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 *(IL1RAP)* 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 *IL1RAP* 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*

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

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