Predicting asthma phenotypes: characterization of IL1RL1 in asthma

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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

Chapter 3

Abstract

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 (rs10513864), 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.
# Abbreviations

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

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 \textit{IL33} and \textit{IL1RL1/IL18R1} loci are important for asthma development.\textsuperscript{1} Genetic studies have not been able to disentangle which gene(s) at the \textit{IL1RL1/IL18R1} locus cause(s) asthma due to strong linkage disequilibrium (LD) in this region. However, recent Bayesian network analyses of asthma-associated SNPs that regulate gene expression in lung tissue suggest that \textit{IL1RL1} is most likely causally implicated in asthma development.\textsuperscript{2}

Proteins encoded by \textit{IL33} and \textit{IL1RL1} are part of the \textit{IL33-IL1RL1} pathway (Figure 1). Interleukin 33 (\textit{IL33}) has been implicated as an alarm signal for epithelial damage, and is released in response to triggers as allergens or infectious agents.\textsuperscript{1,3,4} After release of \textit{IL33}, it binds to its receptor Interleukin-1 Receptor-Like 1 (IL1RL1-a), which forms a receptor complex with Interleukin-1 Receptor-Associated Protein (IL1RACP). This receptor complex induces, via activation of signaling proteins like Myeloid Differentiation Primary Response Gene 88 (MYD88), Toll-Interleukin 1 Receptor (TIR) Domain Containing Adaptor Protein (TIRAP), Interleukin-1 Receptor-associated Kinase 1 and 4 (IRAK1 and IRAK4) and TNF Receptor-associated Factor 6 (TRAF6) release of allergic and eosinophilic mediators, like IL-5, IL-13, resulting in eosinophilic inflammation.\textsuperscript{1,3,4} Alternatively, \textit{IL33} can bind to the soluble receptor IL1RL1-a, which acts as a decoy receptor for \textit{IL33}, resulting in attenuation of the \textit{IL33} signal.\textsuperscript{3} These data show that involvement of the \textit{IL33-IL1RL1} pathway in asthma is biologically plausible. However, besides the ligand \textit{IL33} and its receptor \textit{IL1RL1}, genes encoding other proteins in this pathway may also play a role in asthma. Moreover, genes in this pathway might well interact to contribute to asthma development. So far this has not been studied.

The period early in life is important for asthma development, and certain gene variants may be associated with asthma or wheezing phenotypes with a specific age of onset.\textsuperscript{5} In the GABRIEL consensus GWA meta-analysis, polymorphisms in \textit{IL1RL1} and \textit{IL33} were more strongly associated with early-onset asthma (<16 years) than late-onset asthma (≥16 years), although the difference was not significant.\textsuperscript{4} As 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)\textsuperscript{7}, may provide insight in 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.\textsuperscript{8,9} Our aims are therefore to investigate the association and gene-gene interaction of \textit{IL33-IL1RL1} pathway SNPs with longitudinal wheezing phenotypes in childhood and asthma at 8 years.
Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood

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

Methods

Study cohorts: PIAMA and ALSPAC
The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study is a Dutch multicenter birth cohort that invited 2779 allergic and 5083 non-allergic women to participate in the study; 4146 agreed (53%) and gave written informed consent (1327 allergic and 2819 non-allergic). There were 3963 live-born children. Parents were sent ISAAC-based questionnaires about their child’s health including asthma symptoms at 3, 12, 24, 36, 48, 60, 72, 84 and 96 months after birth. All high-risk children and a sample of low-risk children were invited for a clinical examination at age 4 and/or 8 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. The study protocol was approved by medical ethics committees of the participating institutions and informed parental consent was obtained for each participant.

Avon Longitudinal Study of Parents And Children (ALSPAC) is a population-based birth cohort that recruited 14541 pregnant women resident in Avon, UK, during 1991-1992. There were 14062 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. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.
Phenotypes

In PIAMA, wheezing phenotypes were identified by LLCA based on questionnaire responses about 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 parental confirmative answers to the questions at 8 years: “Did a doctor ever diagnose your child with asthma?” and if so, “Has your child had asthma in the last 12 months?”.

In ALSPAC, longitudinal wheezing phenotypes were identified by LLCA based on questionnaire responses about 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 confirmative answer to the question “Has your child had asthma in the past year?” at 8 years.

SNP selection and genotyping

Eight genes (IL33, IL1RL1, IL1RAP, MYD88, TIRAP, IRAK1, IRAK4 and TRAF6) from the IL33-IL1RL1 pathway were selected based on the published data of their involvement in the pathway.1,3,4 Although AP-1, ERK, MAPK and IKK are also part of the pathway (Figure 1), these were not selected because each of these signaling proteins were encoded by multiple genes which would increase the number of SNPs analyzed and reduce the power of our analysis due to multiple testing. In 8 selected genes, 104 SNPs were chosen for genotyping based on their potential functionality15,16, their reported association with asthma, eosinophils or other atopy-related diseases6,7-21, or their LD with other SNPs (as haplotype-tagging SNPs). SNPs were only selected when they had a minor allele frequency of ≥0.1 and haplotype-tagging SNPs were selected if r²<0.8 based on reference data from HapMap phase II (Haploview 4.1).22,23

In PIAMA, DNA was extracted from blood or buccal swab and amplified by primer extension pre-amplification (PEP) or Repli-G procedure.24 All genotyping was performed in Caucasian children by Competitive Allele-Specific PCR using KASPar™ genotyping chemistry, performed under contract by KBiosciences (Hoddesdon, UK) with quality control as described previously.25

In ALSPAC, genotyping was carried out on an Illumina HumanHap 550 quad array. Quality control for the GWA study was described elsewhere.26 Autosomal genotypic data were imputed using Markov Chain Haplotyping software (MACH v.1.0.16) with the reference data from CEU individuals (Hapmap release 22, Phase II NCBI B36) based on 8365 individuals and 500527 SNPs after quality control. After imputation, all SNPs with poor quality of imputation (r²<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 PIAMA, 3 SNPs deviated from Hardy-Weinberg equilibrium (p<0.01) and were excluded (rs3939286, rs10937442, rs6796131). In PIAMA and ALSPAC, multinomial logistic regression analyses were performed to calculate the association of SNPs with each longitudinal wheezing phenotype, compared to 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 using a fixed-effect model, because the estimate of between-studies variance would have poor precision with two studies. Results were not reported when heterogeneity between studies was present (Cochrane’s Q-statistic p<0.05, I² >75.0%).
Longitudinal wheezing phenotypes described in the manuscript were based on data of complete data of wheeze (all 8 observations) to identify the phenotypes. Longitudinal wheezing phenotypes based on minimal 2 observations were also analyzed, and were comparable to the presented results. Analyses were performed using SPSS 20.0, STATA MP 11.0, Plink v1.07 and R 2.15 (packages design, rpart, globaltest, mbmdr).

Interaction of SNPs was analyzed for longitudinal wheezing phenotypes and asthma using Model-Based Multifactor Dimensionality Reduction (MB-MDR) on PIAMA-data.\(^{27}\) MB-MDR involves a dimensionality reduction strategy that reduces a potentially high dimensional problem to a lower-dimensional one by pooling multi-locus genotypes into three 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 Online Repository. Genetic interaction models in the MB-MDR output were ranked by adjusted p-value (i.e. for multiple testing of all possible SNP pairs). The 10 most promising interactions were selected for further evaluation in PIAMA by regression analyses. SNP pairs that showed a tendency for interaction in PIAMA (p<0.1), were also studied in ALSPAC and subsequently meta-analyzed.

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

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

**Results**

In PIAMA, n=2099 children had DNA available for genetic analysis and after removal of samples of poor quality, n=2007 (96%) had genotypic data of the IL33-IL1RL1 pathway (Table 1). In this study population, 51.6% were male, 38.3% had an allergic mother. In ALSPAC, n=7247 children (87% of the total population with genotypes) had white ethnic background reported by questionnaire and had genotypic data available of the IL33-IL1RL1 pathway, constituting the study population of ALSPAC (Table 1). Of these children, 51.2% were male and 44.3% had an allergic mother.
Table 1. Study population in the PIAMA study and ALSPAC.

<table>
<thead>
<tr>
<th></th>
<th>PIAMA (N = 2007)</th>
<th>ALSPAC (N = 7242)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male; % (n/total)</td>
<td>51.6 (1035/2007)</td>
<td>51.2 (3714/7247)</td>
</tr>
<tr>
<td>Maternal history of allergy; % (n/total)</td>
<td>38.3 (769/2007)</td>
<td>44.3 (3074/6944)</td>
</tr>
<tr>
<td>Maternal history of asthma; % (n/total)</td>
<td>9.4 (180/2002)</td>
<td>11.2 (791/7042)</td>
</tr>
<tr>
<td>Wheezing phenotypes (8 observations; % (n/total))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/infrequent wheeze</td>
<td>74.0 (1252/1691)</td>
<td>61.2 (2457/4078)</td>
</tr>
<tr>
<td>Transient early wheeze</td>
<td>17.1 (288/1691)</td>
<td>15.6 (677/4078)</td>
</tr>
<tr>
<td>Prolonged early wheeze</td>
<td>NP</td>
<td>9.3 (278/4078)</td>
</tr>
<tr>
<td>Intermediate onset wheeze</td>
<td>3.3 (55/1691)</td>
<td>2.3 (95/4078)</td>
</tr>
<tr>
<td>Late onset wheeze</td>
<td>1.7 (29/1691)</td>
<td>4.8 (197/4078)</td>
</tr>
<tr>
<td>Persistent wheeze</td>
<td>3.9 (66/1691)</td>
<td>5.7 (235/4078)</td>
</tr>
<tr>
<td>Asthma in last 12 months at 8 yrs; % (n/total)</td>
<td>4.0 (75/1669)</td>
<td>11.2 (595/5332)</td>
</tr>
<tr>
<td>Use of asthma medication in last 12 months at 8 yrs; % (n/total)</td>
<td>12.4 (231/1870)</td>
<td>12.6 (662/5265)</td>
</tr>
<tr>
<td>Positive skin prick test against HDM at 8 yrs; % (n/total)</td>
<td>12.8 (112/872)</td>
<td>12.2 (597/4806)</td>
</tr>
<tr>
<td>Specific IgE levels ≥0.35 IU/L against HDM, cat or dog at 8 yrs; % (n/total)</td>
<td>23.6 (361/1552)</td>
<td>NP</td>
</tr>
<tr>
<td>Number of eosinophils ×10^9/L, median (10th - 90th percentile)</td>
<td>0.30 (0.11 - 0.79) (n=800)</td>
<td>NP</td>
</tr>
</tbody>
</table>

Longitudinal wheezing phenotypes were highly comparable between PIAMA and ALSPAC, yet prolonged early wheeze was not present in PIAMA but had a prevalence of 9.3% in ALSPAC. The prevalences of late onset wheeze and persistent wheeze were lower in PIAMA compared to ALSPAC (1.7% and 3.9% vs. 4.8% and 5.7% respectively). The prevalence of asthma at 8 years was lower in PIAMA than in ALSPAC (4.0% vs. 11.2% respectively), possibly due to a more stringent definition in PIAMA. A description of all 104 SNPs is reported in Table E1 of the Online Repository.

**Association IL33-IL1RL1 pathway SNPs with wheezing phenotypes**

Table 2 and Figure 2 show nominally significant meta-analyses results of the longitudinal wheezing phenotypes. Association analyses of all SNPs in PIAMA and ALSPAC, and the meta-analysis results are reported per phenotype in Table E2 of the Online Repository.

We observed that different wheezing phenotypes had different effect estimates of association with IL33-IL1RL1 pathway SNPs in the meta-analyses after correction for multiple testing. Intermediate onset wheeze was associated with SNPs of three genes in the IL33-IL1RL1 pathway; two IL33 SNPs (rs4742170, rs7037276), one IL1RAP SNP (rs10513854, Figure 2) and one TRAF6 SNP (rs5030411). Late onset wheeze was associated with two IL1RL1 SNPs (rs10208293, rs13424006), and persistent wheeze was significantly associated with one IL33 SNP (rs1342326) and one IL1RAP SNP (rs9290936). Eleven associations did not remain significant after correction for multiple testing. Transient early wheeze was nominally associated with two IRAK4 SNPs (rs14251520, rs4251513). Intermediate onset wheeze was additionally associated with 4 IL33 SNPs and 4 SNPs in IL1RAP. Three IL1RL1 SNPs (rs10204137, rs13424006, rs10208293) showed a trend for association (p<0.1) with late onset wheeze in both PIAMA and ALSPAC (Table E2).
Association of IL33-IL1 receptor-like 1 (IL1RL1) pathway SNPs with asthma

Three IL33 and four IL1RL1 SNPs were associated with asthma at 8 years (p < 0.05), yet they did not remain significant after correction for multiple testing. Rs10208293 (IL1RL1) was nominally associated with asthma in both PIAMA and ALSPAC (Table 3). The results of all SNPs in PIAMA and ALSPAC, and the meta-analysis are reported in Table E3 of the Online Repository.

Interaction of SNPs in the IL33-IL1RL1 pathway

Within 10 most promising interactions for longitudinal wheezing phenotypes identified by MB-MDR, four SNP pairs showed a tendency for interaction with one of the longitudinal wheezing phenotypes in regression analysis in PIAMA (p<0.08). None of these SNP pairs showed significant interaction for wheezing phenotypes in ALSPAC, nor in the meta-analysis (Table E4 of the Online Repository).

Within the most promising interactions for asthma identified by MB-MDR, three IL1RAP SNP pairs showed evidence of interaction in regression analysis in PIAMA (p<0.05); 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 two other SNP pairs was heterogeneous between PIAMA and ALSPAC (Table E5 of the Online Repository).

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 2), as described previously. Selected SNPs were genotyped in both cohorts and were not in high LD (r²>0.8) with each other (rs1342326, rs17498196, rs13431828, rs10208293, rs10204137). Individuals with a recessive genotype for rs10204137 (IL1RL1) and rs1342326 (IL33) had a higher risk for asthma than other combinations of both SNPs, in both PIAMA and ALSPAC, and in the meta-analysis (OR (95% CI) = 4.03 (0.88 - 18.41), 2.42 (1.30 - 4.52) and 2.60 (1.46 - 4.64) respectively; Figure 3).
Table 2. Significant meta-analysis results of IL33-IL1RL1 pathway SNPs with wheezing phenotypes.

<table>
<thead>
<tr>
<th>SNP (gene)</th>
<th>Transient early wheeze</th>
<th>Intermediate onset wheeze</th>
<th>Late onset wheeze</th>
<th>Persistent wheeze</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs42551520 (IL1RA4)</td>
<td>0.83 (0.72 - 0.94)</td>
<td>0.96 (0.81 - 1.11)</td>
<td>1.22 (1.04 - 1.43)</td>
<td>0.90 (0.79 - 1.03)</td>
</tr>
<tr>
<td>rs4255151 (IL1RA4)</td>
<td>0.80 (0.72 - 0.94)</td>
<td>0.97 (0.83 - 1.12)</td>
<td>1.19 (1.02 - 1.39)</td>
<td>0.88 (0.78 - 1.00)</td>
</tr>
<tr>
<td>rs1542336 (IL1RL1)</td>
<td>0.99 (0.87 - 1.12)</td>
<td>0.97 (0.83 - 1.12)</td>
<td>1.00 (0.88 - 1.12)</td>
<td>0.98 (0.86 - 1.10)</td>
</tr>
</tbody>
</table>

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 Cochrane Q statistic, I2 > 75.0%, or both).

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

Table 3. Significant associations of IL33-IL1RL1 pathway SNPs with asthma in the PIAMA study and ALSPAC or in the meta-analysis.

<table>
<thead>
<tr>
<th>SNP (gene)</th>
<th>Risk allele</th>
<th>PIAMA asthma</th>
<th>ALSPAC asthma</th>
<th>Meta-analysis asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>Pval</td>
<td>N</td>
</tr>
<tr>
<td>rs1342336 (IL1RL1)</td>
<td>A</td>
<td>0.89 (0.79 - 1.01)</td>
<td>0.12</td>
<td>1620</td>
</tr>
<tr>
<td>rs2064362 (IL1RL1)</td>
<td>C</td>
<td>0.99 (0.88 - 1.11)</td>
<td>0.11</td>
<td>1670</td>
</tr>
<tr>
<td>rs17411646 (IL1RL1)</td>
<td>A</td>
<td>0.97 (0.86 - 1.10)</td>
<td>0.20</td>
<td>1350</td>
</tr>
<tr>
<td>rs4710971 (IL1RL1)</td>
<td>C</td>
<td>0.99 (0.88 - 1.10)</td>
<td>0.11</td>
<td>1670</td>
</tr>
</tbody>
</table>

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 heterop, P value for Cochrane Q statistic to test heterogeneity between the studies in the meta-analysis.
Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood

Discussion

This study in two 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 onset 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. Secondly, asthma was also associated with SNPs in IL33 and IL1RL1, but not with other genes of this pathway. Thirdly, we utilized a new method implementation of MB-MDR that show 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, we did observe replicable evidence for additive interaction between IL33 and IL1RL1 SNPs for asthma.

Intermediate onset and late onset wheeze were both associated with several IL33-IL1RL1 polymorphisms after correction for multiple testing. A common factor between these phenotypes is that they are associated with early sensitization (≤ age 4), as >70% of the children with intermediate onset and late onset wheeze had elevated specific IgE levels against common allergens at age 4 in PIAMA. The strong relation of intermediate onset wheeze with atopy was recently 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 positive skin prick test results.
against common allergens at age 1 year while late onset wheeze and persistent wheeze were associated with positive skin prick test results against common allergens at age 3 years but not at age 1 year.\textsuperscript{30}

We therefore speculate that \textit{IL33-IL1RL1} pathway polymorphisms might be affecting the development of wheeze and subsequent asthma through sensitization in early childhood.

Persistent wheeze was associated with one \textit{IL33} and one \textit{IL1RAP} SNP. This is the first study showing that a polymorphism in the gene encoding the co-receptor of \textit{IL1RL1} is associated with childhood wheeze. Several factors contribute to persistent wheeze, including atopy, reduced airway growth and 17q12-21 polymorphisms.\textsuperscript{7,9,30} This suggests that different combinations of pathophysiological processes are represented in different longitudinal wheezing phenotypes and \textit{IL33-IL1RL1} pathway polymorphisms are important for some but not all such phenotypes. Remarkably, we observed larger effect sizes on specific wheezing phenotypes compared to effects on asthma. For instance, rs13424006 was more strongly associated with late onset wheeze than with asthma (OR = 0.74 vs. 0.84 respectively). This suggests that specific wheezing phenotypes, particularly intermediate onset wheeze and late onset wheeze, are more homogeneous than asthma for studying the \textit{IL33-IL1RL1} pathway. Our results in PIAMA confirm previous investigations in ALSPAC that \textit{IL1RL1} and \textit{IL33} SNPs partly predict longitudinal wheezing phenotypes.\textsuperscript{8} Our reported associations with asthma were comparable to published GWAS results of asthma, with consistent direction and similar effect estimates.\textsuperscript{6,31} The high LD-region \textit{IL1RL1}/\textit{IL18R1}, containing SNPs of \textit{IL1RL1} (rs10204137, rs13424006) and \textit{IL18R1} (rs3771166), was associated with asthma in our study, confirming previous GWA studies.\textsuperscript{6,32,33} Within this LD-block, rs10204137 has been identified as eQTL associated with \textit{IL1RL1} mRNA levels in lymphoblasts and as eQTL for \textit{IL18R1} mRNA levels in fat tissue.\textsuperscript{1} Furthermore, rs13424006 has been associated with \textit{IL1RL1}-a levels in serum.\textsuperscript{1,28} Part of this LD-block encodes the TIR-domain in the intracellular part of \textit{IL1RL1}. The TIR domain plays a crucial role in signal transduction, since it connects to \textit{IL1RacP} and interacts with MyD88 and TIRAP for signal transduction (Figure 1).\textsuperscript{1} Haplotype-controlled functional genetic studies may only give further insight which polymorphisms in the high-LD region \textit{IL1RL1}/\textit{IL18R1} contribute to asthma.

Polymorphisms associated with asthma or longitudinal wheezing phenotypes known to result in asthma were located mainly in genes encoding the ligand \textit{IL33}, the receptor \textit{IL1RL1} and the receptor-associated protein \textit{IL1RacP} and not the adaptors or downstream signaling proteins. This suggests that genetic variation in the ligand-receptor-co-receptor complex, but not the downstream adaptor or signaling molecules, is driving the association with asthma. We think that this has implications for developing asthma treatments based on this pathway, i.e. this should focus on correcting the effects of genetic variance in the ligand receptor complex. A recent functional study showed that the membrane receptor \textit{IL1RL1}-b can be degraded by proteosomal activity in response to IL-33 through binding of the specific ubiquitin ligase FBXL19.\textsuperscript{34} This process is facilitated by phosphorylation of \textit{IL1RL1}-b at Ser442-Ser446. 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 humans.\textsuperscript{34}

We did not observe replicable multiplicative gene-gene interaction of \textit{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.\textsuperscript{6} The lack of multiplicative interactions in regression analyses of promising interactions identified by MB-MDR may 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 \textit{IL33-IL1RL1} pathway, or that multiplicative interaction effect of risk alleles is not the right statistical model to analyze interactions in this pathway.\textsuperscript{35,36} Our
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Observations of 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 of two large European birth cohorts with extensive data collection and longitudinally defined, highly comparable phenotypes. Indeed, our findings of stronger genetic risks of IL33-IL1RL1 pathway SNPs in wheezing phenotypes than asthma suggest that these detailed phenotypes better reflect the biological pathways leading to early asthma development. Moreover, we developed and applied the innovative method MB-MDR for multinomial traits to detect gene-gene interaction. 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. It is therefore even more remarkable that we find strong associations when we combine PIAMA and ALSPAC in the meta-analyses.

Finally, 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. We therefore 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 the intermediate onset wheeze. We speculate that the IL33-IL1RL1 pathway may 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. Since the IL33-IL1RL1 pathway has been proposed as a potential drug target, future studies may give insight how polymorphisms in the IL33-IL1RL1 pathway affect asthma development in childhood.

**Acknowledgements**

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 points**

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


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Chapter 3

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Methods

Model-Based Multifactor Dimensionality Reduction (MB-MDR) was used to select 10 SNP pair interactions in the IL33-IL1RL1 pathway for asthma and wheezing phenotypes.1,2 MB-MDR is a data mining technique that enables the fast identification of gene-gene interactions among thousands of SNPs, without the need to make restrictive assumptions about the genetic modes of inheritance. Main effects are easily adjusted for in the analysis, so as to ensure that strong lower-order effects do not give rise to spurious statistically significant epistasis signals. The recommended main effects adjustment is co-dominant, and was also the method of choice in this study.3 We furthermore restricted attention to 2-order interactions between bi-allelic SNPs. Note that 2 bi-allelic SNPs give rise to 9 possible multi-locus genotype combinations.

Since asthma is a binary trait, MB-MDR uses chi-square tests with 1 degree of freedom to label the aforementioned multilocus genotype combinations into high-risk (H), low-risk (L) or no evidence for risk (O).4,5 The later applies when the test is not significant at a given liberal threshold (default: 0.10). Odds ratios are used to distinguish between H (odds ratio>1) and L (odds ratio<1). Pooling all multilocus genotypes with the same label allows association testing between the trait of interest and a lower-dimensional construct with factor levels H, L and O. In particular, MB-MDR considers the maximum of H versus {L,O} and L versus {H,O} chi-square association tests. Overall significance is assessed via the permutation-based step-down maxT multiple testing correction of Westfall and Young, using 999 replicates.6 Under the condition of subset pivotality, this approach guarantees strong control of type I error. The final output of MB-MDR is a list of SNP-pairs with multiple testing corrected p-values, which can be compared to a 5% significance level.

For wheezing (categorical) phenotypes, we extended binary MB-MDR to accommodate more than 2 categories in the outcome. First, explanatory environmental factors (=non-genetic factors) were selected for the wheezing phenotypes by constructing classification trees in R (rpart package).7,8 Hence, the trees were grown with the wheezing phenotypes as the (categorical) response and environmental factors as the potential covariates. Second, multinomial regression was performed to estimate the contribution of environmental factors (and optionally genetic main effects) in the different wheezing phenotypes. Wheezing phenotypes were not weight for posterior probabilities of phenotype membership at this stage. Model fitting was tested as described in Goeman and Le Cessie.9 Vectors of the residuals describe the remaining unexplained variance. Third, identification of genetic interaction was performed with MB-MDR, in which the residual vectors were submitted as new (qualitative) traits. Multilocus genotype labeling was achieved via MANOVA’s Hotelling’s T test, a generalization of the Student’s t-test when more than one trait is involved. As for univariate quantitative MB-MDR, multilocus genotypes labeling involved consecutive comparison of mean trait vectors between two multilocus genotype groups.10 Non-significant association results at the liberal 0.10 criterion led to “no evidence” or “O” labeling. For significant results at the same threshold, the distinction between H and L labelings was achieved by exploiting the relationship between MANOVA and discriminant analysis (DA). DA distinguishes between two groups (the group variable) on the basis of multivariate data using Fisher’s discriminant functions/scores: positive mean of discriminant scores (negative mean of discriminant scores) refers to H(L) category. As before, overall significance is assessed via the permutation-based step-down maxT multiple testing correction of Westfall and Young, using 999 replicates.6 The final output of MB-MDR is a list of SNP-pairs with multiple testing corrected p-values, which can be compared to a 5% significance level. The most promising 10 SNP pairs were selected for further investigation in PIAMA. More details about theoretical power and type I error control of MB-MDR with respect to different outcome types in the presence or absence of noisy data are described in the literature.3,4,10,11
After MB-MDR the 10 most promising SNP pairs were studied in a regression analysis in PIAMA. An genetic additive model was assumed. SNP pairs with a tendency for interaction in regression analyses in PIAMA (p<0.1), were further analyzed in ALSPAC, and the interaction effect was meta-analyzed to describe the summarized effect estimate. When analyzing the 10 most promising interacting SNP pairs in PIAMA and eventually in ALSPAC, the wheezing phenotypes were weighted for their posterior probabilities of phenotype membership.

**Results**

See attached supplemental Excel files, which are available at [http://bit.ly/Chapter_3_Online_Repository_Tables_E1_E5](http://bit.ly/Chapter_3_Online_Repository_Tables_E1_E5)

**Table E1.** Studied SNPs in ALSPAC and PIAMA: Table E1_descriptives_SNPs_IL1RL1pathway.xlsx.

**Table E2.** Association and meta-analysis of all IL1RL1 pathway SNPs and wheezing phenotypes based on 8 observations: TableE2_association&meta-analysis_wheezing_phenotypes.xlsx with at each tab a different phenotype (transient early wheeze, intermediate onset wheeze, late onset wheeze and persistent wheeze).

**Table E3.** Association and meta-analysis of all IL1RL1 pathway SNPs and asthma: TableE3_association&meta-analysis_asthma.xlsx.

**Table E4.** Interaction of IL33-IL1RL1 SNP pairs for wheezing phenotypes at 8 years in ALSPAC, PIAMA and a meta-analysis: TableE4_interaction&meta-analyses_SNPpairs_wheezingphenotypes.xlsx.

**Table E5.** Interaction of IL33-IL1RL1 SNP pairs for asthma at 8 years in ALSPAC, PIAMA and a meta-analysis: TableE5_interaction&meta-analyses_SNPpairs_asthma.xlsx.
References


