## Online Supplement

# Genetic regulation of *IL1RL1* methylation and IL1RL1-a protein levels in asthma.

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## Supplemental methods

### Study populations

*(Overview studies and summary data provided in Table 1,Table S1 and Table S2)*

*DAG cohort*

The DAG cohort consists of 469 trios ascertained through a proband with asthma, combined with an additional case-control study of 452 asthmatics and 511 controls. We selected 469 asthma patients and 104 unaffected spouse controls from these studies, of whom serum and DNA was available for IL1RL1-a analysis.

These subjects participated in a family study on the genetics of asthma in Beatrixoord, Haren, the Netherlands (n= 152 asthma cases and 104 spouse controls) (1), a trio study (n= 297 asthma patients) (2), and a family study as part of the GAIN consortium (n=20 asthma patients) (3), all performed in Beatrixoord Hospital Haren, the Netherlands. For the genetic studies, unrelated cases and if available controls were selected from these families.

*Beatrixoord families*

Between 1962 and 1975, asthma patients in adolescence or early adulthood were admitted to a local asthma referral center, the Beatrixoord Hospital, Haren, the Netherlands. Patients with symptomatic asthma and no current asthma exacerbation were referred to this hospital and admitted for a standardized, comprehensive evaluation for asthma and atopy. From these patients, two hundred probands were selected who were 45 years of age or younger, had characteristic asthma symptoms, and were hyperresponsive to histamine (PC20 histamine ≤ 32 mg/ml).

These 200 probands, together with their spouses, children, children's spouses and grandchildren older than 6 years of age were restudied between 1990 and 1999.

From one hundred eight families investigated between 1996-2001, we selected 141 patients that had available IL1RL1a-data. Ninety three families were asked to participate in a second evaluation between 1999 and 2002. From these participants IL1RL1-a serum levels were measured in 115 participants.

All participants were asked to discontinue asthma and allergy medication before the clinical testing, if possible. There was no history of an exacerbation or treatment with oral corticosteroids during the 6 weeks before the clinical evaluation.

Data on respiratory symptoms, allergic status, use of medication and smoking were obtained by a modified version of the British Medical Research Council (MRC) questionnaire (4).

Asthma was defined as a doctor's diagnosis of asthma, asthma symptoms, and bronchial hyperresponsiveness (BHR) (4). BHR was defined when the provocative concentration producing a 20% fall to baseline in $FEV_1$ was <32 mg/ml histamine (30 seconds inhalation protocol). In controls, neither asthma nor BHR was present. The forced expiratory volume in 1 second ($FEV_1$) was measured using a water-sealed spirometer (Lode Spirograph type DL, Lode b.v., Groningen, The Netherlands). Total peripheral blood eosinophils were counted in a counting chamber and total Immunoglobulin E (IgE) levels were measured in serum by an enzyme-linked fluorescence assay (Mini Vidas, Biomerieux Inc., Marcy, France). In subjects older than 12 years intracutaneous tests with 16 common aeroallergens were performed. In children younger than 12 years, a skin prick test was performed with 10 allergens. Subjects with a positive response to one or more intracutaneous or skin prick tests (SPT) were considered to be atopic.

*Family trios study*

A second, independent trios (proband and both parents) study of 407 patients with asthma has been ascertained through local hospitals and media appeals between 1998 and 2003.

From this study, serum IL1RL1-a levels were available in 297 asthma patients.

All probands have been characterized using the standardized study protocol used in the Beatrixoord families as described above, in the same research center in Beatrixoord in Haren, The Netherlands.

*Dutch families of the GAIN study*

Twenty asthma patients with IL1RL1-a serum levels were selected from the families that have been recruited as part of a multinational genetics study of families with asthma, the Genetics of Asthma International Network (GAIN). Asthma was defined as previously mentioned but BHR was in this study population defined as a 20% fall to baseline in $FEV_1$ when nebulizing with a concentration < 9.8 mg/ml methacholine bromide (30 second protocol). Atopy was defined as the presence of at least one positive SPT.

Clinical investigations and measurements were all performed in the Beatrixoord Hospital, using the using standardized study protocol as described previously. Briefly, eosinophils were measured in peripheral blood and total Immunoglobulin E (IgE) levels were measured in serum by an enzyme-linked fluorescence assay (Mini Vidas, Biomerieux Inc., Marcy, France). SPT was performed according to European guidelines, with a positive SPT defined as mean wheal diameter of at least 3 mm (larger than the negative control), read after 15 minutes.

*PIAMA cohort*

The PIAMA study is a multicenter birth cohort, which was initiated in 1996. 7862 women (2779 with allergy and 5083 without allergy) were invited to participate in the study; 3963 live-born children participated the study (1327 with a mother with allergy were defined as high-risk, and 2726 children with a mother without allergy were defined as low-risk). Questionnaires for parental completion, partly based on the International Study of Asthma and Allergies in Childhood core questionnaires, were sent to the parents during pregnancy, when the children were aged 3 and 12 months, yearly thereafter up to the age of 8 years, at the age of 11/12 years and at the age of 16 years. All 1327 high-risk children and a random sample of 663 low-risk children were selected for an extensive medical examination at age 4 and 8 years. Blood or a buccal brush was used for DNA extraction. IL1RL1-a protein serum levels were measured in 343 children at the age of 4 years and in 323 different children at the age of 8 years. Informed parental consent was obtained for each participant. A detailed description of the cohort outline has been published previously (5).

We defined asthma in the PIAMA cohort by the published classical asthma definition of MeDALL (6), with two of three criteria present: 1) doctor diagnosis of asthma ever, 2) use of asthma medication in the past 12 months and 3) wheezing/breathing difficulties in the past 12 months.

For this study, asthma was assessed at the age of 4 or 8 year, depending on and at the age of the IL1RL1-a measurement. Aeroallergen sensitization was assessed as any specific IgE level ≥0.35 kU/L for Alternaria alternata, birch, cat, Dactylis glomerata, dog or house dust mite (*Dermatophagoides pteronyssinus*) at the age 4 or 8 years.

Eosinophil counts were measured in 2 mL blood with anticoagulants (EDTA) through an automatic cell counter XE-21000 (Sysmex Corp, Kobe, Japan) at age 4 years. No lung function measurements were available for both age groups.

*BAMSE*

Between 1994 and 1996, 4,089 newborn infants were recruited in the BAMSE study, and questionnaire data on baseline study characteristics were obtained (7). The recruitment area included central and north-western parts of Stockholm. At approximately one, two, four, and eight years of age, parents completed questionnaires on their children's symptoms related to asthma and other allergic diseases. The response rates were 96%, 94%, 92% and 84%, respectively. In BAMSE asthma was defined by the published classical asthma definition of MeDALL (6). DNA was extracted at age 4 from peripheral blood, and serum IL1RL1a was measured at the same age.

*INMA*

The INMA cohort was initiated in Sabadell between 2004 and 2006, when pregnant women were recruited at their first routine antenatal care visit in the main city public hospital or health care centre. A total of 622 women (out of 1097 eligible women) enrolled. After the child's birth, interviewer-administered questionnaires that included questions on wheezing and asthma symptoms were completed by the parents, including when the child was 4.4 years (SD 0.2) old. Because information on doctor-diagnosed asthma was not available in INMA, for this cohort the asthma definition included a positive answer to the two remaining questions from the MeDALL asthma

definition (6): 1) use of asthma medication in the past 12 months and 2) wheezing/breathing difficulties in the past 12 months.

Cord blood was collected at birth. At the year 4 survey, an additional blood sample was collected and serum processed and cryopreserved.

### *IL1RL1* locus and linkage disequilibrium (LD) pattern

*(Data provided in Figure 1, Table S7 and Figure S1)*

IL1RL1-a, IL1RL1-b and IL1RL1-c transcript annotation was calculated with the use of Ensemble (release 84) (8). Annotated gene SNP location and function was determined with the use of HaploReg v4.1 (9). For LD pattern calculation we first selected *IL1RL1* SNPs with a minor allele frequency (MAF>0.01) based on data from the 1000 Genomes CEU panel (version 3, March 2012) (10). We then assessed LD patterns between those SNPs and SNPs in a genomic region spanning 200kb up- and downstream from the *IL1RL1* gene (GRCh37/hg19;chr2:102,728,004-103,168,041) with MAF>0.01. LD blocks were calculated using $r^2$> 0.7 with the use of Plink (11).

In the selected *IL1RL1* region, 3,062 genetic variants were available in the imputed dataset of DAG, 3,055 in PIAMA, 2,568 in BAMSE and 2,711 in INMA.

All (epi)genetic data were aligned to assembly GRCh37/hg19.

### Genotyping and imputation

*(Data provided in Table 2, Table 3, Figure 2, Figure 3 and Figure 4)*

*DAG cohort*

Participants in the DAG cohort were genotyped on two platforms, the Illumina 317 Chip and the Illumina 370 Duo Chip (Illumina Inc, San Diego, CA).

Quality control (QC) was performed per chip with exclusion of individuals with missing genotype call rate >0.01, related individuals (identity by descent sharing (IBS) >0.125) and non-Caucasian subjects, as assessed by principal components analysis performed with EIGENSTRAT (12).

SNPs were excluded with a missing genotype rate >0.01, a Hardy-Weinberg equilibrium p-value $<10^{-7}$ and a MAF <0.01. Markers with Mendelian errors in phase I were excluded from analysis. After QC the chips were merged and SNPs not available in both cohorts were excluded from the dataset. 294,775 SNPs remained. Imputation was performed using IMPUTE 2.0 against the reference data set of the CEU panel of the 1000 Genomes project (version March 2012) (10). A total of 16,932,896 SNPs were analyzed.

*PIAMA cohort*

Children from the PIAMA cohort were genotyped on three different platforms. 1377 children were genotyped with the Illumina Omni Express Exome (OEE) Chip, whereas 288 children were genotyped with the Illumina Omni Express (OE) chip (Illumina Inc, San Diego, CA), both with the use of an Illumina BeadArray Reader and Iscan at the Genomics Facility of the University Medical Center Groningen, Groningen, The Netherlands. DNA of 404 children was genotyped with the Illumina Human610 (HM610) quad array and the use of the Ilumina Beadarray reader and Iscans at the Centre National de Génotypage (CNG, Evry, France) as part of the GABRIEL consortium (13).

Quality control inclusion measures per chip on the individuals included a missing genotype call rate <0.03, IBS <0.1875 and a heterozygosity rate deviating <4SD from the mean. Males with >1% heterozygote SNPs on chromosome X were excluded.

Ethnicity was assessed using principal component analyses with HapMap CEU, CHB+JPT, and YRI reference panels, only Caucasians subject were included (11). QC measures per SNP included missing genotype call rate <0.05, MAF >0.05 and Hardy-Weinberg equilibrium p-value $>10^{-6}$. SNPs being >1% heterozygous in males on chromosome X were excluded.

Base pair positions of SNPs on the HM610 chip were converted to genome build 37, in accordance with the OEE chip and the OE chip.
The strand was determined of each SNP and on the different platforms, and if necessary converted to the positive strand. SNPs with unknown strand orientation were removed. Discordant genotypes of duplicate SNPs were set to missing. SNPs that showed large differences in allele frequencies between platforms (>15 %) were either recoded (i.e. alleles were swapped) in case of an A/T or C/G SNP (and rechecked) or removed in other cases.
Duplicate individuals between the platforms were considered sampling errors and both individuals were removed.
The single chips were matched to the 1000G reference set with respect to basepair positions Resemblance between the chip and the 1000G European panel (EUR) of rs-numbers, alleles, and allele frequencies of SNPs on the autosomal chromosomes were checked and if discrepant deleted.

After quality control, a total of 1968 individuals remained and imputation was performed per platform using IMPUTE 2.0 (14) against the reference data set of the ALL panel of 1000G (version 3, March 2012) (10). After imputation, only SNPs of

high quality (info-score IMPUTE ≥ 0.7) were selected per chip. We removed SNPs that showed discrepancy between chips in allele frequency (> 15 %) (N=1795). Rs-numbers and insertions or deletions were separately merged using GTOOL (http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html) due to potential localization at the same base-pair position. The obtained files were combined into one dataset (SNPs N=11,713,219) that was used for further analyses.

*BAMSE*

Genotyping was done on the Illumina Human610 Quad platform at the Centre National de Génotypage in Evry, France under the GABRIEL project framework For imputation, the genotyped SNPs were filtered at - call rate >95%, Hardy Weinberg p-value > 1x10-6 and MAF > 0.01; and sample call rate > 95%; and 515'445 SNPs remained after quality control. These were imputed using MiniMac release stamp 2012-11-16 and the GIANT ALL reference panel, phase 1 v3.20101123 onto n=30'061'897 variants.

*INMA*

DNA was obtained from cord blood, whole blood collected at 4y, or saliva using the Chemagen protocol at the Spanish National Genotyping Centre (CEGEN). Children whose parents reported to be white and to be born in Spain or in European countries and who were not lost during follow-up were selected for genotyping. Genome-wide genotyping was performed using the HumanOmni1-Quad Beadchip (Illumina) at CEGEN. Genotype calling was done using the GeneTrain2.0 algorithm based on HapMap clusters implemented in the GenomeStudio software. SNP coordinates were reported on human reference genome 18 (hg18, b36) and on F strand. PLINK was

used for the genetic data quality control (11). First, SNPs were flipped to the human genome + strand. The following initial quality control thresholds were applied: sample call rate>98% and/or LRR SD<0.3. Then, sex, relatedness (excluded: one duplicated sample and the younger brother of two brother-pairs detected), heterozygosity and population stratification were checked. Genetic variants were filtered for SNP call rate >95%, MAF>1% and HWE p-value $>10^{-6}$.

After genetic analyses results of high quality were selected based on post-analyses SNPtest info-score >0.03, MAF ≥0.01 for continue variables and MAF ≥0.05 for binary traits (15,16).

**Epigenome (wide) analyses**

*(Data provided in Table 2, Figure 2, Figure 4, Table S3. Table S4, Table S6, Table S8 and Figure S3)*

We performed epigenome wide genotyping of 496 blood DNA samples of PIAMA (N=226), BAMSE (N=88) and INMA (N=182) as part of the MeDALL study, as described previously (17). Samples were selected from subjects of whom also serum IL1RL1a levels were available.

*DNA extraction, bisulphite treatment and DNA methylation measurement*

In the MeDALL study, peripheral blood samples were collected from all consenting cohort participants and DNA from peripheral and cord blood samples was extracted using the QIAamp blood kit (Qiagen or equivalent protocols), followed by precipitation-based concentration using GlycoBlue (Ambion).

to obtain DNA samples with equal purity. DNA concentration was determined by Nanodrop measurement and Picogreen quantification. After normalization of the concentration, the samples were randomized to avoid batch effects. Finally, paired

samples were hybridized on the same chip. Standard male and female DNA samples were included in this step for quality control.500 ng of DNA was bisulphite-converted using the EZ 96-DNA methylation kit (Zymo Research. Irvine, USA), following the manufacturer's standard protocol. After verification of the bisulphite conversion step using Sanger Sequencing, genome-wide DNA methylation was measured using the Infinium HumanMethylation450 BeadChip (Illumina).

*Quality control and pre-processing of microarray data*

DNA methylation data were pre-processed in R with Bioconductor package Minfi (18), using the original IDAT files extracted from the HiScanSQ scanner. We had a total of 1,748 blood samples from four birth cohorts in the MeDALL epigenetics study. Samples that did not provide significant methylation signals in more than 10% of probes (detection P=0.01) were excluded from further analysis. Samples were also excluded in cases of low staining efficiency, low single base extension efficiency, low stripping efficiency of DNA from probes after single base extension, poor hybridization performance, poor bisulphite conversion or high negative control probe staining. Further, we used the 65 SNP probes to check for concordance between paired DNA samples and assessed the methylation distribution of the X-chromosome to verify gender. Paired samples with Pearson correlation coefficients <0.9 were regarded as sample mix-ups and were excluded from the study. In total, we excluded 16 samples due to poor quality and 24 samples due to apparent sample mix-up. In probe filtering (19), we excluded probes on sex chromosomes, probes that mapped to multiple loci, the 65 random SNPs assay and probes that contained SNPs at the target 5'-C-phosphate-G-3' (CpG) sites with a MAF >10%. The allele frequencies of a list of SNPs were obtained from 1000 Genomes, release 20110521 for CEU

population. Finally, we implemented “DASEN” (20) to perform signal correction and normalization. After QC 1,708 samples and 439,306 autosomal probes remained. From these, we selected 1,264 samples in pairs from the population of randomly selected children for further analysis.

*Differential methylation analysis*

Methylation levels (beta values, β) at a given CpG site were derived from the ratio of the methylated probe intensity to overall intensity (sum of methylated and unmethylated probe intensities): β is equal to M/(U + M+ α), where M is intensity of the methylated probe, U is the intensity of the unmethylated probe, and α is the constant offset with the default value of 100. To remove bias in methylation profiles due to technical variation, we implemented a correction procedure based on 613 negative control probes (21) present in HM450K arrays because these negative control probes did not relate to biological variation. First, we implemented principal component analysis (PCA) on control probe data according to the method proposed by Zhang et al (22). Then, we permuted the control probe data 10000 times and applied PCA to each of these permuted datasets. We then selected principal components with a p-value defined to get the P(number of var(random pc)>var(pc))/(number of permutations)<10-4. The methylation data for each CpG were the residuals from a linear model incorporating the five significant principal components that reflected technical variation. We adjusted the residuals by cohort, gender, bisulphite conversion kit batch number, position of array and the percentage of monocytes, B cells, NK cells, CD4+ T cells, CD8+ T cells and granulocytes predicted by Houseman algorithm (23).

*SNP under the probe*

IL1RL1 methylation CpG sites cg20060108 and cg25869196 both potentially had a SNP under the probe, but only cg20060108 included a SNP with a MAF>0.01 (rs985523) (19). This SNP only gave a significant result with cg25869196 and lies in a distinct LD block from the top hit associated SNPs. No further corrections were therefore performed.

Subsequent genotypic QC removed SNPs with (MAF <0.01, those with Hardy Weinberg equilibrium (HWE) *P* <1 x 10-6, and genotype call rate < 0.95, the minimum MACH R2 measure to include SNPs (rsq< 0.3) for BAMSE data, and SNPtestinfo score < 0.3 for PIAMA and INMA data. All the genotypes have been aligned to GIANT release of 1000G to facilitate further data integration and meta-analysis by genotype Harmonizer 1.4.9 (24).

The association between 47 CpG sites in the selected *IL1RL1* region and *IL1RL1* SNPs in the MEDALL cohorts was assessed in R (version 3.2.3) (25). Meta-analyses of the association results were performed in METAL (26) using weights effect size estimates using the inverse of the corresponding standard errors.

The non-cell type corrected and Houseman cell-type corrected (23) candidate CpG meta-analysis of the association between nine *IL1RL1* CpG sites and asthma and the epigenome-wide association study (EWAS) on IL1RL1-a levels at age 4 years were performed in R (version 3.2.3) (25).

**Serum IL1RL1-a levels analyses**

*(Data provided in Table 2, Table 3, Table 4, Figure 3 Figure 4, Table S5, Table S6, Table S8, and Figure S3)*

IL1RL1-a protein serum levels in the PIAMA cohort were measured with an ST2 ELISA kit (Medical & Biological Laboratories Co, Woburn, Massat the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands. The intra-assay coefficient of variation (C.V.) and the inter-assay C.V. were 2.9% and 6.9%, respectively. We discarded the same MBL kit for the IL1RL1-a calculations in the other cohorts, since subsequent pilot studies showed that sensitivity was too low and the test-to-test variation was unacceptably high (median C.V.) 60.29%, range 31.35-83.16%). In DAG, BAMSE and INMA we measured the IL1RL1-a protein levels with the ST2/IL-1 R4 Quantikine R&D kit (R&D Systems, Inc, Minneapolis, MN). DAG samples were measured in the University Medical Center Groningen and samples form BAMSE and INMA in the Center for Research in Environmental Epidemiology (CREAL) in Barcelona. In DAG this kit was chosen based on its high reproducibility, from the n=580 serum samples 573 with a CV<10% were included in the association analysis which show a mean intra-plate C.V. of 3.4% with a SD of 2.5%. In BAMSE and INMA, the corresponding mean intra-plate C.V. was 4.8% (SD 4.2%).

GWA analyses on logarithmically transformed serum IL1RL1-a levels in DAG and PIAMA and candidate *IL1RL1* gene association analyses in INMA were performed with SNPtest v2.5β (27), and in BAMSE with PLINK v1.07 (11). Meta-analyses were performed using METAL (26) combining p-values across studies taking into account the sample size and direction of effect.

Before phenotypic analyses IL1RL1-a levels, eosinophil and IgE data was logarithmically transformed to obtain a normal distribution. Asthma related phenotypes in the DAG cohort were only investigated in asthma patients, with correction for age and gender. In PIAMA age was included as a co-variate. A p-value ≤ 0.05 was considered significant. Associations of IL1RL1-a levels with asthma and

asthma-related outcomes were investigated using linear regression in SPSS 22.0 (IBN, Armonk, NY).

In the DAG cohort we performed conditional analysis using a multivariate model with a backward step wise regression analysis in SPSS 22.0 (IBN, Armonk, NY) to assess if the SNPs in the different LD blocks had independent effects on IL1RL1-a serum levels. We selected one genotyped SNP from each of the four LD blocks with the most significant associated SNPs, based on previously reported *IL1RL1* expression or asthma associations (rs1420101, rs11685424, rs13015714 and rs1035130) and adjusted for age and sex.

**Causal inference testing**

*(Data provided in Table S9)*

To assess whether genetic variation and methylation levels were independently related to IL1RL1-a serum levels causal inference tests (CIT) (28,29) were used in 120 children from PIAMA who had complete data. We selected our methylation and protein significant associated SNP rs420101 and the four significantly associated CpG sites (cg11916609, cg19795292, cg25869196, cg20060108). CIT was performed using R (version 3.2.3) (25).

**Additional methods**

*Data provided in Figure 2, Figure 3, Table S10, Figure S1 and Figure S2)*

The LD plot among SNPs was created with Haploview (30) and Manhattan plots were generated with R (25). LocusZoom (31) was used to represent the -log10(pvalue) for the association between the selected *IL1RL1* region SNPs and CpGsites or IL1RL1-a serum levels, respectively.

We used the GTEx consortium data (V7) (40) to identify if important *IL1RL1* meQTLs and pQTLs were also reported as eQTLs in whole blood and lung tissue. In addition, we searched for lung *IL1RL1* eQTLs, to see if trans-SNPs were also important in regulating *IL1RL1* gene expression.

## Supplemental references


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## Supplemental tables

**TABLE S1** Clinical characteristics of the DAG Cohort

| | DAG cohort | | DAG subset with IL1RL1-a levels* | |
|---|---|---|---|---|
| | Cases (n = 909) | Controls (n = 976) | Cases (n = 469) | Controls (n = 104) |
| Age (y), mean (SD) | 33.9 (16.2) | 50.9 (8.3) | 38.8 (14.0) | 54.1 (8.9) |
| Male Sex, n (%) | 426 (46.9) | 45 (37.8) | 212 (45.2) | 37 (35.6) |
| Smoking, n (%) | | | | |
| *Current smoker* | 141 (15.6) | 45 (37.8) | 75 (16.0) | 39 (37.5) |
| *Never smoker* | 537 (59.3) | 32 (26.9) | 243 (51.9) | 26 (25.0) |
| *Ex smoker* | 228 (25.1) | 42 (35.3) | 150 (32.1) | 39 (37.5) |
| ICS use, n (%) | | | | |
| *Oral steroids* | 51 (5.7) | 1 (0.8) | 1 (0.2) | 1 (1.0) |
| *Inhaled steroids* | 502 (56.1) | - | 325 (69.6) | 1 (1.0) |
| *Oral and inhaled steroids* | 22 (2.5) | - | 11 (2.4) | - |
| *No steroids* | 319 (35.7) | 118 (99.2) | 130 (27.8) | 102 (98.0) |
| FEV1(%pred), mean (SD) | 86.4 (20.6) | 100.8 (12.7) | 82.6 (19.9) | 99.9 (12.3) |
| Eosinophils (10e7/L), median (range) | 16.5 (0.0 - 190.0) | 7.0 (0.0 - 63.8) | 14.0 (1.0 - 121.0) | 8.0 (0.0 - 36.0) |
| Total IgE (kU/L), median (range) | 130.0 (0.0 - 12400.0) | 20 (1.0 - 1040.0) | 118 (0.5 - 12400.0) | 17.0 (1.0 - 1465.0) |
| Atopic, n (%) | 576 (85.3) | 30 (25.4) | 390 (8.5) | 26 (25.2) |
| IL1RL1-a levels (ng/ml), median (range) | | | 7.2 (1.47 - 44.1) | 7.7 (2.73 - 23.8) |

DAG: Dutch Asthma Genome-wide Association Study; %: pred, percentage predicted.
*: in 573 participants of the asthma patients cohort serum IL1RL1-a levels were measured. These individuals are included in the clinical characteristics description of the whole cohort, as well described separately.

**Table S2** Clinical characteristics of the MeDALL cohorts

a)

| | PIAMA cohort | | PIAMA subset with IL1RL1-a levels* | |
|---|---|---|---|---|
| | Cases (n = 194) | Controls (n = 1719) | Cases (n = 70) | Controls (n = 562) |
| Age (y), mean (SD) | 4.1 (0.19) | 4.1 (0.2) | 5.8 (2.2) | 6.3 (2.3) |
| Male Sex, n (%) | 123 (63.4) | 846 (49.2) | 44 (62.9) | 309 (55.0) |
| Sensitization at age 4 years | 47 (58.0) | 194 (37.3) | 30 (58.8) | 180 (39.4) |
| Eosinophils (10e7/L), median (range) | 34.0 (5.0-116.0) | 26.0 (1.0 - 395.0) | 34.0 (5.0 - 116.0) | 26.0 (1.0 - 395.0) |
| Total IgE (kU/L), median (range) | 65.0 (1.0 - 4473.0) | 36.0 (0.0 - 2000) | 108 (4.0 - 4300.0) | 46 (0.0 - 2600.0) |
| IL1RL1-a levels (ng/ml), median (range) | | | 0.13 (0.1 - 0.2) | 0.12 (0.0 - 0.3) |

b)

| | BAMSE cohort | | BAMSE subset with IL1RL1-a levels* | |
|---|---|---|---|---|
| | Cases (n = 110) | Controls (n = 275) | Cases (n = 42) | Controls (n = 142) |
| Age (y), mean (SD) | 4.3 (0.2) | 4.3 (0.2) | 4.3 (0.2) | 4.2 (0.2) |
| Male Sex, n (%) | 78 (70.9%) | 136 (49.5) | 24 (57.1) | 74 (52.1) |
| Sensitization at age 4 years | 40 (41.7%) | 18 (6.6%) | 17 (40.5) | 42 (29.6) |
| Eosinophils (10e7/L), median (range) | NA | NA | NA | NA |
| Total IgE (kU/L), median (range) | NA | NA | NA | NA |
| IL1RL1-a levels (ng/ml), median (range) | | | 6.0 (0.76 - 12.8) | 5.2 (0.63 - 20.9) |

c)

| | INMA cohort | | INMA subset with IL1RL1-a levels* | |
|---|---|---|---|---|
| | Cases (n = 59) | Controls (n = 263) | Cases (n = 49) | Controls (n = 236) |
| Age (y), mean (SD) | 4.5 (0.1) | 4.4 (0.2) | 4.4 (0.1) | 4.4 (0.2) |
| Male Sex, n (%) | 38 (64.4) | 132 (50.2) | 30 (61.2) | 115 (48.7) |
| Sensitization at age 4 years | 3 (11.1) | 12 (9.2) | 10 (26.3) | 17 (9.8) |
| Eosinophils (10e7/L), median (range) | NA | NA | NA | NA |
| Total IgE (kU/L), median (range) | NA | NA | NA | NA |
| IL1RL1-a levels (ng/ml), median (range) | | | 9.7 (4.3 - 20.1) | 9.6 (2.5 - 35.0) |

MeDALL: Mechanisms of the Development of Allergy; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; BAMSE: Children/Barn, Allergy, Milieu, Stockholm, an Epidemiological survey; INMA: Infancia y Medio Ambiente.
*: in **a)** 666 PIAMA, **b)** 184 BAMSE and **c)** 285 INMA cohort participants with asthma serum IL1RL1-a levels were measured.
These individuals are included in the clinical characteristics description of the whole cohort, as well described separately.

**TABLE S3** Significant associations of rs1420101 with CpG sites located in *IL1RL2*, *IL18RAP* and *SLC9A4* in MeDALL meta-analysis

| SNP | Position | CpG site | Position | Gene | CpG UCSC RefGene Group | A1 | A2* | Zscore | p-value |
|---|---|---|---|---|---|---|---|---|---|
| rs1420101 | 102957716 | cg08023416 | 102803787 | IL1RL2 | 5'UTR | T | C | 4.46 | 8.20E-06 |
| rs1420101 | 102957716 | cg13315345 | 102803985 | IL1RL2 | 5'UTR | T | C | 6.69 | 2.20E-11 |
| rs1420101 | 102957716 | cg26788216 | 102844110 | IL1RL2 | Body | T | C | -5.62 | 1.92E-08 |
| rs1420101 | 102957716 | cg03938978 | 103052716 | IL18RAP | Body | T | C | -6.20 | 5.70E-10 |
| rs1420101 | 102957716 | cg02552255 | 103104330 | SLC9A4 | Body | T | C | -4.43 | 9.33E-06 |

MeDALL: Mechanisms of the Development of Allergy; SNP: single nucleotide polymorphism; CpG: 5'-C-phosphate-G-3'.
*: A2 was used as the reference allele.

**TABLE S4** *IL1RL1* region top hits associated with CpG sites located in *IL1R1*, *IL1RL2*, *IL18R1*, *IL18RAP* and *SLC9A4* in the MeDALL meta-analysis

| SNP | Position | Gene | CpG site | Position | Gene | CpG UCSC RefGene Group | A1 | A2* | Zscore | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| rs10208542 | 102732315 | IL1R1 | cg20515672 | 102851687 | IL1RL2 | Body | G | C | -4.31 | 1.65E-05 |
| rs11883987 | 102761240 | IL1R1 | cg14837955 | 102803435 | IL1RL2 | 1st exon; 5'UTR | C | T | -4.31 | 1.67E-05 |
| rs12474258 | 102816695 | IL1RL2 | cg13315345 | 102803985 | IL1RL2 | 5'UTR | C | T | -14.93 | 1.97E-50 |
| rs12474258 | 102816695 | IL1RL2 | cg08023416 | 102803787 | IL1RL2 | 5'UTR | C | T | -15.63 | 4.82E-55 |
| rs1922292 | 102829013 | IL1RL2 | cg24708332 | 102794246 | IL1R1 | 3'UTR | C | A | 4.26 | 2.03E-05 |
| rs2310241 | 102841949 | IL1RL2 | cg00674896 | 102804576 | IL1RL2 | Body | A | C | 4.78 | 1.75E-06 |
| rs2302621 | 102842124 | IL1RL2 | cg22797169 | 102803407 | IL1RL2 | TSS200 | G | T | -6.87 | 6.61E-12 |
| rs1997502 | 102844249 | IL1RL2 | cg26788216 | 102844110 | IL1RL2 | Body | A | G | 15.29 | 9.10E-53 |
| rs6543113 | 102911057 | 5' of IL1RL1 | cg11335172 | 103033799 | IL18RAP | TSS1500 | C | T | -4.35 | 1.39E-05 |
| rs114879104 | 102948404 | IL1RL1 | cg01376829 | 102804405 | IL1RL2 | Body | T | C | 4.48 | 7.43E-06 |
| rs2160203 | 102960824 | IL1RL1 | cg01969473 | 102978461 | IL18R1 | TSS1500 | A | G | 4.19 | 2.79E-05 |
| rs17027071 | 103012674 | IL18R1 | cg21282997 | 103035835 | IL18RAP | 5'UTR | C | T | -6.13 | 8.97E-10 |
| rs4851007 | 103024813 | 3' of IL18R1 | cg13897122 | 103039542 | IL18RAP | 5'UTR | T | G | 11.44 | 2.73E-30 |
| rs3755265 | 103052816 | IL18RAP | cg03938978 | 103052716 | IL18RAP | Body | C | A | -32.52 | 5.40E-232 |
| rs2141781 | 103082906 | 5' of SLC9A4 | cg04239558 | 103089729 | SLC9A4 | TSS200 | G | A | -12.45 | 1.36E-35 |
| rs11692304 | 103095404 | SLC9A4 | cg16237262 | 103095386 | SLC9A4 | Body | G | A | 6.44 | 1.24E-10 |
| rs6543145 | 103096436 | SLC9A4 | cg02552255 | 103104330 | SLC9A4 | Body | C | A | 5.97 | 2.33E-09 |

MeDALL: Mechanisms of the Development of Allergy; SNP: single nucleotide polymorphism; CpG: 5'-C-phosphate-G-3'.
Houseman cell type corrected (23) results are represented.
*: A2 was used as the reference allele.

**TABLE S5** Significant trans-effects in the genome wide pQTL analysis on IL1RL1-a levels in the PIAMA cohort

| | | | | | | | | PIAMA | | | DAG | | | Meta-analysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | Position | Chr | A1 | A2* | MAF | (nearest) Gene | Location relative to gene | Beta | SE | p-value | Beta | SE | p-value | p-value |
| rs115185111 | 8115415 | 6 | C | T | 0.01 | EEF1E1 | 3' region | **-1.59** | **0.26** | **2.71E-09** | - | - | - | - |
| rs192895284 | 64171882 | 8 | T | C | 0.01 | YTHDF3 | 3' region | **-0.65** | **0.11** | **2.46E-08** | - | - | - | - |
| rs139311039 | 9998058 | 11 | C | T | 0.01 | SBF2 | Intronic | **0.78** | **0.13** | **2.32E-09** | - | - | - | - |
| rs141140572 | 17728223 | 12 | A | G | 0.01 | MIR3974 | 5' region | **0.91** | **0.16** | **2.01E-08** | - | - | - | - |
| rs35469243 | 53594821 | 12 | T | C | 0.01 | ITGB7 | Intronic | **-0.49** | **0.09** | **4.15E-08** | 0.007 | 0.13 | 0.95 | 9.88E-05 |
| rs145614409 | 32032919 | 18 | T | C | 0.01 | DTNA | 5' region | **0.9** | **0.16** | **3.23E-08** | - | - | - | - |
| rs73015994 | 21265357 | 19 | G | A | 0.03 | ZNF708 | 3' region | **-0.54** | **0.09** | **1.87E-08** | -0.17 | 0.07 | 0.02 | 1.20E-07 |
| rs73015995 | 21448160 | 19 | G | T | 0.03 | ZNF708 | 3 region | **-0.54** | **0.09** | **1.87E-08** | -0.17 | 0.07 | 0.02 | 1.20E-07 |

PIAMA: Prevention and Incidence of Asthma and Mite Allergy; DAG: Dutch Asthma Genome-wide Association Study; SNP: single nucleotide polymorphism.

Results of significant PIAMA polymorphisms who were also present in the DAG cohort pQTL analysis are also provided. Bold faced results are genome wide significant asscociations ($P < 5x10^{-8}$).

*: A2 was used as the reference allele.

**TABLE S6** LD pattern between selected SNPs from the five most important LD blocks regulating whole blood *IL1RL1* DNA methylation and serum IL1RL1-a levels

| SNP | Position | LD block* | rs1420101 | rs11685424 | rs13015714 | rs1035130 | rs10192157 |
|---|---|---|---|---|---|---|---|
| rs1420101 | 102957716 | 1 | 1 | | | | |
| rs11685424 | 102926981 | 2 | 0.61 | 1 | | | |
| rs13015714 | 102971865 | 3 | 0.11 | 0.16 | 1 | | |
| rs1035130 | 103001402 | 4 | 0.68 | 0.42 | 0.04 | 1 | |
| rs10192157 | 102968356 | 5 | 0.40 | 0.10 | | 0.34 | 1 |

MeDALL: Mechanisms of the Development of Allergy; SNP: single nucleotide polymorphism; LD: linkage disequillibrum.
LD values ($r^2$) are calculated with the use of Plink (11) and based on 1000 Genomes CEU panel data (version 3, March 2012) (10).
*: LD block annotation is described in in this article's online supplement.

**TABLE S7** Combined results of the association between *IL1RL1* CpG sites and asthma and between *IL1RL1* CpG sites and IL1RL1-a levels in the MeDALL meta-analysis

| | | | | Asthma | | | | | | IL1RL1-a levels | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Non cell corrected | | | Cell corrected | | | Non cell corrected | | Cell corrected | |
| CpG site | Position | Gene | Gene location | Beta | SE | p-value | Beta | SE | p-value | Zscore | p-value | Zscore | p-value |
| cg17738684 | 102927278 | IL1RL1 | Distal promoter | **-0.006** | **0.002** | **0.03** | -0.003 | 0.002 | 0.23 | 0.369 | 0.71 | 0.578 | 0.56 |
| cg16386158 | 102927397 | IL1RL1 | Distal promoter | -0.006 | 0.002 | 0.03 | -0.001 | 0.002 | 0.53 | 1.048 | 0.29 | 1.853 | 0.06 |
| cg11916609 | 102927488 | IL1RL1 | Distal promoter | -0.004 | 0.009 | 0.67 | -0.001 | 0.003 | 0.74 | 1.367 | 0.17 | **2.234** | **0.03** |
| cg19969733 | 102927898 | IL1RL1 | Distal promoter | 0.002 | 0.007 | 0.77 | 0.006 | 0.004 | 0.18 | -0.339 | 0.73 | -0.380 | 0.70 |
| cg19795292 | 102952285 | IL1RL1 | Intron 1A | -0.004 | 0.005 | 0.43 | -0.004 | 0.003 | 0.34 | 1.285 | 0.20 | **2.154** | **0.03** |
| cg25869196 | 102952419 | IL1RL1 | Intron 1A | -0.003 | 0.007 | 0.72 | -0.007 | 0.004 | 0.14 | 1.048 | 0.29 | **2.429** | **0.02** |
| cg01254707 | 102953421 | IL1RL1 | Proximal promoter | -0.001 | 0.002 | 0.62 | 0.000 | 0.001 | 0.92 | 0.081 | 0.94 | -0.053 | 0.96 |
| cg20060108 | 102954350 | IL1RL1 | Intron 1B | -0.001 | 0.005 | 0.84 | 0.002 | 0.004 | 0.69 | 1.980 | **0.05** | 1.071 | 0.28 |
| cg07020540 | 102968194 | IL1RL1 | Exon 11 | 0.000 | 0.001 | 0.92 | -0.001 | 0.001 | 0.61 | -1.048 | 0.29 | -1.593 | 0.11 |

MeDALL: Mechanisms of the Development of Allergy; CpG: 5'-C-phosphate-G-3'.
Bold faced results are nominal significant associations ($P < 0.05$). Non-cell type corrected and Houseman cell type corrected (23) results are represented.

**TABLE S8** Results of causal inference testing on rs1420101, cg11916609, cg19795292, cg25869196, cg20060108 and IL1RL1-a levels in PIAMA at 4 years of age

| | Non cell corrected | | | Cell corrected | | |
|---|---|---|---|---|---|---|
| CpG site | Causal model p-value | Reactive model p-value | Outcome | Causal model p-value | Reactive model p-value | Outcome |
| cg11916609 | 0.75 | 0.48 | Independent effect | 0.50 | 0.50 | Independent effect |
| cg19795292 | 0.37 | 0.35 | Independent effect | 0.64 | 0.64 | Independent effect |
| cg25869196 | 0.43 | 0.40 | Independent effect | 0.27 | 0.27 | Independent effect |
| cg20060108 | 0.92 | 0.89 | Independent effect | 0.89 | 0.93 | Independent effect |

PIAMA: Prevention and Incidence of Asthma and Mite Allergy; CpG: 5'-C-phosphate-G-3'.
Results with the use of non-cell type corrected and Houseman cell type corrected (23) methylation data are represented.

**TABLE S9** Asthma associated *IL1RL1* SNPs reported in the literature combined with results from asthma, *IL1RL1* methylation and IL1RL1-a protein analyses

| | | | | | | | | | Asthma | | Blood DNA methylation | Serum IL1RL1-a levels | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | Position | Annotation | Transcript | LD block* | A1 | A2[†] | Asthma[‡] | Reference | DAG | MeDALL[§] | MeDALL[§] | DAG | Meta-analysis[¶] |
| rs10173081 | 102957348 | intronic | Intron 5 | | C | T | Risk | (9) | ↓ | | ↑↑↓↑ | ↑ | |
| rs1420089 | 102938389 | intronic | Intron 1 | | T | C | Protection | (42) | ↑ | ↑ | ↑↑↑↑ | ↑ | ↓ |
| rs3771180 | 102953617 | intronic | Proximal promoter | | G | T | Risk | (9) | ↓ | | ↑↑↓↑ | ↑ | |
| rs13431828 | 102954653 | 5'-UTR | Exon 2 | | C | T | Protection | (2,16) | ↓ | ↓ | ↑↑↓↑ | ↑ | ↑ |
| rs13408661 | 102955082 | intronic | Intron 2 | | G | A | Protection | (43) | ↓ | | ↑↑↓↑ | ↑ | |
| rs1041973 | 102955468 | missense | Exon 3 | | C | A | Protection | (26) | ↓ | ↓ | ↓↑↓↑ | ↓ | ↓ |
| rs1420101 | 102957716 | intronic | Intron 5 | 1 | C | T | Risk | (10,11) | ↑ | | ↓↓↓↓ | ↓ | ↓ |
| rs1946131 | 102961929 | intronic | Exon 8 | | C | T | Risk (42) | (42) | ↑ | ↑ | ↓↓↓↓ | ↓ | |
| rs1921622 | 102966067 | intronic | Intron 10 | | G | A | Risk (10) | (10) | ↑ | | ↓↓↓↓ | ↓ | ↓ |
| rs10197862 | 102966549 | intronic | Intron 10 | | A | G | Protection | (3) | ↓ | ↓ | ↑↑↓↑ | ↑ | |
| rs1861246 | 102966783 | intronic | Intron 10 | 3 | T | C | Risk | (10) | ↑ | | ↓↓↓↑ | ↓ | |
| rs10204137 | 102968212 | missense | Exon 11 | 5 | A | G | Protection (2,16) | (2,16) | ↓ | | ↑↑↑↑ | ↑ | |
| rs10192157 | 102968356 | missense | Exon 11 | 5 | C | T | Protection | (2,16) | ↓ | ↓ | ↑↑↑↑ | ↑ | ↑ |
| rs10206753 | 102968362 | missense | Exon 11 | 5 | T | C | Protection (2,16) | (2,16) | ↓ | | ↑↑↑↑ | ↑ | |

DAG: Dutch Asthma Genome-wide Association Study; MeDALL: Mechanisms of the Development of Allergy; SNP: single nucleotide polymorphism; LD: linkage disequillibrum; CpG: 5'-C-phosphate-G-3'.

Positive associations found in the current study are marked with an upward arrow, suggesting a higher risk for asthma, more *IL1RL1* methylation or more IL1RL1-a levels. Negative associations are marked with a downward arrow, suggesting a protective effect on asthma, less *IL1RL1* methylation or less IL1RL1-a levels. Green arrows are significant associations. Blank spots mean no data available.
*: LD block annotation is described in in this article's online supplement.
†: A2 was set as the reference allele.
‡: SNP association with asthma previously found in the literature.
§: results from MeDALL meta-analysis, signs are belonging successively to *IL1RL1* CpG sites; cg11916609, cg19795292, cg25869196 and cg20060108.
¶: results from meta-analysis in DAG and MeDALL cohorts.

**TABLE S10** Whole blood and lung eQTL data from selected *IL1RL1* SNPs from the five most important LD blocks regulating whole blood *IL1RL1* DNA methylation and serum IL1RL1-a levels

| SNP | A2* | Gene | Gencode | Beta† | p-value† | Tissue |
|---|---|---|---|---|---|---|
| rs1420101 | T | IL1RL1 | ENSG00000115602.12 | -0.36 | 8.80E-22 | Lung |
| rs1420101 | T | AC007278.3 | ENSG00000234389.1 | -0.14 | 5.80E-05 | Lung |
| rs11685424 | A | IL1RL1 | ENSG00000115602.12 | -0.50 | 1.90E-53 | Lung |
| rs11685424 | A | IL18R1 | ENSG00000115604.6 | -0.27 | 2.40E-15 | Lung |
| rs11685424 | A | IL18RAP | ENSG00000115607.5 | 0.15 | 1.30E-09 | Whole Blood |
| rs11685424 | A | AC007278.2 | ENSG00000236525.1 | 0.11 | 3.70E-05 | Whole Blood |
| rs13015714 | T | IL18RAP | ENSG00000115607.5 | 0.29 | 5.40E-32 | Whole Blood |
| rs13015714 | T | IL1RL1 | ENSG00000115602.12 | -0.38 | 4.30E-18 | Lung |
| rs13015714 | T | AC007278.2 | ENSG00000236525.1 | 0.23 | 3.90E-15 | Whole Blood |
| rs13015714 | T | AC007278.3 | ENSG00000234389.1 | 0.27 | 1.40E-14 | Whole Blood |
| rs13015714 | T | IL18R1 | ENSG00000115604.6 | -0.27 | 1.50E-10 | Lung |
| rs13015714 | T | AC007278.3 | ENSG00000234389.1 | 0.21 | 2.40E-07 | Lung |
| rs13015714 | T | IL18RAP | ENSG00000115607.5 | 0.19 | 4.00E-07 | Lung |
| rs13015714 | T | AC007278.2 | ENSG00000236525.1 | 0.17 | 4.10E-05 | Lung |
| rs1035130 | T | AC007278.3 | ENSG00000234389.1 | -0.30 | 1.50E-15 | Whole Blood |
| rs1035130 | T | AC007278.3 | ENSG00000234389.1 | -0.26 | 1.30E-12 | Lung |
| rs1035130 | T | IL1RL1 | ENSG00000115602.12 | -0.29 | 2.30E-12 | Lung |
| rs10192157 | T | AC007278.3 | ENSG00000234389.1 | 0.34 | 9.60E-27 | Whole Blood |
| rs10192157 | T | AC007278.3 | ENSG00000234389.1 | 0.31 | 4.40E-18 | Lung |
| rs10192157 | T | IL18RAP | ENSG00000115607.5 | 0.20 | 5.20E-16 | Whole Blood |

SNP: single nucleotide polymorphism.
*: A2 was used as the reference allele. †: data is provided from the GTEx Consortium V7 (40)

## Supplemental figure legends

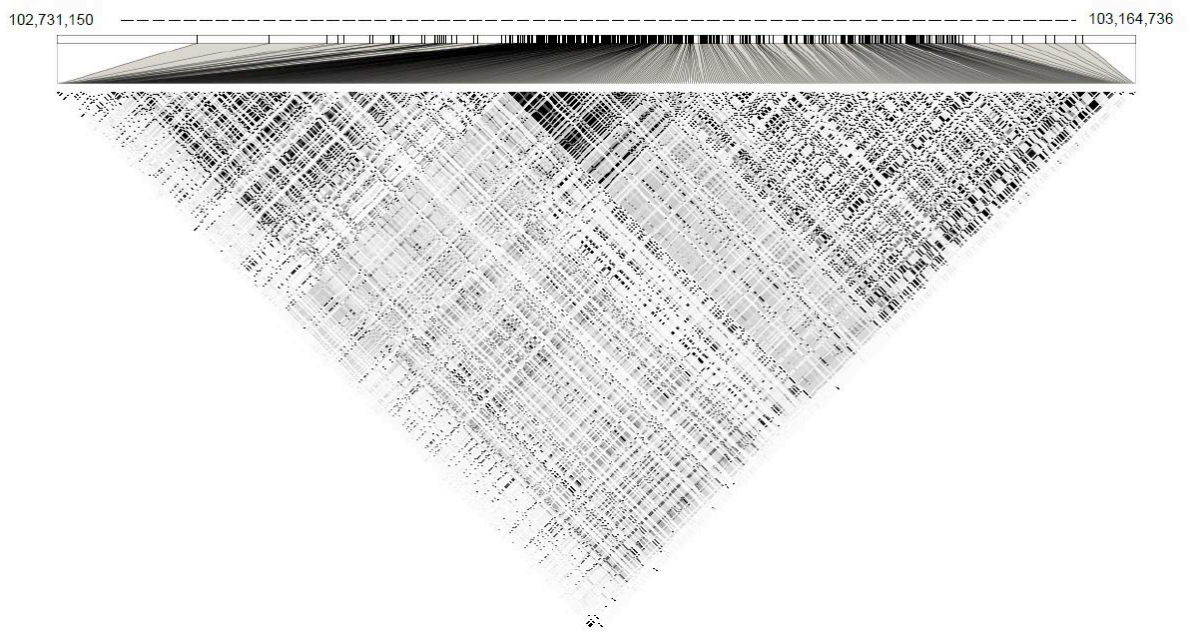


**FIGURE S1** LD matrix of the selected *IL1RL1* region (200kb up- and downstream from the *IL1RL1* gene) with inclusion of single nucleotide polymorphisms with a MAF >0.01. LD ($r^2$) was calculated based on data from the 1000 Genomes CEU panel (version 3, March 2012) (10).

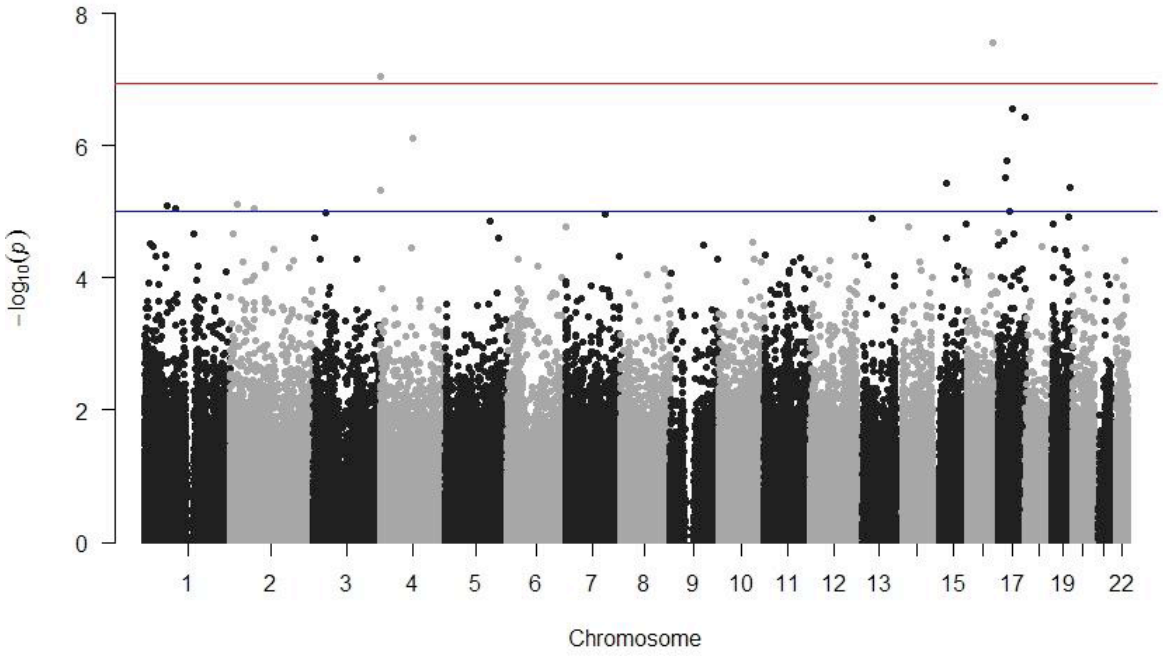


**FIGURE S2** Manhattan plots showing the Houseman cell type corrected (23) results of the epigenome-wide association meta-analysis on serum IL1RL1-a levels in the MeDALL cohorts. The red line indicates the genome-wide significance threshold of a p-value of $5x10^{-8}$, the blue line indicates a less stringent p-value of $1x10^{-5}$.

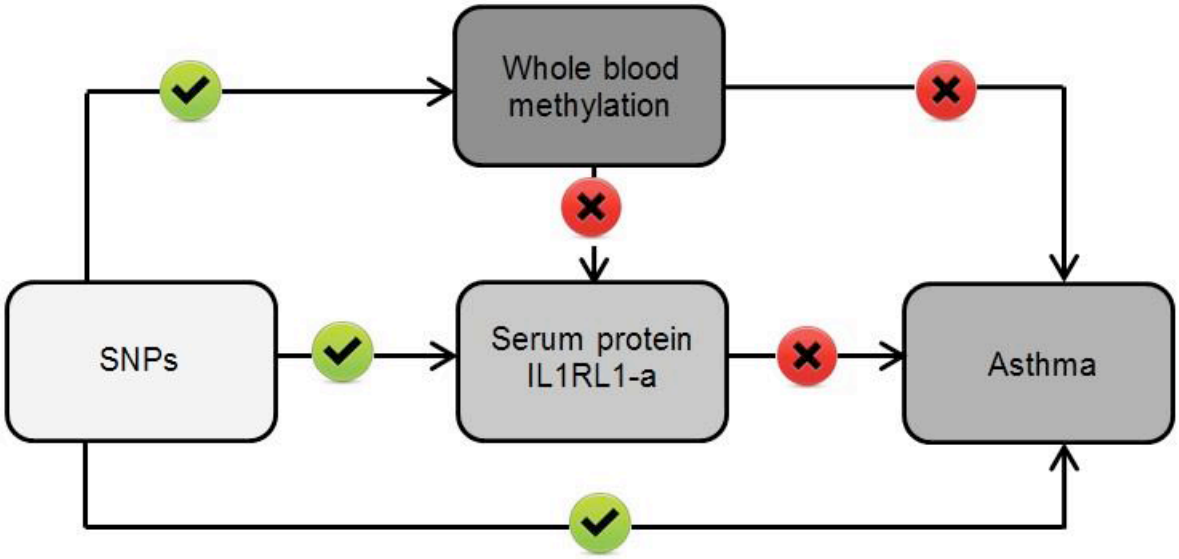


**FIGURE S3** Genetic and epigenetic association model of *IL1RL1* with asthma. *IL1RL1* polymorphisms are associated with asthma, *IL1RL1* blood methylation and serum IL1RL1-a levels (green dots). *IL1RL1* methylation is not associated with asthma and IL1RL1-a levels. IL1RL1-a levels are not associated with asthma (red dots).