

Decoding asthma: Translating genetic variation in *IL33* and *IL1RL1* into disease pathophysiology

Néomi S. Grotenboer, MSc,^{a,b,c} Maria E. Ketelaar, MSc,^{a,c} Gerard H. Koppelman, MD, PhD,^{b,c,*} and Martijn C. Nawijn, PhD^{a,c,*} Groningen, The Netherlands

Asthma is a complex disease that results from the interaction between genetic predisposition and environmental factors. Recently, genome-wide association studies have identified a number of genes that significantly contribute to asthma. Two of these genes, *IL33* and IL-1 receptor–like 1 (*IL1RL1*), act in one signal transduction pathway. *IL33* encodes a cytokine released on damage of cells, whereas *IL1RL1* encodes part of the IL-33 receptor complex. Recent progress made in functional studies in human subjects and mouse models of allergic airway disease indicate a central role of IL-33 signaling in driving T_H2 inflammation, which is central to eosinophilic allergic asthma. Here, IL-33 acts on cells of both the adaptive and innate immune systems. Very recently, a novel population of IL-33–responsive innate immune cells, the type 2 innate lymphoid cells, was found to produce hallmark T_H2 cytokines, such as IL-5 and IL-13. The relevance of these cells for asthma is underscored by the identification of retinoic acid–related orphan receptor α (*RORA*), the gene encoding the transcription factor critical for their differentiation, as another asthma gene in genome-wide association studies. This review describes the mechanisms through which genetic variation at the *IL33* and *IL1RL1* loci translates into increased susceptibility for asthma. We propose that genetic variation associated with asthma at the *IL33* and *IL1RL1* loci can be dissected into independent signals with distinct functional consequences for this pathway that is central to asthma pathogenesis. (J Allergy Clin Immunol 2013;131:856–65.)

Key words: *IL-33*, IL-1RL1, *ST2*, genome-wide association study, nuocytes, innate helper cells, innate type 2 lymphoid cells, expression quantitative trait locus

From ^athe Laboratory of Allergology and Pulmonary Diseases, Department of Pathology and Medical Biology, and ^bthe Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, and ^cGRIAC Research Institute, University of Groningen, University Medical Center Groningen.

*These authors contributed equally to this work.

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Corresponding author: Martijn C. Nawijn, PhD, Laboratory of Allergology and Pulmonary Diseases, Pathology and Medical Biology, IPC EA11, University Medical Center Groningen, GRIAC Research Institute, University of Groningen, Hanzeplein 1, PO Box 30.001, 9700 RB, Groningen, The Netherlands. E-mail: m.c.nawijn@umcg.nl. 0091-6749/\$36.00

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Abbreviations used

ASW:	African ancestry in Southwest United States
CEU:	Utah residents with Northern and Western European ancestry from the CEPH collection (referred to as non-Hispanic white)
CHB:	Han Chinese in Beijing, China
GWA:	Genome-wide association
ILC2:	Type 2 innate lymphoid cell
<i>IL1RL1</i> :	IL-1 receptor–like 1
<i>IL18RI</i> :	IL-18 receptor 1
LD:	Linkage disequilibrium
MEX:	Mexican ancestry in Los Angeles, California (referred to as Mexican population)
MyD88:	Myeloid differentiation primary response gene–88
NF- κ B:	Nuclear factor κ B
<i>RORA</i> :	Retinoic acid–related orphan receptor α
SNP:	Single nucleotide polymorphism
TIR:	Toll-like/IL-1 receptor
TLR:	Toll-like receptor
YRI:	Yoruban in Ibadan, Nigeria

Asthma is a complex, chronic inflammatory disease of the airways currently affecting more than 300 million persons worldwide, with approximately 250,000 annual deaths as a result.¹ It is estimated that by 2025, the number of asthmatic patients will increase by more than 100 million.²

Asthma is characterized by respiratory symptoms, variable airway obstruction, and airway hyperresponsiveness. The clinical expression of asthma is dependent on the interaction between genetic predisposition and environmental factors. The number of identified asthma susceptibility genes has increased rapidly over the last 5 years, especially with the application of the genome-wide association (GWA) study approach. In a GWA study 300,000 to more than a million DNA polymorphisms covering the genome are investigated for association with asthma in large samples of cases and control subjects.

Genetic variation in the *IL33* and IL-1 receptor–like 1 (*IL1RL1*) genes has reproducibly been found to be associated with asthma in GWA studies, identifying IL-33–induced signaling through IL-1RL1 as one of the central pathways in asthmatic patients. Although numerous functional studies have revealed a central role for IL-33–induced signaling in T_H2-driven inflammation, which plays a crucial role in allergic asthma, few studies have directly assessed the functional consequences of genetic variation in the *IL33* and *IL1RL1* genes for the activity of this pathway. Such studies are currently limited by the large number of asthma-associated single nucleotide polymorphisms (SNPs) in these genes and the complex genetic structure of the *IL1RL1* locus.

Therefore this review aims to offer an interpretation of asthma-associated polymorphisms in *IL33* and *IL1RL1* as a limited number of discrete genetic signals with distinct functional consequences and to discuss these in the context of a newly identified

population of innate IL-33–responsive, IL-1RL1⁺ immune cells, the type-2 innate lymphoid cells (ILC2s), in the pathophysiology of asthma.

GENETIC ASSOCIATION OF THE IL-33/IL-1RL1 PATHWAY WITH ASTHMA SUSCEPTIBILITY

IL-33 is an IL-1 family member and the ligand of the IL-1RL1 (ST2) receptor. The *IL33* gene was initially found to be associated with Cedar pollinosis in a Japanese population.³ One year later, a GWA study in an Icelandic population identified SNPs flanking *IL33* to be suggestively associated with blood eosinophils.⁴ This finding was followed up by a large case-control study of asthmatic patients and control subjects, indicating significant association of *IL33* SNPs with asthma.⁴ Subsequent GWA study meta-analyses by the European GABRIEL consortium,⁵ which was recently combined with the Analysis in Population-based Cohorts for Asthma Traits consortium meta-analysis,⁶ and the North American EVE consortium⁷ identified *IL33* as one of the top hits for asthma.

Eight *IL33* SNPs have been reported to be associated with asthma phenotypes (Table I and see the Methods section in this article's Online Repository at www.jacionline.org).⁴⁻¹⁵ Several of these SNPs are in close proximity to each other and are observed more frequently together than would be expected by chance based on the allele frequencies in the population. This phenomenon is termed linkage disequilibrium (LD) and indicates that the SNPs are correlated in populations. In case the SNPs are in full LD, they are always inherited together and therefore represent a single genetic signal. A series of such highly correlated SNPs form an LD block, and their alleles form a haplotype. Although a single SNP within this LD block might be responsible for the functional alteration that contributes to disease, all SNPs within the LD block will be associated with asthma in a genetic association analysis. When the LD structure of *IL33* in the Utah residents with Northern and Western European ancestry from the CEPH collection (CEU; referred to as non-Hispanic white) ethnic background, in which most association analyses have been performed, is taken into account (Fig 1, A), it becomes apparent that 4 of the asthma-associated SNPs are located in 2 LD blocks, whereas the remaining 4 asthma-associated SNPs are not in strong LD with other polymorphisms (Table II [and see the Methods section in this article's Online Repository] and Fig 1, A).⁴⁻²¹ Interestingly, in the Yoruban in Ibadan, Nigeria (YRI) population and, to some extent in the African ancestry in Southwest United States (ASW) and Han Chinese in Beijing (CHB) ethnic backgrounds, the LD patterns of *IL33* are different (see Fig E1 in this article's Online Repository at www.jacionline.org), in that the SNPs clustering into LD blocks within the CEU population are independent SNPs in the studied African and Asian populations, indicating that association analyses in patients of these ethnic backgrounds might reveal the causal SNP retained within the 2 LD blocks.

IL1RL1 was first described as a candidate gene for atopic dermatitis.²² Our group was the first to report *IL1RL1* as an asthma susceptibility locus by taking a candidate gene approach.¹⁰ In total, 7 candidate gene studies^{4,9-14} and 4 GWA studies^{5,6,7,16} have reported an association of *IL1RL1* SNPs with asthma.

Thus far 15 different *IL1RL1* SNPs have been reported to be associated with asthma (Table I). These SNPs lie scattered

throughout the *IL1RL1* gene (Fig 1, B). Analysis of the LD structure of *IL1RL1* and its surrounding genomic region in patients of the CEU ethnic background reveals a complex LD pattern in which 5 LD blocks containing asthma SNPs and 1 independent asthma SNP can be distinguished, each of which might separately contribute to asthma susceptibility (Table II). Importantly, several SNPs in *IL1RL1* are in LD with SNPs in the genes encoding IL-18 receptor 1 (*IL18R1*) and IL-18 receptor accessory protein, 2 genes juxtaposed to *IL1RL1* on chromosome 2q.^{10,24} Thus genetic associations in the CEU population cannot conclusively determine which of these genes is implicated in asthma. Moreover, analysis of the LD pattern of *IL1RL1* in different ethnic backgrounds reveals that the LD structure of the *IL1RL1/IL18R1* locus remains highly complex in all these populations (see Fig E2 in this article's Online Repository at www.jacionline.org). The *IL1RL1* SNP rs1041973 represents an independent SNP in populations of Africans, Asian, and Mexican ethnicity, whereas it is part of LD block 2 in non-Hispanic white subjects (Fig 1, B, and see Fig E2). In the Mexican ancestry in Los Angeles, California, population (MEX; Mexican population) 2 SNPs, rs1041973 and rs13431828 ($r^2 = 0.66$ in MEX; both in LD block 2 in CEU) were in fact both found to be significantly associated with asthma,¹¹ indicating that there might be 2 independent asthma signals present within the non-Hispanic white LD block 2. Therefore association analyses in other ethnic backgrounds might aid the identification of the causal asthma-associated SNPs, but experimental validation will remain key to proving the individual contribution of the identified causal variants. Potential experimental approaches include *in vitro* studies on the functional effects of asthma-associated haplotypes in primary cells or *in vivo* studies using mouse models that have been specifically engineered to carry the risk or protective haplotype of either *IL1RL1* or *IL18R1*.

FUNCTIONAL GENETICS OF IL33 AND IL1RL1

The *IL33* gene, which is located on chromosome 9, spans approximately 42.2 kb in length, harboring 8 exons. All *IL33* asthma-associated SNPs are located 5' of the gene or in the first intron. Therefore it is tempting to speculate that these SNPs affect *IL33* transcription and that asthma susceptibility alleles are associated with increased IL-33 production. At present, however, no published data support this function, warranting further exploration of the functional consequences of these SNPs.

The *IL1RL1* gene, which is located at chromosome 2q12, spans approximately 40.5 kb in length, harboring 13 exons and a distal and a proximal promoter. The *IL1RL1* gene encodes for proteins with an extracellular region carrying 3 immunoglobulin-like domains, a transmembrane domain, and an intracellular region harboring a Toll-like/IL-1 receptor (TIR) domain. Three transcripts are expressed through alternative splicing: a short isoform encoding the soluble protein IL-1-RL1-a (also called sST2); a long isoform encoding the full transmembrane receptor IL-1-RL1-b (ST2L); and a less well-known variant that encodes a truncated protein with 2 immunoglobulin-like domains and a hydrophobic tail called IL-1-RL1-c (ST2V). Functionally, IL-1-RL1-b acts to transduce the IL-33 signal to the intracellular compartment, whereas the soluble IL-1-RL1-a functions as a decoy receptor, capturing IL-33 and inhibiting its function.^{25,26}

IL1RL1 asthma-associated SNPs can translate into functional alterations of the IL-33/IL-1RL1 pathway through several

TABLE I. Asthma-associated polymorphisms located within the *IL33* and *IL1RL1* gene regions

SNP	Chromosome position	Reported allele	SNP class	OR,* RR,† P value	Study approach	Population	Reference
<i>IL33</i>							
rs1342326	6.190.076	C ^z	5'	1.20 (1.13-1.28),* P = 9.20E-10 ⁿ	Meta-analysis (GWAS)	White (Eur, North-Am)	5
		T (with rs928413G)		P = 1.8E-02 ^p	Candidate gene study, 2-locus haplotype TDT	White (NE-Italy)	8
rs2381416	6.193.455	C	5'	P = 1.3E-06 ^f	Meta-analysis (GWAS)	Mixed: North-Am (Eur, Am, Afr-Am, Afr-Car, Lat)	7
rs3939286	6.210.099	A ^z	5'	1.12 (1.07-1.17),* P = 5.3E-06 ⁿ	Candidate gene study	Mixed (Eur, E-Asia)	4
rs928413	6.213.387	A G (with rs1342326T)	5'	P = 1.8E-03 ^f P = 1.8E-02 ^p	Candidate gene study Candidate gene study, 2-locus haplotype TDT	White (non-Hispanic) White (NE-Italy)	15 8
rs2066362	6.219.176	T ^z	Intron	1.21 (1.13-1.29),* P = 1.39E-08 ⁿ	Meta-analysis (GWAS)	White (Eur, North-Am)	5
rs16924159	6.229.417	A ^y	Intron	0.82 (0.68-0.99),* P = 3.7E-02 ^t	Candidate gene study	White (non-Hispanic)	9
rs12551256	6.231.239	G ^y	Intron	0.82 (0.69-0.98),* P = 3.E-02 ^t	Candidate gene study	White (non-Hispanic)	9
rs7025417	6.240.084	C ^z	Intron	1.23 (1.01-1.51),* P = 4.5E-02 ^t	Candidate gene study	White (non-Hispanic)	9
<i>IL1RL1</i>							
rs1420089	102.938.389	T	Intron	P = 3.3E-02 ^{p,‡}	Candidate gene study	White (Eur, North-Am)	12
rs3771180	102.953.617	G	Intron	P = 1.93E-08 ^{n,r}	Meta-analysis (GWAS)	Mixed: North-Am (Eur, Am, Afr-Am, Afr-Car, Lat)	7
rs13431828	102.954.653	T ^y	5' UTR	0.45 (0.29-0.70),† P = 2.0E-04 ^o	Candidate gene study	Mexicans	11
		T ^y		0.63 (0.47-0.83),* P = 8.49E-04 ^t	Candidate gene study	White (non-Hispanic)	9
		C		P = 6.2E-03 ^f and P = 2.7E-02 ^f	Candidate gene study	White (non-Hispanic)	15
rs13408661	102.955.082	G ^z	Intron	1.23 (1.15-1.31),* P = 1.1E-09 ^{n,v}	Meta-analysis (GWAS)	White (Eur)	6
rs1041973	102.955.468	A ^y	Coding, nonsynonymous	0.58 (0.43-0.78),† P = 3.5E-04 ^o	Candidate gene study	Mexicans	11
		A ^y		0.80 (0.65-0.99),* P = 4.3E-02 ^t	Candidate gene study	White (non-Hispanic)	9
		A ^y		0.70 (0.54-0.91),* P = 8E-03 ^q	Candidate gene study	White (Dutch)	13
rs10173081	102.957.348	C ^z	Intron	1.2 (1.11-1.29),* P = 1.42E-08 ⁿ	Meta-analysis (GWAS)	Mixed: North-Am (Eur, Am, Afr-Am, Afr-Car, Lat)	7
rs1420101	102.957.716	A ^z	Coding, synonymous	1.16 (1.11-1.21),* P = 5.5E-12 ⁿ	Candidate gene study	Mixed (Eur, E-Asia)	4
		A		P = 3.9E-02 ^p	Candidate gene study	White (Dutch)	10
rs1946131	102.961.929	T	Intron	P = 1.5E-02 ^{p,‡}	Candidate gene study	White (Eur, North-Am)	12
rs1921622	102.966.067	A	Intron	P = 3.8E-02 ^p	Candidate gene study	White (Dutch)	10
rs10197862	102.966.549	G ^y	Intron	0.75 (-),* P = 1.22E-02 ^q and 0.58 (-),* P = 1.5E-05 ^s	Candidate gene study	White (Aus-Eur)	14
rs1861246	102.966.783	G	Intron	P = 5E-02 ^p	Candidate gene study	White (Dutch)	10
rs1861245	102.966.906	G	Intron	P = 3.2E-02 ^{p,‡}	Candidate gene study	White (Eur, North-Am)	12
rs10204137	102.968.212	G ^y	Coding, nonsynonymous	0.87 (0.83-0.91),* P = 5.10E-09 ⁿ	Meta-analysis (GWAS)	White (Eur, North-Am)	5
		G ^y		0.80 (0.67-0.97),* P = 2.1E-02 ^t	Candidate gene study	White (non-Hispanic)	9

(Continued)

TABLE I. (Continued)

SNP	Chromosome position	Reported allele	SNP class	OR,* RR,† P value	Study approach	Population	Reference
rs10192157	102.968.356	T ^y	Coding, nonsynonymous	0.87 (0.83-0.91),* P = 9.32E-09 ⁿ	Meta-analysis (GWAS)	White (Eur, North-Am)	5
		T ^y		0.80 (0.67-0.97),* P = 2.1E-02 ^t	Candidate gene study	White (non-Hispanic)	9
rs10206753	102.968.362	C ^y	Coding, nonsynonymous	0.87 (0.83-0.91),* P = 6.84E-09 ⁿ	Meta-analysis (GWAS)	White (Eur, North-Am)	5
		C ^y		0.80 (0.67-0.97),* P = 2.1E-02 ^t	Candidate gene study	White (non-Hispanic)	9

SNPs in the *IL33* and *IL1RL1* genes are reported to have a significant association with asthma, with “Y” representing the protective allele and “Z” representing the risk allele. The significance level as a P value after ^aBonferroni correction or as a ^bfalse discovery rate q value level, ^cuncorrected P value, or ^dcorrected P value; ^ein the replication cohort or ^fthe follow-up study; as a ^gnominal P value; or ^hin the meta-analysis and replication combined. The Methods section in this article’s Online Repository describes Table I’s construction method.

Afr-Am, African American; *Afr-Car*, African Caribbean; *Am*, American; *Aus-Eur*, Australian-European; *E-Asia*, East Asian; *Eur*, European; *Lat*, Latino; *NE-Italy*, Northeastern Italy; *North-Am*, North American; *TDI*, transmission disequilibrium test; *UTR*, untranslated region.

*Odds ratio (OR) with 95% CI or † relative risk (RR) with 95% CI.

‡Unlike the initial observed significance in the SLSJ study, the signal did not replicate in any of the other replication study cohorts (SAGE, CAPPS, or BHS).

mechanisms, such as changes in the level of expression or altered protein function through amino acid substitutions (Table II). The distal promoter region, for instance, carries several SNPs experimentally validated to affect *IL1RL1* gene transcriptional activity. This was evidenced by studies using a *Luciferase* reporter in a human mast cell line, showing that the haplotype consisting of the SNPs rs11685480 (G), rs6543115 (C), and rs6543116 (A) resulted in higher *IL1RL1* transcriptional activity compared with the AGG haplotype.²² Although the GCA haplotype was found to be associated with atopic dermatitis²² and not with asthma, these studies do reveal the presence of functional SNPs within the promoter region of *IL1RL1*, making it worthwhile to investigate asthma-associated SNPs located within this region for their effect on gene transcription as well. Further support for the transcriptional activity of these SNPs was provided in a Dutch birth cohort, in which the same alleles were associated with soluble IL-1RL1-a levels in serum.¹³

Within the complex LD pattern of the *IL1RL1* gene, we identify 5 LD blocks carrying asthma-associated SNPs (Fig 1, B). Each of these LD blocks constitutes a discrete genetic signal. Three of these *IL1RL1* LD blocks (blocks 2, 3, and 5) will be discussed in detail based on their genome-wide significant association with asthma and their potential functional effects.

The second LD block of *IL1RL1* harbors multiple asthma-associated polymorphisms, one of which has potential functional implications. SNP rs1041973 is located in exon 3, which encodes the first immunoglobulin-like domain of the extracellular part of the IL-1RL1 protein. Rs1041973 is a nonsynonymous SNP that results in an amino acid substitution from a neutral alanine residue into a negatively charged glutamic acid residue. Lingel et al²⁶ elegantly showed that amino acid substitutions affecting charged residues at the IL-33/IL-1RL1 binding interface strongly influence the affinity of the IL-33/IL-1RL1 interaction, although the specific effect of the amino acid substitution encoded by the rs1041973 SNP was not tested. Nevertheless, it is tempting to speculate that rs1041973 affects IL-33 binding to the IL-1RL1 protein.

The third LD block contains the synonymous asthma SNP rs1420101 in exon 5E, which is only retained in the transcript encoding the truncated third isoform IL-1RL1-c. This might indicate an effect of this SNP on this specific transcript. Surprisingly, an association of this SNP with *IL18R1* mRNA levels has been identified in human peripheral blood CD4⁺ T lymphocytes,

identifying rs1420101 as an *IL18R1* expression quantitative trait locus.¹⁹ Thus this *IL1RL1* SNP or an *IL18R1* SNP in strong LD could contribute to asthma development by altering *IL18R1* transcription levels.¹⁹

The fifth LD block contains a large number of SNPs in full LD and is positioned at the 3’ end of the gene around exon 11 and extends into the *IL18R1* gene also harboring *IL18R1* SNPs. Five SNPs within this LD block have been found to be associated with asthma (Table II), 4 of which (rs10204137, rs10192157, rs10206753, and rs3771166) have been identified as expression quantitative trait loci significantly associated with both *IL1RL1* mRNA levels in lymphoblasts¹⁹ and *IL18R1* mRNA levels in fat tissue.^{18,20,21,27} Therefore it can be hypothesized that these SNPs contribute to asthma development by altering the transcription levels of either the *IL1RL1* gene, the *IL18R1* gene, or both. Further experimental evaluation is required to determine which SNPs contained within this LD block represent causal variants affecting *IL1RL1* or *IL18R1* mRNA levels. Intriguingly, this LD block also contains 5 nonsynonymous coding SNPs, 2 of which affect the same codon and thereby the same amino acid residue. The net result of these 5 nonsynonymous SNPs is a change of 4 amino acids in the intracellular part of IL-1RL1 between the risk and the protective haplotype. These nonsynonymous SNPs are located within exon 11, which, together with exon 10, encodes for the TIR domain of the intracellular part of the IL-1RL1 protein. The TIR domain plays a crucial role in signal transduction because binding of IL-33 induces heterodimerization of IL-1RL1 with the coreceptor IL-1RAcP and subsequent interaction of the intracellular TIR domains of these 2 receptor subunits with signaling adaptor proteins as myeloid differentiation primary response gene-88 (MyD88) or Mal. This triggers the signaling cascade that eventually results in the activation of downstream mitogen-activated protein kinases and transcription factors, such as nuclear factor κB (NF-κB) and activator protein 1 (Fig 2). Activation of this signaling pathway might modulate cytokine production and gene expression but also cell survival, differentiation, and motility, depending on the cell type involved.²⁵

The TIR/TIR domain interaction of IL-1RL1 and the adaptor proteins Mal and MyD88 has been modeled in considerable detail.²⁸ Interestingly, the T433 residue in IL-1RL1-b was identified as an interfacing residue of the IL-1RL1/Mal complex.²⁸

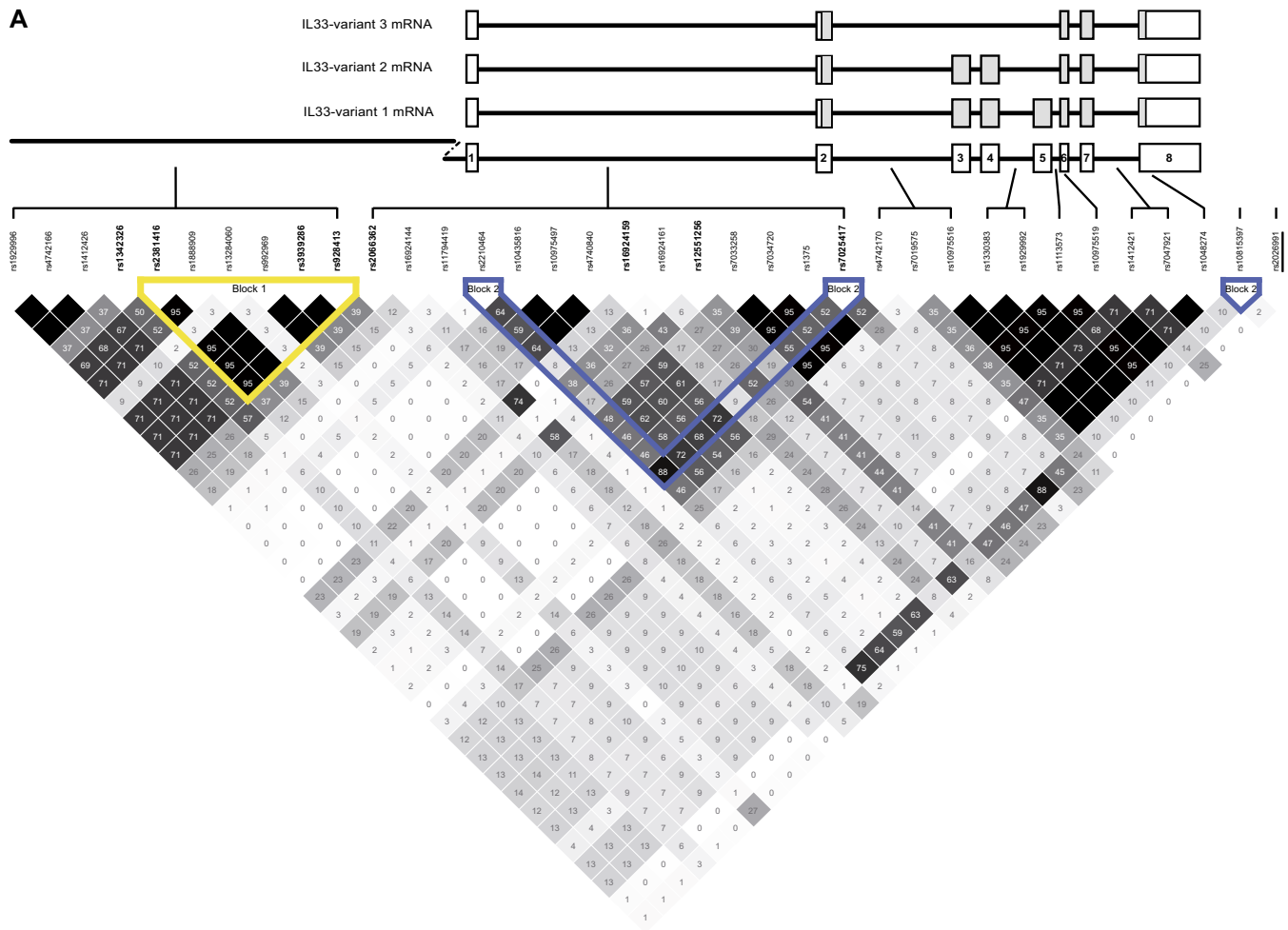


FIG 1. LD of the polymorphisms located within the *IL33* region (chromosome 9) and the *IL1RL1/IL18R1* region (chromosome 2). **A**, LD plot of the *IL33* region in the CEU population (see the [Methods](#) section in this article's Online Repository for more details). **B**, LD plot of the *IL1RL1/IL18R1* region in the CEU population (see the [Methods](#) section in this article's Online Repository for more details). Asthma-associated polymorphisms are depicted in **boldface**, and expression quantitative trait loci are underlined.

Because the nonsynonymous coding SNP rs4988956 (LD block 5, [Table II](#)) results in a change of this polar threonine residue into a nonpolar alanine residue,²⁹ it can be hypothesized that this SNP has a direct effect on the affinity of the interaction between the TIR domains of IL-1RL1-b and Mal. This might directly affect IL-33-induced signaling through IL-1RL1. These data warrant further evaluation of the effect of these 4 amino acid substitutions for IL-33-induced activation of downstream signaling. We propose that amino acid substitutions contained within the TIR domain of IL-1RL1 affect coupling of IL-1RL1 to the coreceptor IL-1RAcP and adaptor proteins, such as Mal, and thus affect downstream signaling.

In addition to direct effects on IL-33-induced signaling, the TIR domain-dependent interaction of IL-1RL1-b with the adaptor proteins MyD88 and Mal also plays a role in the inhibition of Toll-like receptor (TLR) signaling by IL-1RL1 ([Fig 2](#)).^{30,31} IL-1RL1/Mal or MyD88 complex formation may prevent the interaction between MyD88, Mal, or both with activated TLRs.^{28,30} Thus the presence of multiple amino acid substitutions within the TIR domain of IL-1RL1-b could result in altered inhibition of TLR activation. This possibility is especially intriguing given the genetic

interaction we have previously reported for IgE sensitization between polymorphisms in *IL1RL1* and *TLR4*.³²

In summary, the *IL1RL1* gene carries multiple independent genetic signals with possible functional consequences that are highly divergent, including effects on expression of *IL1RL1* and its neighboring gene *IL18R1*, as well as effects on IL-33/IL-1RL1 binding affinity, TIR domain interactions, and downstream signaling.

FROM GENETICS TO BIOLOGY: IL-33/IL-1RL1 SIGNALING IN THE PATHOPHYSIOLOGY OF ASTHMA

Genetic studies strongly implicate the IL-33/IL-1RL1 pathway in asthmatic patients. This is further corroborated by mechanistic studies in experimental animal models of asthma and clinical studies, which will be reviewed below. Very recently, it has been shown that the IL-33/IL-1RL1 pathway can act through the activation of a novel subset of innate immune cells called ILC2s or nuocytes that produce cytokines such as IL-5 and IL-13 in the lungs.³³⁻³⁵

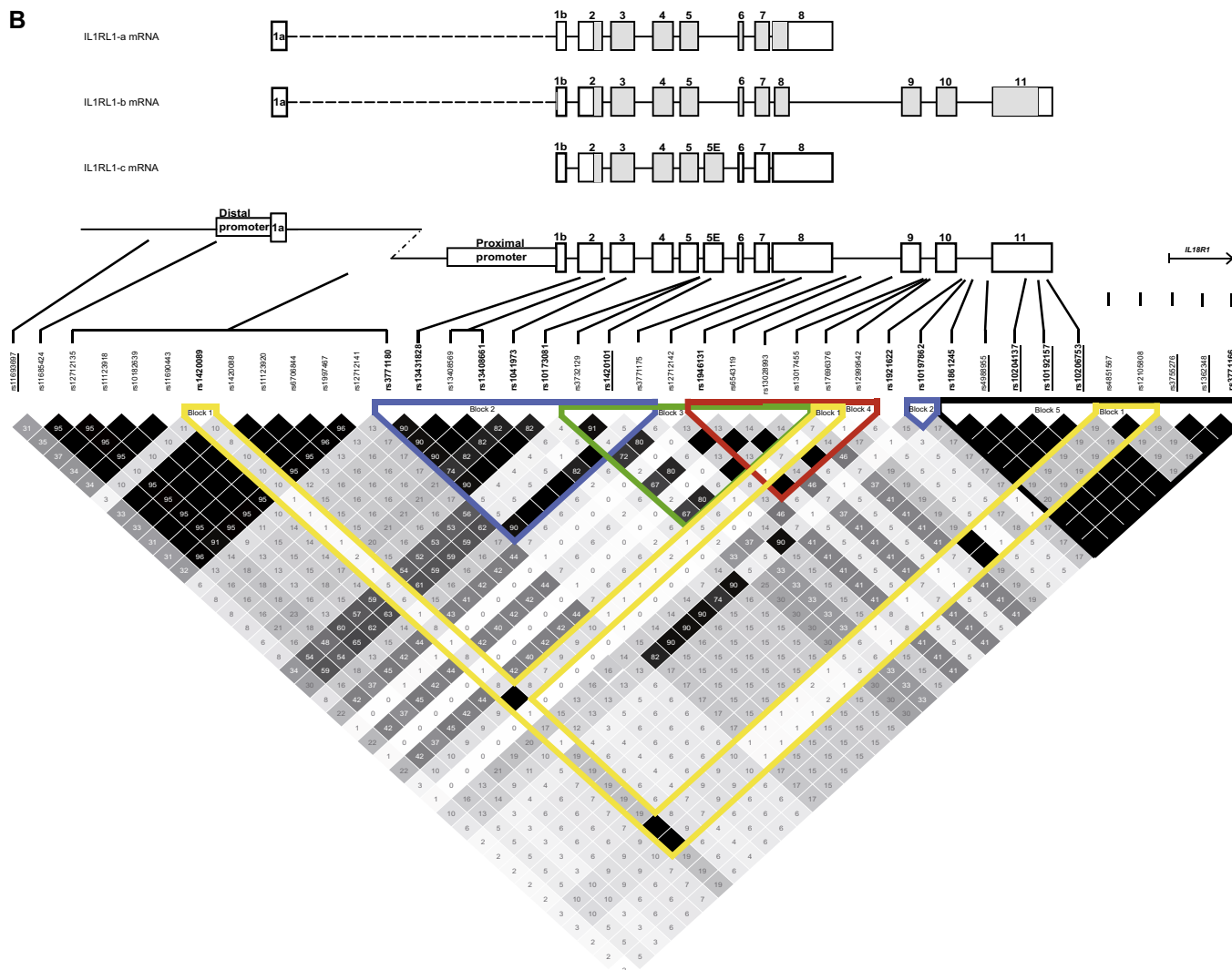


FIG 1. (Continued)

IL-33 has a dual effect as a transcription factor and a cytokine. First, IL-33 is capable of acting as a transcription factor, thereby altering gene expression in a cell-autonomous, IL-1RL1-independent fashion. IL-33 can translocate to the nucleus and bind directly to the chromatin,^{36,37} as well as to the NF- κ B proteins p50 and p65.³⁸ In doing so, IL-33 is capable of regulating the expression of proinflammatory genes, such as *IL6*, *IL8*, and the p65 subunit of NF- κ B,^{39,40} and thus might be a direct regulator of the inflammatory response.

Second, IL-33 signals through binding to the heterodimeric receptor complex consisting of IL-1RL1 and the accessory receptor subunit IL-1RAcP.^{26,41} Binding of IL-33 to the IL-1RL1/IL-1RAcP heterodimeric receptor complex will recruit signaling adaptor proteins, such as Mal or MyD88. Mal and MyD88 are signaling proteins that express a TIR domain⁴² through which they bind to the intracellular TIR domain of IL-1RL1-b and IL-1RAcP and subsequently trigger a signaling cascade culminating in activation of mitogen-activated protein kinases and transcription factors, such as NF- κ B and activator protein 1 (Fig 2). Several recent reviews offer detailed overview of IL-33-induced signal transduction by IL-1RL1.^{25,43} IL-33 is constitutively expressed in lymphoid organs, epithelial barrier tissues, and the brain,

whereas its expression is induced in inflamed tissues and injured epithelia, such as the lung alveoli, during papain-induced allergic airway inflammation.⁴⁴ Because IL-33 is released on injury or damage, it has been coined to act as an “alarmin,” translating damage into an activation of the inflammatory response.

A large number of cell types relevant to asthma pathogenesis have been shown to express IL-1RL1 and to be responsive to IL-33,⁴⁵ including T_H2 cells,⁴⁶ mast cells,^{47,48} invariant natural killer T cells,⁴⁹ eosinophilic and basophilic granulocytes,⁵⁰⁻⁵² and epithelial cells.⁵³ By activating these cells, IL-33 has been shown to mediate a wide range of responses, including T_H17 -mediated airway inflammation⁵⁴ and neutrophil influx,⁵⁵ as well as antiviral CD8⁺ T-cell responses⁵⁶ and lung tissue homeostasis on influenza virus infection.⁵⁷ However, the best described activity of IL-33 is the activation of innate and adaptive immune responses characterized by the production of IL-4, IL-5, and IL-13.

In the first studies on its biological activities, systemic administration of IL-33 to mice induced production of IL-4, IL-5, and IL-13 in multiple organs accompanied by blood eosinophilia, increased IgE titers, and hypertrophy of airway epithelia and increased mucus production.⁴¹ IL-33 administration to the airways also induces a rapid influx of eosinophils and production

TABLE II. Discrete genetic signals and predicted function of SNPs associated with asthma in the *IL33* and *IL1RL1* gene regions

LD block	RS no.	Association reference	SNP location	Predicted function	P value	Tissue	Source database
<i>IL33</i>							
—	rs1342326	5, 8	5' of <i>IL33</i>	—			
1	rs2381416	7	5' of <i>IL33</i>				
	rs3939286	4, 15	5' of <i>IL33</i>				
	rs928413	8	5' of <i>IL33</i>				
—	rs2066362	5	Intron 1	—			
—	rs16924159	9	Intron 1	—			
—	rs12551256	9	Intron 1	—			
2	rs7025417	9	Intron 1	—			
—	<i>rs2026991</i>		3' of <i>IL33</i>	eQTL*	1.00E-24	Liver	GTEEx 17
<i>IL1RL1</i>							
—	<i>rs11693697</i>		5' of <i>IL1RL1</i>	eQTL†	4.95E-04	Fat tissue	GeneVar 18
1	rs1420089	12	Intron 1a	Unknown			
2	rs3771180	7	Proximal promoter				
	rs13431828	9, 11, 15	Exon 2 (5'UTR)				
	rs13408661	6	Intron 2				
	rs1041973	9, 11, 13	Exon 3 (nonsynonymous)	Amino acid substitution			
	rs10173081	7	Intron 5				
	rs10197862	14	Intron 10				
3	rs1420101	4, 10	Exon 5E (synonymous)	eQTL‡	6.88E-04	Primary peripheral blood CD4 ⁺ lymphocytes	(Murphy 19)
	<i>rs12998521</i>		3' of <i>IL1RL1</i>	eQTL‡	1.23E-04	Primary peripheral blood CD4 ⁺ lymphocytes	(Murphy 19)
4	rs1946131	12	Intron 8	Unknown			
—	rs1921622	10	Intron 10	Unknown			
5	rs1861245	12	Intron 10				
	<i>rs4988955</i>		Intron 10		3.05E-04	Fat tissue	GeneVar 18
	<i>rs4988956</i>		Exon 11 (nonsynonymous)		3.05E-04	Fat tissue	GeneVar 18
	<i>rs4988957</i>		Exon 11 (synonymous)		3.78E-10	Monocytes	SeeQTL 20, 21
	<i>rs10192036</i>		Exon 11 (nonsynonymous)		3.05E-04	Fat tissue	GeneVar 18
	rs10204137	5, 9	Exon 11 (nonsynonymous)	Amino acid substitution and eQTL‡	3.05E-04	Fat tissue	GeneVar 18
	<i>rs4988958</i>		Exon 11 (synonymous)		3.05E-04	Fat tissue	GeneVar 18
	rs10192157	5, 9	Exon 11 (nonsynonymous)		3.05E-04	Fat tissue	GeneVar 18
	rs10206753	5, 9	Exon 11 (nonsynonymous)		3.05E-04	Fat tissue	GeneVar 18
	<i>rs3755276</i>		5' of <i>IL18R1</i>		3.05E-04	Fat tissue	GeneVar 18
	rs3771166	5, 9, 16	Intron 2 of <i>IL18R1</i>		3.05E-04	Fat tissue	GeneVar 18

Polymorphisms with a reference in column 3 have been associated with asthma. eQTLs are depicted in italics, whereas *IL1RL1* polymorphisms associated with asthma are depicted in boldface. The Methods section in this article's Online Repository describes Table II's construction method.

eQTL, Expression quantitative trait locus; UTR, untranslated region.

*eQTLs associated with *IL33* mRNA levels.

†eQTLs associated with *IL1RL1* mRNA levels.

‡eQTLs associated with *IL18R1* mRNA levels.

of IL-5 and IL-13 by innate immune cells independently of IL-4 and T_H2 lymphocytes.^{35,58} This innate response characterized by high levels of IL-5 and IL-13 is now generally referred to as an innate type 2 response.

In murine models IL-33 has been found to be released promptly after activation or damage of lung resident cells by, for instance, protease-active or glycolipid allergens^{35,59,60} or influenza infection,⁶¹ as well as after allergen challenge in sensitized mice.²³ On release, IL-33 activates an innate population of IL-5- and IL-13-producing cells, which were originally identified in mouse models by multiple groups as "natural helper cells" or "nuocytes"^{62,63} and now commonly referred to as ILC2s.⁶⁴ Also, in the lung ILC2s have been shown to be present and to respond to IL-33 and to contribute to airway inflammation³⁴ and airway hyperresponsiveness.³³ In fact, ILC2s were found to be required

and sufficient in experimental models of allergic airway disease for IL-5 and IL-13 production and mucus hypersecretion on protease treatment.⁵⁹ Importantly, the induction of airway hyperresponsiveness and goblet cell hyperplasia by IL-33 has been found to be dependent on IL-1RL1 signaling and MyD88-TIR interaction.⁶⁵ In experimental mouse models of allergic airway inflammation, ILC2s were found to be a major source of IL-5 and IL-13 but not of IL-4.³⁴ Importantly, the existence of these IL-33-responsive innate immune cells producing large quantities of IL-5 and IL-13 has recently also been described in human subjects.^{57,66} Further support for a central role of the ILC2 cell population in the pathogenesis of asthma stems from the identification of retinoic acid-related orphan receptor α (*RORA*) as an asthma gene in the GABRIEL GWA study⁵ and the meta-analysis of the Analysis in Population-based Cohorts for Asthma

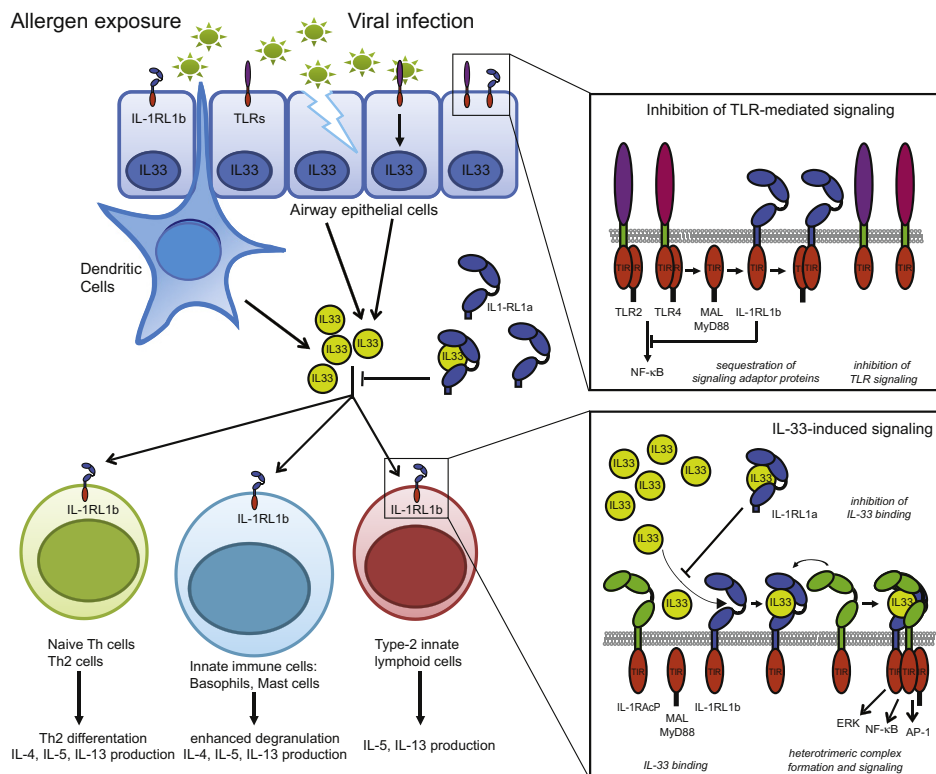


FIG 2. Biological activity of the IL-33/IL-1RL1 signaling pathway. IL-33–producing and responsive cells in the lung and the IL-33/IL-1RL1 signaling pathway (in part based on Lingel et al²⁶) are shown. Airway epithelial damage will induce IL-33 release. IL-33 binding to a receptor complex of IL-1RL1-b and IL-1RAcP initiates recruitment of Mal or MyD88 signaling adaptor molecules, leading to activation of downstream pathways (*lower insert*). IL-1RL1-a can neutralize IL-33. In addition to its role in IL-33 signal transduction, IL-1RL1b can inhibit TLR signaling through TIR domain–dependent sequestration of the adaptor proteins MyD88 and Mal (*top insert*).

Traits consortium with GABRIEL data⁷ because ROR α was recently shown to be critically required for the differentiation of ILC2s *in vivo*.⁶⁷ Taken together, a central role in the pathogenesis of asthma for the IL-33/IL-1RL1 pathway, acting in part through activation of ILC2s, is supported by the genetic evidence stemming from multiple independent GWA studies identifying 3 asthma genes operating in this pathway.

Next to ILC2s, IL-33 also activates T_H2 cells, mast cells, and basophils, inducing the production of IL-4, as well as IL-5 and IL-13.⁶⁸ For instance, IL-33 was shown to act as a chemoattractant for T_H2 cells,⁴⁶ and activation of T_H2 cells in the presence of IL-33 greatly enhanced their production of IL-5 and IL-13 in an IL-1RL1- and MyD88-dependent but IL-4-independent fashion.^{49,58} Basophils responded to IL-33 through enhanced basal and IgE cross-linking–induced production of IL-4, IL-5, and IL-13.^{49,52} Additionally, mast cells responded to IL-33 with enhanced survival and an increased basal and IgE cross-linking–induced production of IL-4, IL-5, and IL-13.^{47,69–71} Of note, unlike ILC2s, these innate cells are capable of producing IL-4 on IL-33 stimulation, a response that has recently been shown to also occur *in vivo* in a T cell–independent fashion.⁷²

From these *in vitro* and *in vivo* studies, a picture is emerging that higher levels of IL-33, higher activity of IL-1RL1-dependent signal transduction, or both will result in an aggravated innate type 2 response mediated by ILC2s, mast cells, and basophils, as well as an enhanced adaptive T_H2 response. The relevance of these observations from experimental mouse models

of asthma for human disease is underscored by studies in asthmatic patients: IL-33 protein was found to be increased in the bronchoalveolar lavage fluid and in airway epithelial cells⁷³ and airway smooth muscle cells of asthmatic patients compared with those of healthy control subjects, which positively correlated with asthma severity.^{74,75} Moreover, several cell types derived from allergic subjects, including mast cells, basophils, and eosinophils, could be matured and activated to release IL-4, IL-5, and/or IL-13 on IL-33 stimulation, which was increased compared with that seen in cells isolated from healthy control subjects.^{47,49}

CONCLUSION AND FUTURE DIRECTIONS

SNPs within the *IL33* and *IL1RL1* genes are reproducibly found to associate with asthma in different populations, and evidence from experimental models strongly supports a functional role for IL-33/IL-1RL1 signaling in asthma pathogenesis. Therefore we conclude that the IL-33/IL-1RL1 axis plays a critical role in the susceptibility for this chronic inflammatory disease. On the basis of currently published data, multiple discrete genetic signals can be distinguished within the *IL33* and *IL1RL1* loci, each of which might have an independent contribution to asthma pathology. However, the complex LD structure at the *IL1RL1/IL18R1* locus precludes identification of causal asthma polymorphisms by genetic studies. This hurdle could possibly be tackled by performing association studies in populations characterized with less LD, such as the African (American) population,⁷⁶ or

in well-powered meta-analyses with conditional analyses to investigate independence of SNP effects on asthma. Finally, whereas GWA studies typically focus on prevalent variants and are able to explain only a small part of the asthma heritability, it has been shown that asthma susceptibility genes might also harbor rare variants with potential large effects on gene function.⁷⁷ Rare variants are therefore thought to explain a part of the “missing heritability” of asthma.⁷⁸ Hence resequencing of *IL33* and *IL1RL1* within the context of large-scale sequencing projects might answer the question of whether these genes indeed harbor rare variants, thereby allowing the analysis of their contribution to asthma susceptibility.

In addition to association studies, functional studies focused on a single SNP or a complete haplotype could also shed light on the biological relevance of the different genetic signals located within the IL-33/IL-1RL1 axis for asthma pathophysiology. One such study performed by Shimizu et al¹⁹ revealed that SNPs located within the distal promoter region of *IL1RL1* contribute to altered *IL1RL1* transcription levels. Clearly, more work needs to be done in this area to fully appreciate the relevance of the diversity of *IL33* and *IL1RL1* genetic signals in the context of asthma pathogenesis.

The recently identified ILC2 is a critical IL-33–responsive cellular intermediate in the pathogenesis of asthma, which contributes to the asthma phenotype through the production of IL-5 and IL-13. The role of the ILC2s is further supported by the identification of *RORA*, encoding the transcription factor critically required for ILC2 differentiation, as another asthma GWA study gene.^{5,7} The relevance of the innate ILC2s, next to the adaptive and allergen-specific T_H2 cells, for the pathogenesis of asthma will be subject of intense research in the coming years. This research will also need to dissect the relative contributions of ILC2s and other effector cells of the immune system, such as basophils and mast cells, to the IL-33/IL-1RL1–driven responses. Such data will likely guide the rational design of novel interventions for this chronic inflammatory disease. Moreover, identification of causal asthma variants and unraveling of the functional relevance of these genetic signals in the appropriate cells and tissues might lead to a future prospect of personalized therapeutic intervention based on individual genetic risk factors. This might be beneficial to understand the clinical variety of asthma phenotypes and offer directions for a rational personalized intervention strategy.

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METHODS

Methods used for assembling Table I

Polymorphisms located in the *IL33* and *IL1RL1* genes reported to have a significant association with asthma were included in the table by using the criterion of significance as defined in each study. SNPs retrieved from candidate studies, genome-wide association studies, and meta-analyses are presented (see references in the table).

Methods used for assembling Table II

Table II includes the reported asthma-associated SNPs from Table I classified according to LD blocks, as defined and depicted in Fig 1, A and B, based on the LD structure found in the CEU population (HapMap release 28, phase I-, II-, and III-merged genotypes and frequencies, based on National Center for Biotechnology Information B36 assembly).^{E1} An analysis of publically available expression quantitative trait locus databases was performed that included datasets from the Pritchard Laboratory,^{E2} GeneVar,^{E3-E6} and GenTex,^{E2,E7} and expression data were retrieved for SNPs from Caucasians.^{E2-E9} A search up to 100 kb 5' of the genes *IL33*, *IL1RL1*, and *IL18R1* was performed, considering an expression quantitative trait locus with a *P* value of less than .001 as significant.

Methods used for defining the LD plots of Fig 1

LD of the polymorphisms located within the *IL33* region (chromosome 9) and the *IL1RL1-IL18R1* region (chromosome 2). Structural information of the genomic region of *IL33* and *IL1RL1*, as well as information regarding mRNA transcript variants, was derived from the National Center for Biotechnology Information Reference Sequence NC_000009.11 and NC000002.11, respectively (Homo sapiens, GRCh37.p5 Primary Assembly, available at <http://www.ncbi.nlm.nih.gov>). LD plots were constructed with HaploView (version 4.2) software, with SNP genotyping data downloaded from the international HapMap Project (available at <http://hapmap.ncbi.nlm.nih.gov/index.html.en>)^{E1} for the CEU population. HapMap release 28 (phase I-, II-, and III-merged genotypes and frequencies) was used and areas {chr9:6170,000..6260,000} and {chr2:102280,000..102370,000} were downloaded for *IL33* and *IL1RL1*, respectively. For reasons of clarity, a limited number of 40 SNPs was included within each LD plot. SNP selection was done by prioritizing asthma-associated SNPs from Table I (main text) while faithfully reporting the underlying LD pattern of the loci. Subsequently, LD blocks were defined by using an r^2 value of 0.8 or greater with at least 1 asthma-associated SNP as a cut-off to include SNPs in a certain LD block.

Methods used for defining the LD plots of Figs E1 and E2

We determined the extent to which LD patterns with asthma-associated SNPs in the *IL33* and *IL1RL1* genomic regions differ among distinct populations worldwide. To this end, SNP genotype data were downloaded for populations of African origin (ASW and YRI), Asian origin (CHB), and Latin American origin (MEX) from the international HapMap Project,^{E1} release 27 (phase I-, II-, and III-merged genotypes and frequencies) was used, and area {chr9:6170,000..6260,000} was selected for *IL33*, whereas area {chr2:102280,000..102370,000} was selected for *IL1RL1*. SNP genotype data of *IL33* and *IL1RL1* were analyzed by using HaploView (version 4.2) software. LD plots were constructed by including the selected asthma-associated SNPs shown in Fig 1 (main body text) from the CEU reference population. SNPs with missing genotypes were excluded from the LD plots. Subsequently, for each of the analyzed populations, the LD pattern for the asthma-associated SNPs (see Tables I and II and the main text) was compared with that reported in the CEU population by using an r^2 value of 0.8 or greater as a cutoff value for each SNP to be considered part of an LD block.

Interpretation of Figs E1 and E2

For Fig E1, the LD pattern of the *IL33* genomic region of several populations was compared with the LD blocks defined in the CEU population. LD block 1, as observed in the CEU population, could be broken down into independent signals in the ASW, YRI, and CHB populations but not in the MEX population. This resulted in the identification of multiple independent SNPs in these ethnic backgrounds: SNPs rs3939286 (ASW population), rs928413 (ASW and CHB populations), and rs2381416 (YRI and CHB populations) constituted independent signals with an LD of less than 0.8 (r^2), SNPs that have been associated with asthma in several white populations (Table II). In addition, LD block 2, as observed in the CEU ethnic background, are all independent SNPs in the YRI population, including asthma-associated SNP rs7025417. For the asthma-associated SNPs that were independent signals in the CEU ethnic background, some SNPs (eg, rs16924159 and rs12551256 in the YRI or rs1342326 and rs2066362 in the MEX) now form a new LD block, making their individual contributions harder to dissect in those ethnic backgrounds.

For Fig E2, the LD pattern of asthma-associated SNPs of the *IL1RL1/IL18R1* genomic region of several distinct populations was compared with the LD blocks defined in the CEU population. For all 4 populations studied, some differences in LD pattern were apparent with regard to the asthma-associated SNPs, although overall, (unlike the *IL33* gene), the LD pattern was remarkably similar in all ethnic backgrounds, with a high degree of LD throughout the locus. However, it is worth noting that the nonsynonymous SNP rs1041973 (LD block 2 in the CEU, see Tables I and II) represents an independent SNP within all 4 studied populations (ASW, YRI, CHB, and MEX), an SNP that Wu et al^{E10} associated with asthma in the Mexican population. Furthermore, an additional asthma-associated SNP (rs1861246, see also Table I) that is not present in the HapMap dataset (release 27) of the other populations is present in the CHB population and is in LD with block 3 SNPs.

Overall, functional experiments are required to further establish true causal SNPs in the discussed areas, whereby it should be noted that not only the LD pattern of SNPs can differ among populations but also the relationship with asthma can prove distinct.

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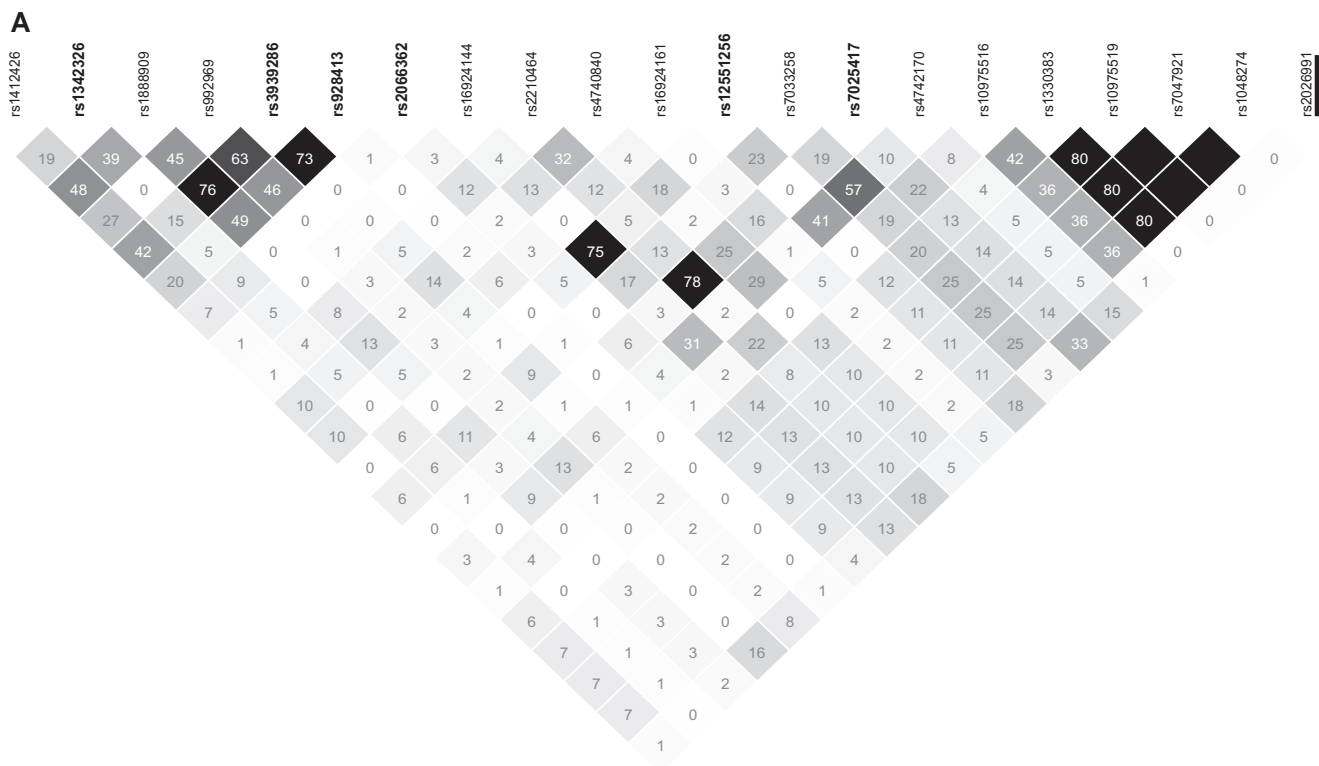


FIG E1. LD of the *IL33* genomic region for the ASW, YRI, CHB, and MEX populations. **A**, ASW population. **B**, YRI population. **C**, CHB population. **D**, MEX population. **Boldface**, Asthma-associated SNPs; **underlined**, expression quantitative trait loci.

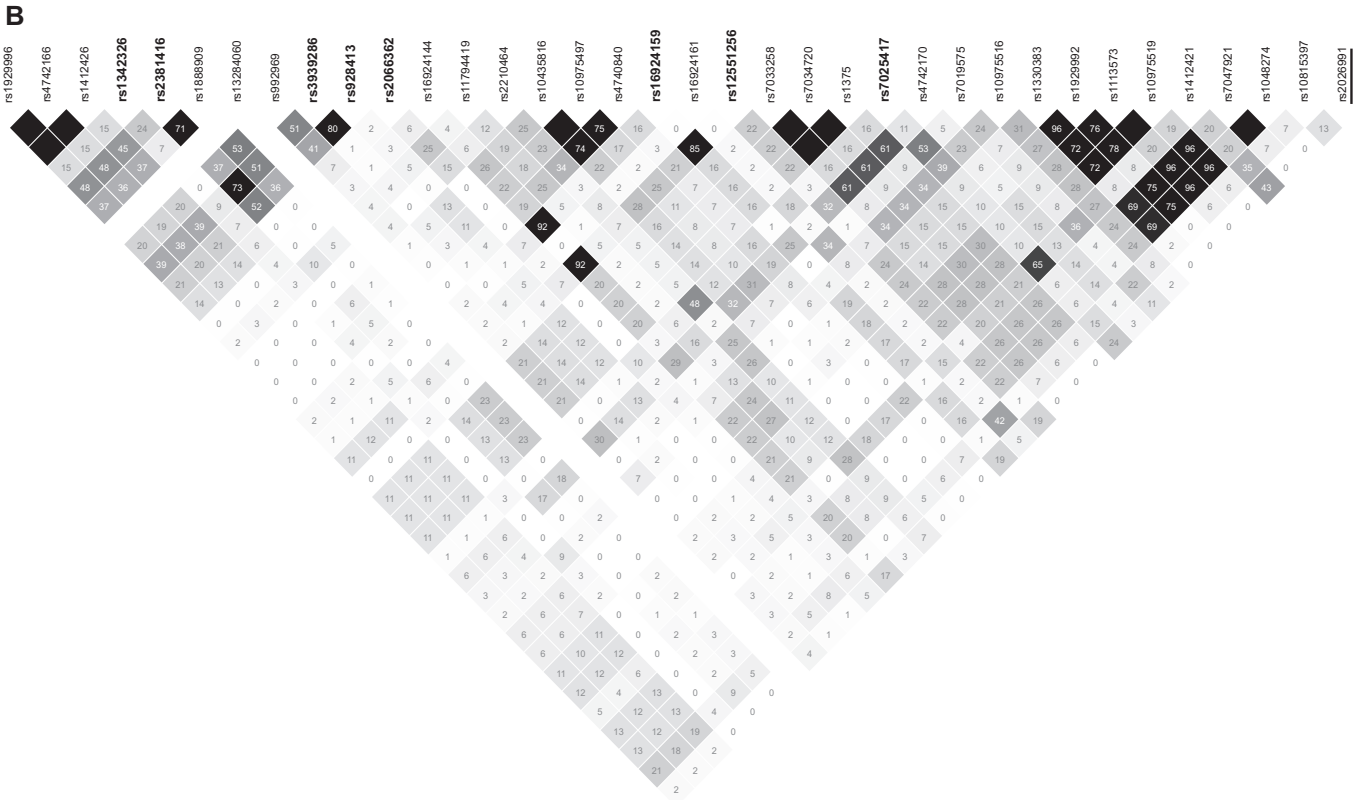


FIG E1. (Continued)

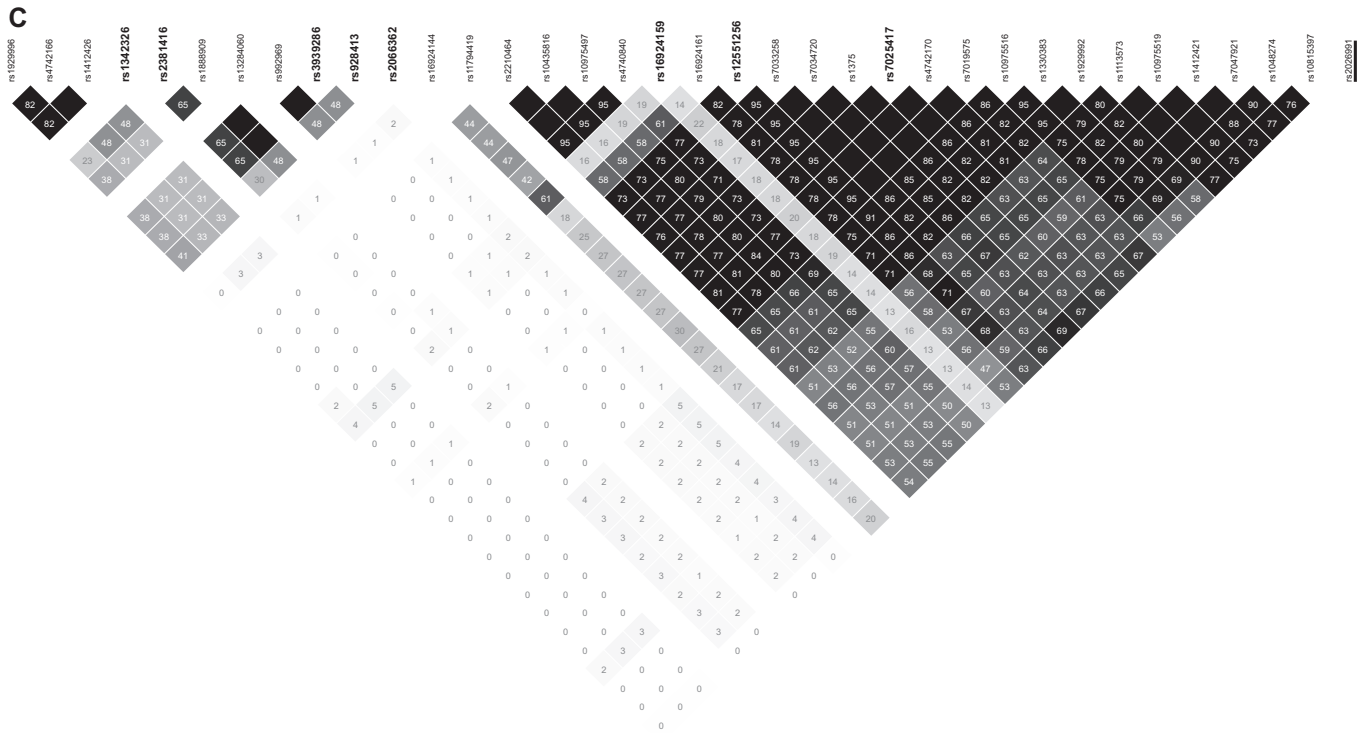


FIG E1. (Continued)

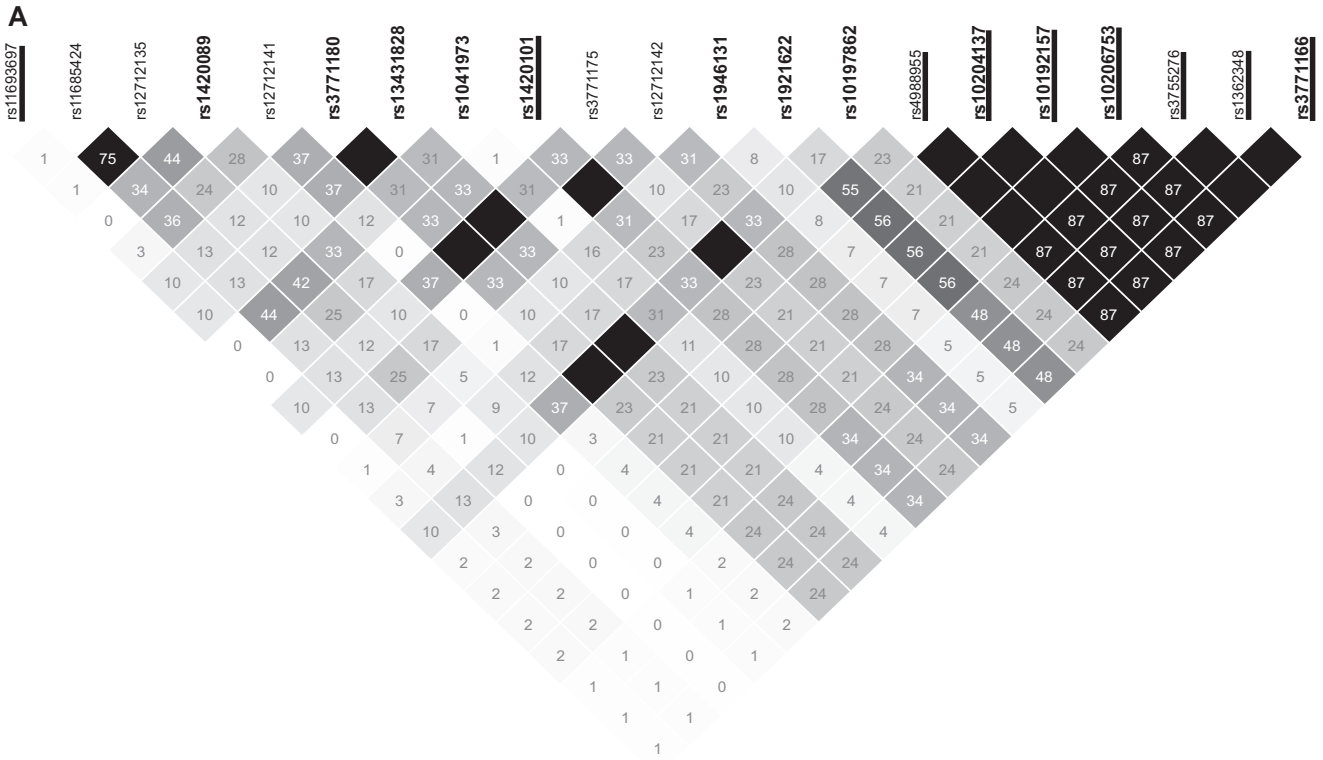


FIG E2. LD of the *IL1RL1* genomic region for the ASW, YRI, CHB, and MEX populations. **A**, ASW population. **B**, YRI population. **C**, CHB population. **D**, MEX population. *Boldface*, Asthma-associated SNPs; *underlined*, expression quantitative trait loci.

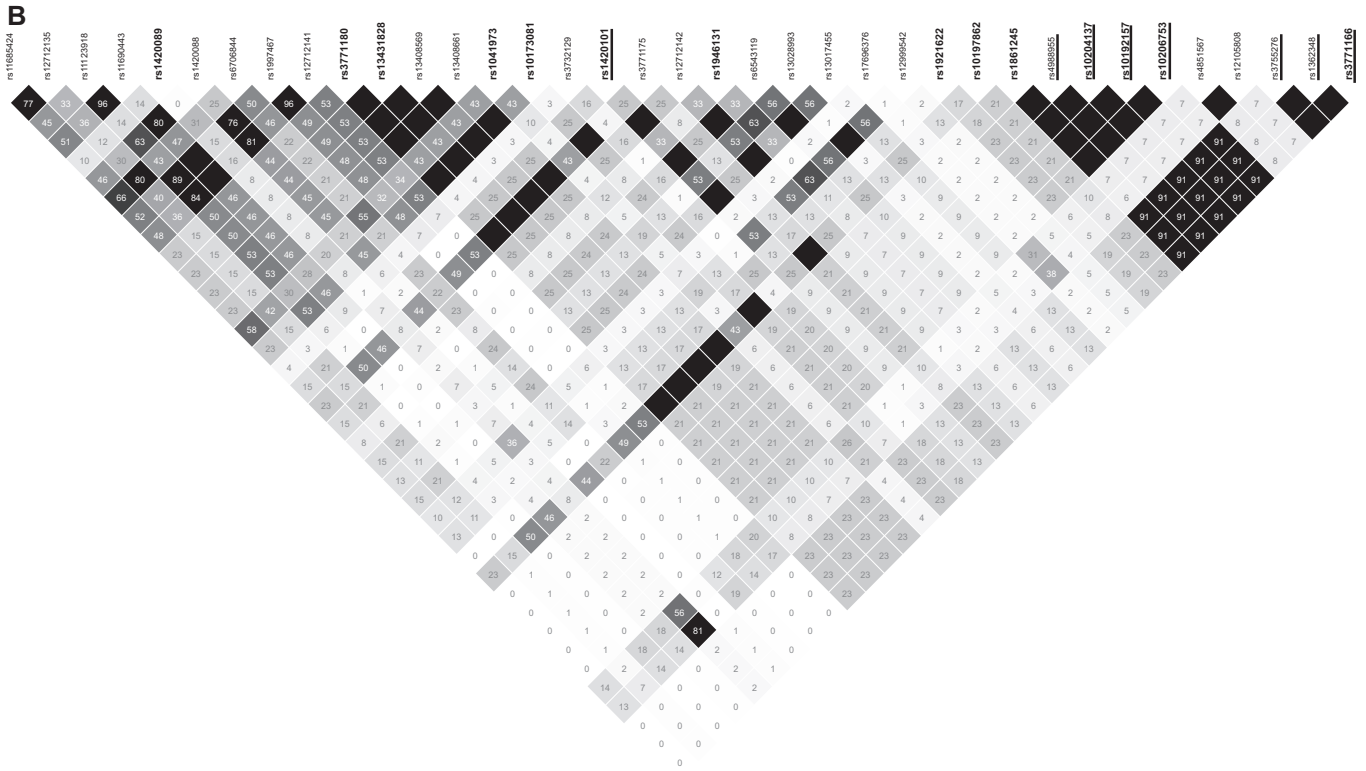


FIG E2. (Continued)

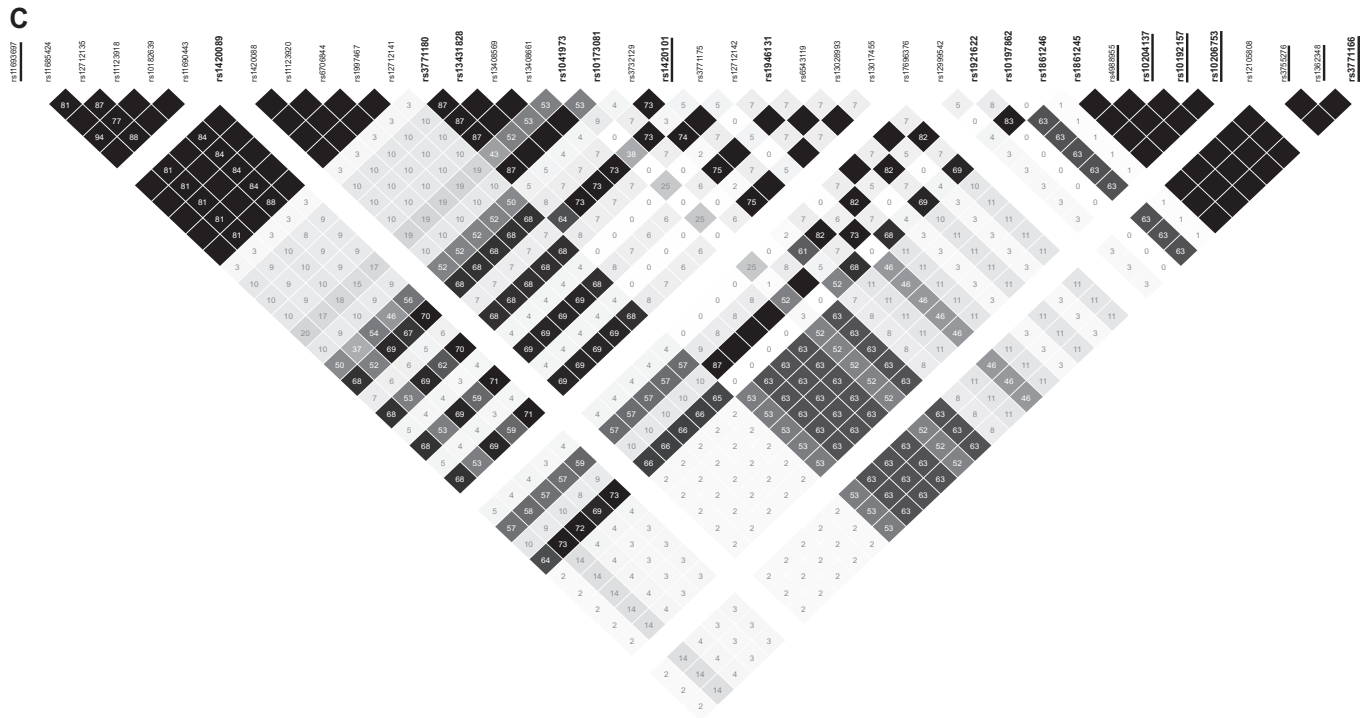


FIG E2. (Continued)

