

Association of *IL33*–IL-1 receptor–like 1 (*IL1RL1*) pathway polymorphisms with wheezing phenotypes and asthma in childhood

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Background: Genome-wide association studies identified *IL33* and IL-1 receptor–like 1 (*IL1RL1*)/*IL18R1* as asthma susceptibility loci. *IL33* and *IL1RL1* constitute a single ligand–receptor pathway.

Objective: In 2 birth cohorts, the Prevalence and Incidence of Asthma and Mite Allergy (PIAMA) study and Avon Longitudinal Study of Parents and Children (ALSPAC), we analyzed associations of longitudinal wheezing phenotypes and

asthma with single nucleotide polymorphisms (SNPs) of 8 genes encoding IL-33, *IL1RL1*, its coreceptor *IL1RAcP*, its adaptors myeloid differentiation primary response gene 88 (MyD88) and Toll–IL-11 receptor domain containing adaptor protein (TIRAP), and the downstream IL-1 receptor–associated kinase 1, IL-1 receptor–associated kinase 4, and TNF receptor–associated factor 6 (TRAF6). Furthermore, we investigated whether SNPs in this pathway show replicable evidence of gene–gene interaction.

Methods: Ninety-four SNPs were investigated in 2007 children in the PIAMA study and 7247 children in ALSPAC.

Associations with wheezing phenotypes and asthma at 8 years of age were analyzed in each cohort and subsequently meta-analyzed. Gene–gene interactions were assessed through model-based multifactor dimensionality reduction in the PIAMA study, and gene–gene interactions of 10 SNP pairs were further evaluated.

Results: Intermediate-onset wheeze was associated with SNPs in several genes in the *IL33*–*IL1RL1* pathway after applying multiple testing correction in the meta-analysis: 2 *IL33* SNPs (rs4742170 and rs7037276), 1 IL-1 receptor accessory protein (*ILIRAP*) SNP (rs10513854), and 1 *TRAF6* SNP (rs5030411).

Late-onset wheeze was associated with 2 *IL1RL1* SNPs (rs10208293 and rs13424006), and persistent wheeze was associated with 1 *IL33* SNP (rs1342326) and 1 *ILIRAP* SNP (rs9290936). *IL33* and *IL1RL1* SNPs were nominally associated with asthma. Three SNP pairs showed interaction for asthma in the PIAMA study but not in ALSPAC.

Conclusions: *IL33*–*IL1RL1* pathway polymorphisms are associated with asthma and specific wheezing phenotypes; that is, most SNPs are associated with intermediate-onset wheeze, a phenotype closely associated with sensitization. We speculate that *IL33*–*IL1RL1* pathway polymorphisms affect development of wheeze and subsequent asthma through sensitization in early childhood. (J Allergy Clin Immunol 2014;■■■:■■■–■■■.)

Key words: *IL33*–*IL1RL1* pathway, asthma, wheezing phenotypes, children, *IL1RL1*, *IL33*, Avon Longitudinal Study of Parents and Children study, Prevalence and Incidence of Asthma and Mite Allergy study

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Asthma is a complex disease in which genetic and environmental factors and their interactions lead to airway inflammation and variable airflow limitation. Candidate gene studies and genome-wide association (GWA) studies have shown that the *IL33* and IL-1 receptor–like 1 (*IL1RL1*)/*IL18R1* loci are important for asthma development.¹ Genetic studies have not

Abbreviations used

ALSPAC:	Avon Longitudinal Study of Parents and Children
eQTL:	Expression quantitative trait locus
GWA:	Genome-wide association
<i>IL1RAP</i> :	IL-1 receptor accessory protein
<i>IL1RL1</i> :	IL-1 receptor–like 1
<i>IL1RL1-b</i> :	IL-1 receptor–like 1 receptor
<i>IRAK1</i> :	IL-1 receptor–associated kinase 1
<i>IRAK4</i> :	IL-1 receptor–associated kinase 4
LD:	Linkage disequilibrium
LLCA:	Longitudinal latent class analysis
MB-MDR:	Model-based multifactor dimensionality reduction
<i>MYD88</i> :	Myeloid differentiation primary response gene 88
PIAMA:	Prevalence and Incidence of Asthma and Mite Allergy
SNP:	Single nucleotide polymorphism
TIR:	Toll–IL-1 receptor
<i>TIRAP</i> :	Toll–IL-11 receptor domain containing adaptor protein
<i>TRAF6</i> :	TNF receptor–associated factor 6

been able to disentangle which gene or genes at the *IL1RL1/IL18R1* locus cause asthma because of strong linkage disequilibrium (LD) in this region. However, recent Bayesian network analyses of asthma-associated single nucleotide polymorphisms (SNPs) that regulate gene expression in lung tissue suggest that *IL1RL1* is most likely causally implicated in asthma development.²

Proteins encoded by *IL33* and *IL1RL1* are part of the IL-33–IL1RL1 pathway (Fig 1). IL-33 has been implicated as an alarm signal for epithelial damage and is released in response to triggers, such as allergens or infectious agents.^{1,3,4} After release of IL-33, it binds to its receptor, IL-1 receptor–like 1 (IL1RL1-b), which forms a receptor complex with IL-1 receptor-associated protein (IL1RAcP). This receptor complex induces, through activation of signaling proteins, such as myeloid differentiation primary response gene 88 (MYD88), Toll–IL-11 receptor domain containing adaptor protein (TIRAP), IL-1 receptor–associated kinase 1 (IRAK1), IL-1 receptor–associated kinase 4 (IRAK4), and TNF receptor–associated factor 6 (TRAF6), release of allergic and eosinophilic mediators, such as IL-5 and IL-13, resulting in eosinophilic inflammation.^{1,3,4} Alternatively, IL-33 can bind to the soluble receptor IL1RL1-a, which acts as decoy receptor for IL-33, resulting in attenuation of the IL-33 signal.³ These data show that involvement of the IL33–IL1RL1 pathway in asthma is biologically plausible. However, in addition to *IL33* and *IL1RL1*, other genes in this pathway might also play a role in asthma. Moreover, genes in this pathway might well interact to contribute to asthma development. Thus far, this has not been studied.

The period early in life is important for asthma development, and certain gene variants might be associated with asthma or wheezing phenotypes with a specific age of onset.⁵ In the GABRIEL study polymorphisms in *IL1RL1* and *IL33* were more strongly associated with early-onset asthma (<16 years) than late-onset asthma (≥16 years), although the difference was not significant.⁶ Because asthma symptoms are heterogeneous in young children, more detailed phenotypes of asthma in early childhood, such as longitudinal wheezing phenotypes defined by longitudinal latent class analysis (LLCA),⁷ might provide insight into the early origins of asthma. Distinct biological origins of wheezing phenotypes are suggested if certain DNA variants are

associated with specific wheezing phenotypes, as was shown for 17q12-21 variants and intermediate-onset and persistent wheeze.^{8,9}

Our aim is to investigate the association and gene-gene interaction of *IL33*–*IL1RL1* pathway SNPs with longitudinal wheezing phenotypes in childhood and asthma at 8 years.

METHODS**Study cohorts: Prevalence and Incidence of Asthma and Mite Allergy study and Avon Longitudinal Study of Parents and Children**

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study is a Dutch multicenter birth cohort that invited allergic and nonallergic women to participate in the study; 4146 (53%) agreed and provided written informed consent (1327 allergic and 2819 nonallergic subjects).¹⁰ There were 3963 live-born children. Parents were sent International Study of Asthma and Allergies in Childhood–based questionnaires about their child's health, including asthma symptoms at 3, 12, 24, 36, 48, 60, 72, 84, and 96 months after birth.¹¹ All high-risk children and a sample of low-risk children were invited for a clinical examination at age 4 years, 8 years, or both with collection of blood for DNA extraction. Children who did not participate in a clinical examination were invited to send a buccal swab by mail. Details of the study have been published previously.¹² The study protocol was approved by the medical ethics committees of the participating institutions, and informed parental consent was obtained for each participant.

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort that recruited 14,541 pregnant women resident in Avon, United Kingdom, during 1991–1992. There were 14,062 live-born children. Study mothers were sent a questionnaire about the health of their child, including asthma symptoms, at 6, 18, 30, 42, 57, 69, 81, and 91 months after birth. Cord blood and venous blood taken at age 7 years were used for DNA extraction and creation of lymphoblastoid cell lines. Details of the study have been published previously.¹³ Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Phenotypes

In the PIAMA study wheezing phenotypes were identified by using LLCA based on questionnaire responses about the presence of wheeze in the last 12 months from birth to 8 years and will further be addressed as longitudinal wheezing phenotypes.⁷ Asthma was defined as confirmatory answers to the following questions at 8 years: “Did a doctor ever diagnose your child with asthma?” If so, “Has your child had asthma in the last 12 months?”

In ALSPAC longitudinal wheezing phenotypes were identified by using LLCA based on questionnaire responses about the presence of wheeze in the last 12 months (6 months in the first questionnaire) from birth to 8 years. A complete description and validation of these longitudinal wheezing phenotypes has been published previously.^{7,14} Asthma was defined as a confirmatory answer to the following question at 8 years: “Has your child had asthma in the past year?”

SNP selection and genotyping

Eight genes (*IL33*, *IL1RL1*, IL-1 receptor accessory protein [*IL1RAP*], *MYD88*, *TIRAP*, *IRAK1*, *IRAK4*, and *TRAF6*) from the *IL33*–*IL1RL1* pathway were selected based on data of their involvement in the pathway.^{1,3,4} Although activator protein 1, extracellular signal-regulated kinase, mitogen-activated protein kinase, and inhibitor of nuclear factor κ B (IKK) are also part of the pathway (Fig 1), these were not selected because each of these signaling proteins was encoded by multiple genes, which would increase the number of SNPs analyzed and reduce the power of our analysis because of multiple testing. In total, 104 SNPs were chosen for genotyping based on their potential functionality^{15,16}; their reported association with asthma, eosinophils, or other atopy-related diseases^{6,17–21}; or their LD with other

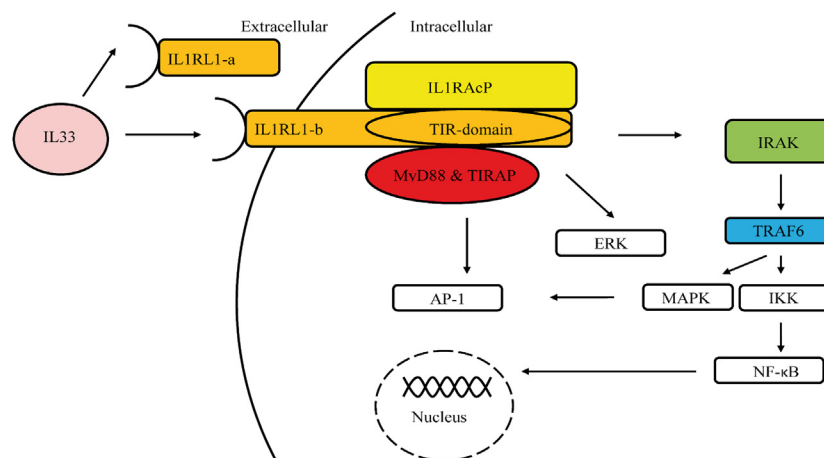


FIG 1. Schematic overview of the *IL33-IL1RL1* pathway, as adapted from Kakkar and Lee.³ Genes of colored proteins are genotyped in this study. *AP-1*, Activator protein 1; *ERK*, extracellular signal-regulated kinase; *IKK*, inhibitor of nuclear factor κ B; *MAPK*, mitogen-activated protein kinase; *NF- κ B*, nuclear factor κ B.

SNPs (as haplotype-tagging SNPs). SNPs were only selected when they had a minor allele frequency of 0.1 or greater, and haplotype-tagging SNPs were selected if the r^2 value was less than 0.8 based on reference data from HapMap phase II (Haploview 4.1).^{22,23}

In the PIAMA study DNA was extracted from blood or buccal swabs and amplified by means of primer extension before amplification or the Repli-G procedure.²⁴ Genotyping was performed in white children by using competitive allele-specific PCR with KASPar genotyping chemistry, which was performed under contract by KBiosciences (Hoddesdon, United Kingdom) with quality control, as described previously.²⁵

In ALSPAC genotyping was carried out on an Illumina HumanHap 550 Quad array (Illumina, San Diego, Calif). Quality control for the GWA study was described elsewhere.²⁶ Autosomal genotypic data were imputed by using Markov Chain Haplotyping software (MACH version 1.0.16), with the reference data from CEU subjects (HapMap release 22, Phase II NCBI B36) based on 8,365 subjects and 500,527 SNPs after quality control. After imputation, all SNPs with poor quality of imputation ($r^2 < 0.3$) were removed. Ninety-four *IL33-IL1RL1* pathway SNPs were selected in which the dosage for genetic variants was used for analysis.

Statistical analyses

In the PIAMA study 3 SNPs deviated from Hardy-Weinberg equilibrium ($P < .01$) and were excluded (rs3939286, rs10937442, and rs6796131). In the PIAMA study and ALSPAC multinomial logistic regression analyses were performed to calculate the association of SNPs with each longitudinal wheezing phenotype compared with never/infrequent wheeze. Multinomial regression analyses were weighted for posterior probabilities of phenotype membership, a probability to account for the uncertainty of phenotype membership. Logistic regression analyses were performed to analyze the association of SNPs with asthma. Additive models were assumed for all SNPs in regression analyses. Meta-analyses were performed per outcome by using a fixed-effects model because the estimate of between-studies variance would have poor precision with 2 studies. Results were not reported when heterogeneity between studies was present (Cochrane Q statistic, $P < .05$; $I^2 > 75.0\%$).

Longitudinal wheezing phenotypes described in the article were based on data of all 8 observations of wheeze to identify the phenotypes. Analyses were performed with SPSS 20.0 (SPSS, Chicago, Ill), STATA MP 11.0 (StataCorp, College Station, Tex), Plink version 1.07, and R 2.15 (packages design, rpart, globaltest, mbmdr) software.

Interaction of SNPs was analyzed for longitudinal wheezing phenotypes and asthma in PIAMA data by using model-based multifactor dimensionality reduction (MB-MDR).²⁷ MB-MDR involves a dimensionality reduction strategy that reduces a potentially high-dimensional problem to a

lower-dimensional one by pooling multilocus genotypes into 3 groups based on association test results (high, low, or no evidence for association with the trait). Initially developed for binary and quantitative traits, MB-MDR was adapted to qualitative traits for this study. More details about MB-MDR are provided in the [Methods](#) section in this article's Online Repository at www.jacionline.org. Genetic interaction models in the MB-MDR output were ranked by adjusted P value (ie, for multiple testing of all possible SNP pairs). The 10 most promising interactions were selected for further evaluation in the PIAMA study by means of regression analyses. SNP pairs that showed a tendency for interaction in the PIAMA study ($P < .1$) were also studied in ALSPAC and subsequently meta-analyzed.

Although this study contains hypothesis-based aims, false discovery rate correction was performed for multiple testing for the number of independent genetic signals ($r^2 < 0.8$), which included 83 independent signals per phenotype. Results that did not withstand multiple testing correction were also reported to minimize the chance of a type II error.

A subset of the data has been analyzed previously. Twenty-eight SNPs have been studied in 1037 children of the PIAMA study to study gene-gene interactions in the Toll-like receptor pathway for asthma and atopy as part of a combination of 3 cohorts.¹⁸ Fifteen SNPs have been analyzed in the PIAMA study to study the associations of *IL1RL1* and its gene product (IL1RL1-a), the number eosinophils in blood, and asthma.²⁸ Furthermore, 359 children in the PIAMA study and 1216 children in ALSPAC were part of the GABRIEL study.⁶

RESULTS

In the PIAMA study 2099 children had DNA available for genetic analysis, and after removal of samples of poor quality, 2007 (96%) had genotypic data on the *IL33-IL1RL1* pathway (Table I). In this study population 51.6% were male, and 38.3% had an allergic mother. In ALSPAC 7247 children (87% of the total population with genotypes) were of a white ethnic background, as reported by questionnaire, and had genotypic data available for the *IL33-IL1RL1* pathway, constituting the study population of ALSPAC (Table I). Of these children, 51.2% were male, and 44.3% had an allergic mother.

Longitudinal wheezing phenotypes were highly comparable between the PIAMA study and ALSPAC, yet prolonged early wheeze was not present in the PIAMA study but had a prevalence of 9.3% in ALSPAC.⁷ The prevalences of late-onset and persistent wheeze were lower in the PIAMA study compared with ALSPAC (1.7% and 3.9% vs 4.8% and 5.7%, respectively). The prevalence

TABLE I. Study population in the PIAMA study and ALSPAC

	PIAMA (n = 2007)		ALSPAC (n = 7247)	
Male sex (% [n/total])	51.6	(1035/2007)	51.2	(3714/7247)
Maternal history of allergy (% [n/total])	38.3	(769/2007)	44.3	(3074/6944)
Maternal history of asthma (% [n/total])	9.4	(189/2003)	11.2	(791/7042)
Wheezing phenotypes (8 observations; % [n/total])				
Never/infrequent wheeze	74.0	(1252/1691)	61.2	(2497/4078)
Transient early wheeze	17.1	(288/1691)	16.6	(677/4078)
Prolonged early wheeze	NA	NA	9.3	(378/4078)
Intermediate-onset wheeze	3.3	(55/1691)	2.3	(95/4078)
Late-onset wheeze	1.7	(29/1691)	4.8	(197/4078)
Persistent wheeze	3.9	(66/1691)	5.7	(233/4078)
Asthma in last 12 mo at 8 y (% [n/total])	4.0	(75/1869)	11.2	(595/5332)
Use of asthma medication in last 12 mo at 8 y (% [n/total])	12.4	(231/1870)	12.6	(662/5263)
Positive skin prick test response against HDM at 8/7 y (% [n/total])	12.8	(112/872)	12.2	(597/4898)
Specific IgE levels ≥ 0.35 IU/L against HDM, cat, or dog at 8 y (% [n/total])	23.6	(367/1552)	NA	NA
No. of eosinophils $\times 10^9$ /L (median [10th-90th percentile])	0.30 (0.11-0.79)		NA	
	n = 800			

HDM, House dust mite; NA, data not available.

of asthma at 8 years was lower in the PIAMA study than in ALSPAC (4.0% vs 11.2%, respectively), possibly because of a more stringent definition in the PIAMA study. All 104 SNPs are described in Table E1 in this article's Online Repository at www.jacionline.org.

Association *IL33-IL1RL1* pathway SNPs with wheezing phenotypes

Table II and Fig 2 show nominally significant meta-analysis results of the longitudinal wheezing phenotypes. Association analyses of all SNPs in the PIAMA study and ALSPAC and the meta-analysis results are reported per phenotype in Table E2 in this article's Online Repository at www.jacionline.org.

We observed that different wheezing phenotypes had different effect estimates of association with *IL33-IL1RL1* pathway SNPs in meta-analyses after correction for multiple testing. Intermediate-onset wheeze was associated with SNPs of 3 genes in the *IL33-IL1RL1* pathway: 2 *IL33* SNPs (rs4742170 and rs7037276), 1 *IL1RAP* SNP (rs10513854, Fig 2), and 1 *TRAF6* SNP (rs5030411). Late-onset wheeze was associated with 2 *IL1RL1* SNPs (rs10208293 and rs13424006), and persistent wheeze was significantly associated with 1 *IL33* SNP (rs1342326) and 1 *IL1RAP* SNP (rs9290936). Eleven associations did not remain significant after correction for multiple testing. Transient early wheeze was nominally associated with 2 *IRAK4* SNPs (rs4251520 and rs4251513). Intermediate-onset wheeze was additionally associated with 4 *IL33* SNPs and 4 *IL1RAP* SNPs. Three *IL1RL1* SNPs (rs10204137, rs13424006, and rs10208293) showed a trend for association ($P < .1$) with late-onset wheeze in both the PIAMA study and ALSPAC (see Table E2).

Association *IL33-IL1RL1* pathway SNPs with asthma

Three *IL33* and 4 *IL1RL1* SNPs were associated with asthma at age 8 years ($P < .05$), yet they did not remain significant after correction for multiple testing. Rs10208293 (*IL1RL1*) was nominally associated with asthma in both the PIAMA study and ALSPAC (Table III). The results of all SNPs in the PIAMA study and ALSPAC and the meta-analysis are reported in Table E3 in this article's Online Repository at www.jacionline.org.

Interaction of SNPs in the *IL33-IL1RL1* pathway

Within the 10 most promising interactions for longitudinal wheezing phenotypes identified by using MB-MDR, 4 SNP pairs showed a tendency for interaction with a longitudinal wheezing phenotype in regression analysis in the PIAMA study ($P < .08$). None of these SNP pairs showed significant interaction for wheezing phenotypes in ALSPAC or in the meta-analysis (see Table E4 in this article's Online Repository at www.jacionline.org).

Within the most promising interactions for asthma identified by using MB-MDR, 3 *IL1RAP* SNP pairs showed evidence of interaction in regression analysis in the PIAMA study ($P < .05$): rs1988743*rs9847868, rs3773980*rs4687153, and rs1988743*rs4320092. One SNP pair could not be analyzed in ALSPAC because rs3773980 was not available. The interaction effect of the 2 other SNP pairs was heterogeneous between the PIAMA study and ALSPAC (see Table E5 in this article's Online Repository at www.jacionline.org).

Given the strong biological rationale for interaction of *IL33* and *IL1RL1*, we determined the increased risk for asthma in children carrying both risk alleles of *IL33* and *IL1RL1* (Table III), as described previously.²⁹ Selected SNPs were genotyped in both cohorts and were not in high LD ($r^2 < 0.8$) with each other (rs1342326, rs17498196, rs13431828, rs10208293, and rs10204137). Subjects with a recessive genotype for rs10204137 (*IL1RL1*) and rs1342326 (*IL33*) had a higher risk for asthma than other combinations of both SNPs in both the PIAMA study and ALSPAC and in the meta-analysis (odds ratios of 4.03 [95% CI, 0.88-18.41], 2.42 [95% CI, 1.30-4.52], and 2.60 [95% CI, 1.46-4.64], respectively; Fig 3).

DISCUSSION

This study in 2 large European birth cohorts showed evidence for association of specific longitudinal wheezing phenotypes with distinct *IL33-IL1RL1* pathway polymorphisms. Most significant associations were observed for intermediate and late-onset wheeze. Associations with longitudinal wheezing phenotypes mainly involved polymorphisms in the genes encoding the ligand and receptor complex (*IL33*, *IL1RAP*, and *IL1RL1*) but not genes encoding adaptor or signaling molecules. Second, asthma was also associated with SNPs in *IL33* and *IL1RL1* but not with other genes of this pathway. Third, we used a new implementation method for MB-MDR that shows evidence for gene-gene interaction, yet none of the selected SNP pairs showed multiplicative interaction in the meta-analysis of asthma and longitudinal wheezing phenotypes. However, for the first time, we observed replicable evidence for additive interaction between *IL33* and *IL1RL1* SNPs for asthma.

TABLE II. Significant meta-analysis results of *IL33-IL1RL1* pathway SNPs with wheezing phenotypes

SNP (gene)	Transient early wheeze			Intermediate-onset wheeze			Late-onset wheeze			Persistent wheeze		
	OR (95% CI)	P value	No. of cases	OR (95% CI)	P value	No. of cases	OR (95% CI)	P value	No. of cases	OR (95% CI)	P value	No. of cases
rs4251520 (<i>IRAK4</i>)	0.83 (0.72-0.94)	.006	957	0.86 (0.62-1.19)	.359	149	1.12 (0.82-1.54)	.490	226	1.02 (0.79-1.33)	.864	297
rs4251513 (<i>IRAK4</i>)	0.90 (0.82-0.99)	.022	961	0.98 (0.78-1.23)	.875	150	0.94 (0.79-1.13)	.545	224	0.94 (0.80-1.09)	.418	298
rs1342326 (<i>IL33</i>)	0.96 (0.84-1.09)	.502	959	0.76 (0.61-0.95)	.016	149	X	X	X	0.75 (0.63-0.89)	.001*	298
rs2066362 (<i>IL33</i>)	0.94 (0.83-1.07)	.376	962	0.77 (0.61-0.97)	.024	149	X	X	X	0.89 (0.72-1.08)	.235	298
rs17498196 (<i>IL33</i>)	0.93 (0.84-1.05)	.221	958	0.76 (0.62-0.93)	.006	150	0.83 (0.68-0.99)	.039	226	0.97 (0.79-1.19)	.736	299
rs4742170 (<i>IL33</i>)	1.00 (0.89-1.11)	.977	962	0.72 (0.59-0.86)	.001*	150	X	X	X	1.02 (0.86-1.22)	.805	298
rs7037276 (<i>IL33</i>)	0.89 (0.75-1.07)	.212	965	0.53 (0.39-0.72)	<.001*	149	0.88 (0.64-1.22)	.441	226	0.81 (0.62-1.06)	.121	299
rs8172 (<i>IL33</i>)	0.97 (0.81-1.16)	.741	959	0.66 (0.47-0.93)	.016	150	0.99 (0.70-1.38)	.936	226	0.92 (0.69-1.22)	.548	298
rs9290936 (<i>IL1RAP</i>)	0.92 (0.80-1.06)	.265	964	0.93 (0.67-1.28)	.642	150	1.06 (0.78-1.46)	.702	225	0.72 (0.61-0.85)	<.001*	299
rs10513854 (<i>IL1RAP</i>)	0.93 (0.81-1.06)	.250	958	0.67 (0.55-0.83)	<.001*	150	1.02 (0.76-1.36)	.902	225	1.02 (0.79-1.31)	.885	298
rs9813227 (<i>IL1RAP</i>)	0.99 (0.86-1.13)	.849	961	0.71 (0.55-0.92)	.010	150	0.94 (0.73-1.22)	.658	226	1.11 (0.86-1.43)	.428	299
rs7626795 (<i>IL1RAP</i>)	0.97 (0.84-1.13)	.716	961	0.72 (0.57-0.92)	.008	150	1.14 (0.80-1.64)	.467	226	0.95 (0.74-1.22)	.688	298
rs9847868 (<i>IL1RAP</i>)	1.02 (0.90-1.16)	.780	961	0.79 (0.63-0.98)	.036	150	1.09 (0.83-1.44)	.539	226	0.87 (0.73-1.04)	.132	298
rs9831803 (<i>IL1RAP</i>)	1.03 (0.89-1.18)	.728	961	0.75 (0.60-0.93)	.008	149	1.19 (0.84-1.69)	.319	225	0.86 (0.71-1.04)	.127	298
rs5030411 (<i>TRAF6</i>)	0.94 (0.86-1.04)	.251	957	0.77 (0.64-0.93)	.005*	148	1.02 (0.83-1.25)	.871	226	0.96 (0.81-1.13)	.584	299
rs10208293 (<i>IL1RL1</i>)	0.98 (0.88-1.09)	.715	962	0.83 (0.66-1.04)	.105	150	0.74 (0.62-0.87)	<.001*	226	X	X	X
rs13424006 (<i>IL1RL1</i>)	0.99 (0.89-1.10)	.871	954	0.90 (0.73-1.13)	.373	149	0.74 (0.63-0.87)	<.001*	224	X	X	X

Nominal significant associations are shown in boldface.

OR, Odds ratio; X, associations with significant heterogeneity between the studies in the meta-analysis ($P < .05$ for Cochran Q statistic, $I^2 > 75.0\%$, or both).

*Associations that were significant after correction for multiple testing with false discovery rate.

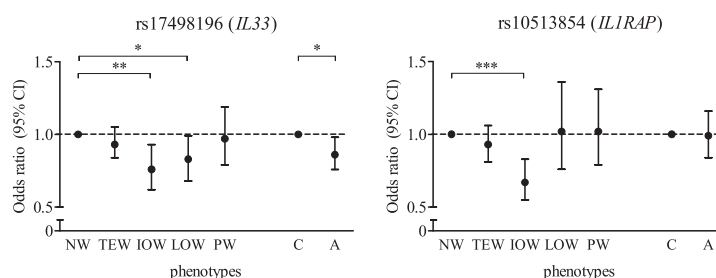


FIG 2. Odds ratios of meta-analyses of association of rs17498196 (*IL33*) and rs10204137 (*IL1RL1*) with longitudinal wheezing phenotypes and asthma at 8 years. A, Asthmatic children at age 8 years; C, children without asthma at age 8 years; IOW, intermediate-onset wheeze; LOW, late-onset wheeze; NW, never wheeze; PW, persistent wheeze; TEW, transient early wheeze. * $P < .1$, ** $P < .01$, and *** $P < .001$.

Our results confirm previous findings that *IL1RL1* and *IL33* SNPs partly predict longitudinal wheezing phenotypes.⁸ Intermediate-onset and late-onset wheeze were associated with several *IL33-IL1RL1* polymorphisms. A common factor between these phenotypes is that they are associated with early sensitization (4 years old or less) because greater than 70% of the children with intermediate- and late-onset wheeze had increased specific IgE levels against common allergens at age 4 years in the PIAMA study.⁷ The strong relation of intermediate-onset wheeze with atopy was confirmed in the Southampton Women's Study cohort, which allocated longitudinal wheezing phenotypes to 926 children based on their wheeze patterns in childhood. Intermediate-onset wheeze was already associated with sensitization at age 1 year, whereas late-onset wheeze and persistent wheeze were associated with sensitization at age 3 years but not at age 1 year.³⁰ Therefore we speculate that *IL33-IL1RL1* pathway polymorphisms might be affecting the development of wheeze, leading to intermediate- or late-onset wheeze and subsequent asthma through sensitization in early childhood.

Persistent wheeze was associated with 1 *IL33* SNP and 1 *IL1RAP* SNP. This is the first study showing that a polymorphism

in the gene encoding the coreceptor of *IL1RL1* is associated with childhood wheeze. Several factors contribute to persistent wheeze, including atopy, reduced airway growth, and 17q12-21 polymorphisms.^{7,9,30} This suggests that different combinations of pathophysiologic processes are represented in different longitudinal wheezing phenotypes and that *IL33-IL1RL1* pathway polymorphisms are important for some, but not all, such phenotypes. Remarkably, we observed larger effect sizes on specific wheezing phenotypes compared with effects on asthma. This suggests that specific wheezing phenotypes are more homogeneous phenotypes than asthma for studying the *IL33-IL1RL1* pathway. Definitions of more severe asthma, such as severe doctor-diagnosed asthma in the last year based on a minimum of 3 attacks per year, might further contribute to study the *IL33-IL1RL1* pathway.

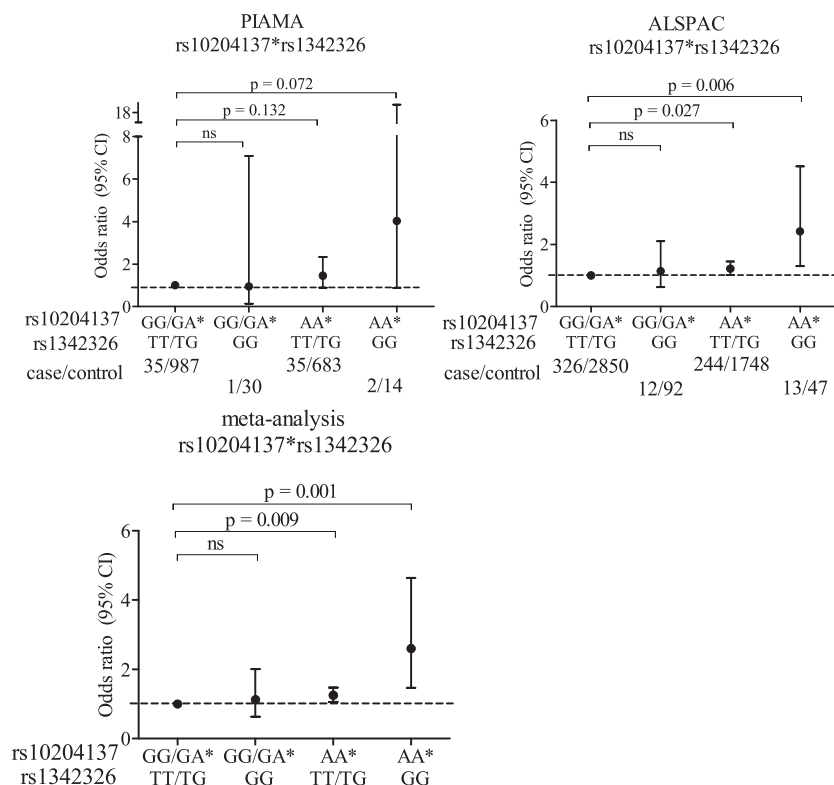
Our reported associations with asthma were comparable with published GWA results of asthma, with consistent direction and similar effect estimates.^{6,31} The high-LD region *IL1RL1/IL18R1*, containing SNPs of *IL1RL1* (rs10204137 rs13424006) and *IL18R1* (rs3771166), was associated with asthma in our study, confirming previous GWA studies.^{6,32,33} Within this LD block,

TABLE III. Significant associations of *IL33-IL1RL1* pathway SNPs with asthma in the PIAMA study and ALSPAC or in the meta-analysis

SNP (gene)	Risk allele	PIAMA asthma			ALSPAC asthma			Meta-analysis asthma		
		OR (95% CI)	P value	No.	OR (95% CI)	P value	No.	OR (95% CI)	P value	P value, heterog
rs1342326 (<i>IL33</i>)	A	1.13 (0.70-1.82)	.609	1825	0.78 (0.67-0.90)	.001	5332	0.80 (0.70-0.93)	.003	.139
rs2066362 (<i>IL33</i>)	G	1.18 (0.72-1.93)	.508	1810	0.83 (0.71-0.97)	.019	5332	0.86 (0.74-1.00)	.042	.180
rs17498196 (<i>IL33</i>)	A	1.11 (0.74-1.66)	.625	1832	0.84 (0.73-0.96)	.010	5332	0.86 (0.76-0.98)	.022	.199
rs13431828 (<i>IL1RL1</i>)	C	2.61 (1.13-5.99)	.024	1832	1.17 (0.97-1.40)	.098	5332	1.21 (1.01-1.45)	.036	.064
rs10208293 (<i>IL1RL1</i>)	A	0.59 (0.38-0.93)	.022	1844	0.85 (0.74-0.97)	.017	5332	0.82 (0.72-0.94)	.003	.135
rs13424006 (<i>IL1RL1</i>)	C	0.79 (0.55-1.13)	.198	1813	0.85 (0.75-0.96)	.011	5332	0.84 (0.75-0.95)	.005	.712
rs10204137 (<i>IL1RL1</i>)	A	1.30 (0.90-1.86)	.157	1831	1.18 (1.04-1.34)	.011	5332	1.19 (1.06-1.34)	.004	.624

Nominal significant associations are shown in boldface. None of the SNPs remained significantly associated with asthma after correction for multiple testing with false discovery rate.

OR, Odds ratio; *P* value heterog, *P* value for Cochran Q statistic to test heterogeneity between the studies in the meta-analysis.

**FIG 3.** Additive interaction of rs10204137 (*IL1RL1*) and rs1342326 (*IL33*) genotypes in a recessive model for asthma at age 8 years. *ns*, Not significant.

rs10204137 has been identified as an expression quantitative trait locus (eQTL) associated with *IL1RL1* mRNA levels in lymphoblasts and as eQTL locus for *IL18R1* mRNA levels in fat tissue.¹ Part of this LD block encodes the Toll-IL-1 receptor (TIR) domain in the intracellular part of *IL1RL1*, which plays a crucial role in signal transduction because it connects to *IL1RAcP* and interacts with MyD88 and TIRAP for signal transduction (Fig 1).¹ A recent study has shown that missense SNPs in the TIR domain of *IL1RL1* (eg, rs10204137) increase the *IL1RL1*-a level directly and through induction of *IL-33*. MyD88 and MAL are necessary for this *IL1RL1*-b-mediated *IL1RL1*-a production. These SNPs also enhance the proximal and distal promoter activity of *IL1RL1*.³⁴ Taken together, missense SNPs in the TIR domain of *IL1RL1*, such as rs10204137, enhance the *IL33-IL1RL1* pathway, leading to increased *IL1RL1*-a production (Fig 1).

Polymorphisms associated with asthma or longitudinal wheezing phenotypes known to result in asthma were located mainly in genes encoding the ligand *IL-33*, the receptor *IL1RL1*, and the receptor-associated protein *IL1RAcP* and not the adaptors or downstream signaling proteins. These results suggest that genetic variation in the ligand receptor-coreceptor complex, but not the downstream adaptor or signaling molecules, is driving the association with asthma. We think that this finding has implications for developing asthma treatments based on the *IL33-IL1RL1* pathway and that future studies should focus on the effects of genetic variance in the ligand receptor complex that can diminish the lung inflammation in asthma. A functional study showed that the membrane receptor *IL1RL1*-b can be degraded by proteosomal activity in response to *IL-33* through binding of the specific ubiquitin ligase FBXL19.³⁵ This process

is facilitated by means of phosphorylation of IL1RL1-b. Overexpression of FBXL19 effectively attenuated pulmonary infiltration induced by intratracheal challenge with IL-33 and blocked apoptosis in a mouse model, indicating that a small molecule that enhances or mimics the actions of the specific ubiquitin ligase in the airways might be a medical treatment for asthma in human subjects.³⁵

We did not observe replicable multiplicative gene-gene interaction of *IL33-IL1RL1* pathway SNPs for asthma or longitudinal wheezing phenotypes. We applied MB-MDR and developed a new application to study multinomial outcomes. Our finding is in line with the results of the GABRIEL study.⁶ The lack of multiplicative interactions in regression analyses of promising interactions identified by means of MB-MDR might have several reasons. It could be that the power of our study was insufficient to detect these interactions, that no genetic interactions were present within the *IL33-IL1RL1* pathway, or that the multiplicative interaction effect of risk alleles is not the right statistical model to analyze interactions in this pathway.^{36,37} Our observations of an additive interaction of rs10204137 and rs1342326 for asthma should be further replicated and supplemented by knowledge of their precise biological mechanisms before we can draw definitive conclusions about their biological interaction.

There are strengths and limitations to this study. A strength is that it contains data from 2 large European birth cohorts with extensive data collection and longitudinally defined, highly comparable phenotypes.^{7,12,13} Moreover, we developed and applied the innovative method MB-MDR for multinomial traits to detect gene-gene interaction. We hypothesized that SNPs in *IL33* might act as eQTLs,¹ but for other genes in this pathway, the biological significance of SNPs remains unknown because the function of these SNPs has not been studied thus far. The prevalence of intermediate-onset and late-onset wheeze is low, especially in the PIAMA birth cohort, leading to limited power to detect significant genetic associations. Therefore it is even more remarkable that we find strong associations when we combine the PIAMA study and ALSPAC in the meta-analyses.

Some of our findings did not withstand multiple testing correction. Nevertheless, our aims and hypotheses were strongly driven by previous findings and literature, which reduces the probability of false-positive findings. SNPs and outcomes (wheezing phenotypes and asthma) were related to each other, indicating that a conservative statistical procedure based on assumption of independence will underestimate true interaction. Therefore we adjusted for the correlation of SNPs by the number of genetic signals ($r^2 < 0.8$). Adjustment for multiple testing increases the chance of a type II error, so that a true association is not found, which is an undesirable situation.

In conclusion, this study confirms that *IL33-IL1RL1* pathway polymorphisms are associated with asthma and adds to the knowledge that *IL33-IL1RL1* pathway polymorphisms are associated with specific wheezing phenotypes, especially intermediate-onset wheeze. We speculate that the *IL33-IL1RL1* pathway might affect wheeze and subsequent asthma development through allergic sensitization development in childhood. Finally, we provide evidence for additive gene-gene interaction between SNPs in *IL33* and *IL1RL1* for asthma. Because the *IL33-IL1RL1* pathway has been proposed as a potential drug target,³ future studies might provide insight into how

polymorphisms in the *IL33-IL1RL1* pathway function and affect asthma development in childhood.

We thank all the families who took part in the PIAMA or ALSPAC birth cohorts and all the persons working to collect, measure, and manage the data in the cohorts. We thank Alison Teyhan for help with the ALSPAC data management for this study.

Key messages

- *IL33* SNPs are mostly associated with intermediate-onset wheeze, and *IL1RL1* SNPs are mostly associated with late-onset wheeze.
- *IL33-IL1RL1* pathway SNPs that are associated with wheezing phenotypes are mostly located in *IL33*, *IL1RL1*, and *IL1RAP*.

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