

Doublesex and mab-3 related transcription factor 1 (*DMRT1*) is a sex-specific genetic determinant of childhood-onset asthma and is expressed in testis and macrophages

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Background: Asthma is a disease affecting more boys than girls in childhood and more women than men in adulthood. The mechanisms behind these sex-specific differences are not yet understood. **Objective:** We analyzed whether and how genetic factors contribute to sex-specific predisposition to childhood-onset asthma.

Methods: Interactions between sex and polymorphisms on childhood asthma risk were evaluated in the Multicentre Asthma Genetics in Childhood Study (MAGICS)/Phase II International Study of Asthma and Allergies in Childhood (ISAAC II) population on a genome-wide level, and findings

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were validated in independent populations. Genetic fine mapping of sex-specific asthma association signals was performed, and putatively causal polymorphisms were characterized *in vitro* by using electrophoretic mobility shift and luciferase activity assays. Gene and protein expression of the identified gene doublesex and mab-3 related transcription factor 1 (*DMRT1*) were measured in different human tissues by using quantitative real-time PCR and immunohistochemistry. **Results:** Polymorphisms in the testis-associated gene *DMRT1* displayed interactions with sex on asthma status in a population of primarily clinically defined asthmatic children and nonasthmatic control subjects (lowest $P = 5.21 \times 10^{-6}$). Replication of this interaction was successful in 2 childhood populations clinically assessed for asthma but showed heterogeneous results in other population-based samples. Polymorphism rs3812523 located in the putative *DMRT1* promoter was associated with allele-specific changes in transcription factor binding and promoter activity *in vitro*. *DMRT1* expression was observed not only in the testis but also in lung macrophages. **Conclusion:** *DMRT1* might influence sex-specific patterns of childhood asthma, and its expression in testis tissue and lung macrophages suggests a potential involvement in hormone or immune cell regulation. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: Asthma, genetic association, *DMRT1*, interaction, rs3812523, sex, single nucleotide polymorphism

During childhood, asthma prevalence is higher in boys than in girls, with a ratio of up to 2:1.¹ However, female sex is a risk factor for the persistence of asthma symptoms into adulthood,² whereas remission seems to be more pronounced in boys during puberty.³ During adolescence and adulthood, more female than male subjects acquire asthma,⁴ resulting in a female predominance in asthma prevalence among adults.⁵ Changes in physiology and the hormonal milieu during puberty are suggested mechanisms for sex-specific disease susceptibility.⁶ Furthermore, sex-specific differences in social behavior, exposure to environmental disease triggers, and disease awareness can exist.^{6,7}

However, sex-specific differences in age of onset and persistence of asthma can also result from distinct dissimilarities in genetic susceptibility and in mechanisms of disease development between girls and boys. Identifying the genetic basis of sex differences in asthma onset and its course is urgently needed to develop accurate prognostic markers and a more personalized approach to therapeutic intervention. In this study we conducted a genome-wide search for sex-specific associations with childhood asthma, performed replication studies in 7 different study populations, analyzed the functional relevance of associated polymorphisms, and approached the potential role of the associated gene in asthma pathogenesis.

METHODS

Genome-wide study of sex by SNP interactions on childhood-onset asthma risk

Interactions between sex and single nucleotide polymorphisms (SNPs) on childhood-onset asthma risk were analyzed on a genome-wide level in the Multicentre Asthma Genetics in Childhood Study (MAGICS)/Phase II International Study of Asthma and Allergies in Childhood (ISAAC II) population (see the [Methods](#) section and [Table E1](#) in this article's Online

Abbreviations used

ALSPAC:	Avon Longitudinal Study of Parents and Children
Ankara:	Clinical study population recruited in Ankara, Turkey
AP-1:	Activator protein 1
BAMSE:	Children, Allergy, Milieu, Stockholm an Epidemiological Study
Ct:	Cycle threshold
DAG:	Dutch Asthma Genome-wide Association Study
DMRT:	Doublesex and mab-3 related transcription factor
EMSA:	Electrophoretic mobility shift assay
Freiburg:	Clinical study population recruited in Freiburg, Germany
ISAAC II:	Phase II International Study of Asthma and Allergies in Childhood
LD:	Linkage disequilibrium
MAF:	Minor allele frequency
MAGICS:	Multicentre Asthma Genetics in Childhood Study
MCPI:	Monocyte chemotactic protein 1
PIAMA:	Prevention and Incidence of Asthma and Mite Allergy
SNP:	Single nucleotide polymorphism
Tomsk:	Clinical study population primarily recruited in Tomsk, Russia

Repository at www.jacionline.org for population details). A total of 1361 subjects (703 asthmatic subjects with 65.3% male subjects and 658 nonasthmatic control subjects with 49.7% male subjects) with chip-based SNP genotypes were available (Sentrix HumanHap300 BeadChip; Illumina, San Diego, Calif).⁸ All calculations were carried out with the PLINK software package, version 1.07, by using the following filtering parameters: minor allele frequency (MAF) of 0.05 or greater, SNP genotyping rate of 0.95 or greater, and Hardy-Weinberg disequilibrium P value in the control population of .0001 or greater.⁹ Logistic regression was used to model dominant SNP effects on asthma status in the complete MAGICS/ISAAC II data set, as well as in male and female subsets. For the interaction analysis, an additional sex-SNP interaction term was introduced in the regression model.

Replication of selected interaction signals was performed in 7 independent populations with childhood-onset asthma phenotypes (the Avon Longitudinal Study of Parents and Children [ALSPAC]; the clinical study population recruited in Ankara, Turkey [Ankara]; the Children, Allergy, Milieu, Stockholm an Epidemiological Study [BAMSE]; the Dutch Asthma Genome-wide Association Study [DAG]; the clinical study population recruited in Freiburg, Germany [Freiburg]; the Prevention and Incidence of Asthma and Mite Allergy [PIAMA] study; and the clinical study population primarily recruited in Tomsk, Russia [Tomsk]; see the [Methods](#) section and [Table E1](#) in this article's Online Repository for population details).

Genetic fine mapping of the *DMRT1* locus

Fine mapping and linkage disequilibrium (LD) analyses were carried out in the MAGICS/ISAAC II data set to determine the extent of sex-specific asthma associations in the doublesex and mab-3 related transcription factor 1 (*DMRT1*) locus. Genetic fine mapping was performed by using a tagging SNP approach based on HapMap data and enriched with SNPs from the 1000 Genomes Project and the SNPper database (see the [Methods](#) section in this article's Online Repository for details).¹⁰⁻¹² Polymorphisms not present in the MAGICS/ISAAC II data set of chip- and imputation-based genotypes were genotyped by using mass spectrometry, as described previously.^{13,14} LD structure and tagging bins of *DMRT1* in the MAGICS/ISAAC II population were calculated with Haploview software (*DMRT1* $\pm$ 10 kb; MAF $\geq$ 0.05; pairwise LD threshold, $r^2 \geq$ 0.8).¹⁵ Dominant SNP effects on asthma status were determined for both sexes by using PLINK. Male-specific asthma associations were investigated for independence in a

set of conditional analyses in which the strongest associated SNP from every tagging bin was individually used as a covariate in logistic regression.

In silico analysis of putative SNP function

Localization of SNPs to conserved coding regions of *DMRT1* was determined by means of alignment of amino acid sequences of all human *DMRT* gene family members (Vector NTI Advance 11; Invitrogen, Life Technologies, Carlsbad, Calif). The functional effect of nonsynonymous SNPs was assessed with the PolyPhen-2 software tool.¹⁶ Phylogenetic shadowing between human subjects and mice was investigated with the Vista Genome Browser (settings: *DMRT1* ±10 kb flanking sequences; 70% sequence conservation in window size 50 bp).¹⁷ Allele-specific changes in transcription factor binding were predicted with AliBaba2.1.¹⁸

Electrophoretic mobility shift assay

Allele-specific differences in DNA-protein interaction were investigated by using electrophoretic mobility shift assays (EMSAs; see the [Methods](#) section and [Table E6](#) in this article's Online Repository at www.jacionline.org for details). Briefly, isotope-labeled DNA probes carrying the SNP allele of interest were incubated with nuclear protein extracts from HEK-293 cells, and resulting DNA-protein complexes were resolved on gels. Competition experiments with unlabeled probes or addition of specific antibodies were used to identify DNA-protein complexes.

Dual luciferase reporter assay

Allele-specific effects on activity of the putative *DMRT1* promoter were investigated in dual luciferase reporter assays (see the [Methods](#) section in this article's Online Repository for details). In short, approximately 2 kb of genomic sequence upstream of *DMRT1* was cloned into the pGL3-Basic vector upstream of the Firefly Luciferase gene (see [Fig E2](#) and [Table E7](#) in this article's Online Repository at www.jacionline.org; Promega, Mannheim, Germany). Vectors carrying the SNP allele of interest were used for transfection of HEK-293 cells, and Firefly Luciferase activity was used as a readout of promoter activity. Cotransfection of Renilla Luciferase driven by the thymidine kinase promoter was used for normalization of the Firefly Luciferase signal.

DMRT1 expression in human tissues

Current literature describes considerable expression of *DMRT1* in the testes only.¹⁹ To identify *DMRT1* expression in further potentially relevant tissues, we conducted quantitative real-time PCR on a cDNA library of human tissues and cell lines (see the [Methods](#) section in this article's Online Repository for more details). Expression levels of *18S rRNA* and the Δ cycle threshold (Ct) method (Ct_{*DMRT1*}–Ct_{*18S rRNA*}) were used to calculate relative gene expression.²⁰

DMRT1 immunohistochemistry was performed on human tissue sections from testis and lung tissue. Details on ethical guidelines for sample collection and methods for immunohistochemical and hematoxylin staining can be found in the [Methods](#) section in this article's Online Repository.

RESULTS

Genome-wide analysis of sex-specific effects on asthma risk and *DMRT1* fine mapping

The strongest sex-SNP interaction on asthma status was observed for rs2187366 ($P = 3.29 \times 10^{-6}$), which is located 8 kb upstream of the uncharacterized gene *LOC100505817* on chromosome 18 ([Fig 1](#)). Almost identical significance levels were observed for rs4500131 and rs2146532 ($P = 5.21 \times 10^{-6}$ and $P = 7.45 \times 10^{-6}$, respectively), which are located in the *DMRT1* gene on chromosome 9p24, and an additional 2 polymorphisms in the *DMRT1* locus (rs3812523 and rs1323271) were among the 100 strongest interaction signals ([Fig 1](#) and see [Table E2](#), A, in this article's Online Repository at www.jacionline.org). Other interaction signals were distributed

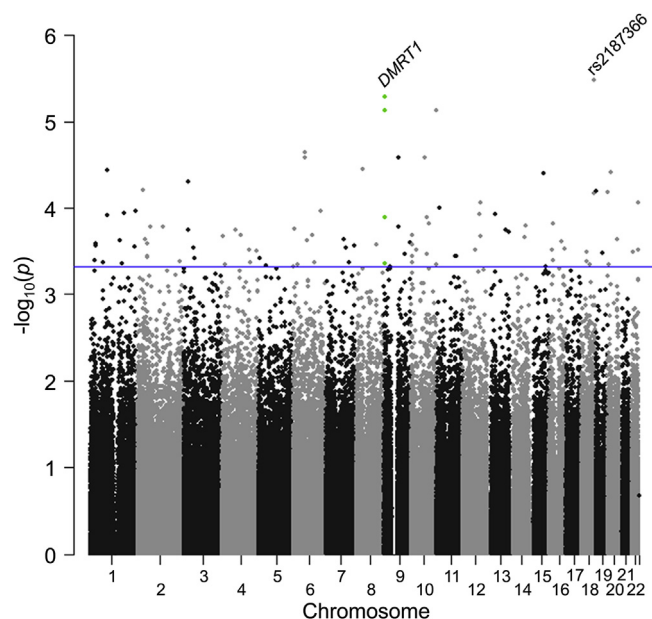


FIG 1. Genome-wide interaction analysis between sex and polymorphisms on asthma risk. The Manhattan plot depicts P values of interactions between sex and SNPs on childhood asthma associations in the MAGIC/ISAAC II population. The 100 most significant observations are indicated above the blue line, and polymorphism rs2187366 on chromosome 18 obtained the lowest P value ($P = 3.29 \times 10^{-6}$). The top 100 interaction signals were distributed over the genome, with not more than 2 signals within 1000 kb (details available from the authors on request), except the *DMRT1* locus on chromosome 9, which contained 4 interaction signals within 5 kb (highlighted in green: rs4500131, $P = 5.21 \times 10^{-6}$; rs2146532, $P = 7.45 \times 10^{-6}$; rs3812523, $P = 1.28 \times 10^{-4}$; rs1323271, $P = 4.37 \times 10^{-4}$).

over the genome, with no more than 2 signals within 1000 kb. Because of the known male-specific biological function of *DMRT1*, this locus was selected for further analyses, despite ranging slightly below a genome-wide significance level at a P value of less than 1.6×10^{-7} .¹⁹ Asthma association data for *DMRT1* polymorphisms revealed protective effects for the minor allele in male subjects with odds ratios of less than 1, whereas female subjects displayed no or opposite effects ([Fig 2](#) and see [Table E2](#), B and C).

Replication analyses of *DMRT1* polymorphisms rs4500131 and rs3812523 ($r^2 = 0.43$ in MAGIC/ISAAC II) were performed in 6 (rs4500131) and 7 (rs3812523) independent populations with childhood-onset asthma. Polymorphisms rs2146532 and rs1323271 were not investigated during replication because of their high LD with rs4500131 ($r^2 = 0.98$ and $r^2 = 0.80$, respectively). Polymorphism rs4500131 exhibited a trend for interaction in clinical studies from Tomsk and Freiburg ([Fig 2](#) and see [Table E3](#) in this article's Online Repository at www.jacionline.org). Comparable with the MAGIC/ISAAC II population, the rs4500131 minor allele displayed protective effects in male asthmatic subjects in the clinical Tomsk and DAG cohorts, as well as a trend in the birth cohort PIAMA. For female asthmatic subjects, a trend for replication of risk effects was observed in Freiburg, whereas DAG displayed a protective trend. For rs3812523, significant replications of the sex interaction were observed in Tomsk and Freiburg, as well as a significant protective effect in male asthmatic subjects in Tomsk and a trend in Ankara and the PIAMA cohort. As for rs4500131, rs3812523 results for female asthmatic subjects were inhomogeneous, with a

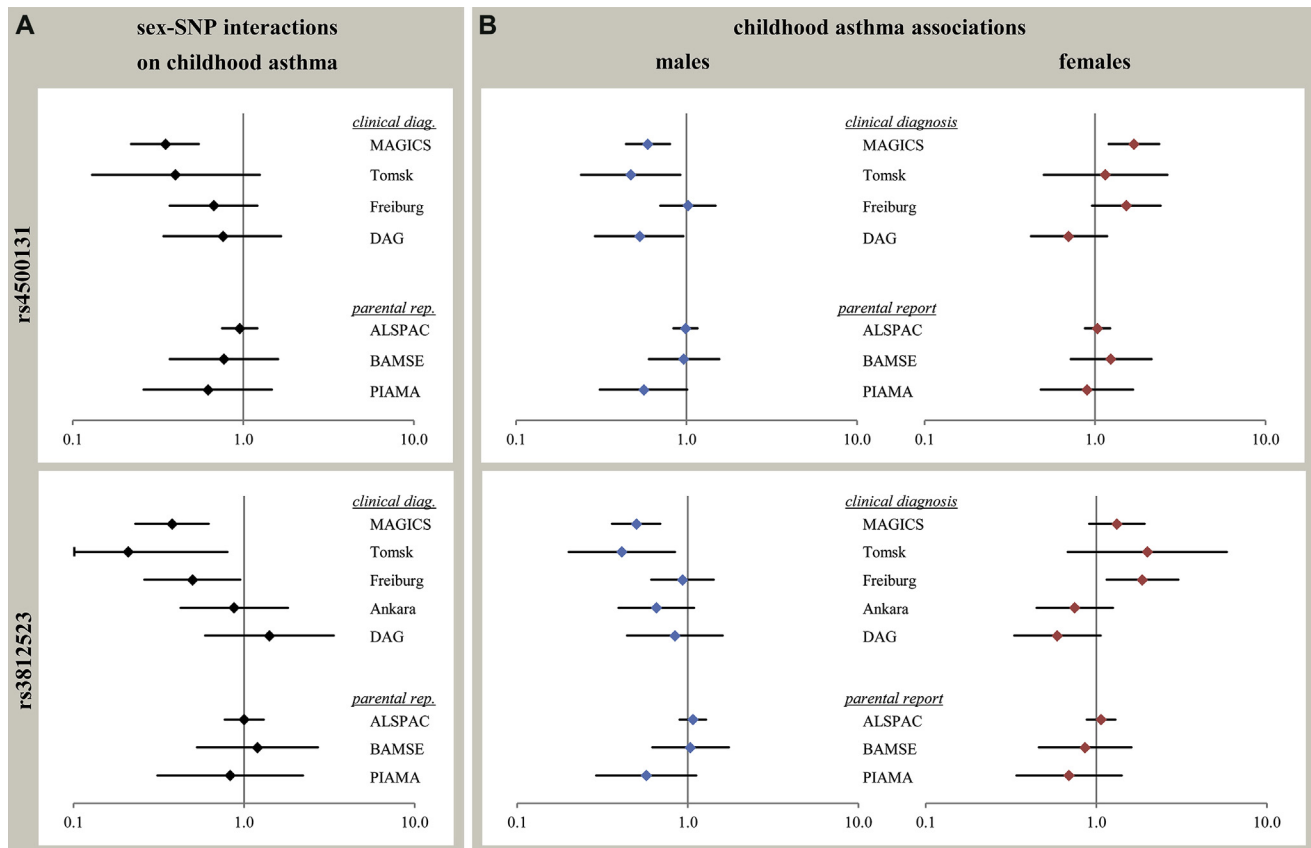


FIG 2. *DMRT1* sex-specific effects on asthma associations. Polymorphisms identified in the MAGICS/ISAAC II discovery population were analyzed in up to 7 independent populations. Results for rs2146532 and rs1323271 are not shown because of high LD with rs4500131. **A**, Sex-SNP interactions on asthma association. Polymorphism rs4500131 displayed a trend for interaction in Tomsk, whereas replication of interaction was observed for rs3812523 in Tomsk and Freiburg. **B**, Sex-specific asthma associations. The minor allele of rs4500131 displayed protective effects in male subjects in Tomsk and DAG and a trend in the PIAMA cohort. Polymorphism rs3812523 replicated this protective effect in male subjects in Tomsk, as well as a trend in Ankara and the PIAMA cohort. Female subjects showed inhomogeneous results, with risk effects in some populations and a trend for protection in other populations.

significant risk effect in Freiburg and a trend for risk in Tomsk, whereas Ankara and DAG displayed trends for protective effects.

Genetic fine mapping of the 147 kb *DMRT1* locus in the MAGICS/ISAAC II population identified 182 SNPs in 58 tagging bins (*DMRT1* ± 10 kb; MAF ≥ 0.05 ; and $r^2 \geq 0.8$). Detailed information on all genotyped and analyzed SNPs is provided in Table E4 in this article's Online Repository at www.jacionline.org. Significant male-specific effects ($P_{\text{male}} < .01$) clustered to 23 polymorphisms in the 5' gene region. These SNPs belonged to 5 different tagging bins, with the strongest signal originating from rs3812523 (Fig 3 and Table I). Male-specific association signals based on imputation for rs7022951, rs2025309, rs4338164, and rs3739583 with asthma and rs7040024 and rs6477321 with eczema were verified by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (eczema data are not shown but are available from the authors on request).

In silico and conditional analyses identify rs3812523 as a candidate for functional investigation

All polymorphisms with male-specific asthma associations at a P value of less than .01 were analyzed *in silico*. Alignment of the human *DMRT* protein family revealed high similarity in the amino

acid sequence in the DM domain for DNA binding. Polymorphism rs3739583 lies outside of this domain, and the amino acid exchange from serine to threonine was predicted to be benign (Table I). Comparison between human and mouse sequences identified 5 SNPs to be located in phylogenetically conserved regions (rs7022951, rs7858180, rs3812523, rs3739583, and rs2146532), and allele-specific effects on transcription factor binding were predicted for 4 polymorphisms (rs7022951, rs3812523, rs6477293, and rs2181402; Table I). Conditional analysis using the strongest associated SNP from every tagging bin with male-specific associations as a covariate revealed that associations of the remaining 22 SNPs are dependent on rs3812523 (Table I and see Table E5 in this article's Online Repository at www.jacionline.org; detailed data on *in silico* analyses are available on request from the authors). In conclusion, *in silico* and conditional analyses suggested rs3812523 to be the driving force for the male-specific asthma association.

rs3812523 demonstrates allele-specific effects on transcription factor binding and promoter activity in vitro

EMSA identified a specific DNA-protein complex for the nonrisk G allele of rs3812523 (conferring a lower risk for asthma

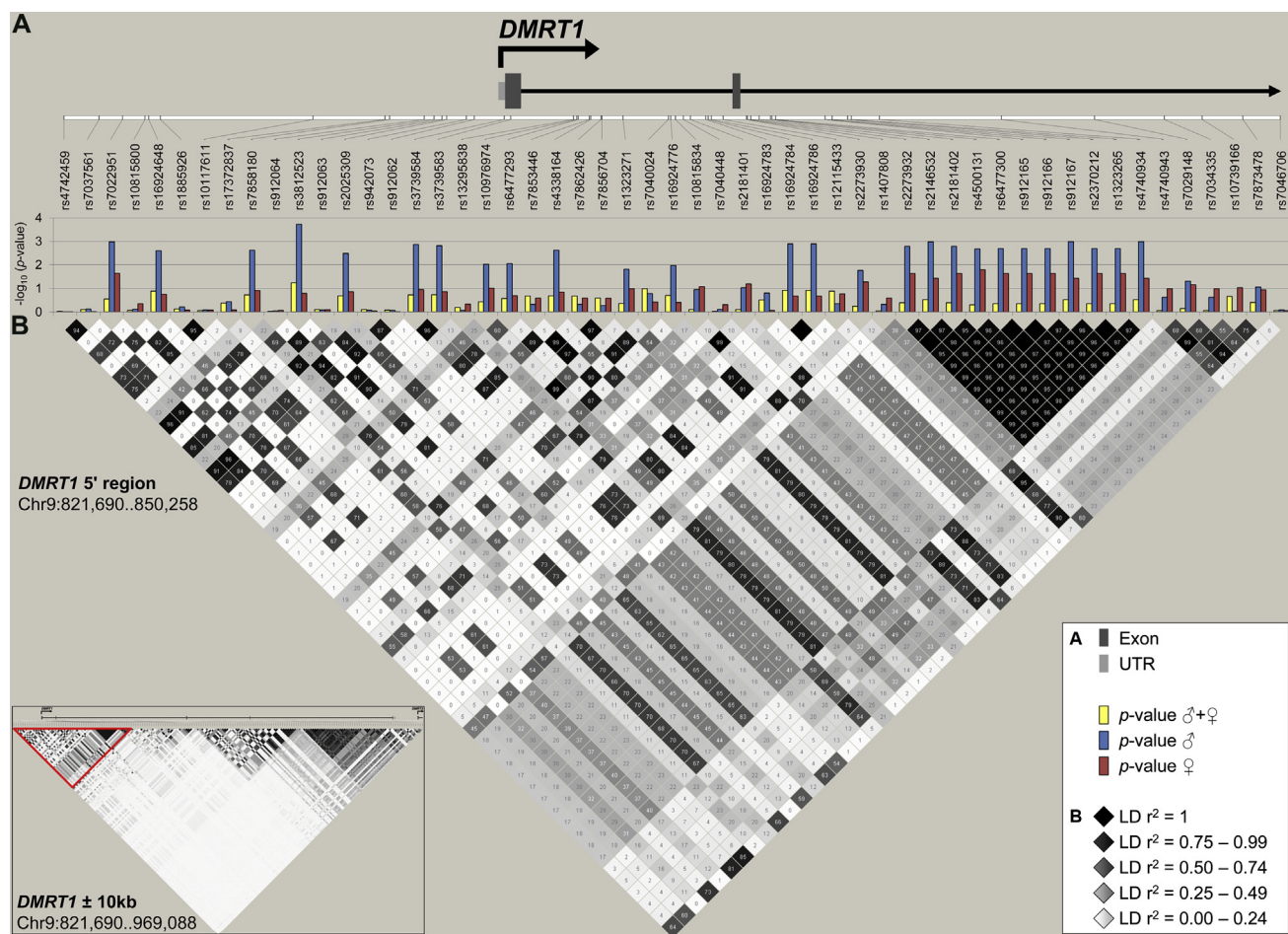


FIG 3. Fine mapping of male-specific asthma associations in *DMRT1*. Genome-wide analysis identified clustering of sex-SNP interactions on asthma risk to the *DMRT1* locus. Subsequently, *DMRT1* was genetically fine mapped to determine sex-specific asthma associations. **A**, Male-specific asthma associations clustered to the 5' region of *DMRT1*. In female subjects weaker but opposite effects were observed (see Table I for effect directions). The localization of all analyzed SNPs within this area is depicted. Colored bars represent P values ($-\log_{10}$) for sex-combined (yellow), male-specific (blue), and female-specific (red) analyses for asthma associations in the MAGICS/ISAAC II data set. **B**, The LD structure between polymorphisms was measured by using r^2 analysis.

in male subjects) in nuclear protein extract from HEK-293 cells, which was not present for the risk A allele (Fig 4, complex I, lanes 3-6). Competition experiments demonstrated a high allele specificity of this DNA-protein complex (Fig 4, lanes 7-8). Competition with a high-affinity activator protein 1 (AP-1) consensus site successfully abrogated the DNA-protein complex (Fig 4, lane 9). The dimeric AP-1 transcription factor can be formed by different members of the Jun, Fos, activating transcription factor, and Jun dimerization protein families.²¹ Antibody-specific supershift experiments revealed a supershift of the DNA-protein complex after incubation with a c-Jun antibody (Fig 4, lane 11) and negative results for c-Fos (Fig 4, lane 12), activating transcription factor 7 (Fig 4, lane 13), and Jun dimerization protein 2 (data not shown).

A second DNA-protein complex at a lower molecular weight also displayed allele-specific effects (Fig 4, complex II, lanes 3-6). However, for both alleles, the cross-competitions were incomplete (Fig 4, lanes 1 and 8), illustrating that different DNA-protein complexes are overlaid at this height of the EMSA gel. This finding is supported by competition with the

AP-1 consensus site (see Fig E1, complex II, lanes 5 and 14 in this article's Online Repository at www.jacionline.org), leading to complete abrogation of this complex only in the nonrisk allele. Antibody-specific supershifts in both the nonrisk and risk alleles confirmed that c-Jun and c-Fos participate in this complex (see Fig E1, complex II, lanes 2, 3, 16, and 17). However, even after successful c-Jun and c-Fos supershifts, an unidentified complex remains visible in the risk allele (see Fig E1, complex II, lanes 2 and 3).

Dual luciferase reporter assays were conducted to test whether the allele-specific binding of transcription factors to rs3812523 observed in EMSA has an influence on its activity as a promoter. A strong increase in luciferase signal between the empty pGL3-Basic vector and the 2 pGL3-*DMRT1* vectors demonstrates the functionality of this putative *DMRT1* promoter region (Fig 5). Comparison between the rs3812523 risk and nonrisk alleles displayed a significantly lower expression of the Firefly Luciferase gene under the control of the nonrisk allele in all tested conditions, with the strongest effect in unstimulated HEK-293 cells ($P = .0008$).

TABLE I. Male-specific asthma associations in the *DMRT1* locus and *in silico* analyses for SNP function

SNP	Tagging bin	r^2	Localization	MAF	Asthma association*			
					Sex-combined analysis		Male-specific analysis	
					Odds ratio (95 % CI)	P value	Odds ratio (95 % CI)	P value
rs7022951‡	1		5' Upstream	0.13	0.88 (0.69-1.12)	.282	0.58 (0.42-0.80)	.0010
rs16924648‡	2		5' Upstream	0.12	0.82 (0.63-1.06)	.129	0.58 (0.41-0.83)	.0025
rs7858180	6	0.93	5' Upstream	0.19	0.86 (0.68-1.08)	.188	0.62 (0.46-0.85)	.0023
rs2025309‡	6		5' Upstream	0.19	0.87 (0.70-1.08)	.206	0.64 (0.48-0.86)	.0032
rs3812523	8	0.82	5' Upstream	0.14	0.79 (0.61-1.01)	.058	0.53 (0.38-0.74)	.00019
rs3739584	8	0.87	Exon 1 5' UTR	0.13	0.85 (0.67-1.08)	.189	0.60 (0.43-0.82)	.0013
rs3739583	8	0.85	Exon 1 (S45T)	0.13	0.85 (0.67-1.08)	.185	0.60 (0.44-0.82)	.0015
rs6477293	8	0.90	Intron 1	0.14	0.87 (0.68-1.11)	.267	0.65 (0.47-0.90)	.0088
rs4338164‡	8		Intron 1	0.13	0.86 (0.67-1.09)	.206	0.61 (0.45-0.84)	.0023
rs16924776	8	0.80	Intron 1	0.13	0.85 (0.66-1.09)	.199	0.65 (0.47-0.90)	.010
rs16924784	8	0.85	Intron 1	0.12	0.82 (0.63-1.06)	.120	0.57 (0.41-0.80)	.0013
rs16924786	8	0.85	Intron 1	0.12	0.82 (0.63-1.06)	.120	0.57 (0.41-0.80)	.0013
rs10976974	14	0.80	Intron 1	0.22	0.90 (0.72-1.13)	.374	0.67 (0.50-0.91)	.0094
rs1323271	14	0.81	Intron 1	0.22	0.92 (0.73-1.15)	.438	0.69 (0.51-0.93)	.015
rs2273932	14	0.96	Intron 2	0.23	0.91 (0.73-1.14)	.410	0.62 (0.46-0.84)	.0016
rs2146532	14	0.99	Intron 2	0.23	0.89 (0.71-1.11)	.296	0.61 (0.45-0.82)	.0010
rs2181402	14	0.96	Intron 2	0.23	0.91 (0.73-1.14)	.410	0.62 (0.46-0.84)	.0016
rs4500131‡	14		Intron 2	0.23	0.93 (0.74-1.16)	.497	0.62 (0.46-0.84)	.0021
rs6477300	14	0.96	Intron 2	0.23	0.92 (0.74-1.14)	.443	0.63 (0.47-0.84)	.0020
rs912165	14	0.96	Intron 2	0.23	0.92 (0.74-1.14)	.443	0.63 (0.47-0.84)	.0020
rs912166	14	0.96	Intron 2	0.23	0.92 (0.74-1.14)	.443	0.63 (0.47-0.84)	.0020
rs912167	14	0.99	Intron 2	0.23	0.89 (0.71-1.11)	.296	0.61 (0.45-0.82)	.0010
rs2370212	14	0.96	Intron 2	0.23	0.92 (0.74-1.14)	.443	0.63 (0.47-0.84)	.0020
rs1323265	14	0.96	Intron 2	0.23	0.92 (0.74-1.14)	.443	0.63 (0.47-0.84)	.0020
rs4740934	14	0.99	Intron 2	0.23	0.89 (0.71-1.11)	.296	0.61 (0.45-0.82)	.0010

+/-, Positive or negative outcome; NA, analysis method not applicable; TF, transcription factor; UTR, untranslated region.

*After fine mapping of *DMRT1*, a total of 23 polymorphisms with male-specific effects on asthma status ($P_{\text{male}} < .01$; in boldface) were identified in the MAGIC/ISAAC II data set. Information is given on pairwise LD between SNPs and their tagging SNPs (§). The SNP localization within the *DMRT1* gene structure and the MAFs in the MAGIC/ISAAC II population are listed.

‡*In silico* analyses for functional relevance of SNPs were carried out at the protein and DNA level, but also statistical effects were considered to identify a candidate SNP for functional *in vitro* analysis. Polymorphism rs3812523 in the putative promoter of *DMRT1* was identified as a target for functional *in vitro* analysis.

***DMRT1* is expressed in testis tissue and macrophages in the lung**

We screened a cDNA library of human tissue samples and human cell lines for *DMRT1* expression (see Table E8 in this article's Online Repository at www.jacionline.org). As expected, testis tissue displayed strong *DMRT1* expression, but lower mRNA levels were also observed in immunologically relevant cell lines (Jurkat and YT cells) and in HEK-293 cells.

Immunohistochemistry for *DMRT1* presented strong nuclear staining of adult testis Sertoli cells and possibly some germ cells (Fig 6).¹⁹ Lung tissue from patients with normal lung function displayed weak *DMRT1* staining in alveolar macrophages. Furthermore, lung tissue from adults with asthma, desquamative interstitial pneumonia, chronic obstructive pulmonary disease, or idiopathic pulmonary fibrosis presented strong staining for *DMRT1* in all alveolar macrophages and also less prominent staining in interstitial macrophages. In contrast to staining in testis tissue, *DMRT1* appears to primarily locate to the cytoplasm of macrophages in the lung. There was no staining of granulocytes, lymphocytes, or plasma cells. The appendix, thymus, lymph node, and spleen indicated only staining in a subset of macrophages, mainly in the germinal center (so-called tingible body macrophages) and vascular structures, morphologically

high endothelial venules (see Fig E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Our genome-wide analysis for interactions between sex and SNP effects on childhood asthma risk identified a number of strong interactions close to the genome-wide significance level set for simple association studies. Our analysis did not replicate sex-SNP interaction results from a previous study on asthma in European American subjects in which the polymorphism rs2549003, which is located near interferon regulatory factor 1 (*IRF1*) on chromosome 5q31, displayed a significant sex-SNP interaction on asthma with male-specific effects.²² In our data set no trend for interaction ($P = .89$) or asthma association in male subjects ($P_{\text{male}} = .39$) could be observed for rs2548997, which was described to be in complete LD with rs2549003 in the same study.

Polymorphisms in *DMRT1* displayed strong asthma associations in male subjects and weaker but opposite effects in female subjects. Replication of rs4500131 and rs3812523 ($r^2 = 0.43$) was successful in some but not all populations. Interestingly, the clinical Toms and Freiburg populations

TABLE I. (Continued)

Female-specific analysis		<i>In silico</i> analyses [†]				
Odds ratio (95 % CI)	P value	Conserved AA sequence (human DMRT family)/ influence on protein function	Conserved genomic region (human and mouse)	Allele-specific effect on TF binding	Conditional association analysis	Strongest association signal
1.54 (1.06-2.23)	.023	NA	+	+	—	—
1.31 (0.88-1.95)	.179	NA	—	—	—	—
1.32 (0.93-1.88)	.124	NA	+	—	—	—
1.30 (0.92-1.82)	.138	NA	—	—	NA	—
1.31 (0.90-1.93)	.162	NA	+	+	+	+
1.36 (0.93-1.97)	.113	NA	—	—	NA	—
1.33 (0.91-1.95)	.137	—/—	+	—	NA	—
1.28 (0.87-1.88)	.203	NA	—	+	NA	—
1.32 (0.91-1.93)	.142	NA	—	—	NA	—
1.19 (0.80-1.76)	.394	NA	NA	NA	NA	NA
1.29 (0.86-1.93)	.211	NA	—	—	NA	—
1.29 (0.86-1.93)	.211	NA	—	—	NA	—
1.34 (0.95-1.90)	.097	NA	—	—	NA	—
1.33 (0.94-1.89)	.103	NA	NA	NA	NA	NA
1.49 (1.06-2.11)	.023	NA	—	—	NA	—
1.44 (1.02-2.03)	.037	NA	+	—	NA	—
1.49 (1.06-2.11)	.023	NA	—	+	NA	—
1.54 (1.08-2.18)	.016	NA	—	—	NA	—
1.49 (1.06-2.11)	.023	NA	—	—	NA	—
1.49 (1.06-2.11)	.023	NA	—	—	NA	—
1.49 (1.06-2.11)	.023	NA	—	—	NA	—
1.44 (1.02-2.03)	.037	NA	—	—	—	—
1.49 (1.06-2.11)	.023	NA	—	—	NA	—
1.49 (1.06-2.11)	.023	NA	—	—	NA	—
1.44 (1.02-2.03)	.037	NA	—	—	—	—

presented the best replication of our findings, whereas the clinically recruited DAG population with an algorithm-based asthma diagnosis replicated part of the observations. Effects in birth cohorts with parental report of doctor's asthma diagnosis were weaker or not present (ALSPAC, BAMSE, and PIAMA). This might indicate that sex-specific effects of DMRT1 on asthma pathogenesis might be less pronounced in a population-based milder asthma phenotype compared with that in populations recruited from tertiary clinical centers. Additionally, opposite effect directions between male and female subjects, as well as ambiguous effects in female subjects from different studies, could indicate that DMRT1 function in asthma pathogenesis might be modulated by yet unknown population-specific environmental factors.²³

Our fine mapping focused on male subjects because of the known biological function of DMRT1 in testis tissue, and this approach identified that asthma associations are defined to 23 polymorphisms in the 5' region of *DMRT1*. Conditional analysis and *in silico* prediction suggested rs3812523, which is located in the putative promoter of *DMRT1*, as the best candidate that can drive the sex-specific asthma association. EMSA displayed allele-specific binding of c-Jun to the nonrisk allele of rs3812523, whereas a second DNA-protein complex containing

heterodimeric AP-1 (c-Jun/c-Fos) exhibited quantitative effects in binding affinity between risk and nonrisk alleles. Additionally, the risk allele specifically binds another yet unidentified transcription factor. In line with these observations, our promoter activity assays also demonstrated significant allele-specific effects for rs3812523. Interestingly, stimulation with phorbol 12-myristate 13-acetate leads to a relatively lower promoter activity, which is in line with observations of inhibited *DMRT1* expression in primary mouse Sertoli cells after phorbol 12-myristate 13-acetate stimulation.²⁴ These *in vitro* analyses allow us to assume that also the *in vivo* expression of *DMRT1* might be influenced by rs3812523, which could contribute to the functional relevance of the association with asthma.

With *DMRT1*, we propose a potential factor for genetically driven differences in the cause of childhood asthma between sexes. *DMRT1* belongs to a family of transcriptional regulators containing the DM domain DNA-binding motif, which was first identified in a comparison of the *Drosophila melanogaster* gene doublesex and the *Caenorhabditis elegans* gene male abnormal 3.²⁵ This DM domain can be found in a wide spectrum of animal taxa, and its function in sexual differentiation has been reviewed in detail by Matson and Zarkower.¹⁹ For mammals, Matson and Zarkower describe exclusive expression of *DMRT1* in the gonads,

Probe	risk allele				nonrisk allele										
Antibody											c-Jun	c-Fos	ATF-7	‡	
Competition	non-risk allele	risk-allele					non-risk allele	risk-allele	AP1	†					
Nuclear extract	HEK P/I	HEK P/I	HEK P/I	HEK unst.	HEK unst.	HEK P/I	HEK P/I	HEK P/I	HEK P/I	HEK P/I	HEK P/I	HEK P/I	HEK P/I	HEK P/I	
Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

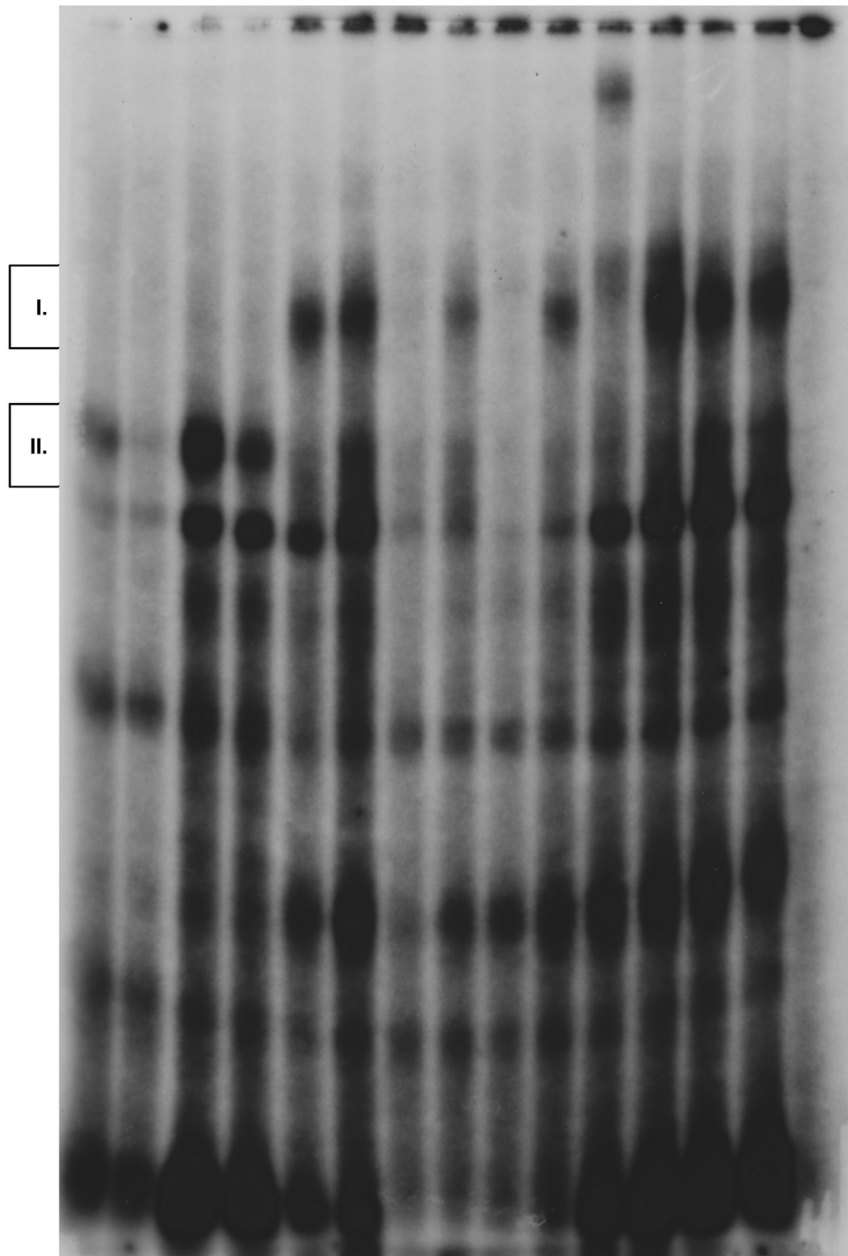


FIG 4. Allele-specific binding of c-Jun to rs3812523. EMSAs were carried out for the risk and nonrisk alleles of rs3812523 by using nuclear protein extracts from HEK-293 cells without stimulation and after stimulation with phorbol 12-myristate 13-acetate-ionomycin (P/I). *Complex I*, Distinct binding of proteins to the nonrisk allele was observed for unstimulated and P/I-stimulated conditions (*lanes 3-6*). Competition with the risk allele proved specificity of protein binding to the nonrisk allele (*lanes 7 and 8*). Competition with an AP-1 consensus site suggested involvement of this transcription factor (*lane 9*). Incubation with a c-Jun antibody proved c-Jun binding by means of retardation of the DNA-protein complex (*lane 11*). Antibody tests for the additional AP-1-associated molecules c-Fos and activating transcription factor 7 (ATF-7) showed no effect (*lanes 12 and 13*). *Complex II*, Further allele-specific effects were observed (*lanes 3-6*), which were shown to involve different overlaying DNA-protein complexes because of incomplete cross-competitions (*lanes 1 and 8*). Further information on the identity of these complexes is given in [Fig E1](#). †Unrelated double-stranded DNA probe. ‡Unrelated antibody.

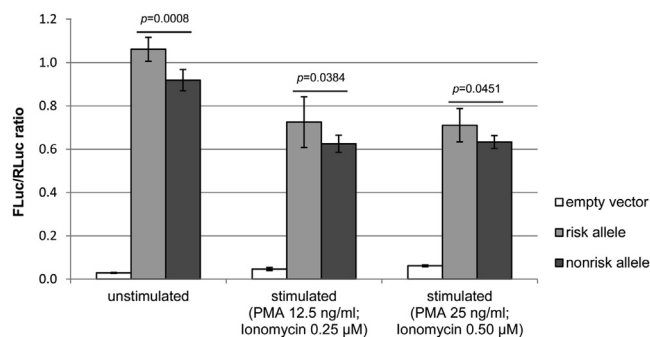


FIG 5. Influence of rs3812523 on promoter activity. Expression of Firefly Luciferase proved the functionality of the genomic sequence upstream of *DMRT1* as a promoter compared with the empty vector. Substitution of the rs3812523 risk allele with the nonrisk allele caused a significant decrease in promoter activity in all tested conditions. Firefly Luciferase expression was normalized to expression of Renilla Luciferase in the same sample (*FLuc/RLuc* ratio). The conditions *unstimulated* and *PMA25ng/ml; Ionomycin0.5μM* represent 6 samples. *PMA12.5ng/ml; Ionomycin0.25μM* represents 8 samples. *PMA*, Phorbol 12-myristate 13-acetate.

which becomes male specific during fetal testes differentiation. Human adult testes express *DMRT1* in Sertoli cells and spermatogonia but not in most other testicular cells.²⁶ Genetic studies linked human *DMRT1* with testicular germ cell cancer and XY gonadal disorders of sexual development.^{27,28} Deletion of *DMRT1* in male adult mice caused testicular Sertoli cells to transdifferentiate toward female ovarian granulosa cells.²⁹ In the same study specific deletion of *DMRT1* in fetal Sertoli cells caused increased estradiol levels and reduced androgen activity. It has been shown that *DMRT1* is continuously expressed in human testis tissue from fetal stages until puberty and thereafter. However, this expression appears to follow a spatiotemporal expression pattern in different testis cell types, whereas in human ovaries *DMRT1* expression is only present in early fetal stages and downregulated in later fetal stages.²⁶ One could speculate that rs3812523-driven regulation of *DMRT1* expression might influence, to some extent, the timing of testis development and, as a consequence, also the levels and timing of sexual hormone expression. It is an attractive notion that boys carrying the nonrisk allele benefit from an effect that might normally not occur until puberty, when “outgrowing” of asthma symptoms is frequently observed.³

However, it is possible that *DMRT1* exerts its influence on asthma from another tissue than testis. Our cDNA screen detected *DMRT1* expression in lymphoid cell lines, whereas immunohistochemistry data revealed low levels of *DMRT1* in alveolar macrophages in control lung tissue. In contrast, strong *DMRT1* staining was observed in the cytoplasm of alveolar macrophages in patients with interstitial lung disease (desquamative interstitial pneumonia and idiopathic pulmonary fibrosis) and patients with chronic obstructive pulmonary disease, which might indicate a disease-associated function of *DMRT1*. Bilateral lung explants harvested during lung transplantation from adult asthmatic patients displayed *DMRT1* staining similar to that seen in patients with other lung diseases. However, samples derived from adult patients with asthma accompanied by smoking-induced end-stage lung disease (displaying emphysematous changes and anthracosis in addition to the histologic findings typically associated with asthma, such as chronic inflammation of the

small airways, hypertrophy of bronchial smooth muscles, and mucous plugs) present a considerably different phenotype than childhood asthma. Unfortunately, childhood asthma samples were not available. The sporadic staining of subsets of macrophages and high endothelial venules in the appendix, thymus, lymph node, and spleen suggests that *DMRT1* has a function also in these tissues outside the lung (see Fig E3). Importantly, also female patients displayed positive staining of macrophages, and for now and in contrast to male testis tissue, this cell type appears to be the only site of *DMRT1* expression that could be causative for the genetic association signals observed in female subjects in our analyses. Furthermore, sex-specific gene regulation has been suggested for *DMRT1*, with repression of *Stra8* expression in male mice but enhancement of *Stra8* expression in female mice.³⁰ The underlying mechanism is unknown, but sex-specific gene regulation in human subjects by *DMRT1* might explain the opposing effect directions of asthma associations observed between sexes in our study.

Localization of *DMRT1* to the cytoplasm of macrophages might indicate inactivation of its transcriptional function. Considering the regulatory function of alveolar macrophages in inflammatory responses of the lung,³¹ detailed studies on *DMRT1* function in these cells need to follow, such as the potential of *DMRT1* translocation to the nucleus upon macrophage activation. To date, little is known on the nuclear import of human *DMRT1*, but an importin-β1-dependent mechanism has been suggested, and the potential role of importins in the regulation of allergic immune responses has recently been reviewed.^{32,33} Interestingly, in an explorative study we observed significant correlations between rs3812523 and cytokines secreted from cultured PBMCs ($n = 61$ adults). Among others, protein levels of monocyte chemotactic protein 1 (MCP1), which is also produced by macrophages, were significantly lower in homozygous carriers of the rs3812523 risk allele compared with those in heterozygous carriers ($P = .037$). Moreover, we were able to support this observation with a trend for lower *MCP1* mRNA levels in risk allele carriers ($P = .101$, for details, see the Methods section and Fig E4 in this article's Online Repository at www.jacionline.org).³⁴ The explorative character of this analysis with a relatively small sample size needs to be acknowledged, as well as the unexpected downregulation of the proinflammatory cytokine MCP1 in carriers of the risk allele for asthma, but this effect might serve as a first indication for transcriptional regulation of *DMRT1* in immune cells, which warrants further investigation.

Finally, we also conducted sex-specific analyses for asthma associations in the MAGIC/ISAAC II population for all known human *DMRT* genes (details are provided in Tables E9 and E10 and Fig E5 in this article's Online Repository at www.jacionline.org) and observed weak but significant associations in male subjects in *DMRT2* and *DMRTB1*. This identification of further sex-specific associations within the *DMRT* gene family supports our results for *DMRT1* and suggests a possible role of further DM domain genes in childhood asthma. Additionally, in our sex-combined analysis we observed an asthma association of *DMRTA1*, which is a gene recently identified in a genetic study on atopic dermatitis.³⁵

In conclusion, we present *DMRT1* as a novel candidate to explain sex-specific asthma effects during childhood. Its role in

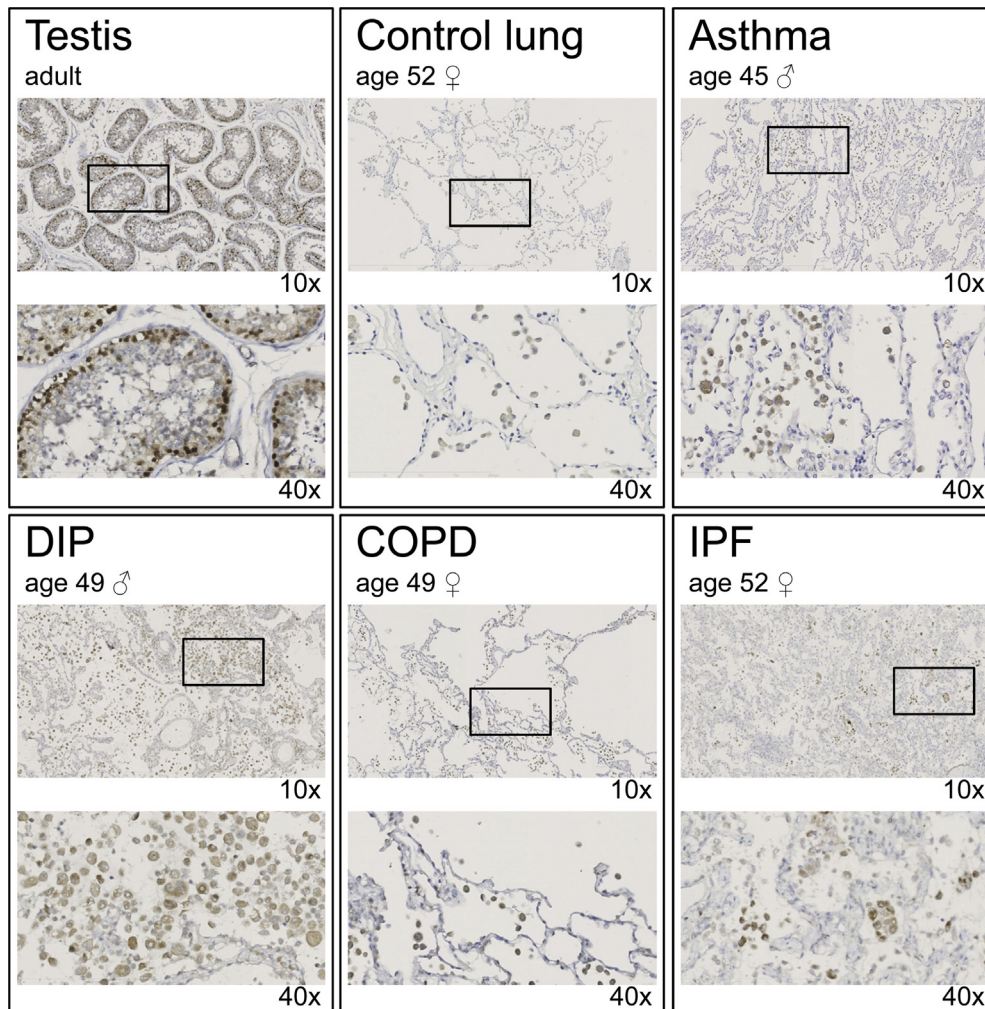


FIG 6. Immunohistochemistry for DMRT1. DMRT1 staining in adult testis tissue was present in nuclei of Sertoli cells and possibly immature germ cells. Lung tissue from patients with normal lung function showed weak DMRT1 staining in alveolar macrophages, whereas tissue from patients with different lung diseases displayed strong DMRT1 staining in all alveolar macrophages and, to a lesser extent, also in interstitial macrophages. Staining in the lung was compatible with localization of DMRT1 to the cytosolic compartment. No staining was observed in granulocytes, lymphocytes, or plasma cells. *Asthma*, Asthma bronchiale (adult patient with accompanying emphysematous changes); *COPD*, chronic obstructive pulmonary disease; *DIP*, desquamative interstitial pneumonia; *IPF*, idiopathic pulmonary fibrosis.

Tissue samples from lungs of asthmatic subjects were kindly provided by the Institute of Pathology (Hannover Medical School, Germany). All other tissue samples were kindly provided by the Department of Pathology and Medical Biology (University of Groningen, The Netherlands).

testis development, as well as our demonstration of *DMRT1* expression in alveolar macrophages, opens potential for further functional investigations. The precise analysis of spatiotemporal gene expression of *DMRT1* will have to follow to elucidate whether and how *DMRT1* expression in testis tissue or in cells of the immune system affects asthma pathogenesis in male and female subjects.

Clinical implications: Sex-specific genetic associations identified *DMRT1* as a candidate for sex-specific effects in childhood asthma. Expression of DMRT1 in testis tissue and alveolar macrophages suggests its involvement in hormone or immune cell regulation.

REFERENCES

1. Almqvist C, Worm M, Leynaert B. Impact of gender on asthma in childhood and adolescence: a GA2LEN review. *Allergy* 2008;63:47-57.
2. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N Engl J Med* 2003;349:1414-22.
3. Soto-Ramirez N, Ziyab AH, Karmaus W, Zhang H, Kurukulaaratchy RJ, Ewart S, et al. Epidemiologic methods of assessing asthma and wheezing episodes in longitudinal studies: measures of change and stability. *J Epidemiol* 2013;23:399-410.
4. Mandhane PJ, Greene JM, Cowan JO, Taylor DR, Sears MR. Sex differences in factors associated with childhood- and adolescent-onset wheeze. *Am J Respir Crit Care Med* 2005;172:45-54.
5. Schatz M, Camargo CA Jr. The relationship of sex to asthma prevalence, health care utilization, and medications in a large managed care organization. *Ann Allergy Asthma Immunol* 2003;91:553-8.

6. Townsend EA, Miller VM, Prakash YS. Sex differences and sex steroids in lung health and disease. *Endocr Rev* 2012;33:1-47.
7. Genuneit J. Sex-specific development of asthma differs between farm and nonfarm children: a cohort study. *Am J Respir Crit Care Med* 2014;190:588-90.
8. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007;448:470-3.
9. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559-75.
10. The International HapMap Consortium. The international HapMap project. *Nature* 2003;426:789-96.
11. Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, et al. A map of human genome variation from population-scale sequencing. *Nature* 2010;467:1061-73.
12. Riva A, Kohane IS. A SNP-centric database for the investigation of the human genome. *BMC Bioinformatics* 2004;5:33.
13. Michel S, Liang L, Depner M, Klopp N, Ruether A, Kumar A, et al. Unifying candidate gene and GWAS Approaches in Asthma. *PLoS One* 2010;5:e13894.
14. Suttner K, Ruoss I, Rosenstiel P, Depner M, Pinto LA, Schedel M, et al. HLX1 gene variants influence the development of childhood asthma. *J Allergy Clin Immunol* 2009;123:82-8.e6.
15. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263-5.
16. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248-9.
17. Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I. VISTA: computational tools for comparative genomics. *Nucleic Acids Res* 2004;32:W273-9.
18. Grabe N. AliBaba2: context specific identification of transcription factor binding sites. *In Silico Biol* 2002;2(suppl):S1-15.
19. Matson CK, Zarkower D. Sex and the singular DM domain: insights into sexual regulation, evolution and plasticity. *Nat Rev Genet* 2012;13:163-74.
20. Schieck M, Sharma V, Michel S, Toncheva AA, Worth L, Potaczek DP, et al. A polymorphism in the TH2 locus control region is associated with changes in DNA methylation and gene expression. *Allergy* 2014;69:1171-80.
21. Hess J, Angel P, Schorpp-Kistner M. AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci* 2004;117:5965-73.
22. Myers RA, Scott NM, Gauderman WJ, Qiu W, Mathias RA, Romieu I, et al. Genome-wide interaction studies reveal sex-specific asthma risk alleles. *Hum Mol Genet* 2014;23:5251-9.
23. Meyers DA, Bleecker ER, Holloway JW, Holgate ST. Asthma genetics and personalised medicine. *Lancet Respir Med* 2014;2:405-15.
24. Chen JK, Heckert LL. Dmrt1 expression is regulated by follicle-stimulating hormone and phorbol esters in postnatal Sertoli cells. *Endocrinology* 2001;142:1167-78.
25. Raymond CS, Shamu CE, Shen MM, Seifert KJ, Hirsch B, Hodgkin J, et al. Evidence for evolutionary conservation of sex-determining genes. *Nature* 1998;391:691-5.
26. Jorgensen A, Nielsen JE, Jensen MB, Graem N, Rajpert-De Meyts E. Analysis of meiosis regulators in human gonads: a sexually dimorphic spatio-temporal expression pattern suggests involvement of DMRT1 in meiotic entry. *Mol Hum Reprod* 2012;18:523-34.
27. Turnbull C, Rapley EA, Seal S, Pernet D, Renwick A, Hughes D, et al. Variants near DMRT1, TERT and ATF7IP are associated with testicular germ cell cancer. *Nat Genet* 2010;42:604-7.
28. Barbaro M, Balsamo A, Anderlid BM, Myhre AG, Gennari M, Nicoletti A, et al. Characterization of deletions at 9p affecting the candidate regions for sex reversal and deletion 9p syndrome by MLPA. *Eur J Hum Genet* 2009;17:1439-47.
29. Matson CK, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D. DMRT1 prevents female reprogramming in the postnatal mammalian testis. *Nature* 2011;476:101-4.
30. Krentz AD, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D. DMRT1 promotes oogenesis by transcriptional activation of Stra8 in the mammalian fetal ovary. *Dev Biol* 2011;356:63-70.
31. Balhara J, Gounni AS. The alveolar macrophages in asthma: a double-edged sword. *Mucosal Immunol* 2012;5:605-9.
32. Ying M, Chen B, Tian Y, Hou Y, Li Q, Shang X, et al. Nuclear import of human sexual regulator DMRT1 is mediated by importin-beta. *Biochim Biophys Acta* 2007;1773:804-13.
33. Aggarwal A, Agrawal DK. Importins and exportins regulating allergic immune responses. *Mediators Inflamm* 2014;2014:476357.
34. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* 2009;29:313-26.
35. Schaarschmidt H, Ellinghaus D, Rodriguez E, Kretschmer A, Baurecht H, Lipinski S, et al. A genome-wide association study reveals 2 new susceptibility loci for atopic dermatitis. *J Allergy Clin Immunol* 2015;136:802-6.

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DMRT1 is a sex-specific genetic determinant of childhood onset asthma and is expressed in testis and macrophages

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Description of childhood onset asthma populations

The Multicentre Asthma Genetics In Childhood Study (MAGICS, cases)^{E1} and the International Study of Asthma and Allergy in Childhood, phase II (ISAAC II, reference population)^{E2} share a genetically homogeneous background of German origin and in both studies similar tools and definitions were used. In MAGICS, asthma was diagnosed in clinical asthma centres by paediatric pulmonologists based on physical examination, case history and objective tests of lung function. In the German arm of ISAAC II, asthma and atopy were assessed in a cross sectional study design in 4th graders from Munich and Dresden by questionnaire, lung function, skin and blood tests. Parental reports of doctor's diagnosis of asthma once or of spastic or asthmatic bronchitis more than once were used to define asthma cases in ISAAC II. Asthmatics from ISAAC II were then added to the large set of MAGICS asthmatics and the remaining ISAAC II subjects were defined as healthy controls for subsequent asthma association analyses. In total, 1361 subjects with genome-wide chip genotyping data were available in MAGICS/ISAAC II (asthmatics: ISAAC II n=72 and MAGICS n=631; controls: ISAAC II n=658).^{E3} Please note that for genetic fine mapping of the *DMRT1* association signal this original cohort of n=1361 was extended to n=1446 with further subjects from MAGICS (asthmatics n=60) and ISAAC II (controls n=25; Table E1). The study protocol was approved by ethic committees of participating centres and written informed consent was given by the parents of all children.

Replication of sex-specific asthma associations in the *DMRT1* locus was approached in seven independent populations with childhood onset asthma phenotypes (population characteristics are summarized in Table E1). In brief, ALSPAC, BAMSE and PIAMA are birth cohorts from Europe with a questionnaire based parental report of a doctor's diagnosis of asthma ever.^{E4-E7} Ankara from Turkey, Freiburg from Germany and Tomsk from Russia are studies with recruitment of children at hospitals and in these studies asthma diagnosis was based on clinical examinations.^{E8-E10} Comparably, DAG is a Dutch study also with recruitment at hospitals and clinical examination. However, in DAG asthma diagnosis was made through an algorithm considering bronchial hyperresponsiveness, respiratory symptoms, smoking, airway obstruction, and bronchodilator response.^{E11-E13} Please note that MAGICS and Freiburg both used an independent set of ISAAC II subjects as controls.

Population	Region	Recruitment	Childhood asthma diagnosis	Males		Females		Total
				Asthmatics	Controls	Asthmatics	Controls	
MAGICS/ISAAC II	Germany and Austria	hospital	clinical examination MAGICS n=631 (n=691) [†] parental reported ISAAC II n=72	459 (501)	327 (337)	244 (262)	331 (346)	1361 (1446)
ALSPAC	England	birth cohort	parental reported doctor's diagnosis (ever)	1009	1523	803	1618	4953
Ankara	Turkey	hospital	clinical examination	198	90	129	111	528
BAMSE	Sweden	birth cohort	parental reported doctor's diagnosis (ever)	156	125	95	129	505
DAG	Netherlands	hospital	clinical examination algorithm based diagnosis	137	67	193	87	484
Freiburg	Germany	hospital	clinical examination	201	295	109	289	894
PIAMA	Netherlands	birth cohort	parental reported doctor's diagnosis (ever)	101	86	71	100	358
Tomsk	Russia	hospital	clinical examination	106	50	50	40	246

Table E1. Characteristics of childhood asthma populations

The MAGICS/ISAAC II population was used for genome-wide analysis of sex-SNP interactions on childhood asthma risk, as well as for genetic fine mapping of the *DMRT1* locus. Replication analysis of sex-SNP interactions on asthma risk were conducted in seven independent populations ALSPAC, Ankara, BAMSE, DAG, Freiburg, PIAMA and Tomsk.

†: A total of 1361 subjects with genome-wide chip genotyping data were available in MAGICS/ISAAC II. Numbers in brackets represent the original cohort extended by further subjects used for MALDI-TOF MS during SNP fine mapping of the *DMRT1* association signal.

a. Interactions between sex and SNP on asthma association:		
SNP	Odds Ratio (95% CI)	p-value
rs4500131	0.35 (0.22-0.55)	0.0000052
rs2146532	0.36 (0.23-0.57)	0.0000075
rs3812523	0.38 (0.23-0.62)	0.00013
rs1323271	0.45 (0.29-0.70)	0.00044

b. Asthma associations in males		
SNP	Odds Ratio (95% CI)	p-value
rs4500131	0.59 (0.44-0.80)	0.00052
rs2146532	0.58 (0.44-0.78)	0.00025
rs3812523	0.50 (0.36-0.69)	0.000030
rs1323271	0.65 (0.49-0.87)	0.0039

c. Asthma associations in females		
SNP	Odds Ratio (95% CI)	p-value
rs4500131	1.69 (1.20-2.38)	0.0025
rs2146532	1.60 (1.15-2.25)	0.0058
rs3812523	1.32 (0.91-1.92)	0.146
rs1323271	1.45 (1.03-2.03)	0.031

Table E2. Sex-SNP interactions on asthma risk in the *DMRT1* locus identified in genome-wide analysis

a. Genome-wide interaction analysis between sex and polymorphisms on childhood asthma associations in the MAGIC/ISAAC II population identified four polymorphisms in the *DMRT1* locus. **b.-c.** Asthma associations were strong in males with protective effects for the minor alleles (OR < 1). Females displayed none or weaker associations with opposite effects (OR > 1).

SNP	Population	Asthma		male-specific analysis		female-specific analysis	
		sex-SNP interaction					
		Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value
rs4500131	MAGICS	0.35 (0.22-0.55)	0.0000052	0.59 (0.44-0.80)	0.00052	1.69 (1.20-2.38)	0.0025
	Tomsk	0.40 (0.13-1.25)	0.116	0.47 (0.24-0.92)	0.044	1.15 (0.50-2.66)	0.738
	Freiburg	0.67 (0.37-1.21)	0.182	1.02 (0.70-1.48)	0.935	1.53 (0.96-2.42)	0.073
	DAG	0.76 (0.34-1.67)	0.489	0.53 (0.29-0.96)	0.037	0.70 (0.42-1.18)	0.181
	ALSPAC	0.95 (0.75-1.21)	0.693	0.99 (0.84-1.16)	0.859	1.03 (0.87-1.23)	0.707
	BAMSE	0.77 (0.37-1.60)	0.491	0.96 (0.60-1.56)	0.878	1.24 (0.72-2.15)	0.434
	PIAMA	0.62 (0.26-1.47)	0.278	0.56 (0.31-1.01)	0.054	0.90 (0.48-1.67)	0.735
rs3812523	MAGICS	0.38 (0.23-0.62)	0.00013	0.50 (0.36-0.69)	0.00003	1.32 (0.91-1.92)	0.146
	Tomsk	0.21 (0.05-0.80)	0.022	0.41 (0.20-0.84)	0.016	1.99 (0.68-5.83)	0.226
	Freiburg	0.50 (0.26-0.95)	0.035	0.93 (0.61-1.42)	0.737	1.86 (1.15-3.03)	0.012
	Ankara	0.88 (0.42-1.81)	0.718	0.65 (0.39-1.09)	0.102	0.75 (0.45-1.25)	0.268
	DAG	1.41 (0.59-3.36)	0.438	0.84 (0.44-1.60)	0.591	0.59 (0.33-1.06)	0.078
	ALSPAC	1.00 (0.77-1.30)	0.984	1.07 (0.89-1.28)	0.462	1.07 (0.88-1.29)	0.509
	BAMSE	1.20 (0.53-2.71)	0.658	1.03 (0.62-1.74)	0.900	0.86 (0.46-1.61)	0.638
	PIAMA	0.83 (0.31-2.21)	0.705	0.57 (0.29-1.12)	0.101	0.69 (0.34-1.41)	0.309

Table E3. Replication of sex-specific effects on asthma associations

Polymorphisms identified in the MAGIC/ISAAC II discovery population were analyzed in up to seven independent populations. Considering the high LD ($r^2 \geq 0.8$) of rs4500131 with rs2146532 and rs1323271, results are only presented for rs4500131. Due to the different population sizes (Table E1) presented *p*-values have to be interpreted with caution and the OR 95% CI should be regarded.

Genetic fine mapping of the *DMRT1* locus in the MAGIC/ISAAC II population

A HapMap data based tagging SNP approach was used for genetic fine mapping of the *DMRT1* locus, including ± 10 kilobase pairs (kb) of flanking sequences (release #24; CEU panel; $MAF \geq 0.05$).^{E14} LD calculations and tagging SNP analyses were carried out with the Haploview software version 4.2 (pairwise LD threshold $r^2 \geq 0.8$).^{E15} The SNPper database and the 1,000 genomes project dataset (n=60 CEU subjects) were screened for additional SNPs for fine mapping.^{E16,E17} Data from SNPper was used to identify SNPs in exonic regions (parameters: exons ± 100 bp; $MAF \geq 0.03$) while 1,000 genomes data was used to screen the conserved DM DNA binding domain. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Sequenom Inc., San Diego, USA) was used to genotype SNPs not present in the MAGIC/ISAAC II dataset.^{E18} Genotyping quality control assessed call rates of genotypes within the whole study population and deviations from Hardy-Weinberg equilibrium (HWE) within the control subjects. After completion of genotyping, the final structure of LD and tagging bins was determined in the MAGIC/ISAAC II population (*DMRT1* ± 10 kb; $MAF \geq 0.05$; $r^2 \geq 0.8$). Logistic regression was used to identify dominant SNP effects on asthma status (PLINK software package version 1.07).^{E19}

Polymorphisms excluded from *DMRT1* fine mapping

The following SNPs were excluded from analysis due to a low MAF (< 0.05) in the MAGIC/ISAAC II population.

rs10114238	rs10114393	rs1033833	rs1033836	rs10491582	rs10491585	rs10976978
rs10977467	rs10977617	rs10977646	rs10977668	rs10977752	rs10977815	rs10977857
rs11790381	rs12336792	rs12340514	rs12340997	rs12341117	rs12348273	rs1322983
rs16924644	rs16925225	rs16925320	rs16925359	rs16925374	rs16925404	rs16925431
rs16925457	rs16925460	rs16925475	rs16925495	rs16925505	rs16925720	rs16925792
rs16925796	rs16925887	rs16926097	rs16926231	rs16926340	rs16926432	rs16926656
rs1885771	rs1998437	rs279902	rs279906	rs279908	rs34946058	rs35846503
rs3739582	rs3739585	rs41298220	rs4510916	rs4742549	rs4742562	rs55905583
rs7018970	rs7023831	rs7025380	rs7044108	rs7852019	rs7867920	rs9299086

Asthma														
SNP	Tagging bin	r ²	Position on chr. 9	Localisation	MAF	Alleles	HWE p-value	Genotype source	sex-combined analysis		male-specific analysis		female-specific analysis	
									Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value
rs7022951*	1		823,238	5' upstream	0.13	G > A	0.26	MALDI-TOF MS	0.88 (0.69-1.12)	0.282	0.58 (0.42-0.80)	0.0010	1.54 (1.06-2.23)	0.023
rs16924648*	2		823,828	5' upstream	0.12	G > A	1.00	Imputation	0.82 (0.63-1.06)	0.129	0.58 (0.41-0.83)	0.0025	1.31 (0.88-1.95)	0.179
rs10815800	3	0.85	823,740	5' upstream	0.07	C > G	0.24	Imputation	1.03 (0.75-1.40)	0.865	0.94 (0.61-1.43)	0.762	1.20 (0.75-1.92)	0.443
rs1885926	3	0.96	824,091	5' upstream	0.07	G > C	0.10	Imputation	0.95 (0.68-1.31)	0.748	0.89 (0.57-1.38)	0.607	1.06 (0.64-1.73)	0.831
rs10117611*	3		827,514	5' upstream	0.06	T > C	0.10	Imputation	0.97 (0.70-1.35)	0.875	0.95 (0.61-1.48)	0.816	1.06 (0.64-1.73)	0.831
rs7046706	3	0.86	849,262	Intron 2	0.07	G > A	0.10	Imputation	0.98 (0.71-1.34)	0.879	0.95 (0.62-1.47)	0.826	1.04 (0.64-1.69)	0.877
rs17372837*	4		829,141	5' upstream	0.29	C > T	1.00	Imputation	1.09 (0.88-1.36)	0.422	1.14 (0.86-1.53)	0.364	1.04 (0.74-1.46)	0.823
rs4742459	5	0.96	821,919	5' upstream	0.05	G > A	0.41	Imputation	0.99 (0.69-1.41)	0.935	1.00 (0.61-1.62)	0.988	1.00 (0.58-1.73)	0.990
rs7037561	5	0.91	822,711	5' upstream	0.06	G > A	0.71	Imputation	0.95 (0.67-1.36)	0.795	0.93 (0.58-1.48)	0.748	1.01 (0.59-1.74)	0.969
rs912064*	5		830,023	5' upstream	0.05	A > G	0.40	Imputation	0.98 (0.69-1.41)	0.934	1.03 (0.63-1.68)	0.899	0.95 (0.55-1.66)	0.857
rs912063	5	0.89	830,420	5' upstream	0.06	C > G	0.25	Imputation	1.05 (0.74-1.48)	0.784	1.06 (0.66-1.69)	0.819	1.07 (0.63-1.81)	0.804
rs942073	5	0.95	830,971	5' upstream	0.05	A > G	0.71	Imputation	0.95 (0.67-1.36)	0.794	0.96 (0.59-1.54)	0.853	0.97 (0.56-1.67)	0.900
rs912062	5	0.83	831,152	5' upstream	0.06	A > C	0.50	Chip	0.97 (0.69-1.35)	0.837	0.96 (0.61-1.52)	0.872	0.99 (0.59-1.66)	0.980
rs7958180	6	0.93	829,245	5' upstream	0.19	G > C	0.55	Imputation	0.86 (0.68-1.08)	0.188	0.62 (0.46-0.85)	0.0023	1.32 (0.93-1.88)	0.124
rs2025309*	6		830,527	5' upstream	0.19	G > C	0.47	MALDI-TOF MS	0.87 (0.70-1.08)	0.206	0.64 (0.48-0.86)	0.0032	1.30 (0.92-1.82)	0.138
rs13295838*	7		833,382	Intron 1	0.48	G > A	0.82	Imputation	0.94 (0.74-1.21)	0.649	1.03 (0.75-1.42)	0.858	0.87 (0.59-1.27)	0.463
rs3812523	8	0.82	830,247	5' upstream	0.14	A > G	0.66	Chip	0.79 (0.61-1.01)	0.058	0.53 (0.38-0.74)	0.00019	1.31 (0.90-1.93)	0.162
rs3739584	8	0.87	831,825	Exon 1 5' UTR	0.13	C > T	0.88	MALDI-TOF MS	0.85 (0.67-1.08)	0.189	0.60 (0.43-0.82)	0.0013	1.36 (0.93-1.97)	0.113
rs3739583	8	0.85	831,971	Exon 1 (S45T)	0.13	T > A	0.88	MALDI-TOF MS	0.85 (0.67-1.08)	0.185	0.60 (0.44-0.82)	0.0015	1.33 (0.91-1.95)	0.137
rs6477293	8	0.90	833,484	Intron 1	0.14	G > A	0.76	Imputation	0.87 (0.68-1.11)	0.267	0.65 (0.47-0.90)	0.0088	1.28 (0.87-1.88)	0.203
rs4338164*	8		833,763	Intron 1	0.13	C > T	0.75	MALDI-TOF MS	0.86 (0.67-1.09)	0.206	0.61 (0.45-0.84)	0.0023	1.32 (0.91-1.93)	0.142
rs16924776	8	0.80	835,564	Intron 1	0.13	T > C	0.74	Chip	0.85 (0.66-1.09)	0.199	0.65 (0.47-0.90)	0.010	1.19 (0.80-1.76)	0.394
rs16924784	8	0.85	836,407	Intron 1	0.12	C > T	0.74	Imputation	0.82 (0.63-1.06)	0.120	0.57 (0.41-0.80)	0.0013	1.29 (0.86-1.93)	0.211
rs16924786	8	0.85	836,480	Intron 1	0.12	A > G	0.74	Imputation	0.82 (0.63-1.06)	0.120	0.57 (0.41-0.80)	0.0013	1.29 (0.86-1.93)	0.211
rs7853446	9	0.97	833,731	Intron 1	0.25	C > G	0.39	Imputation	1.15 (0.92-1.43)	0.211	1.11 (0.83-1.49)	0.469	1.22 (0.87-1.71)	0.258
rs7862426	9	0.98	834,001	Intron 1	0.25	A > G	0.52	Imputation	1.15 (0.92-1.43)	0.211	1.11 (0.83-1.49)	0.469	1.22 (0.87-1.71)	0.258
rs7856704*	9		834,022	Intron 1	0.25	C > T	0.67	Imputation	1.14 (0.91-1.41)	0.255	1.10 (0.82-1.47)	0.536	1.21 (0.87-1.71)	0.262
rs7040024	9	0.89	835,516	Intron 1	0.26	A > C	0.25	MALDI-TOF MS	1.19 (0.97-1.47)	0.102	1.22 (0.92-1.62)	0.166	1.16 (0.84-1.61)	0.376
rs7040448*	10		835,844	Intron 1	0.09	C > A	1.00	Imputation	1.01 (0.76-1.36)	0.931	0.94 (0.63-1.41)	0.780	1.17 (0.75-1.82)	0.488
rs16924783*	11		836,350	Intron 1	0.10	C > A	0.0042	Imputation	1.16 (0.87-1.54)	0.306	1.33 (0.90-1.96)	0.156	1.04 (0.68-1.60)	0.860
rs12115433*	12		836,742	Intron 1	0.06	G > C	1.00	Imputation	1.31 (0.92-1.86)	0.130	1.20 (0.75-1.90)	0.447	1.47 (0.85-2.52)	0.167

Table E4. Overview on DMRT1 SNPs analyzed for sex-specific effects on asthma status in the MAGIC/ISAAC II population

Details include information on tagging SNPs (*), tagging bins, pairwise LD between tagged SNPs and their tagging SNP (r^2), genetic localization, allele frequency, analysis of Hardy-Weinberg Equilibrium in the ISAAC II control subjects and source of genotyping.

Asthma														
SNP	Tagging bin	r ²	Position on chr. 9	Localisation	MAF	Alleles	HWE p-value	Genotype source	sex-combined analysis		male-specific analysis		female-specific analysis	
									Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value
rs749427	12	0.84	854,122	Intron 2	0.07	G > A	0.72	Imputation	1.23 (0.89-1.71)	0.211	1.04 (0.68-1.59)	0.873	1.53 (0.92-2.55)	0.099
rs1407808*	13		837,292	Intron 2	0.10	T > G	0.66	Chip	1.02 (0.77-1.34)	0.901	0.87 (0.60-1.26)	0.469	1.28 (0.84-1.94)	0.256
rs10976974	14	0.80	833,454	Intron 1	0.22	C > A	0.66	Imputation	0.90 (0.72-1.13)	0.374	0.67 (0.50-0.91)	0.0094	1.34 (0.95-1.90)	0.097
rs1323271	14	0.81	834,487	Intron 1	0.22	T > C	0.65	Chip	0.92 (0.73-1.15)	0.438	0.69 (0.51-0.93)	0.015	1.33 (0.94-1.89)	0.103
rs2273932	14	0.96	837,365	Intron 2	0.23	G > C	0.74	Imputation	0.91 (0.73-1.14)	0.410	0.62 (0.46-0.84)	0.0016	1.49 (1.06-2.11)	0.023
rs2146532	14	0.99	837,547	Intron 2	0.23	G > A	0.59	Chip	0.89 (0.71-1.11)	0.296	0.61 (0.45-0.82)	0.0010	1.44 (1.02-2.03)	0.037
rs2181402	14	0.96	837,806	Intron 2	0.23	G > C	0.74	Imputation	0.91 (0.73-1.14)	0.410	0.62 (0.46-0.84)	0.0016	1.49 (1.06-2.11)	0.023
rs4500131*	14		837,836	Intron 2	0.23	G > A	0.91	Chip	0.93 (0.74-1.16)	0.497	0.62 (0.46-0.84)	0.0021	1.54 (1.08-2.18)	0.016
rs6477300	14	0.96	837,917	Intron 2	0.23	C > T	0.74	Imputation	0.92 (0.74-1.14)	0.443	0.63 (0.47-0.84)	0.0020	1.49 (1.06-2.11)	0.023
rs912165	14	0.96	839,036	Intron 2	0.23	C > T	0.74	Imputation	0.92 (0.74-1.14)	0.443	0.63 (0.47-0.84)	0.0020	1.49 (1.06-2.11)	0.023
rs912166	14	0.96	839,037	Intron 2	0.23	G > C	0.74	Imputation	0.92 (0.74-1.14)	0.443	0.63 (0.47-0.84)	0.0020	1.49 (1.06-2.11)	0.023
rs912167	14	0.99	839,197	Intron 2	0.23	C > G	0.67	Imputation	0.89 (0.71-1.11)	0.296	0.61 (0.45-0.82)	0.0010	1.44 (1.02-2.03)	0.037
rs2370212	14	0.96	839,537	Intron 2	0.23	A > T	0.66	Imputation	0.92 (0.74-1.14)	0.443	0.63 (0.47-0.84)	0.0020	1.49 (1.06-2.11)	0.023
rs1323265	14	0.96	839,625	Intron 2	0.23	C > G	0.66	Imputation	0.92 (0.74-1.14)	0.443	0.63 (0.47-0.84)	0.0020	1.49 (1.06-2.11)	0.023
rs4740934	14	0.99	840,258	Intron 2	0.23	G > A	0.59	Imputation	0.89 (0.71-1.11)	0.296	0.61 (0.45-0.82)	0.0010	1.44 (1.02-2.03)	0.037
rs10815834	15	0.88	835,667	Intron 1	0.47	T > C	0.88	Imputation	0.97 (0.76-1.23)	0.793	0.77 (0.56-1.06)	0.113	1.41 (0.96-2.07)	0.084
rs2181401	15	0.89	836,003	Intron 1	0.47	C > T	0.81	Imputation	0.97 (0.76-1.23)	0.793	0.76 (0.55-1.05)	0.093	1.44 (0.98-2.13)	0.065
rs2273930	15	0.96	837,265	Intron 2	0.48	A > G	0.39	Imputation	0.93 (0.73-1.19)	0.561	0.67 (0.48-0.93)	0.017	1.47 (0.99-2.18)	0.053
rs7029148*	15		845,091	Intron 2	0.49	G > A	0.43	Chip	0.96 (0.75-1.23)	0.726	0.71 (0.51-1.00)	0.049	1.45 (0.97-2.16)	0.069
rs10739166	15	0.81	847,511	Intron 2	0.46	G > T	0.48	Imputation	0.86 (0.68-1.09)	0.218	0.98 (0.72-1.35)	0.912	0.73 (0.5-1.060)	0.094
rs7873478	15	0.94	848,429	Intron 2	0.50	T > C	0.27	Imputation	0.90 (0.70-1.15)	0.394	0.74 (0.53-1.04)	0.087	0.73 (0.50-1.08)	0.115
rs4740943	16	1.00	843,004	Intron 2	0.40	T > C	0.94	Chip	1.02 (0.81-1.28)	0.879	0.83 (0.61-1.13)	0.237	1.35 (0.94-1.92)	0.102
rs7034335*	16		846,658	Intron 2	0.40	A > G	0.94	Imputation	1.02 (0.81-1.28)	0.879	0.83 (0.61-1.13)	0.237	1.35 (0.94-1.92)	0.102
rs912060*	17		853,582	Intron 2	0.09	A > T	0.30	Imputation	1.04 (0.77-1.39)	0.814	0.90 (0.61-1.32)	0.578	1.29 (0.83-2.00)	0.266
rs755383*	18		853,635	Intron 2	0.39	T > C	0.04	Chip	1.10 (0.88-1.38)	0.399	1.07 (0.79-1.44)	0.655	1.22 (0.85-1.75)	0.273
rs4742514*	19		856,587	Intron 2	0.17	A > C	0.49	Imputation	0.96 (0.76-1.22)	0.732	0.84 (0.61-1.15)	0.268	1.17 (0.81-1.68)	0.402
rs4742515	19	1.00	856,617	Intron 2	0.17	C > T	0.49	Chip	0.96 (0.76-1.22)	0.732	0.84 (0.61-1.15)	0.268	1.17 (0.81-1.68)	0.402
rs4742516*	20		856,725	Intron 2	0.46	T > C	0.81	Chip	1.19 (0.93-1.52)	0.160	0.95 (0.68-1.33)	0.770	1.49 (1.02-2.18)	0.038
rs7858689*	21		859,722	Intron 2	0.23	T > C	0.83	MALDI-TOF MS	0.97 (0.78-1.20)	0.771	0.89 (0.67-1.18)	0.415	1.10 (0.79-1.53)	0.583
rs4538942*	22		860,027	Intron 2	0.39	G > A	0.62	Chip	1.09 (0.87-1.36)	0.479	0.93 (0.68-1.26)	0.617	1.28 (0.90-1.82)	0.167
rs6477321*	23		862,364	Intron 2	0.14	A > G	0.31	MALDI-TOF MS	1.07 (0.84-1.37)	0.561	1.01 (0.73-1.39)	0.961	1.12 (0.76-1.64)	0.569

Asthma														
SNP	Tagging bin	r ²	Position on chr. 9	Localisation	MAF	Alleles	HWE p-value	Genotype source	sex-combined analysis		male-specific analysis		female-specific analysis	
									Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value
rs6477322*	24		862,418	Intron 2	0.12	C > T	0.70	Imputation	1.18 (0.91-1.53)	0.208	1.07 (0.76-1.50)	0.695	1.32 (0.88-1.97)	0.181
rs7047524*	25		864,129	Intron 2	0.13	A > G	0.73	Chip	1.01 (0.79-1.30)	0.924	1.13 (0.80-1.60)	0.473	0.92 (0.62-1.36)	0.675
rs912059	26	0.99	865,166	Intron 2	0.26	G > A	0.21	Imputation	1.14 (0.91-1.41)	0.255	1.13 (0.84-1.51)	0.418	1.17 (0.83-1.64)	0.375
rs2370214*	26		865,364	Intron 2	0.26	T > C	0.15	Imputation	1.14 (0.91-1.41)	0.255	1.14 (0.85-1.53)	0.382	1.15 (0.82-1.62)	0.415
rs4742527	26	0.93	869,935	Intron 2	0.27	A > G	0.26	Chip	1.13 (0.91-1.40)	0.279	1.10 (0.82-1.47)	0.521	1.19 (0.84-1.66)	0.327
rs912058*	27		865,947	Intron 2	0.30	C > T	0.18	Imputation	0.91 (0.74-1.14)	0.422	0.79 (0.59-1.05)	0.105	1.13 (0.81-1.59)	0.466
rs7038076	27	0.98	866,463	Intron 2	0.31	C > A	0.18	Imputation	0.93 (0.75-1.16)	0.524	0.81 (0.61-1.09)	0.165	1.12 (0.80-1.57)	0.511
rs2006898	28	0.94	865,134	Intron 2	0.39	G > A	1.00	Imputation	1.12 (0.90-1.41)	0.309	1.39 (1.03-1.87)	0.034	0.84 (0.59-1.19)	0.330
rs10977244	28	0.95	866,308	Intron 2	0.38	G > T	0.056	Chip	1.01 (0.80-1.27)	0.954	1.21 (0.89-1.66)	0.229	0.78 (0.54-1.12)	0.173
rs11790408	28	0.96	866,418	Intron 2	0.38	G > T	0.87	Imputation	1.09 (0.87-1.37)	0.435	1.31 (0.97-1.76)	0.080	0.86 (0.61-1.22)	0.390
rs10491588*	28		867,740	Intron 2	0.38	C > G	0.93	Imputation	1.09 (0.87-1.36)	0.473	1.31 (0.97-1.76)	0.079	0.85 (0.60-1.20)	0.341
rs7875857*	29		867,871	Intron 2	0.05	A > G	0.68	Chip	1.06 (0.74-1.51)	0.769	0.85 (0.53-1.34)	0.478	1.39 (0.78-2.46)	0.261
rs10733547*	30		870,777	Intron 2	0.24	C > T	1.00	Imputation	1.05 (0.84-1.31)	0.677	1.00 (0.74-1.34)	0.993	1.14 (0.81-1.61)	0.442
rs10977270*	31		873,226	Intron 2	0.33	G > A	0.29	Chip	1.15 (0.93-1.44)	0.199	1.15 (0.86-1.54)	0.352	1.17 (0.83-1.65)	0.362
rs10815971	32	0.99	883,446	Intron 2	0.23	G > C	0.16	Imputation	1.27 (1.02-1.59)	0.035	1.29 (0.96-1.73)	0.096	1.23 (0.87-1.73)	0.246
rs7859987*	32		887,949	Intron 3	0.22	C > T	0.16	Chip	1.26 (1.01-1.57)	0.043	1.27 (0.95-1.71)	0.110	1.21 (0.85-1.71)	0.286
rs7875002	32	0.95	890,015	Intron 3	0.23	C > A	0.21	Imputation	1.26 (1.01-1.57)	0.041	1.29 (0.96-1.73)	0.093	1.19 (0.85-1.68)	0.315
rs13298202	32	0.90	890,186	Intron 3	0.24	A > G	0.51	Imputation	1.23 (0.99-1.54)	0.062	1.27 (0.95-1.70)	0.111	1.17 (0.83-1.65)	0.362
rs2057429*	33		892,302	Intron 3	0.29	A > G	0.39	Chip	0.99 (0.80-1.24)	0.958	1.00 (0.74-1.34)	0.984	1.07 (0.76-1.50)	0.700
rs10816007*	34		892,888	Intron 3	0.24	C > T	0.59	Imputation	1.07 (0.86-1.33)	0.559	1.07 (0.80-1.43)	0.671	1.05 (0.75-1.48)	0.775
rs10491587*	35		894,106	Intron 3	0.09	C > G	0.82	Imputation	1.02 (0.77-1.36)	0.875	1.00 (0.69-1.45)	1.000	1.02 (0.66-1.60)	0.915
rs10977402	35	0.91	895,068	Intron 3	0.10	A > G	1.00	Imputation	1.02 (0.78-1.34)	0.878	0.98 (0.68-1.40)	0.899	1.07 (0.70-1.64)	0.760
rs1885775	36	0.87	895,584	Intron 3	0.13	G > T	1.00	Imputation	1.06 (0.82-1.37)	0.637	0.96 (0.68-1.35)	0.799	1.24 (0.84-1.84)	0.277
rs10977412*	36		895,849	Intron 3	0.12	A > G	0.33	Imputation	1.04 (0.80-1.36)	0.776	0.93 (0.65-1.32)	0.669	1.22 (0.81-1.83)	0.344
rs4742545	36	0.96	897,561	Intron 3	0.12	A > G	0.27	Chip	1.02 (0.79-1.33)	0.882	0.90 (0.64-1.27)	0.552	1.20 (0.80-1.80)	0.373
rs1885779	36	0.81	899,580	Intron 3	0.12	A > C	1.00	Imputation	1.07 (0.82-1.38)	0.632	0.93 (0.65-1.31)	0.660	1.29 (0.86-1.93)	0.211
rs1885780	36	0.91	899,695	Intron 3	0.11	C > G	0.68	Imputation	1.01 (0.77-1.33)	0.934	0.91 (0.64-1.31)	0.620	1.16 (0.76-1.77)	0.493
rs6477367	36	0.89	900,485	Intron 3	0.10	T > C	0.53	Imputation	1.02 (0.78-1.35)	0.878	0.92 (0.64-1.33)	0.669	1.18 (0.77-1.81)	0.437
rs10739181	36	0.86	900,902	Intron 3	0.10	A > G	0.51	Imputation	1.04 (0.79-1.38)	0.767	0.95 (0.65-1.37)	0.766	1.20 (0.78-1.84)	0.404
rs12553424*	37		895,937	Intron 3	0.08	C > T	1.00	Imputation	1.07 (0.80-1.44)	0.643	1.13 (0.76-1.68)	0.538	0.97 (0.61-1.55)	0.914
rs7042955	37	1.00	897,968	Intron 3	0.08	C > T	1.00	Imputation	1.07 (0.80-1.44)	0.643	1.13 (0.76-1.68)	0.538	0.97 (0.61-1.55)	0.914

Asthma														
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									Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value
rs2146534	37	0.88	901,829	Intron 3	0.09	A > G	0.34	Chip	1.04 (0.78-1.39)	0.768	1.14 (0.77-1.68)	0.516	0.94 (0.60-1.48)	0.798
rs7856502	38	0.84	894,078	Intron 3	0.21	T > C	0.73	Imputation	1.06 (0.85-1.33)	0.593	1.02 (0.76-1.37)	0.908	1.09 (0.77-1.54)	0.638
rs2370218	38	0.92	897,988	Intron 3	0.22	T > G	0.36	Chip	1.12 (0.90-1.40)	0.309	1.06 (0.79-1.42)	0.716	1.19 (0.84-1.68)	0.325
rs1033832*	38	898,583	Intron 3	0.21	C > G	0.20	Imputation	1.06 (0.85-1.33)	0.592	1.00 (0.74-1.34)	0.985	1.14 (0.80-1.61)	0.470	
rs4237171	39	0.87	904,180	Intron 3	0.10	T > A	0.52	Imputation	1.09 (0.83-1.44)	0.522	1.13 (0.78-1.64)	0.503	1.07 (0.70-1.62)	0.771
rs1322984	39	0.92	906,220	Intron 3	0.09	C > T	0.46	Chip	1.07 (0.80-1.44)	0.638	1.13 (0.76-1.68)	0.538	1.02 (0.65-1.60)	0.930
rs10123307	39	1.00	907,015	Intron 4	0.09	T > G	0.47	Imputation	1.08 (0.81-1.44)	0.602	1.13 (0.77-1.65)	0.544	1.03 (0.66-1.61)	0.883
rs7029422*	39	907,635	Intron 4	0.09	G > A	0.63	Imputation	1.08 (0.81-1.44)	0.602	1.13 (0.77-1.65)	0.544	1.03 (0.66-1.61)	0.883	
rs16926063	39	0.85	913,017	Intron 4	0.08	G > T	0.42	Imputation	1.04 (0.77-1.40)	0.810	1.14 (0.76-1.70)	0.531	0.92 (0.58-1.47)	0.730
rs11791208	39	0.86	913,342	Intron 4	0.08	G > A	0.60	Imputation	1.03 (0.76-1.38)	0.869	1.14 (0.76-1.70)	0.531	0.89 (0.55-1.43)	0.625
rs11789525	40	0.85	910,107	Intron 4	0.08	G > C	0.39	Imputation	1.05 (0.77-1.43)	0.744	1.21 (0.79-1.83)	0.378	0.89 (0.55-1.44)	0.623
rs4742561*	40	910,887	Intron 4	0.09	G > C	0.45	Imputation	1.08 (0.81-1.45)	0.594	1.13 (0.76-1.68)	0.538	1.04 (0.66-1.63)	0.874	
rs11792914	40	0.86	913,938	Intron 4	0.08	C > T	0.40	Imputation	1.04 (0.76-1.42)	0.805	1.18 (0.78-1.78)	0.448	0.89 (0.55-1.44)	0.623
rs11794097	40	0.86	914,014	Intron 4	0.08	A > C	0.40	Imputation	1.04 (0.76-1.42)	0.805	1.18 (0.78-1.78)	0.448	0.89 (0.55-1.44)	0.623
rs10977534	40	0.86	914,525	Intron 4	0.08	G > T	0.40	Imputation	1.04 (0.76-1.42)	0.805	1.18 (0.78-1.78)	0.448	0.89 (0.55-1.44)	0.623
rs10491586	41	0.99	907,348	Intron 4	0.30	G > A	0.71	Chip	1.02 (0.82-1.27)	0.867	0.94 (0.70-1.26)	0.696	1.16 (0.82-1.63)	0.405
rs2093602	41	0.97	910,954	Intron 4	0.30	T > G	0.71	Chip	1.04 (0.84-1.29)	0.739	0.97 (0.73-1.30)	0.832	1.17 (0.84-1.65)	0.356
rs10121226*	41	912,574	Intron 4	0.30	T > C	0.78	Imputation	1.03 (0.83-1.29)	0.760	0.97 (0.73-1.30)	0.835	1.16 (0.83-1.63)	0.388	
rs4237170	42	0.83	904,087	Intron 3	0.20	G > A	0.63	Imputation	0.93 (0.74-1.17)	0.536	0.78 (0.58-1.06)	0.113	1.18 (0.83-1.68)	0.355
rs2370220	42	0.93	907,667	Intron 4	0.21	C > T	0.91	Chip	0.96 (0.76-1.20)	0.693	0.85 (0.63-1.14)	0.274	1.14 (0.81-1.62)	0.451
rs7045606	42	0.93	911,597	Intron 4	0.21	C > T	0.64	Imputation	0.97 (0.77-1.21)	0.791	0.87 (0.64-1.17)	0.344	1.16 (0.82-1.64)	0.414
rs10759043	42	0.96	911,872	Intron 4	0.23	C > A	1.00	Imputation	1.01 (0.81-1.26)	0.937	0.90 (0.67-1.21)	0.468	1.21 (0.86-1.71)	0.273
rs10739185	42	1.00	913,402	Intron 4	0.22	G > A	1.00	Imputation	1.00 (0.80-1.24)	0.973	0.88 (0.65-1.18)	0.388	1.21 (0.86-1.71)	0.268
rs4637907	42	1.00	913,562	Intron 4	0.22	G > T	1.00	Imputation	1.00 (0.80-1.24)	0.973	0.88 (0.65-1.18)	0.388	1.21 (0.86-1.71)	0.268
rs4461954	42	1.00	913,709	Intron 4	0.22	G > A	1.00	Imputation	1.00 (0.80-1.24)	0.973	0.88 (0.65-1.18)	0.388	1.21 (0.86-1.71)	0.268
rs10759049	42	1.00	915,162	Intron 4	0.22	T > C	1.00	Imputation	1.00 (0.80-1.24)	0.973	0.88 (0.65-1.18)	0.388	1.21 (0.86-1.71)	0.268
rs10816055*	42	915,305	Intron 4	0.22	G > A	1.00	Imputation	1.00 (0.80-1.24)	0.973	0.88 (0.65-1.18)	0.388	1.21 (0.86-1.71)	0.268	
rs6477370	42	0.94	915,394	Intron 4	0.21	G > C	0.64	Imputation	0.97 (0.77-1.21)	0.791	0.87 (0.64-1.17)	0.344	1.16 (0.82-1.64)	0.414
rs10977543	42	0.99	915,828	Intron 4	0.22	A > G	1.00	Imputation	1.00 (0.80-1.25)	0.982	0.90 (0.67-1.21)	0.477	1.20 (0.85-1.69)	0.301
rs2370161	42	0.93	916,639	Intron 4	0.21	G > C	0.73	Imputation	0.97 (0.78-1.21)	0.791	0.87 (0.65-1.18)	0.380	1.14 (0.81-1.61)	0.457
rs9299089	42	0.93	918,556	Intron 4	0.21	C > T	0.73	Imputation	0.97 (0.78-1.21)	0.791	0.87 (0.65-1.18)	0.380	1.14 (0.81-1.61)	0.457

Asthma														
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									Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value
rs7039918	42	0.91	919,611	Intron 4	0.21	T > C	1.00	Imputation	0.99 (0.79-1.24)	0.926	0.87 (0.64-1.17)	0.349	1.21 (0.85-1.70)	0.291
rs10759051	42	0.93	919,644	Intron 4	0.23	C > T	0.58	Imputation	0.99 (0.79-1.23)	0.929	0.89 (0.66-1.19)	0.433	1.17 (0.83-1.65)	0.362
rs10977581*	43		920,033	Intron 4	0.39	C > T	0.74	MALDI-TOF MS	1.16 (0.93-1.44)	0.184	1.19 (0.89-1.60)	0.231	1.13 (0.81-1.59)	0.472
rs10816063*	44		920,145	Intron 4	0.44	A > G	0.81	MALDI-TOF MS	1.16 (0.92-1.45)	0.209	1.21 (0.90-1.63)	0.214	1.11 (0.78-1.59)	0.559
rs10124480*	45		921,833	Intron 4	0.19	C > G	0.31	Imputation	0.93 (0.74-1.17)	0.534	0.84 (0.62-1.14)	0.263	1.05 (0.73-1.50)	0.801
rs10977643	45	0.88	928,331	Intron 4	0.17	G > A	0.79	Imputation	0.90 (0.71-1.14)	0.379	0.81 (0.59-1.10)	0.179	1.03 (0.71-1.48)	0.893
rs7856742	45	0.83	929,156	Intron 4	0.18	C > T	0.79	Chip	0.90 (0.71-1.13)	0.352	0.81 (0.60-1.11)	0.184	1.01 (0.70-1.45)	0.960
rs10977650	45	0.85	929,738	Intron 4	0.17	G > A	0.89	Imputation	0.93 (0.74-1.18)	0.563	0.87 (0.64-1.18)	0.365	1.01 (0.70-1.46)	0.954
rs4742580	45	0.94	933,950	Intron 4	0.18	C > G	0.44	Imputation	0.92 (0.73-1.15)	0.456	0.82 (0.60-1.11)	0.205	1.05 (0.73-1.50)	0.794
rs12004781	45	0.90	939,942	Intron 4	0.18	C > G	0.29	Imputation	0.91 (0.72-1.15)	0.419	0.83 (0.61-1.13)	0.226	1.03 (0.72-1.47)	0.879
rs12347404	46	0.84	921,254	Intron 4	0.14	G > A	0.74	Imputation	0.96 (0.75-1.23)	0.764	0.98 (0.70-1.36)	0.889	0.93 (0.63-1.37)	0.711
rs10125691	46	0.94	921,815	Intron 4	0.15	A > G	0.54	Imputation	0.99 (0.78-1.26)	0.916	0.98 (0.71-1.35)	0.879	0.99 (0.68-1.45)	0.974
rs10816069	46	0.84	922,251	Intron 4	0.14	A > G	0.74	Imputation	0.97 (0.76-1.24)	0.813	0.99 (0.71-1.38)	0.965	0.93 (0.63-1.37)	0.711
rs10816070	46	0.95	922,504	Intron 4	0.15	A > G	0.65	Imputation	0.99 (0.78-1.26)	0.916	0.98 (0.71-1.35)	0.879	0.99 (0.68-1.45)	0.974
rs7863654	46	0.95	922,818	Intron 4	0.15	A > T	0.65	Imputation	0.99 (0.78-1.26)	0.916	0.98 (0.71-1.35)	0.879	0.99 (0.68-1.45)	0.974
rs7863679	46	0.95	922,875	Intron 4	0.15	A > G	0.65	Imputation	0.99 (0.78-1.26)	0.916	0.98 (0.71-1.35)	0.879	0.99 (0.68-1.45)	0.974
rs12340817	46	0.95	925,130	Intron 4	0.15	T > C	0.65	Imputation	0.99 (0.78-1.26)	0.916	0.98 (0.71-1.35)	0.879	0.99 (0.68-1.45)	0.974
rs7859543	46	0.90	925,672	Intron 4	0.13	G > A	1.00	Imputation	0.96 (0.75-1.24)	0.760	0.95 (0.68-1.34)	0.787	0.97 (0.65-1.43)	0.868
rs12115189	46	0.95	926,289	Intron 4	0.14	G > A	0.87	Imputation	0.94 (0.73-1.21)	0.624	0.93 (0.67-1.29)	0.665	0.96 (0.65-1.41)	0.830
rs12001187	46	0.90	926,455	Intron 4	0.13	A > G	1.00	Imputation	0.96 (0.75-1.24)	0.760	0.95 (0.68-1.34)	0.787	0.97 (0.65-1.43)	0.868
rs7862332	46	0.85	929,023	Intron 4	0.13	A > G	0.86	Imputation	0.99 (0.76-1.27)	0.909	0.98 (0.70-1.37)	0.899	0.98 (0.66-1.47)	0.937
rs10491584	46	0.90	929,653	Intron 4	0.13	A > G	1.00	Chip	0.96 (0.75-1.24)	0.772	0.96 (0.68-1.34)	0.801	0.97 (0.65-1.43)	0.868
rs7853048	46	1.00	931,570	Intron 4	0.15	T > C	0.64	Chip	0.96 (0.75-1.23)	0.768	0.93 (0.67-1.28)	0.640	1.02 (0.70-1.49)	0.929
rs11790375	46	0.90	931,995	Intron 4	0.13	T > C	0.87	Imputation	0.95 (0.74-1.22)	0.665	0.94 (0.67-1.31)	0.713	0.95 (0.64-1.41)	0.804
rs10977669*	46		933,070	Intron 4	0.15	G > A	0.64	Imputation	0.96 (0.75-1.23)	0.768	0.93 (0.67-1.28)	0.640	1.02 (0.70-1.49)	0.929
rs10119951	46	0.95	933,611	Intron 4	0.15	T > G	0.65	Imputation	0.93 (0.73-1.18)	0.548	0.88 (0.64-1.22)	0.443	0.99 (0.68-1.45)	0.974
rs16926504	46	0.88	936,041	Intron 4	0.14	C > T	1.00	Imputation	0.91 (0.71-1.17)	0.453	0.89 (0.64-1.25)	0.512	0.92 (0.62-1.36)	0.683
rs12341935	46	0.83	936,931	Intron 4	0.13	G > T	0.87	Imputation	0.91 (0.70-1.17)	0.449	0.90 (0.65-1.27)	0.554	0.91 (0.62-1.36)	0.655
rs12004928	46	0.85	937,880	Intron 4	0.16	T > C	1.00	Chip	0.93 (0.73-1.19)	0.564	0.91 (0.66-1.25)	0.553	0.96 (0.66-1.40)	0.843
rs16926587	46	0.83	940,227	Intron 4	0.13	C > G	0.87	Imputation	0.91 (0.70-1.17)	0.449	0.90 (0.65-1.27)	0.554	0.91 (0.62-1.36)	0.655
rs10491583	46	0.83	941,954	Intron 4	0.13	G > A	0.87	Imputation	0.91 (0.70-1.17)	0.449	0.90 (0.65-1.27)	0.554	0.91 (0.62-1.36)	0.655

Asthma														
SNP	Tagging bin	r ²	Position on chr. 9	Localisation	MAF	Alleles	HWE p-value	Genotype source	sex-combined analysis		male-specific analysis		female-specific analysis	
									Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value
rs203657	47	1.00	942,908	Intron 4	0.30	G > A	0.30	Imputation	0.97 (0.78-1.21)	0.804	0.95 (0.71-1.28)	0.754	1.00 (0.71-1.40)	0.980
rs279874	47	1.00	943,057	Intron 4	0.30	G > C	0.30	Imputation	0.97 (0.78-1.21)	0.804	0.95 (0.71-1.28)	0.754	1.00 (0.71-1.40)	0.980
rs10759073	47	0.99	943,465	Intron 4	0.30	T > C	0.30	Chip	0.97 (0.78-1.21)	0.783	0.95 (0.71-1.27)	0.731	1.00 (0.71-1.40)	0.980
rs434531	47	0.99	944,943	Intron 4	0.29	G > C	0.40	Imputation	0.98 (0.79-1.22)	0.847	0.96 (0.72-1.28)	0.785	1.01 (0.72-1.41)	0.964
rs442925*	47		946,228	Intron 4	0.30	G > A	0.40	Imputation	0.97 (0.78-1.21)	0.804	0.95 (0.71-1.28)	0.754	1.00 (0.71-1.40)	0.980
rs10123824*	48		948,073	Intron 4	0.22	A > G	0.58	Chip	0.98 (0.79-1.23)	0.883	0.92 (0.69-1.24)	0.605	1.08 (0.77-1.52)	0.662
rs166790	48	0.93	948,746	Intron 4	0.23	T > A	0.66	Imputation	1.00 (0.80-1.25)	0.982	0.88 (0.66-1.19)	0.410	1.19 (0.84-1.69)	0.320
rs12376821*	49		949,176	Intron 4	0.07	T > C	0.36	Imputation	0.94 (0.68-1.29)	0.693	1.00 (0.66-1.53)	0.995	0.83 (0.50-1.39)	0.483
rs17384966*	50		950,221	Intron 4	0.10	G > C	0.68	Imputation	0.94 (0.72-1.24)	0.685	0.94 (0.65-1.35)	0.730	0.96 (0.63-1.47)	0.857
rs279903*	51		952,184	Intron 4	0.24	G > A	0.67	Chip	1.00 (0.80-1.24)	0.974	0.87 (0.65-1.17)	0.363	1.20 (0.85-1.69)	0.291
rs370435	52	0.83	944,997	Intron 4	0.19	C > T	0.38	Imputation	0.98 (0.78-1.23)	0.878	0.98 (0.73-1.34)	0.921	1.00 (0.70-1.42)	0.986
rs364477	52	0.83	945,794	Intron 4	0.19	T > C	0.38	Imputation	0.98 (0.78-1.23)	0.878	0.98 (0.73-1.34)	0.921	1.00 (0.70-1.42)	0.986
rs382037	52	0.90	947,521	Intron 4	0.18	C > G	0.51	Imputation	0.96 (0.76-1.21)	0.738	1.00 (0.74-1.37)	0.980	0.93 (0.65-1.33)	0.687
rs420061	52	0.90	947,951	Intron 4	0.18	G > C	0.51	Imputation	0.96 (0.76-1.21)	0.738	1.00 (0.74-1.37)	0.980	0.93 (0.65-1.33)	0.687
rs279904	52	0.91	950,479	Intron 4	0.18	G > A	0.59	Chip	0.96 (0.76-1.22)	0.751	1.01 (0.74-1.37)	0.968	0.93 (0.65-1.33)	0.687
rs279900*	52		953,897	Intron 4	0.17	C > G	0.40	Imputation	0.97 (0.76-1.22)	0.779	1.05 (0.76-1.43)	0.786	0.91 (0.63-1.31)	0.611
rs279895	52	0.88	957,981	Intron 4	0.16	C > G	0.30	Imputation	0.97 (0.76-1.23)	0.774	0.97 (0.70-1.34)	0.858	1.02 (0.71-1.47)	0.899
rs279894	52	0.91	958,334	Exon 5'UTR	0.17	A > G	0.58	MALDI-TOF MS	0.99 (0.79-1.24)	0.920	1.04 (0.77-1.41)	0.794	0.92 (0.64-1.31)	0.639
rs279899*	53		955,006	Intron 4	0.28	T > A	1.00	Imputation	1.02 (0.82-1.26)	0.891	1.02 (0.76-1.36)	0.906	1.03 (0.73-1.44)	0.868
rs279896	53	0.99	956,685	Intron 4	0.28	A > T	0.77	Imputation	1.02 (0.82-1.26)	0.891	1.02 (0.76-1.36)	0.906	1.03 (0.73-1.44)	0.868
rs12336949	54	1.00	957,606	Intron 4	0.11	G > T	0.84	Imputation	1.01 (0.77-1.32)	0.934	0.97 (0.68-1.39)	0.880	1.08 (0.71-1.64)	0.714
rs4742608*	54		959,044	Exon 5'UTR	0.11	A > G	0.84	Chip	1.01 (0.77-1.32)	0.934	0.97 (0.68-1.39)	0.880	1.08 (0.71-1.64)	0.714
rs408200*	55		962,476	3' downstream	0.31	C > T	0.71	Chip	1.02 (0.82-1.27)	0.870	0.89 (0.67-1.19)	0.436	1.21 (0.86-1.70)	0.268
rs279910*	56		965,112	3' downstream	0.28	A > G	0.63	Chip	1.00 (0.80-1.24)	0.977	0.98 (0.74-1.31)	0.909	1.04 (0.74-1.45)	0.843
rs604973	57	0.96	961,066	3' downstream	0.18	G > A	0.24	Imputation	0.97 (0.77-1.22)	0.785	0.93 (0.68-1.27)	0.653	1.08 (0.76-1.54)	0.654
rs279891*	57		967,556	3' downstream (DMRT3 Intron 1)	0.18	A > C	0.29	Imputation	0.96 (0.76-1.21)	0.739	0.90 (0.66-1.23)	0.498	1.10 (0.77-1.57)	0.595
rs279888*	58		968,833	3' downstream (DMRT3 Intron 1)	0.41	A > G	1.00	Chip	0.98 (0.78-1.23)	0.871	0.89 (0.65-1.21)	0.452	1.10 (0.77-1.57)	0.596

SNP used as covariate in conditional male-specific asthma analysis															
SNP	Tagging bin	rs7022951		rs16924648		rs7858180		rs3812523		rs912167					
		Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value				
rs7022951	1	na	na	0.92 (0.44-1.93)	0.831	0.75 (0.48-1.17)	0.210	0.97 (0.56-1.69)	0.910	0.76 (0.50-1.14)	0.181				
rs16924648	2	0.60 (0.28-1.30)	0.195	na	na	0.72 (0.45-1.15)	0.169	0.90 (0.50-1.61)	0.713	0.74 (0.48-1.14)	0.176				
rs7858180	6	0.73 (0.49-1.09)	0.120	0.75 (0.50-1.12)	0.155	na	na	0.96 (0.55-1.66)	0.877	0.84 (0.51-1.39)	0.491				
rs2025309	6	0.81 (0.54-1.21)	0.308	0.80 (0.53-1.22)	0.301	1.09 (0.37-3.19)	0.881	1.02 (0.61-1.71)	0.931	0.91 (0.56-1.48)	0.708				
rs3812523	8	0.53 (0.31-0.91)	0.021	0.58 (0.34-0.99)	0.046	0.55 (0.31-1.00)	0.049	na	na	0.64 (0.42-0.99)	0.045				
rs3739584	8	0.82 (0.47-1.44)	0.499	0.71 (0.41-1.21)	0.207	0.65 (0.38-1.11)	0.115	1.87 (0.58-6.07)	0.296	0.72 (0.47-1.11)	0.136				
rs3739583	8	0.86 (0.49-1.49)	0.586	0.76 (0.44-1.31)	0.329	0.67 (0.39-1.13)	0.133	1.64 (0.61-4.40)	0.329	0.73 (0.47-1.11)	0.140				
rs6477293	8	0.82 (0.50-1.34)	0.427	0.90 (0.53-1.54)	0.712	0.86 (0.54-1.37)	0.529	1.54 (0.74-3.23)	0.250	0.88 (0.57-1.35)	0.544				
rs4338164	8	0.89 (0.52-1.53)	0.677	0.77 (0.44-1.35)	0.356	0.72 (0.44-1.18)	0.190	1.18 (0.56-2.50)	0.660	0.78 (0.50-1.22)	0.275				
rs16924776	8	0.81 (0.51-1.28)	0.366	0.88 (0.54-1.44)	0.620	0.85 (0.54-1.33)	0.465	1.18 (0.65-2.14)	0.583	0.87 (0.57-1.33)	0.519				
rs16924784	8	0.63 (0.39-1.04)	0.069	0.68 (0.41-1.15)	0.152	0.69 (0.43-1.11)	0.128	0.89 (0.46-1.71)	0.717	0.72 (0.46-1.13)	0.158				
rs16924786	8	0.63 (0.39-1.04)	0.069	0.68 (0.41-1.15)	0.152	0.69 (0.43-1.11)	0.128	0.89 (0.46-1.71)	0.717	0.72 (0.46-1.13)	0.158				
rs10976974	14	0.80 (0.55-1.17)	0.251	0.84 (0.57-1.24)	0.382	1.03 (0.56-1.89)	0.926	0.97 (0.64-1.49)	0.894	1.20 (0.65-2.23)	0.560				
rs1323271	14	0.83 (0.57-1.21)	0.334	0.88 (0.60-1.29)	0.504	1.12 (0.61-2.03)	0.717	1.01 (0.66-1.54)	0.955	1.36 (0.72-2.57)	0.342				
rs2273932	14	0.72 (0.51-1.02)	0.062	0.73 (0.51-1.04)	0.078	0.74 (0.45-1.22)	0.236	0.80 (0.54-1.18)	0.263	1.33 (0.29-6.06)	0.710				
rs2146532	14	0.69 (0.48-0.98)	0.040	0.71 (0.49-1.02)	0.062	0.68 (0.42-1.11)	0.124	0.76 (0.52-1.12)	0.160	na	na				
rs2181402	14	0.72 (0.51-1.02)	0.062	0.73 (0.51-1.04)	0.078	0.74 (0.45-1.22)	0.236	0.80 (0.54-1.18)	0.263	1.33 (0.29-6.06)	0.710				
rs4500131	14	0.72 (0.50-1.03)	0.071	0.73 (0.50-1.05)	0.093	0.74 (0.45-1.21)	0.225	0.79 (0.53-1.17)	0.237	na	na				
rs6477300	14	0.73 (0.51-1.03)	0.074	0.74 (0.52-1.05)	0.092	0.76 (0.46-1.26)	0.288	0.82 (0.55-1.21)	0.311	2.01 (0.36-11.15)	0.423				
rs912165	14	0.73 (0.51-1.03)	0.074	0.74 (0.52-1.05)	0.092	0.76 (0.46-1.26)	0.288	0.82 (0.55-1.21)	0.311	2.01 (0.36-11.15)	0.423				
rs912166	14	0.73 (0.51-1.03)	0.074	0.74 (0.52-1.05)	0.092	0.76 (0.46-1.26)	0.288	0.82 (0.55-1.21)	0.311	2.01 (0.36-11.15)	0.423				
rs912167	14	0.69 (0.48-0.98)	0.038	0.71 (0.49-1.01)	0.060	0.70 (0.43-1.14)	0.150	0.77 (0.52-1.13)	0.185	na	na				
rs2370212	14	0.73 (0.51-1.03)	0.074	0.74 (0.52-1.05)	0.092	0.76 (0.46-1.26)	0.288	0.82 (0.55-1.21)	0.311	2.01 (0.36-11.15)	0.423				
rs1323265	14	0.73 (0.51-1.03)	0.074	0.74 (0.52-1.05)	0.092	0.76 (0.46-1.26)	0.288	0.82 (0.55-1.21)	0.311	2.01 (0.36-11.15)	0.423				
rs4740934	14	0.69 (0.48-0.98)	0.038	0.71 (0.49-1.01)	0.060	0.70 (0.43-1.14)	0.150	0.77 (0.52-1.13)	0.185	na	na				

Table E5. Conditional analysis for independency of male-specific association signals

Conditional analysis, using the strongest associated polymorphism from each tagging bin with male specific-associations as a covariate, revealed that the association signal of rs3812523 is independent of the allelic state of the covariate. Conversely, all other SNPs with former male-specific asthma associations became insignificant after adjustment for rs3812523. Polymorphisms rs912167 and rs4740934 from tagging bin 14 exhibited identical *p*-values in male-specific asthma analysis (Table E4) and results for conditional analysis are presented only for rs912167, due to high LD with rs4740934 ($r^2=0.99$ see also Figure 3). Results of conditional analysis were identical for rs912167 and rs4740934 (data not shown).

na: analysis not applicable due to high LD with covariate

Electrophoretic Mobility Shift Assay

Electrophoretic mobility shift assays (EMSA) were carried out using double-stranded (ds) DNA probes and nuclear protein extracts from unstimulated HEK-293 cells (DSMZ, Braunschweig, Germany) and HEK-293 cells after three hours stimulation with PMA(50ng/ml)-Ionomycin(1μM). Detailed information on the EMSA method was previously published.^{E18,E20} Information on oligonucleotide sequences used for probe preparation can be found in Table E6. DNA probes were isotope-labeled using ³²P-γ-ATP (PerkinElmer, Rodgau, Germany) and labelling efficiency between different probes was validated with scintillation counting. DNA probes were then incubated with nuclear extracts followed by polyacrylamide gel electrophoresis to resolve DNA-protein complexes. Autoradiography on film was used for detection. For specificity testing of DNA-protein binding a 100-fold molar excess of unlabeled probe was incubated with nuclear extracts before addition of isotopic labeled probes. Identification of DNA-protein complexes was achieved by addition of specific antibodies and subsequent retardation of the complex during gel electrophoresis.

Oligonucleotide sequences of DNA probes	
rs3812523_risk_fwd:	5' - AGAACTCAGTGCTGAAAACACCAAAGTCTG - 3'
rs3812523_risk_rev:	5' - CAGCAGTTTGGTGTTTTCAGCACTGAGTTCT - 3'
rs3812523_non-risk_fwd:	5' - AGAACTCAGTGCTGAGAACACCAAAGTCTG - 3'
rs3812523_non-risk_rev:	5' - CAGCAGTTTGGTGTTCTCAGCACTGAGTTCT - 3'
AP-1_fwd: ^{E21}	5' - GGTATCGATAAGCTATGACTCATCCGGGG - 3'
AP-1_rev:	5' - CCCCGGATGAGTCATAGCTTATCGATACC - 3'
Antibodies	
GR	sc-8992X - Santa Cruz Biotechnology, Heidelberg, Germany
c-Jun	sc-1694X - Santa Cruz Biotechnology, Heidelberg, Germany
c-Fos	sc-253X - Santa Cruz Biotechnology, Heidelberg, Germany
ATF-7	sc-19765X - Santa Cruz Biotechnology, Heidelberg, Germany
JDP-2	TA316065 - BioCat, Heidelberg, Germany

Table E6. Overview on DNA probes and antibodies used in EMSA experiments

Figure E1. Analysis of rs3812523 allele-specific effects on transcription factor binding

Complex I: Allele-specific binding of c-Jun to the non-risk allele has been discussed in detail in the main article. **Complex II:** Cross-competitions were incomplete for both rs3812523 alleles (lanes 6 and 13) which indicated that more than one DNA-protein complex is present at this height of the EMSA gel. For the non-risk allele, competition with the AP-1 consensus site led to a complete abrogation of the complex (lane 14) and antibody-specific supershifts with c-Jun and c-Fos proofed AP-1 identity (lanes 16 and 17). AP-1 competition was incomplete for the risk allele (lane 5) and successful supershift can only be estimated by the appearance of shifted complexes for c-Jun antibody (lane 3; strong supershift) and c-Fos (lane 2; weak supershift). The risk-allele specific DNA-protein complex, not shifted by these antibodies, could not be identified yet (Complex II lanes 2 and 3 compared with lanes 16 and 17).

† unrelated ds DNA probe

‡ unrelated antibody

Dual Luciferase Reporter Assay

A total of 1,970 bp of genomic sequence upstream of the *DMRT1* translation start site were cloned into the pGL3-Basic vector (Promega, Mannheim, Germany) using NheI and XhoI restriction sites (primer sequences are listed in Table E7). Presence of major alleles of all secondary polymorphisms was ensured by Sanger sequencing of 40 control subjects of central European ancestry and subsequent site directed mutagenesis (Agilent Technologies, Böblingen, Germany). Vectors carrying the alleles of the SNP of interest rs3812523 at position -1,592 bp from the translation start site were verified by sequencing (Figure E2).

For determination of *DMRT1* promoter activity, HEK-293 cells were seeded at 2×10^4 /well in 96-well plates. The next day, transfection of 50 ng plasmid DNA/well was carried out using FuGene 6TM (Roche Diagnostics, Mannheim, Germany) according to the manufacturers protocol. The following plasmids were transfected: pGL3-Basic-*DMRT1* risk allele or pGL3-Basic-*DMRT1* non-risk allele (45ng/well) both encoding a *Firefly luciferase* together with a *Renilla luciferase* driven by thymidine kinase promoter (5ng/well) (pRL-TK, Clontech Saint-Germain-en-Laye, France). After 24 hours, medium was replaced and cells were stimulated with PMA and Ionomycin (Sigma-Aldrich, Taufkirchen, Germany) for 12 hours. The dual luciferase reporter assay was performed according to the manufacturer's instructions (Promega, Mannheim, Germany) and cell lysates were analyzed on a 96-well microplate reader (Tecan, Crailsheim, Germany).

Figure E2. Vector map of the pGL3-Basic vector containing 1,970 bp of genomic sequence upstream of the *DMRT1* translation start site
Allele-specific differences of rs3812523 on promoter activity were investigated by monitoring the expression of the *Firefly luciferase* gene.

fwd_primer:	5' -GGTACCGCTAGCC CC CAGGCAACAGAGCAAGACT - 3'
	NheI DMRT1
rev_primer:	5' -CTAGATCTCTCGAG GGT GCCCCCTAGGAGAAGTGCG - 3'
	XhoI DMRT1

Table E7. Oligonucleotide sequences used for cloning

Overview on oligonucleotide sequences used for cloning of the genomic sequence of *DMRT1* into the pGL3-Basic vector via *NheI* and *XhoI* restriction sites.

DMRT1 quantitative Real-Time PCR in a human cDNA library

Human tissue samples were collected with approval of the local ethical committee and kindly provided by the Institute for Legal Medicine (Hannover Medical School, Hannover, Germany). Samples were homogenized prior to RNA isolation with Precellys ceramic beads (Pqlab, Erlangen, Germany). Total RNA from cell lines and tissues was isolated according to the manufacturer protocols (Qiagen, Hamburg, Germany and Macherey-Nagel, Düren, Germany). cDNA was prepared from 1 µg total RNA (Qiagen). *DMRT1* expression analysis was conducted with quantitative Real-Time PCR (qRT-PCR) using TaqMan probes (assay Hs00232766_m1; Life Technologies, Darmstadt, Germany). The expression of *18S rRNA* was measured as an endogenous control to allow for relative gene expression quantification using the delta-Ct method ($Ct_{DMRT1} - Ct_{18S rRNA}$), as previously described.²⁰

cDNA	delta Ct ($Ct_{DMRT1} - Ct_{18S rRNA}$)	cDNA	delta Ct ($Ct_{DMRT1} - Ct_{18S rRNA}$)
A549	nd	Brain	nd
BJAB	nd	Big intestine	nd
DG75	nd	Small intestine	nd
H358	nd	Heart	nd
H441	nd	Kidney	nd
HEK-293	24.5	Liver	nd
HepG2	nd	Lymph node	nd
Jurkat	23.4	Lung	nd
YT	18.9	Ovary	nd
primary Fibroblasts	nd	Testis	12.6
		Thymus	21.6

Table E8. DMRT1 expression in human tissues and cell lines

Screening of a human cDNA library displayed strong *DMRT1* expression in testis tissue and weaker expression in cell lines (HEK, Jurkat and YT). Expression in thymus was at the border of the detection limit and only sporadically measurable ($Ct_{DMRT1} > 40$).

nd: *DMRT1* expression not detectable

***DMRT1* immunohistochemistry**

Human tissues were collected according to the Research Code of the University Medical Center Groningen, The Netherlands (<http://www.rug.nl/umcg/onderzoek/researchcode/index>) and national ethical and professional guidelines (“Code of conduct; Dutch federation of biomedical scientific societies”; <http://www.federa.org>).

Immunostaining for DMRT1 was performed using a polyclonal rabbit anti-human DMRT1 antibody, (HPA027850 Sigma-Aldrich, Zwijndrecht, Netherlands). Lung sections were deparaffinized and antigen retrieval was performed in 10 mM citrate (pH=6.0) at 125°C for 15 minutes. After wash steps, slides were incubated with DMRT1 antibody at 1:200 for 1 hour at 20°C. For the second step, peroxidase-conjugated goat-anti-rabbit antibody was used at 1:100 (with 1% human serum) for 30 minutes, followed by a third step rabbit-anti-goat antibody at 1:100 (with 1% human serum). 3,3'-Diaminobenzidine (DAB) was used as a substrate, resulting in a brown reaction product and hematoxylin was used for nuclear counterstaining.

Figure E3. Immunohistochemistry for DMRT1

Staining of lung tissue from two adult females with asthma (accompanied by smoking-induced end-stage lung disease with emphysematous changes) displayed comparable strong DMRT1 staining in alveolar macrophages and interstitial macrophages as in DIP, COPD and IPF (Figure 6). Appendix and thymus indicated staining in a subset of macrophages, mainly in the germinal center (so-called tingible body macrophages) and vascular structures, morphologically high-endothelial venules. DMRT1 staining in lymph node and spleen was comparable but weaker (data not shown).

SNP effects on protein expression

Due to our observation of DMRT1 expression in immune cells from qRT-PCR and immunohistochemistry, we queried our protein expression dataset from human peripheral blood mononuclear cells (PBMC) in an explorative setting in order to investigate allele-specific effects of rs3812523 on cytokine expression. In brief, PBMC were isolated from a cohort of 61 randomly selected adults and stimulated with either PHA (5µg/ml; Sigma-Aldrich ‘L9017’, Taufkirchen, Germany), LpA (0.1µg/ml; Sigma-Aldrich ‘L0774’), Ppg (10µg/ml; Sigma-Aldrich ‘77140’) or Derp1 (30µg/ml; Indoor Biotechnologies ‘NA-DP1-1’, Cardiff, United Kingdom) for 48 hours. Concentrations of 23 different secreted cytokines were measured in supernatants using Bio-Plex technology (Bio-Rad, Munich, Germany). Allele-specific effects of rs3812523 on secreted protein levels were determined with a Wilcoxon rank-sum test for uncensored data and with Tobit regression on ranks for censored data.^{E22} We identified a significant correlation between rs3812523 alleles and the expression of 8 out of 23 measured cytokines at $p < 0.05$ (unpublished data; details available from the authors upon request). Considering the total number of 92 tests (23 measured cytokines in four different sets of stimulated PBMCs) and a total of 11 significant observations ($p < 0.05$), presence of non-random effects can be assumed. In particular, allele-specific effects on MCP1 protein levels in LpA stimulated PBMC raised our attention (Figure E4). This effect could also be observed as a trend on the mRNA level in in qRT-PCR of *MCP1* in the same population (assay Hs00234140_m1; Life Technologies, Darmstadt, Germany).

Figure E4. DMRT1 rs3812523 correlation with MCP1 expression from cultured PBMC

Secretion of MCP1 from LpA stimulated PBMC correlated significantly with rs3812523 genotypes. MCP1 levels were lower in homozygous carriers of the asthma risk allele 'A' compared to heterozygous carriers. No homozygous carriers of the non-risk allele 'G' were present in the analyzed population. *MCP1* mRNA measurement with qRT-PCR also displayed a trend for lower levels in homozygous carriers of the risk allele. The delta-Ct was defined as $Ct_{MCP1} - Ct_{18S rRNA}$.

Sex-specific asthma associations in other human *DMRT* genes

In order to support the finding of sex-specific asthma associations in *DMRT1*, we assessed if comparable effects can be observed in other members of the *DMRT* gene family (Table E9). The MAGICS/ISAAC II chip genotyping dataset contained information on 34 polymorphisms in the *DMRT* family (Table E10) of which three SNP pairs were in high LD ($r^2 > 0.8$; Figure E5). Consequently, 31 independent tests for asthma associations were conducted. Male-specific asthma associations ($p < 0.05$) were observed for one polymorphism downstream of *DMRTB1* on chromosome 1 and for two *DMRT2* polymorphisms. Importantly, there was no detectable LD between associated SNPs in *DMRT1* and *DMRT2* (Figure E5). Association of nearly 10 percent of the analyzed SNPs (3 out of 31 SNPs) suggests presence of non-random effects. It should be noted that additional fine mapping of polymorphisms and functional analyses is needed to identify functionally relevant polymorphisms.

Gene	Chromosome and base pair position incl. ± 10 kb borders
<i>DMRTA2</i>	Chr . 1: 50,645,809-50,671,729
<i>DMRTB1</i>	Chr . 1: 53,687,660-53,715,746
<i>DMRT1</i>	Chr . 9: 821,690- 969,088
<i>DMRT3</i>	Chr . 9: 956,964- 991,732
<i>DMRT2</i>	Chr . 9: 1,030,354- 1,057,552
<i>DMRTA1</i>	Chr . 9: 22,426,840-22,452,472
<i>DMRTC2</i>	Chr . 19: 47,030,926-47,058,238
<i>DMRTC1</i>	Chr . X: 71,903,697-71,995,361

Table E9. Overview on members of the human *DMRT* gene family

Information on chromosomal localizations was obtained from Ensembl (<http://www.ensembl.org>; NCBI36 Release 54, May2009)

Asthma									
Gene	Chr	Position	SNP	sex-combined analysis		male-specific analysis		female-specific analysis	
				Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value	Odds Ratio (95 % CI)	p-value
DMRTA2	1	50,654,746	rs1278537	1.17 (0.92-1.50)	0.199	1.10 (0.80-1.53)	0.554	1.23 (0.84-1.79)	0.286
	1	53,689,164	rs12046664	0.88 (0.69-1.12)	0.305	0.78 (0.57-1.07)	0.129	1.01 (0.69-1.47)	0.963
	1	53,689,986	rs2153944	1.02 (0.74-1.39)	0.927	1.00 (0.66-1.53)	0.995	1.05 (0.65-1.70)	0.842
	1	53,694,743	rs7543581	0.96 (0.77-1.20)	0.708	0.83 (0.62-1.12)	0.228	1.15 (0.82-1.63)	0.420
DMRTB1	1	53,700,768	rs732385	0.97 (0.77-1.21)	0.759	0.81 (0.60-1.09)	0.172	1.21 (0.86-1.71)	0.283
	1	53,709,765	rs1296438	1.00 (0.80-1.25)	0.993	0.86 (0.64-1.15)	0.301	1.17 (0.83-1.66)	0.358
	1	53,715,171	rs11581093	0.86 (0.67-1.10)	0.233	0.69 (0.50-0.96)	0.029	1.16 (0.79-1.70)	0.463
	9	958,334	rs279894	0.99 (0.79-1.24)	0.920	1.04 (0.77-1.41)	0.794	0.92 (0.64-1.31)	0.639
DMRT3	9	959,044	rs4742608	1.01 (0.77-1.32)	0.934	0.97 (0.68-1.39)	0.880	1.08 (0.71-1.64)	0.714
	9	962,476	rs408200	1.02 (0.82-1.27)	0.870	0.89 (0.67-1.19)	0.436	1.21 (0.86-1.70)	0.268
	9	965,112	rs279910	1.00 (0.80-1.24)	0.977	0.98 (0.74-1.31)	0.909	1.04 (0.74-1.45)	0.843
	9	968,833	rs279888	0.98 (0.78-1.23)	0.871	0.89 (0.65-1.21)	0.452	1.10 (0.77-1.57)	0.596
	9	978,817	rs279878	1.05 (0.84-1.32)	0.655	1.01 (0.75-1.38)	0.935	1.07 (0.75-1.52)	0.706
	9	981,114	rs279877	0.99 (0.78-1.25)	0.938	1.08 (0.79-1.47)	0.624	0.90 (0.63-1.30)	0.588
	9	986,439	rs279876	0.95 (0.75-1.21)	0.689	0.94 (0.69-1.30)	0.725	1.02 (0.71-1.46)	0.912
	9	1,031,524	rs2279989	1.22 (0.90-1.67)	0.203	1.07 (0.70-1.63)	0.755	1.52 (0.96-2.42)	0.076
	9	1,032,166	rs2279988	1.14 (0.92-1.42)	0.232	1.14 (0.85-1.53)	0.369	1.17 (0.84-1.65)	0.357
	9	1,032,284	rs2279986	0.91 (0.71-1.16)	0.447	0.83 (0.60-1.16)	0.272	1.00 (0.69-1.46)	0.998
	9	1,033,752	rs6474536	1.09 (0.85-1.39)	0.521	1.08 (0.77-1.51)	0.676	1.16 (0.79-1.69)	0.456
	9	1,035,596	rs2063279	0.88 (0.70-1.11)	0.273	0.72 (0.52-0.98)	0.039	1.07 (0.75-1.53)	0.716
DMRT2	9	1,043,465	rs869939	1.37 (0.96-1.95)	0.087	1.48 (0.89-2.45)	0.133	1.38 (0.82-2.31)	0.227
	9	1,043,910	rs2275516	1.07 (0.85-1.35)	0.580	1.09 (0.80-1.49)	0.570	0.99 (0.69-1.43)	0.966
	9	1,047,204	rs4741029	1.59 (1.12-2.26)	0.009	1.81 (1.10-2.97)	0.019	1.48 (0.89-2.47)	0.133
	9	1,047,318	rs9140	0.98 (0.78-1.24)	0.889	0.75 (0.55-1.03)	0.077	1.30 (0.91-1.86)	0.144
	9	1,047,797	rs10809097	1.16 (0.90-1.49)	0.243	1.17 (0.83-1.66)	0.363	1.30 (0.90-1.87)	0.166
	9	1,052,999	rs959481	1.05 (0.83-1.33)	0.691	1.01 (0.73-1.38)	0.971	1.08 (0.75-1.55)	0.692
	9	22,436,828	rs655497	0.99 (0.79-1.23)	0.893	0.96 (0.72-1.28)	0.783	0.94 (0.67-1.32)	0.725
	9	22,440,566	rs538247	0.99 (0.78-1.25)	0.904	0.97 (0.70-1.33)	0.840	0.97 (0.67-1.42)	0.883
DMRTA1	9	22,444,150	rs6475646	0.76 (0.60-0.95)	0.017	0.83 (0.61-1.14)	0.249	0.70 (0.49-1.00)	0.050
	9	22,446,296	rs10738628	0.84 (0.67-1.05)	0.123	0.95 (0.71-1.28)	0.746	0.71 (0.51-1.01)	0.054
	9	22,450,095	rs665908	0.98 (0.76-1.25)	0.841	1.03 (0.74-1.42)	0.864	0.86 (0.58-1.28)	0.462
	19	47,035,366	rs3922888	1.14 (0.90-1.45)	0.283	1.21 (0.88-1.65)	0.246	1.07 (0.74-1.55)	0.728
DMRTC1	X	71,784,311	rs4585883	0.95 (0.83-1.08)	0.440	1.00 (0.82-1.22)	0.985	1.01 (0.18-5.76)	0.886
	X	72,162,076	rs4557875	0.91 (0.81-1.02)	0.093	1.01 (0.86-1.19)	0.860	0.96 (0.81-1.14)	0.644

Table E10. Asthma association analyses in the human DMRT gene family

Chip genotyping data from the MAGIC/ISAAC II population was used for analyses of sex-specific asthma associations in the human DMRT gene family. Logistic regression for dominant SNP effects was used in all analyses. Associations $p < 0.05$ are printed in bold.

Figure E5. Analysis of the LD structure within the *DMRT* gene family

A number of *DMRT* genes were located on identical chromosomes and the possibility of linkage between associated SNPs was assessed. No LD was detected between male-specific associated SNPs from *DMRT1* fine mapping and the nearby genes *DMRT3* and *DMRT2* on chromosome 9. *DMRTAL*, located 21 Mb from *DMRT1*, did not display LD with *DMRT1*. LD ($r^2 > 0.8$) was observed for three SNP pairs in *DMRT3*, *DMRT2* and *DMRTB1*, respectively. This information was considered in multiple testing for sex-specific asthma associations. Weak LD was observed for SNPs in *DMRTC1* ($r^2=0.41$).

- E1. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007; 448:470-3.
- E2. Weiland SK, von Mutius E, Hirsch T, Duhme H, Fritzsche C, Werner B, et al. Prevalence of respiratory and atopic disorders among children in the East and West of Germany five years after unification. *Eur Respir J* 1999; 14:862-70.
- E3. Michel S, Liang L, Depner M, Klopp N, Ruether A, Kumar A, et al. Unifying candidate gene and GWAS Approaches in Asthma. *PLoS One* 2010; 5:e13894.
- E4. Brunekreef B, Smit J, de Jongste J, Neijens H, Gerritsen J, Postma D, et al. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol* 2002; 13 Suppl 15:55-60.
- E5. Golding J, Pembrey M, Jones R. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatr Perinat Epidemiol* 2001; 15:74-87.
- E6. Kull I, Melen E, Alm J, Hallberg J, Svartengren M, van Hage M, et al. Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. *J Allergy Clin Immunol* 2010; 125:1013-9.
- E7. Scholtens S, Postma DS, Moffatt MF, Panasevich S, Granell R, Henderson AJ, et al. Novel childhood asthma genes interact with in utero and early-life tobacco smoke exposure. *J Allergy Clin Immunol* 2014; 133:885-8.
- E8. Prosperi MC, Sahiner UM, Belgrave D, Sackesen C, Buchan IE, Simpson A, et al. Challenges in identifying asthma subgroups using unsupervised statistical learning techniques. *Am J Respir Crit Care Med* 2013; 188:1303-12.
- E9. Heinzmann A, Ahlert I, Kurz T, Berner R, Deichmann KA. Association study suggests opposite effects of polymorphisms within IL8 on bronchial asthma and respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol* 2004; 114:671-6.
- E10. Freidin MB, Kobayakova OS, Ogorodova LM, Puzyrev VP. Association of polymorphisms in the human IL4 and IL5 genes with atopic bronchial asthma and severity of the disease. *Comp Funct Genomics* 2003; 4:346-50.
- E11. Koppelman GH, Meyers DA, Howard TD, Zheng SL, Hawkins GA, Ampleford EJ, et al. Identification of PCDH1 as a novel susceptibility gene for bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2009; 180:929-35.
- E12. Koppelman GH, Stine OC, Xu J, Howard TD, Zheng SL, Kauffman HF, et al. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. *J Allergy Clin Immunol* 2002; 109:498-506.
- E13. Portelli MA, Siedlinski M, Stewart CE, Postma DS, Nieuwenhuis MA, Vonk JM, et al. Genome-wide protein QTL mapping identifies human plasma kallikrein as a post-translational regulator of serum uPAR levels. *FASEB J* 2014; 28:923-34.
- E14. The International HapMap Consortium. The International HapMap Project. *Nature* 2003; 426:789-96.
- E15. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21:263-5.
- E16. Riva A, Kohane IS. A SNP-centric database for the investigation of the human genome. *BMC Bioinformatics* 2004; 5:33.
- E17. Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, et al. A map of human genome variation from population-scale sequencing. *Nature* 2010; 467:1061-73.
- E18. Suttner K, Ruoss I, Rosenstiel P, Depner M, Pinto LA, Schedel M, et al. HLX1 gene variants influence the development of childhood asthma. *J Allergy Clin Immunol* 2009; 123:82-8 e6.
- E19. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81:559-75.
- E20. Schieck M, Sharma V, Michel S, Toncheva AA, Worth L, Potaczek DP, et al. A polymorphism in the TH2 locus control region is associated with changes in DNA methylation and gene expression. *Allergy* 2014; 69:1171-80.
- E21. Nakabeppu Y, Ryder K, Nathans D. DNA binding activities of three murine Jun proteins: stimulation by Fos. *Cell* 1988; 55:907-15.
- E22. Ballenberger N, Lluís A, von Mutius E, Illi S, Schaub B. Novel statistical approaches for non-normal censored immunological data: analysis of cytokine and gene expression data. *PLoS One* 2012; 7:e46423.

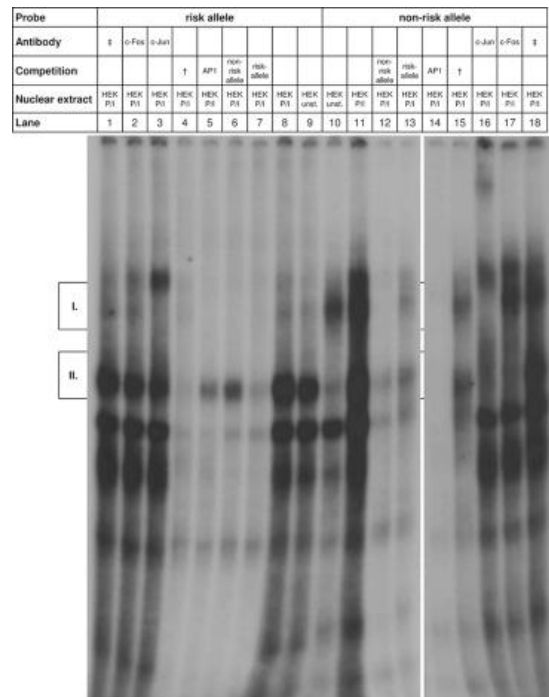


Fig E1.

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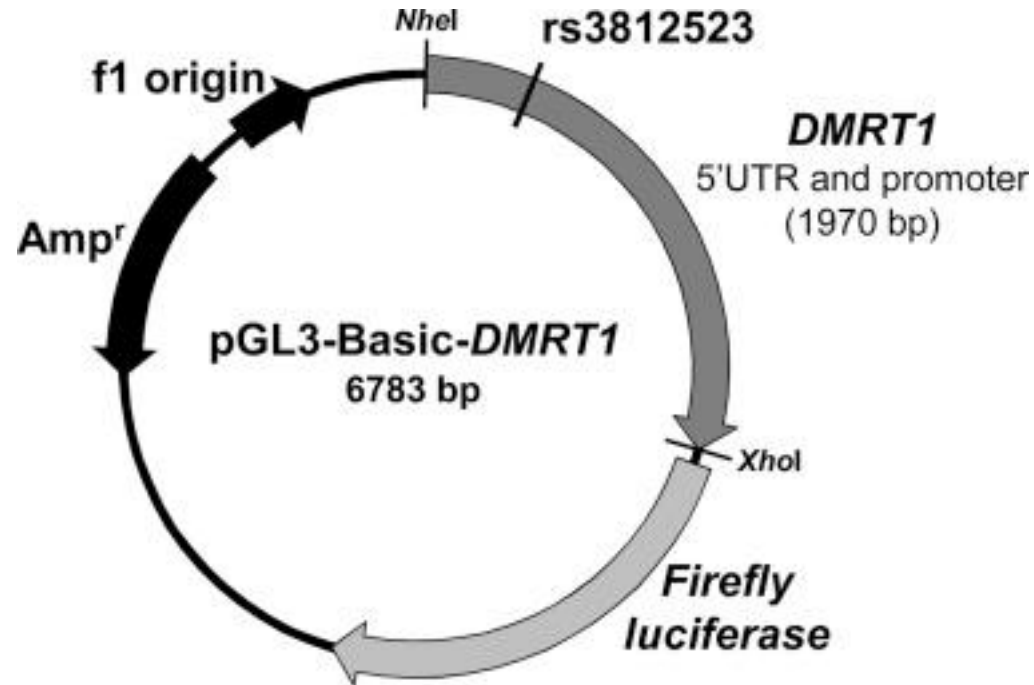


Fig E2.

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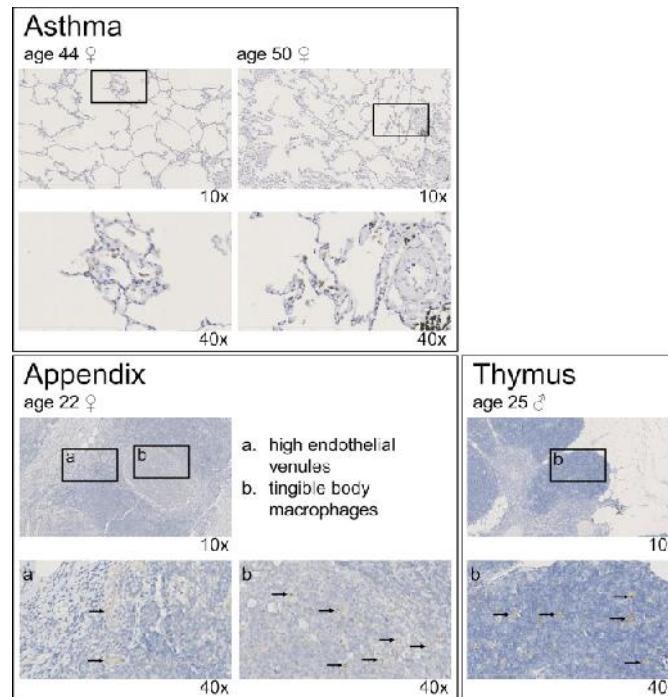


Fig E3.

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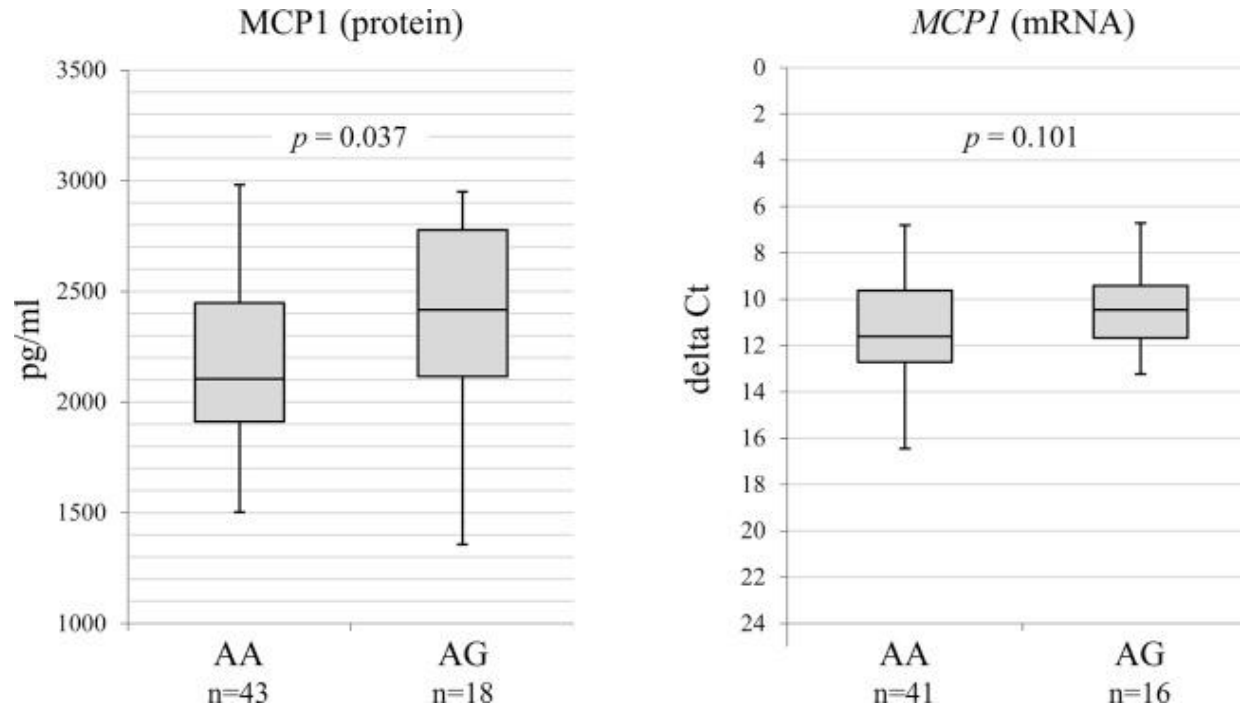


Fig E4.

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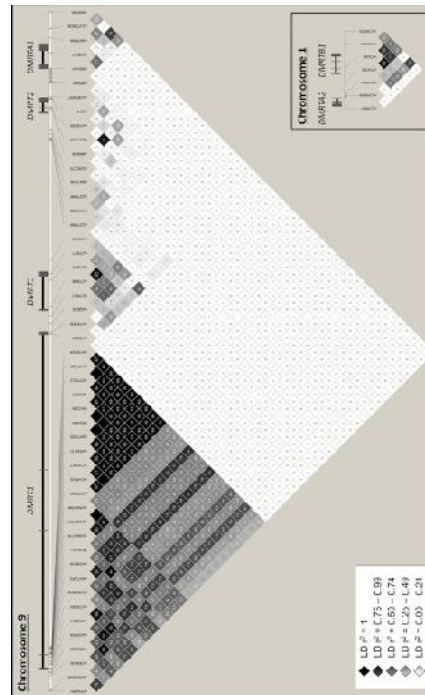


Fig E5.

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