# 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**

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**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 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;138:421-31.)**

***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.

*Abbreviations used*

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| 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 |
| MCP1: | 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 |

## 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 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* ± 10 kb; MAF ≥ 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