthma was determined through previously published GWAS, where association was shown with all asthma (rs11690532) (22) and pulmonary function (rs17027258/rs11690532; FEV1 and FEV1/FVC) (57). More importantly, Signal C was also associated with moderate-to-severe asthma in the largest GWAS of this phenotype published to date, to our knowledge (rs72825929; β: 0.11, P = 0.0016, Allele A) (57,214). In Lifelines, rs72825929 was associated with blood eosinophil counts in the general population ([G] β: 0.05, SE: 0.015, FDR: 0.03, AF general population [G]: 0.10) but not with the other studied phenotypes. Based on these analyses, we selected 3 SNPs that identify key signals of association spanning the region for functional analyses and also included TIR domain SNP rs10192157 (Signal D) from the literature, giving a total of 4 signals for functional follow-up. Overall, these data complement and extend the accumulating data, suggesting a role for the IL-33/IL1RL1 axis that is genetically determined in T2-driven inflammation (211,212) and eosinophilic asthma (15). Taken together, our data suggest that there are multiple independent genetic signals in the *IL1RL1* locus that may be particularly important in driving severe, eosinophilic asthma phenotype with reduced lung function, with limited evidence for genetic variants driving other features of asthma such as atopy, total IgE levels, and age at onset in these cohorts.

eQTLs.

Previous work has established that genetic variation in the *IL1RL1* locus can act as eQTLs for *IL1RL1* mRNA, as methylation QTLs in WBCs and as protein QTL (pQTL) for serum levels of soluble IL1RL1 (27,161,189,206,215). In this study, we specifically investigated the functional effects of our 4 priority signals in (a) lung tissue, (b) airway epithelial brush samples, and (c) cultured HBECs at baseline and in the presence of asthma-relevant stimuli. Lung tissue eQTL identified 3 of the 4 selected signals as eQTLs for *IL1RL1*, with differential SNP effects observed for membrane and soluble isoforms. Interestingly, the presence of the asthma risk allele for the 2 asthma selected *IL1RL1* signals (Signals A and C) presented with contradictory effects of *IL1RL1* mRNA transcripts in lung tissue, suggesting that these 2 independent signals may have different roles/functionality with regard to IL1RL1 and asthma. The effect size of the associations was greatest for the soluble *IL1RL1* mRNA, suggesting that *IL1RL1* eQTL effects are more likely to translate to functional effects through regulation of soluble rather than membrane IL1RL1 levels, with the exception of the TIR domain–effecting SNP rs10192157 (Signal D). This latter SNP has also been identified as an eQTL for IL1RL1 in nonstructural cells (216). Our data are in good agreement with the lung eQTL data found in the GTEx database (https://gtexportal.org/home/) (131,217). However, when comparing our 3 eQTL signals with the strongest *IL1RL1* eQTL signals in this data set, a near-perfect LD pattern ($r2 \geq 0.98$) was only observed for Signal B, which may suggest that the other 2 eQTL signals (Signals C and D) may be LD shadows of the true causative eQTL variant. On the other hand, our data are further supported by being in good agreement with previous work examining genetic variants that are associated with soluble *IL1RL1* mRNA (215) and serum levels of soluble IL1RL1 (27). More specifically, Signals A–D have all recently been associated with plasma IL1RL1 levels (206,215). Searching for our four signals on Open target genetics (https://genetics.opentargets.org/, accessed June 18, 2019), we identified that the risk allele in Signal A (T) was associated with elevated IL1RL1 levels in blood plasma (β: -0.202; $P = 8.3 \times 10–16$), which contrasts to the lack of eQTL reported in our lung and biopsy/brush eQTL data sets. The remaining eQTLs in plasma extracted from whole blood provided additional insight (e.g., the risk allele for Signal B [A] was associated with lower levels of plasma IL1RL1 [rs10179654, β: –0.85, $P = 3.00 \times 10–391$]) (77,218), which supports our findings and the concept that blood levels of soluble ST2 may be driven by epithelial produced protein (211). The risk allele for Signal C (A) was associated with elevated levels (β: –0.469, $P = 5.5 \times 10–34$), and the risk allele of Signal D (C) was associated with lower levels of IL1RL1 (β: –0.288, $P = 1.7 \times 10–30$) in good agreement with our data across data sets.

Importantly, our data extend this work by offering a comprehensive analysis of all IL1RL1 transcripts (total, membrane, and soluble IL1RL1 encoding mRNA) and extends the recently suggested concept that asthma risk alleles essentially lead to a decrease in soluble IL1RL1, and this lack of decoy receptor diminishes the ability to mitigate the effects of IL-33 (199). Importantly, these analyses highlight multiple signals spanning the *IL1RL1* locus that can regulate IL1RL1 and contribute to disease mechanisms via modulation of IL1RL1 receptor levels.

We did not observe SNP eQTL effects in the biopsy and bronchial brush data sets generated from tissue samples obtained from volunteers without respiratory diseases, which is in contrast to a recent study that identified 3 *IL1RL1* SNPs (rs12712135; tagged by Signal B; rs1420088 [r2 = 0.98], rs1041973 and rs10185897), which showed association with membrane IL1RL1 mRNA in bronchial brush samples from asthma patients (215). This can potentially be explained due to differences in sample cell composition and by the fact that the eQTL was run in a nonasthmatic population in our analyses, which would indicate that the eQTL effects are disease specific. To provide greater insight, we therefore completed reductionist eQTL analyses in cultured HBECs isolated from asthma patients. Here, we confirmed the signals tagged by Signals B and C (rs4142132 and rs72825929) as eQTLs, where the observed direction of effect for both signals complemented those seen in our lung tissue data base. Interestingly, we demonstrated that the asthma protective allele (C) for Signal A (rs995514) was an eQTL in cultured epithelial cells, being associated with elevation in soluble protein levels. In contrast, no eQTL could be identified for Signal D (rs10192157). These data provide an additional indication that IL1RL1 SNPs act as an eQTL in a tissue- and cell type–specific manner, potentially with inflammatory cells contributing to the lung tissue findings (e.g., mast cells, basophils, Th2 cells, ILC2s, and eosinophils). These data do not support the hypothesis that presence of asthma risk alleles leads to reduced soluble IL1RL1 to act as a decoy, limiting the mitigation of the biological effects of IL-33; however, they do suggest a complex effect of genetic signals on membrane and soluble IL1RL1 levels that is cell, tissue, and context dependent. For example, we hypothesize that changes in the IL1RL1 TIR signaling domain structure, — driven by the variation in Signal D, which we have shown drives increased receptor signaling — may cause a negative feedback loop that attenuates IL1RL1 and concurrent soluble *IL1RL1* expression.

Inducible eQTLs.

We tested for inducible eQTLs of our priority signals by culturing HBECs in the presence and absence of RV-16, HDM, and human recombinant IL-33. In general, RV-16–stimulated HBECs showed a decrease in soluble *IL1RL1* mRNA; however, no SNP-specific effects were observed, suggesting a limited role of these eQTLs in regulating RV-16–driven effects on soluble *IL1RL1* mRNA.

When considering the aeroallergen HDM, another relevant environmental agent involved in allergic asthma, our data identified that HBECs release soluble IL1RL1 in response to HDM; however, this response was accompanied by a decrease in membrane *IL1RL1* mRNA. This is especially relevant when considering that elevations of circulating IL-33 on HDM stimulation have been reported in mouse models (219,220), with attenuated HDM-induced airway hyperresponsiveness in IL1RL1-KO mice. We therefore suggest that a negative feedback mechanism may exist in HBECs, where attenuation of membrane receptor expression may act as a measure to halt HDM-induced activation of Th2 inflammation.

Stratification of these observations based on genetic signals identified that this mechanism appears to be SNP dependent. Carriers of the asthma protective allele in Signal A (rs995514;

CC) and Signal D (rs10192157; TC/TT) responded to HDM through elevations in soluble IL1RL1 protein. This observation ties in well with the hypothesis that increased levels of circulating soluble IL1RL1 protein may act as a decoy receptor for circulating IL-33, preventing activation of the transmembrane receptor and relates to our earlier observation that asthma risk allele carriers for IL1RL1 variants present with lower soluble IL1RL1 protein. However, homozygote carriers of the asthma risk allele in Signal C (rs17027258; AA) also presented elevated levels of soluble IL1RL1 protein following HDM stimulation, suggesting potential roles of circulating soluble IL1RL1 that may induce asthma under certain conditions.

SNP stratification of IL1RL1 expression at the mRNA level following HDM stimulation suggests that Signal B (rs4142132) is crucial in driving the attenuation of IL1RL1 expression identified in the nongenotype selected experiments, as it was the only region to act as an eQTL in this regard. This effect appears to be driven by the eQTL effects at baseline, where sufficient IL1RL1 levels for a negative feedback mechanism are only present in carriers of the protective allele.

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Considering these findings in relation to IL1RL1 inducible eQTLs, we present the signals at Signal B (rs4142132) and Signal C (rs72825929) as signals of particular importance for IL1RL1 regulation both at baseline in cultured epithelial cells and in response to these allergic triggers. The Signal at position B (rs4142132) is associated with blood eosinophils, childhood asthma, atopy, type 2 inflammation, and asthma (25,28,213), all relevant to allergic disease potentially driven by allergens such as HDM, whereas the Signal C (rs72825929) is associated with general allergic disease (1) and therefore also potentially has relevance to allergens such as HDM.

nterestingly, ENCODE data indicate that the effect on transcription factor motif binding for Signal C (rs72825929) is minimal, with an effect only on the Sox family of transcription factors, which have been linked with the normal development of the trachea and the lung (210,221-223). However, a greater number of motif changes disrupted by the remaining 3 polymorphisms, with significant overlap, can be observed. These include GATA binding motifs, where GATA2 is shown to regulate IL1RL1 expression in mast cells and basophils (145,207) and has been shown to mediate the effects of HDM in the human lung (220). Of note also is that several of the eQTL SNPs resulted in changes in type 2 inflammatory transcription factors that are known to regulate IL1RL1 (e.g., Gfi1). However, a large range of ubiquitously expressed transcription factors indicates that the effect on IL1RL1 expression by different stimuli may be driven through different transcription factor binding motif interactions. While we present a comprehensive analysis of functional effects of IL1RL1 region SNPs for IL1RL1 gene expression and function, we acknowledge that functional effects on other genes (eQTL effects on IL18R1, for example) may be of relevance, as well; however, these were beyond the focus of our current investigations to advance our understanding of the contribution of genetic variants to IL1RL1 biology in the context of asthma.

Coding variants.
Finally, we examined the functional effects of the rs10192157 SNP that is in complete LD with several polymorphisms encoding 4 amino acid changes: Ala433/Gln501/Thr549/Leu551 being the asthma risk haplotype and Thr433/Arg501/Ile549/Ser551 being the protective haplotype. In agreement with other reports, we show that the asthma risk haplotype leads to enhanced NF-κB activity after IL-33–mediated IL1RL1 signaling in a reductionist cell model (48,200). It has been previously suggested that IL1RL1 may also affect TLR2 and TLR4 signaling (88), but we were unable to show supporting evidence for this in vitro. However, we do show that cells carrying the asthma risk IL1RL1 protein are more amenable to the antiinflammatory effects of anti-IL1RL1 and anti–IL-33, which has important therapeutic implications for potential stratified medicine approaches, especially in light of current pharmaceutical development of an IL-33/IL1RL1 antagonist for use in asthma.

Conclusion

This study has significantly advanced our understanding of both the phenotypic and the functional effects of polymorphisms in the *IL1RL1* locus in the context of asthma (Table 4). We have confirmed and extended genetic association to specific features of asthma, identifying 3 independent signals associated with blood eosinophil counts/asthma, lung function (FEV1), and severe asthma. Importantly, we have extended our understanding of Signal C (tagged by rs72825929), a previously reported signal for allergy (1) and self-reported asthma (18), associating it specifically to severe asthma. All 4 of the signals identified for functional analyses show effects on *IL1RL1* regulation, complementing and extending the literature, particularly by examining eQTLs in lung tissue and HBECs under different environments. However, some caveats in the data are observed — in particular, that asthma risk alleles at different signals have opposing effects on *IL1RL1* expression, although this is supported by other studies and potentially highlights the complexity of this locus. Overall, these data suggest that asthma and asthma phenotype–related risk alleles, as part of distinct genetic signals at the *IL1RL1* locus, significantly affect *IL1RL1* mRNA and protein levels in a tissue- and isoform-specific way. Overall, our study therefore highlights the complexity of this susceptibility locus for asthma and identifies multiple signal-driven mechanisms that contribute to the genetic association signals, which, at least in part, explains why this locus represents one of the most reproducible association signals in asthma, to date.

To take home

- SNPs at the *IL1RL1* region associate with a specific asthma phenotype of severe, early-onset eosinophilic asthma with low lung function
- These asthma-phenotype related risk alleles, at multiple loci, significantly affect *IL1RL1* mRNA and protein levels in a tissue and isoform specific way

Supplemental

Supplemental Results online

Association of *IL1RL1* variants with characteristics of asthma

In our Stage 1 analyses (Figure 1) we identified 45 SNPs associated with one or more of the asthma related traits across the association analyses using the initial FDR<0.05 screen. In particular, 29 SNPs showed association with asthma diagnosis and 8 with blood eosinophilic asthma in the LifeLines cohort (Supplemental Tables 8 & 9). This consisted of two signals (r^2>0.1) each contained within its own LD block, where one signal was common to both asthma diagnosis and eosinophilic asthma (Asthma/Eos SNP rs12474258 (*IL1RL2* intronic) (T): Asthma; OR=1.20 (SE 0.05), *P*=3.36E-04, Eos; OR=1.30 (SE 0.07), *P*=9.36E-05; Asthma SNP rs72823628 (*IL1RL1* intronic) (G): OR=1.39 (SE 0.09), *P*=1.30E-04). Similarly, 4 SNPs showed association with atopy in a meta-analysis of the GASP & DAG cohorts (Sentinel SNP: rs2041747 (*IL1R1*) (A) meta-analysis; Beta =-0.63 (SE 0.19), *P*=7.81E-04). This consisted of a single signal (r^2>0.1) (Supplemental Table 7). However this signal did not achieve later criteria of MAF≥0.1 and was therefore not taken forward. An additional two association signal consisting of a single SNP each at FDR<0.05, was also observed for lung function (FEV_1) in the meta-analysis (Sentinel SNP: rs4142132 (*IL1RL1* intronic) (A); Beta=-0.07 (SE 0.02), *P*=2.67E-05; Sentinel SNP: rs113238379 (*SLC9A2* intronic) (A); Beta=-0.21 (SE 0.07), *P*=2.64E-03) (Supplemental Table 11). However the signal tagged by rs113238379 did not achieve later criteria of MAF≥0.1 and was therefore not taken forward.

Overall this identified 3 independent signals across the asthma-subtypes consisting of two or more SNPs in LD. Association testing in the childhood cohort (MAAS) for lung function and atopy phenotypes evaluated 2,206 SNPs for association and identified no signals meeting initial criteria of an FDR less than 5%.

To identify novel variants, including potentially rare variants, we undertook two sequencing approaches. By resequencing the exons and additional regions of *IL1RL1* in 94 asthma patients using Sanger sequencing we identified 56 variants (Supplemental Table 7) with 8 resulting in amino acid changes within *IL1RL1*. Of these 56 SNPs, 40 were common and 16 rare (Minor Allele Frequency [MAF]<0.05) in our adult asthma population (N=94) (see Supplemental Table 7). No novel SNPs were identified. Using a complimentary approach that also integrates intronic and intergenic regions, in parallel we enriched the entire chromosome 2q12 locus for region based sequencing using DNA from 200 severe asthma subjects and 200 non-asthmatic, non-atopic control subjects. These analyses identified 4,107 variants spanning the region (Supplemental Table 5); including 51 variants (37 non-synonymous, 14 synonymous) in the coding regions of *IL18R1, IL18RAP, IL1R1, IL1RL1, IL1RL2* & *SLC9A4* with nine coding region variants in the *IL1RL1* gene (Supplemental Table 5). Additional variation including structural variation e.g. insertion/deletions spanning the region were also identified (Supplemental Table 5).

Using region based sequencing data generated in the case/control cohort we completed an association analysis for severe asthma as an outcome using sequencing allele counts.

Using the same initial screening of association (FDR<0.05) we identified 8 variants meeting criteria (Supplemental Table 6). This included a novel SNP at location chr2:103141056 (C|T) with a population frequency of 0.0271. The most significant variant, a previously unreported signal; rs35404747 (*IL1R1*), generates an insertion (C/CCTT), case MAF: 0.0004; control MAF: 0.1093, *P*=1.87E-11 (Supplemental Table 11). However the only variant to achieve later criteria of (MAF≥0.1) was rs72825929 (5` *SLC9A4*), (A), case MAF: 0.08; control MAF: 0.18, *P*=5.13E-5 (Supplemental Table 11).

Supplemental Methods online

SureSelect bait design for region based next generation sequencing

The SureSelect method requires the design of 120 base pair oligonucleotide 'baits' in order to carry out sample target enrichment. Bait design was carried out using the SureSelect™ e-array design software available at https://earray.chem.agilent.com/earray as directed by the manufacturer.

5

Normal Values of Inflammatory Variables From Healthy Subjects (NORM) study

RNA extraction, Sample preparation and gene expression quantification

Bronchial brushes and biopsies were taken from segmental divisions of the main bronchi in separate bronchoscopy sessions. Brushes were analyzed for RNA expression using micro-array, biopsies were processed for RNAsequencing.

Brushes: bronchial brushes were collected using a Cellebrity brush (Boston Scientific, Massachusetts, USA). After collection, they were immediately placed in RNAprotect Cell Reagent (Qiagen, Venlo, The Netherlands) and stored at −80°C. Total cellular RNA was isolated using the Qiagen total RNA isolation kit. Samples were then randomized, labeled, and run on Affymetrix Human Gene chip ST1.0 arrays as described previously according to manufacturer's instructions (224). Microarray analyses were performed using R (v3.3.2) limma package and normalization was conducted in a single batch using Robust Multi-array Average. (168)

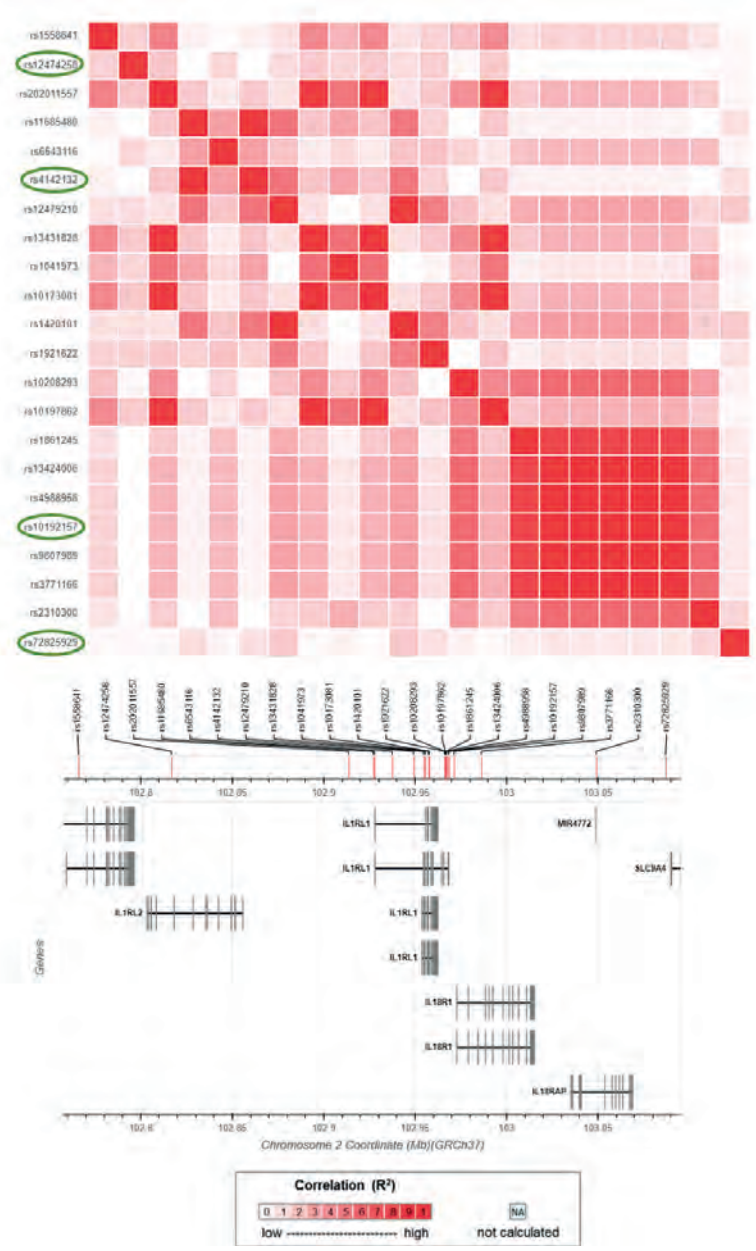
Biopsies: Biopsies frozen in Tissue-Tek (VWR, Radnor, PA) at -80°C were thawed at room temperature and cut from the blocks when they were semi-solid. Total RNA was extracted using AllPrep DNA/RNA Mini kit (Qiagen, Venlo, the Netherlands). Samples were lysed in 600µl RLT-plus buffer using an IKA Ultra Turrax T10 Homogenizer, and RNA was purified according to the manufacturer's instructions. RNA samples were dissolved in 30µl RNAse free water. Concentrations and quality of RNA were assessed using a Nanodrop-1000 and run on a Labchip GX (PerkinElmer, Waltham, MA). RNA samples were further processed using the TruSeq Stranded Total RNA Sample Preparation Kit (Illumina, San Diego, CA), using an automated procedure in a Caliper Sciclone NGS Workstation (PerkinElmer, Waltham, MA). In this procedure, all cytoplasmic and mitochondria rRNA was removed (RiboZero Gold kit). The obtained cDNA fragment libraries were loaded in pools of multiple samples onto an Illumina HiSeq2500 sequencer using default parameters for paired-end sequencing (2 × 100 bp).

The trimmed FastQ files where aligned to build b37 of the human reference genome using HISAT (version 0.1.5) allowing for 2 mismatches (225). Before gene quantification SAMtools (version 1.2) was used to sort the aligned reads (226). The gene level quantification was performed by HTSeq (version 0.6.1p1) using Ensembl version 75 as gene annotation database (227).

Quality control (QC) metrics were calculated for the raw sequencing data, using the FastQC tool (version 0.11.3) (228). QC metrics were calculated for the aligned reads using Picard-tools (version 1.130) (http://picard.sourceforge.net) CollectRnaSeqMetrics, MarkDuplicates, CollectInsertSize-Metrics and SAMtools flagstat. In addition, we checked for concordance between sexlinked (*XIST* and Y-chromosomal genes) gene expression and reported sex. All samples were concordant.

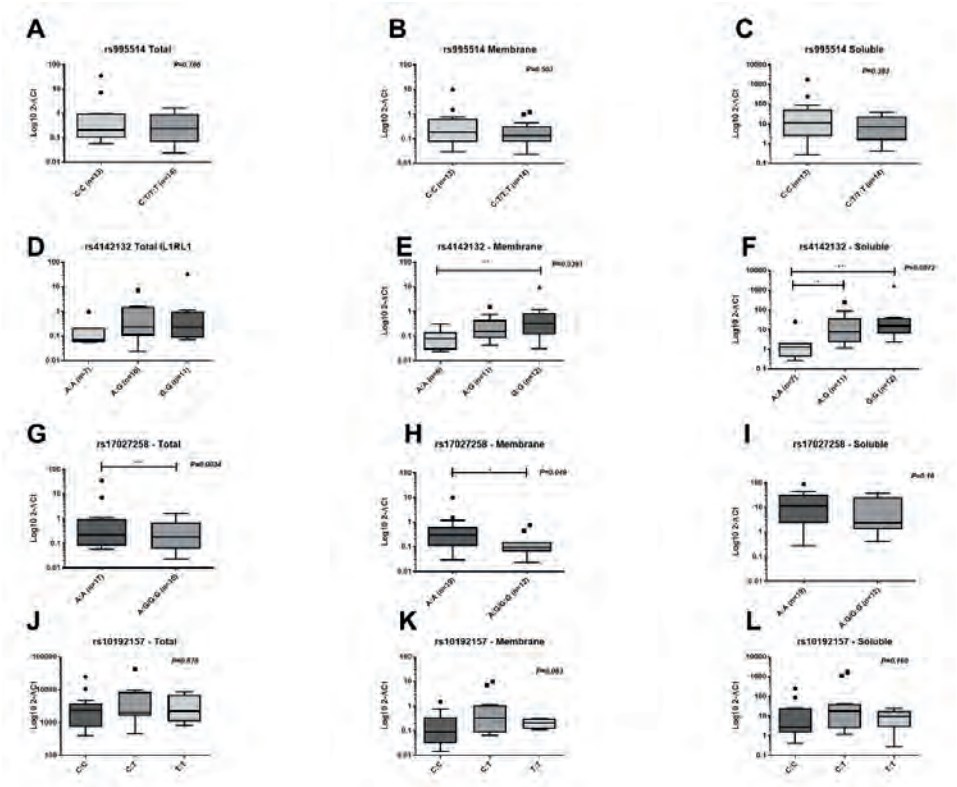
Supplemental Figures

Supplemental Figure 1. Overview of genomic region used for phenotypic association testing.
The region encompasses 400kb up- and downstream of *IL1RL1* (chr2: 102,527,961-103,368,497). Figure was made with the use of Ensembl Data. (186)



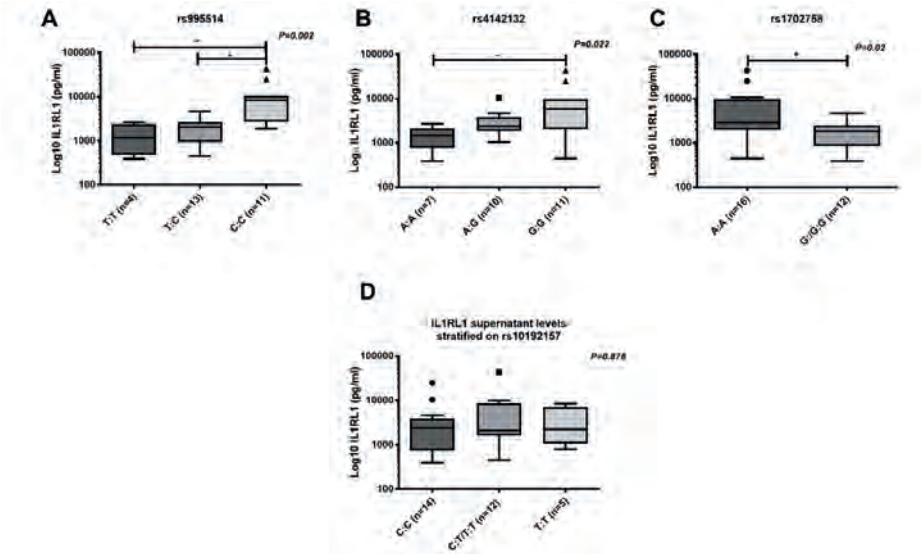
Supplemental Figure 2. Linkage disequilibrium map of the three variants identified in Stages 1-3 and the tagging SNP for the TIR domain haplotype, with previously published asthma associated SNPs in the chromosome 2 region.

SNPs discussed in this manuscript are highlighted in blue. Figure identifies the level of LD between signals identified based on r^2 values. Image generated using the EUR population of the Phase I cohort of the 1000 genomes study via the LDmatrix tab of the online software tool LDlink 3.6, available at https://ldlink.nci.nih.gov/



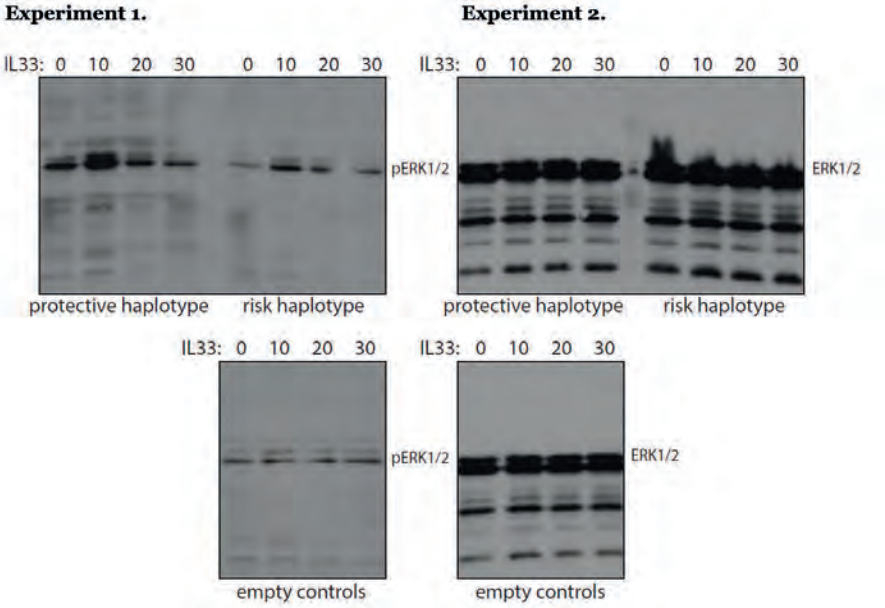
Supplemental Figure 3. Complete analyses of baseline levels of IL1RL1 mRNA in bronchial epithelial cells in vitro.

Cells were cultured in vitro *and IL1RL1 levels were stratified based on the four selected SNPs tagging four loci of association across chromosome 2. Each row represents the mRNA levels (qPCR) of Total – Membrane – Soluble isoforms of IL1RL1 respectively. Panels A-C represent Locus A tagged by rs995514, D-F Locus B tagged by rs4142132, G-I Locus C tagged by rs17027258, J-L Locus D tagged by rs10192157. Statistics run were either Mann-Whitney or Kruskall Wallis as appropriate for two or three group comparisons. *P<0.05, **P<0.01.*



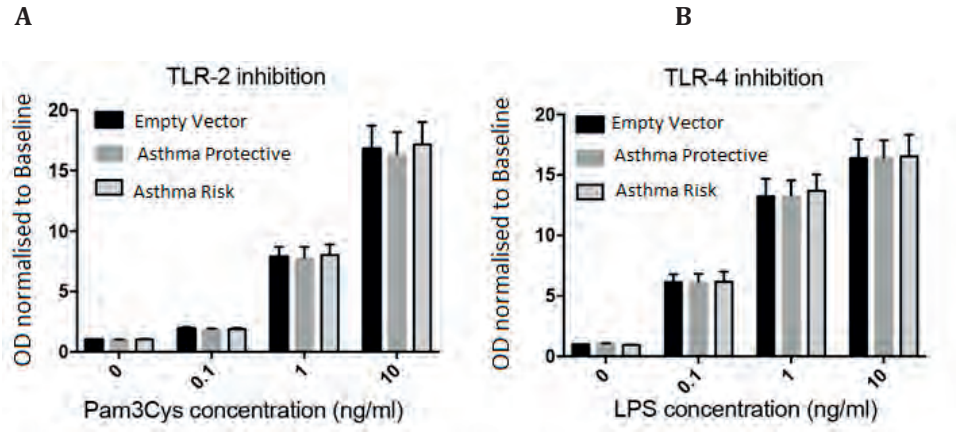
Supplemental Figure 4. Complete analyses of baseline levels of soluble IL1RL1 protein in bronchial epithelial cell supernatants.

*Cells were cultured in vitro and protein levels of IL1RL1 (Luminex) were stratified based on the four selected SNPs tagging four loci of association across chromosome 2. Locus A tagged by rs995514 (A), Locus B tagged by rs4142132 (B), Locus C tagged by rs17027258 (C), Locus D tagged by rs10192157 (D). Statistics run were either Mann-Whitney or Kruskall Wallis as required. *P<0.05, **P<0.01.*



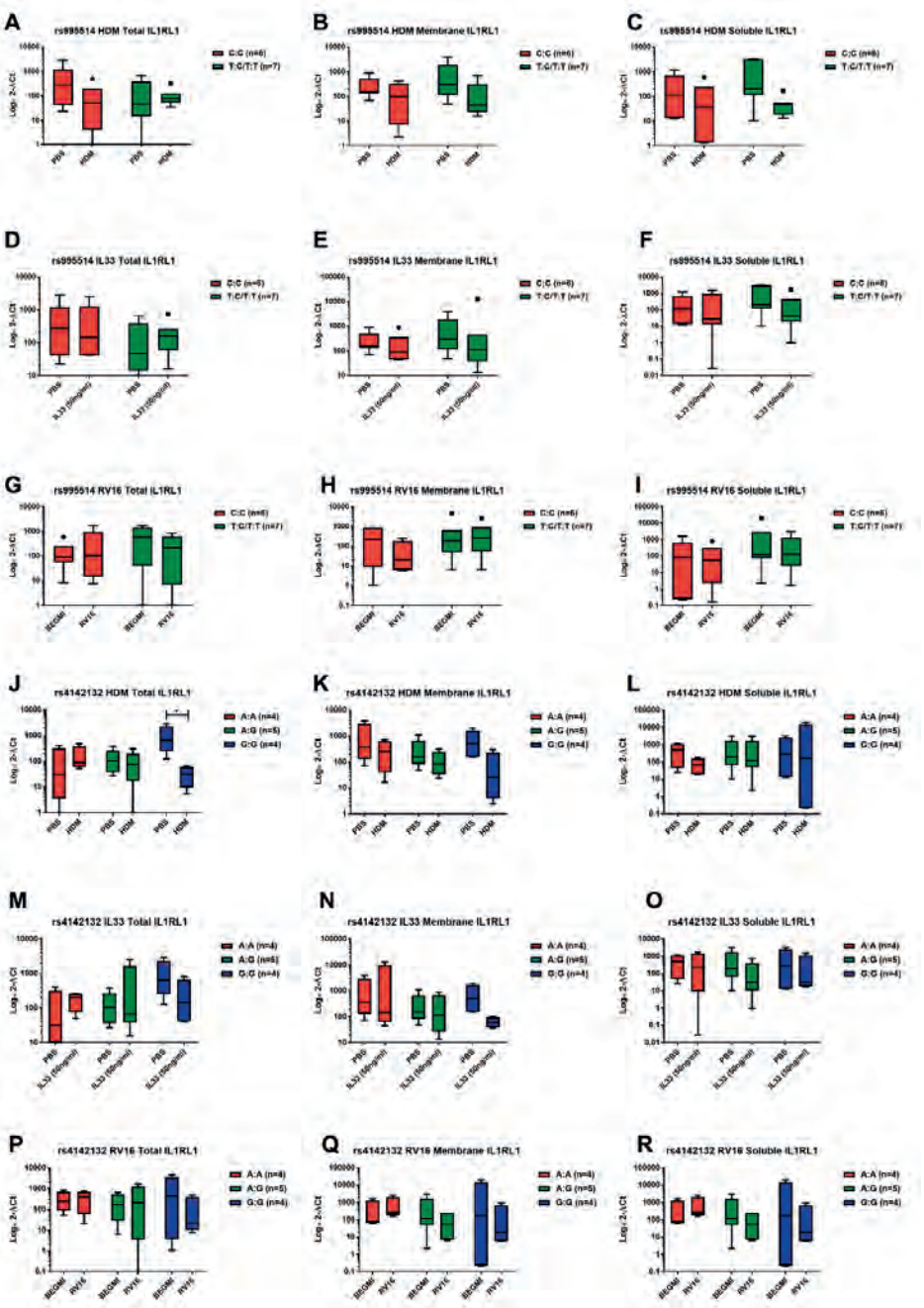
Supplemental Figure 5. Transiently transfected HEK293T cells with IL1RL1 protective or risk haplotype.

Cells were stimulated with recombinant IL33 (30 ng/ml) for 0, 10, 20 or 30 minutes as indicated followed by lysis and Western blot analysis for total and phosphorylated ERK1/2. HEK293T cells transfected with either *IL1RL1* haplotype display an IL33 induced phosphorylation of ERK that is largely absent in empty control cells.



Supplemental Figure 6. Normalized SEAP (NF-kB) activity assay in TLR2 and TLR4 reporter transfected HEK cells.

TLR2 and TLR4 expressing HEK cells transiently transfected with pCDNA3.1 empty vector (negative control) or encoding the asthma risk or non-risk IL1RL1 Ala433Thr/Glu501Arg/Thr549Ile/Leu551Ser haplotypes were stimulated with a dose range of PAM3Cys (Panel A) or LPS (Panel B), respectively. Mean values are presented for cells transiently transfected with the empty vector, Bars represent mean+/- Standard Error of the mean.


A
rs995514 HDM Total IL1RL1
B
rs995514 HDM Membrane IL1RL1
C
rs995514 HDM Soluble IL1RL1
D
rs995514 IL33 Total IL1RL1
E
rs995514 IL33 Membrane IL1RL1
F
rs995514 IL33 Soluble IL1RL1
G
rs995514 RV16 Total IL1RL1
H
rs995514 RV16 Membrane IL1RL1
I
rs995514 RV16 Soluble IL1RL1
J
rs4142132 HDM Total IL1RL1
K
rs4142132 HDM Membrane IL1RL1
L
rs4142132 HDM Soluble IL1RL1
M
rs4142132 IL33 Total IL1RL1
N
rs4142132 IL33 Membrane IL1RL1
O
rs4142132 IL33 Soluble IL1RL1
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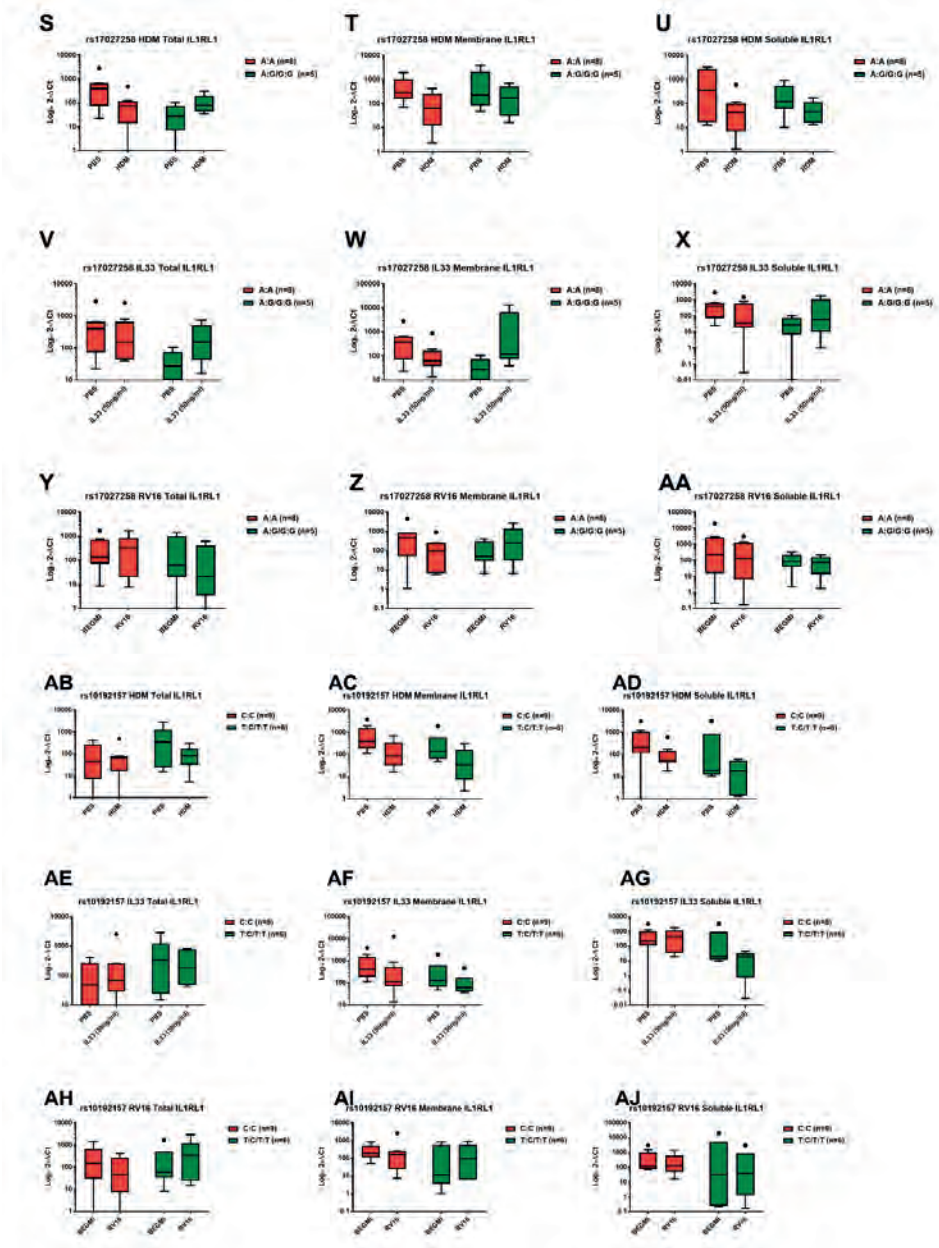
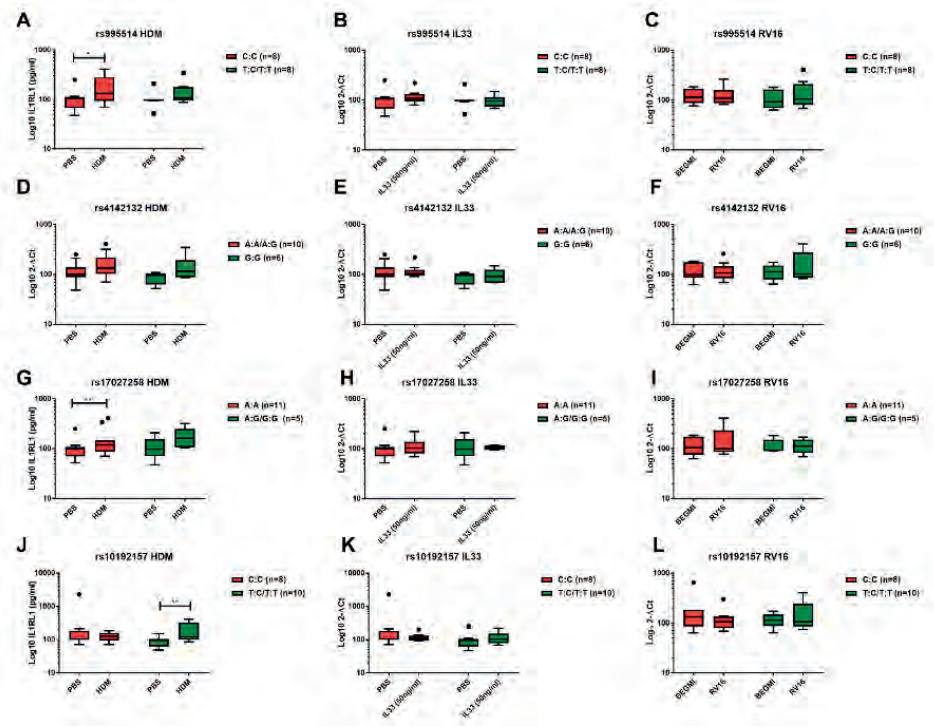
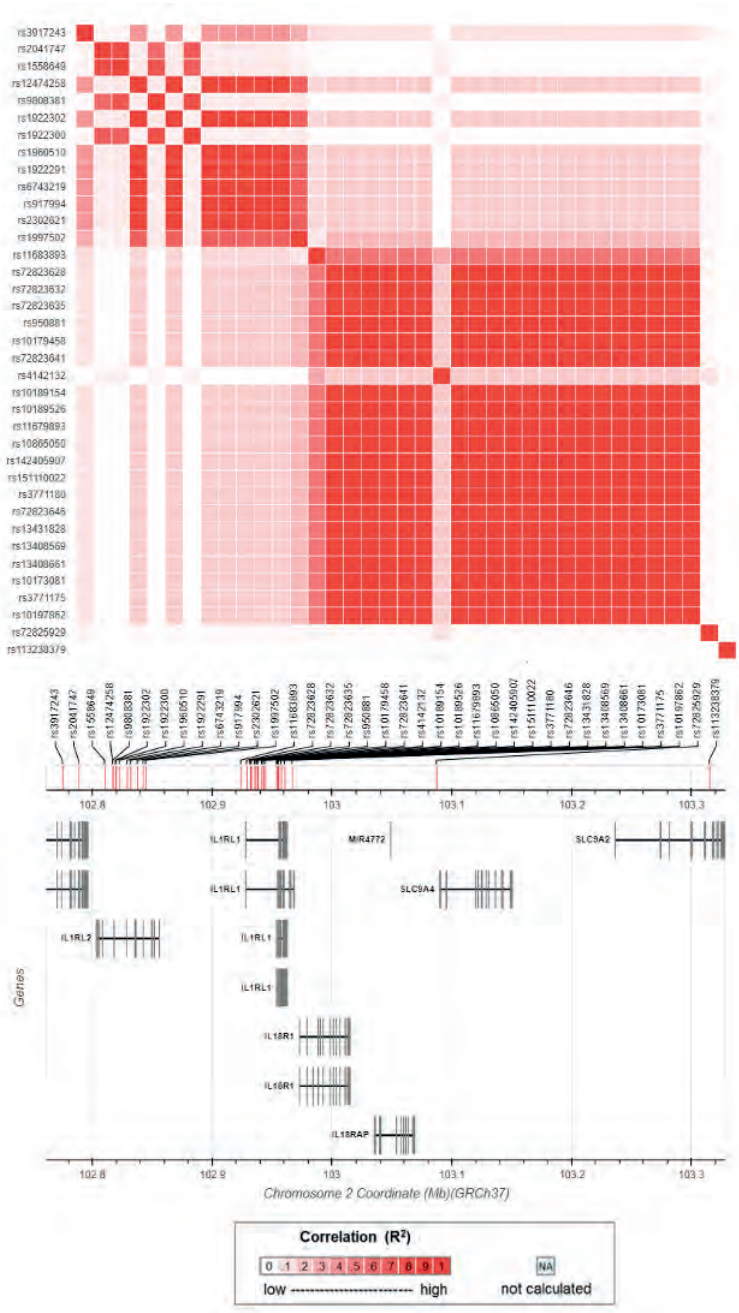
Q
rs4142132 RV16 Membrane IL1RL1
R
rs4142132 RV16 Soluble IL1RL1



Supplemental Figure 7.
Complete analyses of levels of *IL1RL1* mRNA in bronchial epithelial cells isolated from asthma patients and cultured in vitro under different asthma relevant micro-environments then stratified based on the four selected SNPs tagging four loci of association across chromosome 2. Each row represents the mRNA levels of Total – Membrane – Soluble isoforms of IL1RL1, in that order. Panels A-I represent Locus A tagged by rs995514, Panels J-R represent Locus B tagged by rs4142132, Panels S-AA represent Locus C tagged by rs17027258 and Panels AB-AJ represent Locus D tagged by rs10192157. Statistics run were either Mann-Whitney or Kruskall Wallis as appropriate for two or three group comparisons. *P<0.05, **P<0.01.

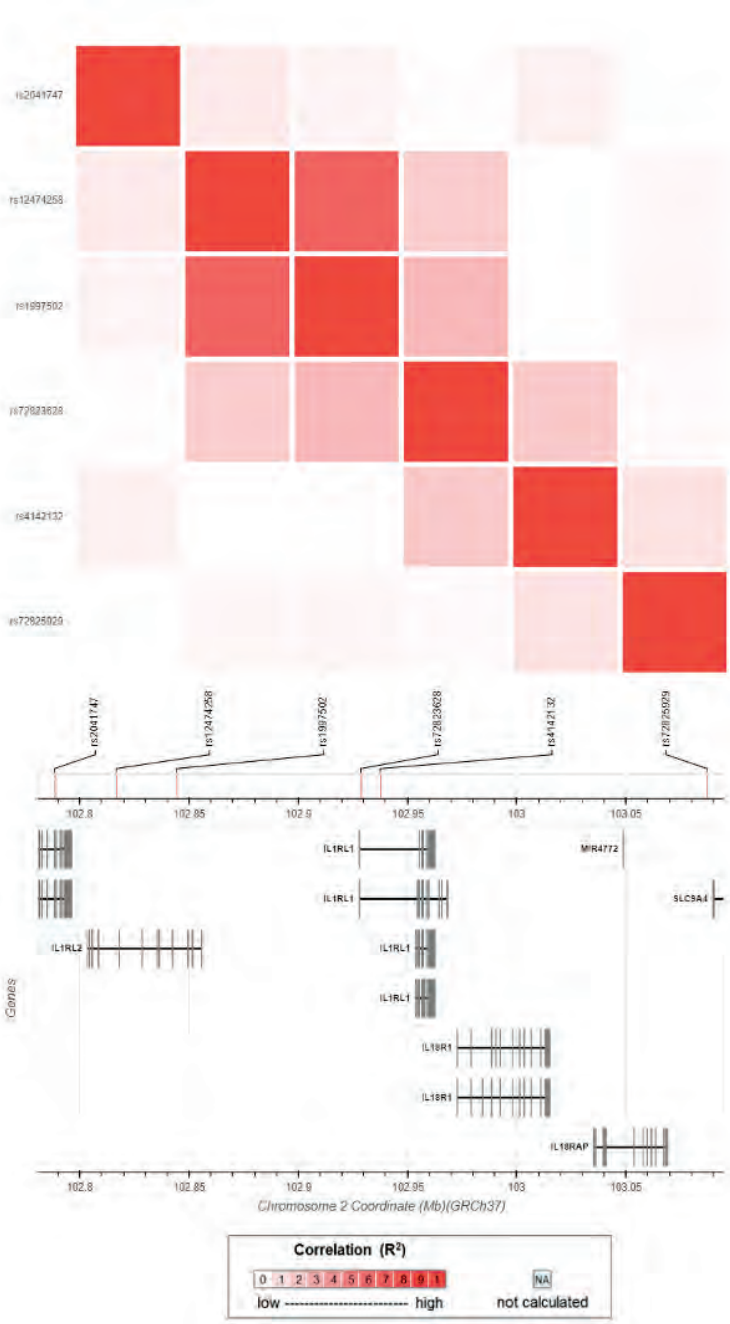


Supplemental Figure 8.
Complete analyses of levels of soluble IL1RL1 protein in bronchial epithelial cell supernatants isolated from asthma patients, cultured *in vitro* under different asthma relevant micro-environments and stratified based on the four selected SNPs tagging four loci of association across chromosome 2. Column 1 presents stratification following HDM stimulation, Column 2 following IL33 stimulation (50ng/ml) and Column 3 following RV16 infection (MOI=1). Panels A-C represent Locus A tagged by rs995514, Panels D-F represent Locus B tagged by rs4142132, Panels G-I represent Locus C tagged by rs17027258 and Panels J-L represent Locus D tagged by rs10192157.Statistics run were either Mann-Whitney or Kruskall Wallis as required. *P<0.05, **P<0.01.



Supplemental Figure 9 Linkage disequilibrium map of chromosome 2 illustrating location of 45 variants showing association (5% FDR) with one or more trait in the association analyses (Stage 1).

Figure identifies the level of LD between signals identified based on r^2. Image generated using the EUR population of the Phase I cohort of the 1000 genomes study via the online software tool LDlink 3.0, available at: https://analysistools.nci.nih.gov/LDlink/?tab=home



Supplemental Figure 10. Linkage disequilibrium map of chromosome 2 illustrating location of 6 variants prioritised through Stage 2 SNP selection.

Figure identifies the level of LD between signals identified based on r^2. Image generated using the EUR population of the Phase I cohort of the 1000 genomes study via the online software tool LDlink 3.0, available at: https://analysistools.nci.nih.gov/LDlink/?tab=home

Supplemental Tables online

Supplemental Table 1. Demographics of the GASP, DAG and LifeLines cohorts used independently and together (GASP & DAG) for genetic association analyses. Percentage values are presented as percent of valid subjects. (56,159,229)

Characteristics	GASP (N=2536)	N	DAG (N=909)	N	LifeLines Asthma (N=1066)	N	LifeLines Control (N=6863)	N
Age (yrs), mean (SD)	47.83 (15.51)	2285	34.78 (15.80)	909	46.2 (10.9)	1066	49.2 (11.9)	6863
Gender, Male (%)	36.0	2534	46.9	909	40.2	1066	46.8	6863
Height (m), mean (SD)	1.66 (0.10)	2095	1.68 (0.16)	905	1.80 (0.10)	1066	1.75 (0.09)	6863
FEV_1 (L), mean (SD)	2.26 (0.85)	2089	2.81 (0.94)	899	3.1 (0.8)	1066	3.4 (0.8)	6863
FEV_1/FVC, mean (SD)	0.69 (0.14)	1931	0.79 (0.10)	262	0.73 (0.09)	1066	0.77 (0.7)	6863
Blood Eosinophils (10-9/L), median (range)	0.31 (0.00-5.42)	1018	0.23 (0.00-1.90)	769	0.20 (0.13-0.30)	1066	0.15 (1.10-0.22)	6863
Total IgE (kU/L), median (range)	406.79 (1.00 – 5000.00)	1379	378.41 (0.00 – 12400.00)	772	ND	ND	ND	ND
Atopy* (%)	1072 (68.5)	1559	578 (85.4)	677	ND	ND	ND	ND
Age of asthma onset (yrs), mean (SD)	23.20 (17.95)	1178	10.07 (10.58)	689	ND	ND	ND	ND
Childhood onset asthma†	578 (46.2)	1284	520 (75.5)	689	ND	ND	ND	ND

N = number of subjects data field available for, SD: Standard of Deviation, ND = Not Determined/Defined

*Atopy was based on at least one positive response to intracutaneous or skin prick tests (SPT) †Childhood onset asthma defined as a diagnosis occurring before the age of 16.

Supplemental Table 1. Demographics for the sub-cohorts taken from GASP (56) (200 cases) and GEDLING (9) (200 non-asthmatic, non-atopic controls) used in the next-generation sequencing of the chromosome 2 locus.

Characteristics	GASP Cohort (cases)	Gedling Cohort (controls)
Age (yrs), mean (SD)	48 (14.88)	57 (12.64)
Gender, Male (%)	30.6	27.0
Height (m), mean (SD)	1.64 (0.08)	1.66 (0.06)
FEV_1 (L), mean (SD	2.17 (0.84)	2.77 (0.79)
Smoking pack/years	11.82 (20.25)	8.40 (18.61)
Never Smokers (%)	52.0	53.5

Supplemental Table 2. Demographics of the Manchester Asthma and Allergy Study (56,214)

Characteristics	MAAS (N=1025)	N
Age (yrs), mean (SD)	7.98 (0.16)	928
Gender, Male (%)	53.6	928
Height (cm) , mean (SD)	128.12 (5.5)	928
FEV_1 (L), mean (SD)	1.58 (0.25)	696
FEV_1/FVC, mean (SD)	0.87 (0.06)	696
Atopy, number (%)	270/826 (32.6%)	826
Asthma, number (%)	206/855 (24%)	855

Supplemental Table 3. Demographics for donors of bronchial epithelial cells contributing to the AHBEC dataset. (214)

Characteristics	AHBEC (N=51)	N
Age (yrs), mean (SD)	50 (13.47)	20
Gender, Male (%)	43.5	23
Height (m), mean (SD)	1.71 (0.10)	13
FEV_1 (L), mean (SD)	2.70 (0.95)	25
FEV_1/FVC , mean (SD)	0.69 (0.11)	19
Atopy*, number (%)	7 (58.3)	12

*Atopy was defined as a positive response to a skin prick test. Data was not available for the full cohort of 51 individuals.

Please see online for Supplemental Tables 5-11.

Supplemental Table 5. Resequencing of the chromosome 2 locus.
Region based resequencing of 200 UK severe asthma cases and 200 UK non-asthmatic, non-atopic controls identifies 4108 variants within the region of which fifteen represent *IL1RL1* coding region variation, rs13431828, rs201837678, rs202037442, rs1041973, rs111970215, rs138892317, rs4988956, rs111942110, rs4988957, rs10192036, rs10204137, rs138992262, rs4988958, rs10192157, rs10206753. Case-control association analyses for variant association with diseases state. Using FDR<0.05 we identify 71 variants that are associated with disease status (SNVer). OR, Odds ratio

Supplemental Table 6. Variants achieving FDR<0.05 from the resequencing of the chromosome 2 locus.
Eight *IL1RL1* coding variants achieve FDR<0.05 with disease status (SNVer), based off region based resequencing of 200 UK severe asthma cases and 200 UK non-asthmatic, non-atopic controls. Variants were identified based on case-control association analyses for variant association with diseases state. OR, Odds ratio

Supplemental Table 7. Exon resequencing of IL1RL1.
Data identifies 56 variants spanning the *IL1RL1* introns, exons and promoter region. MAF, Minor allele frequency.

Supplemental Table 8. Association results for all variants meeting initial criteria (FDR<0.05) for association with asthma diagnosis in LifeLines.
A1: Coding Allele, BP: Basepair position, FRQ: Coding allele frequency in cohort, SE: standard error.

Supplemental Table 9. Association results for all variants meeting initial criteria (FDR<0.05) for association with eosinophilic asthma in LifeLines.
A1: Coding Allele, BP: Basepair position, FRQ: Coding allele frequency in cohort, SE: standard error.

Supplemental Table 10. Association results for all variants meeting initial criteria (FDR<0.05) for association with atopy as defined by a positive response to a skin prick test in a combined meta-analysis of the GASP and DAG cohorts.
MAF, Minor allele frequency; SE, standard error.

Supplemental Table 11. Association results for all variants meeting initial criteria (FDR<0.05) for association with lung function as defined by FEV1 in a combined meta-analysis of the GASP and DAG cohorts.
Allele 1: Coding Allele, Effect: Beta

Supplemental Table 12. Association results of chromosome 2 variation previously reported to be associated with asthma.

SNP	POSITION (BP)	GENE	VARIANT ALLELES	ANCESTRAL ALLELE	ASSOCIATION	FDR	OR	SE	PMID
rs1558641	102765865	5' of IL1RL1	G/A	G	-	-	-	-	26493291
rs202011557	102913643	5' of IL1RL1	-/AAAC/AAACAAAC/AAC	-	-	-	-	-	27182965
rs11685480	102927086	5' of IL1RL1	G/A	-	-	-	-	-	21629437 27699235 21281963 22574108 24568840
rs6543116	102927726	5' of IL1RL1	A/G	A	-	-	-	-	19852851 21629437 24568840 19254249 16118232
rs12479210	102949161	IL1RL1	C/A/T	T	-	-	-	-	30552067 30578877
rs13431828	102954653	IL1RL1	C/T	C	Asthma LifeLines	0.03994842	1.36	0.08	24568840 22357570 20816195 20860503 19910030 22694930 21629437 25932636 23209423
rs1041973	102955468	IL1RL1	C/A	A	-	-	-	-	19910030 19852851 28266165 27699235 21281963 22574108 28273074 25091434 23999434 20816195 20860503
rs10173081	102957348	IL1RL1	C/T	T	Asthma LifeLines	0.03994842	1.36	0.08	23028483 21804549 27699235 26102239

Supplemental Table 12 continued:

SNP	POSITION (BP)	GENE	VARIANT ALLELES	ANCESTRAL ALLELE	ASSOCIATION	FDR	OR	SE	PMID
rs1420101	102957716	IL1RL1	C/T	T	-	-	-	-	18774397 21738479 19910030 23028483 21150878 20833654 19852851 26069107 21629437 27699235 21281963 22574108 21966603 20592918 25091434 24568840 20816195 20860503 19198610
rs1921622	102966067	IL1RL1	G/A	A	-	-	-	-	18774397 22357570 19852851 21629437 27699235 25091434
rs10208293	102966310	IL1RL1	G/A	A	-	-	-	-	27130862 21629437 24568840
rs10197862	102966549	IL1RL1	A/G	A	-	-	-	-	22357570 23028483 21150878 27058054
rs1861245	102966906	IL1RL1	C/T	C	-	-	-	-	19852851 21629437
rs13424006	102967236	IL1RL1	T/C	C	-	-	-	-	24568840

Supplemental Table 12 continued:

SNP	POSITION (BP)	GENE	VARIANT ALLELES	ANCESTRAL ALLELE	ASSOCIATION	FDR	OR	SE	PMID
rs4988958	102968285	IL1RL1	T/C	T	-	-	-	-	22357570
rs10192157	102968356	IL1RL1	C/T	C	-	-	-	-	20816195 20860503 21150878 25409453 27058054
rs9807989	102971200	3` of IL1RL1	T/C	T	-	-	-	-	23755072 22561531
rs3771166	102986222	IL18R1	G/A/T	A	-	-	-	-	19852851 19910030 23886662 20860503 23028483 23755072 21543792 27130862 27658857 21629437 21966603
rs2310300	103049074	IL18RAP	A/G	A	-	-	-	-	20860503 22846752

List of chromosome 2 polymorphisms published in the literature as showing association to asthma traits. Statistically significant association of two SNPs in our datasets are noted above (FDR<0.05).

Supplemental Table 13. Expression quantitative trait loci (eQTL) analysis for tagging SNPS or proxies in a dataset in bronchial biopsy and bronchial brushes from healthy individuals (n=70). (190)

			Bronchial biopsy			Bronchial brushing		
Signal/SNP	**(C/NC*)**	**MAF**	**Beta**	**SE**	**Pval**	**Beta**	**SE**	**Pval**
A rs10167431†	G/A	0.44	-0.01	0.17	0.93	-0.01	0.06	0.87
B rs10178436††	G/A	0.45	0.01	0.19	0.97	-0.07	0.06	0.29
C rs2241116†††	C/A	0.17	-0.11	0.25	0.66	0.04	0.07	0.62
D rs10192157	G/A	0.32	-0.09	0.16	0.55	-0.02	0.05	0.64

*SNPs are reported in relation to the linkage disequilibrium (LD) blocks (r^2>0.8) reported in this study. TAO: Time to asthma onset

†Variants relate to the variants subsequently selected as tagging SNPs for functional studies.

No SNPs were identified as eQTLs in this dataset. *C = coded allele, NC = Non-coded allele, †rs10167431 was used as a proxy for rs12474258 (r2=0.68), ††rs10178436 was used as a proxy for rs4142132 (r2=0.98), †††rs2241116 was used as a proxy for rs72825929 (r2=0.37), MAF= Minor allele frequency

Supplemental Table 14. Detailed ENCODE analyses of four priority tagging SNPs identified several putative regulatory mechanisms.
Detailed ENCODE (162) analyses of the four selected SNPs tagging our 4 regions of association, identified several putative regulatory mechanisms. LD, linkage disequilibrium.

TAGGING SNP	SNPS IN LD*	ENHANCER HISTONE MARKS IN LUNG	DNASE IN LUNG	PROTEINS BOUND	MOTIFS CHANGED
RS12474258	6	Y	N	CTCF, RAD21, MAFK, HAE2F1	BRACHYURY, GATA, MYF, ZFP105, AP-1, HLX1, IRF, NKX6-1, POU3F1, STAT, SP100, TCF12
RS4142132	62	Y	Y	CTCF, IRF4, RAS21, SMC3, GATA2, SETDB1, MAFK, STAT3	IRF, AFP1, AHR::ARNT, AHR::ARNT::HIF1, AIRE, AP-1, AP-2, ARID3A, ARID5A, ASCL2, ATF3, BARHL1, BBX, BCL, BDP1, CART1, CDP, CDX2, CEBPB, CIZ, CTCF, DBX1, DBX2, DMRT2, DMRT3, DMRT7, EN-1, ESX1, ETS, EVI-1, EWSR1-FLI1, FAC1, FOX, FOXA, FOXD3, FOXF1, FOXI1, FOXJ2, FOXL1, FOXO, FOXP1, FOXQ1, FXR, GFI1, GLI, GM397, GR, HAND1, HAND1, HDAC2, HDX, HIF1, HMG-IY, HNF1, HNF4, HNF6, HOXA10, HOXA3, HOXA4, HOXA5, HOXA9, HOXB13, HOXB9, HOXC6, HSF, IK-2, IRC900814, IRF, IRX, ISL2, ISX, LF-A1, LHX4, MAF, MEF2, MRG, MSX-1, MYF, NANOG, NCX, NF-I, NF-KAPPAB, NKX2, NKX3, NKX6-1, NRF-2, NRSF, P300, PAX-4, PAX-5, PAX-6, PAX7, PBX-1, PBX3, PDX1, PHOX2A, PITX2, PLZF, POU1F1, POU2F2, POU3F2, POU3F3, POU3F4, POU4F3, POU5F1, POU6F1, PRDM1, PRRX1, PRRX2, PU.1, RAD21, RAR, RFX5, RREB-1, RXRA, SMAD, SMAD3, SMC3, SOX, STAT, TAL1, TATA, TCF12, TCF4, TGIF1, TLX1::NFIC, VAX2, VDR, YY1, ZBTB12, ZFP105, ZFP161, ZID
RS72825929	0	N	N	NONE	SOX
RS10192157	49	N	N	GATA2, EGR1,POL2, CTCF,SMC3,CEBPB	CTCFL,CTCF,NRSF,PTF1-BETA,STAT,ZNF263, GR,SMAD,YY1, MEF2,TATA,MEF2,RHOX11, FOXA,HDX ,POU2F2, BCL,PRDM1,PU.1,PAX-5,RXRA,NFAT,PRDM1,PU.1,STAT,BCL,PRDM1 ,PU.1,PAX5,CDX,NKX2,NKX3,SPDEF, ZBTB3, AP-1,FAC1,P300,AP1,FAC1,P300,AP3,GATA,P OU3F1,PRDM1,PAX-5,ROAZ,POU2F2,ROAZ,OBOX6, DUXL,ATF3,ATF6,NKX2,FOX,FOXC1,FOXD3,FOXF1,FOXF2,FOXI1,FOXJ1,FOXJ2,F OXK1,FOXL1,FOXO,FOXQ1,HDAC2,SOX,ZFP105,CDX2,DMRT4,DBX1,HNF1,HOXD1 0,NCX,NKX6-1,PAX4,POU2F2,SOX, ,TEF,CDC5,GR, HOXA5,CDP,ZFP691,FAC1, CDP, ,GR,HMG-IY,RREB-1,SOX,ZFP105, ERALPHA-A,GFI1,LRH1,BACH2,CDX,HOXA5,IR F,SIX5, ARID3A,BARHL1,CART1,DBX1,DBX2,EN-1,FOXA,GR,HOXA7,HOXB4,HOX B9,ISL2,MSX-1,NCX,NKX6-1,NKX6-2,PAX-4,PAX7,POU1F1,POU3F4,POU4F3,SOX, CEBPB,ERALPHA-A,PLZF,RAR,RXRA,VDR, AHR,HEY1, GR, ESR2, EBF,SZF1-1,ZEB1, CART1, IRF,POU2F2, IRF,POU2F2, HDAC2,IK-2,NF-AT1,POU2F2,STAT, FOXP3,IRF,STAT, ARID5B,MRG,PLZF,RXR::LXR, HNF1,HNF4,IRF,PAX-4,RREB-1, PU.1,SIN3AK-20, RREB-1,UF1H3BETA, CDP,GR,HNF1, HNF1,IK-2, BBX,POU5F1,SIX5

*LD is based on r2>0.8, †Evidence of IL1RL1 eQTL in lung tissue.