Gene-gene interaction in regulatory T–cell function in atopy and asthma development in childhood

Renske W. B. Bottema, MD,[a,b] Marjan Kerkhof, MD, PhD,[d] Naomi E. Reijmerink, MD,[a,b] Carel Thijs, MD, PhD,[f] Henriette A. Smit, PhD,[h] Constant P. van Schayck, PhD,[g] Bert Brunekreef, PhD,[i] Antoon J. van Oosterhout, PhD,[e] Dirkje S. Postma, MD, PhD,[a] and Gerard H. Koppelman, MD, PhD[c] *Groningen, Maastricht, Bilthoven, and Utrecht, The Netherlands*

Background: Regulatory T–cell dysfunction is associated with development of the complex genetic conditions atopy and asthma. Therefore, we hypothesized that single nucleotide polymorphisms in genes involved in the development and function of regulatory T cells are associated with atopy and asthma development.

Objective: To evaluate main effects and gene-gene interactions of haplotype tagging single nucleotide polymorphisms of genes involved in regulatory T–cell function—*IL6*, *IL6R*, *IL10*, heme-oxygenase 1 *(HMOX1)*, *IL2*, Toll-like receptor 2 *(TLR2)*, *TGFB1*, TGF-β receptor *(TGFBR)–1*, *TGFBR2*, *IL2RA*, and forkhead box protein 3 *(FOXP3)*—in relation to atopy and asthma.

Methods: Single-locus and multilocus associations with total IgE (3rd vs 1st tertile); specific IgE to egg, milk, and indoor allergens; and asthma were evaluated by χ^2 tests and the multifactor dimensionality-reduction method in 3 birth cohorts (Allergenic study).

Results: Multiple statistically significant multilocus associations existed. *IL2RA* rs4749926 and *TLR2* rs4696480 associated with IgE in both age groups tested (1-2 and 6-8 years). *TGFBR2* polymorphisms associated with total and specific IgE in both age groups and with asthma. *TGFBR2* rs9831477 associated with specific IgE for milk at age 1 to 2 years and indoor allergens at age 6 to 8 years. For milk-specific IgE, interaction between *TGFBR2* and *FOXP3* polymorphisms was confirmed by logistic regression and consistent in 2 birth cohorts and when stratified for sex, supplying internal replications.

Conclusion: Genes involved in the development and function of regulatory T cells, specifically *IL2RA*, *TLR2*, *TGFBR2*, and *FOXP3*, associate with atopy and asthma by gene-gene interaction. Modeling of multiple gene-gene interactions is important to unravel further the genetic susceptibility to atopy and asthma. (J Allergy Clin Immunol 2010;126:338-46.)

Key words: *T regulatory cells, atopy, IgE, asthma, MDR, polymorphism, interaction, birth cohort*

Atopy and asthma have a complex genetic background, and it is likely that multiple genes contribute to their development through main effects and through gene-gene interactions. Gene-gene interactions can be investigated by multifactor dimensionality reduction (MDR), a method designed to translate high-dimensional genetic data into a single dimension.[1] MDR selects single nucleotide polymorphisms (SNPs) in an unbiased way; therefore, it is possible to analyze genes in a biologically plausible pathway relevant for the development of atopy and asthma, yet without knowledge beforehand which genes will be important.

In recent years, regulatory T (Treg) cells have been identified to play a key role in balancing immune responses to maintain or acquire tolerance against allergens. Treg cells contribute to the control of allergen-specific immune responses in several ways—for example, through suppression of effector T cells or through suppression of dendritic cells that support the generation of effector T cells. Compromised numbers or function of Treg cells may therefore contribute to development and persistence of allergic disease,[2-5] a concept for which evidence is now accumulating from models of allergic inflammation in mice and various studies in human subjects, and from genetic association studies.

In genetic association studies, variations in genes that encode proteins involved in the development and function of Treg cells, such as Toll-like receptor (TLR)–2, *TGFB1*, *IL10*, and heme-oxygenase 1 *(HMOX1)*, have been reported to associate with atopy and asthma phenotypes.[6-18]

In an ovalbumin-induced mouse model of allergic airway inflammation, Xia et al[19] described that induction of HMOX1 induces forkhead box protein 3 (FOXP3) expression and production of IL10 and membrane-bound TGFB1, thereby enhancing activity of Treg cells. These immunologic alterations correlate with a decrease of serum ovalbumin-specific IgE and eosinophil infiltration in bronchial alveolar lavage fluid, suggesting a protective

From [a]the Department of Pulmonology, [b]the Department of Pediatrics and [c]the Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, [d]the Department of Epidemiology, and [e]the Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen; [f]the Department of Epidemiology, Care and Public Health Research Institute and Nutrition and Toxicology Research Institute Maastricht, and [g]the Department of General Practice and Care and Public Health Research Institute, Maastricht University; [h]the Center for Prevention and Health Services Research, National Institute for Public Health and the Environment, Bilthoven; and [i]the Institute for Risk Assessment Sciences, University of Utrecht, and Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht.

Supported by ZonMW (grant no. 912-03-031).

Disclosure of potential conflict of interest: C. Thijs receives research support from the Netherlands Asthma Foundation, the Netherlands Heart Foundation, and the INO Quality of Life Research Institute. The rest of the authors have declared that they have no conflict of interest.

Received for publication August 6, 2009; revised April 20, 2010; accepted for publication April 26, 2010. Available online July 5, 2010.

Available online July 5, 2010.

Reprint requests: Gerard H. Koppelman, MD, PhD, Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, University Medical Center Groningen, PO Box 196, 9700 AD Groningen, The Netherlands. E-mail: g.h.koppelman@bkk.umcg.nl.

0091-6749/$36.00

doi:10.1016/j.jaci.2010.04.024

Abbreviations used
FDR: False discovery rate
FOXP3: Forkhead box protein 3
KOALA: Dutch acronym for Child, Parent and Health: Lifestyle and Genetic Constitution
HMOX1: Heme-oxygenase 1
MDR: Multifactor dimensionality reduction
OR: Odds ratio
PIAMA: Prevention and Incidence of Asthma and Mite Allergy
PREVASC: PREvention of ASthma in Children
sIgE: Specific IgE
SNP: Single nucleotide polymorphism
TGFBR: TGF-β receptor
TLR: Toll-like receptor
Treg: Regulatory T

role for these proteins in asthma development. Another experiment in an ovalbumin-induced mouse model showed that TGF-β receptor 1 (TGFBR1) is upregulated in lung tissue with allergic airway inflammation.[20]

A contributing role of Treg cells in allergic diseases was further suggested by the observed increase of Treg cells in peripheral blood during the pollen season in children with allergy.[21] Another study demonstrated that the presence of increased numbers of peripheral blood Treg cells associated with spontaneous remission of cow's milk allergy.[22] Furthermore, a quantitative and functional impairment of pulmonary Treg cells has been reported in children with asthma.[23]

Given the important role of the Treg pathway in the development of atopy and asthma, we hypothesize that multiple genes involved in the induction of and suppression by Treg cells are associated with atopy and asthma phenotypes through their main effects, gene-gene interaction, or both. We investigated this by evaluating 11 genes (Fig 1) in relation to atopy and asthma phenotypes in 3062 children participating to the Allergenic study, a prospective birth cohort study composed of 3 Dutch cohorts.[24]

METHODS

Study populations

The Allergenic study includes 3 prospective Dutch birth cohorts: Prevention and Incidence of Asthma and Mite Allergy (PIAMA),[25] PREvention of ASthma in Children (PREVASC),[26,27] and KOALA (Dutch acronym for Child, Parent and Health: Lifestyle and Genetic Constitution).[28] A brief description of the 3 cohorts and their eligibility criteria is included in the Online Repository. Genetic studies were approved by local medical ethics committees of participating institutes. All parents provided written informed consent.

IgE measurements

Total IgE levels were determined in capillary or venous blood collected at ages 1 and 8 years in PIAMA; ages 1, 2, and 6 years in PREVASC; and ages 1 and 2 years in KOALA (Sanquin Research, Amsterdam, The Netherlands). Total IgE levels were measured by radioimmunoassay as described previously.[29-31] Total IgE measurements were clustered at ages 1 to 2 years and 6 to 8 years and analyzed in tertiles (Table I; see this article's Online Repository "Total IgE tertiles and specific IgE measurements" section at www.jacionline.org, including Table E1). Specific IgE (sIgE) to various allergens was tested at different ages in the PIAMA, KOALA, and PREVASC cohorts. To be able to pool data of the separate cohorts, we studied sensitization (defined as sIgE ≥0.35 IU/mL) to food allergens milk and egg clustered at ages 1 to 2 years, and to indoor allergens (to cat, dog, or house dust mite) clustered at ages 6 to 8 years (Table I; see the Online Repository at www.jacionline.org).

Asthma definition

Asthma was clustered at ages 6 and 8 years (PREVASC at age 6 years; PIAMA at age 8 years) and defined as at least 1 episode of wheeze or dyspnea in the last year and/or the use of inhaled steroids.

SNP selection and genotyping

Haplotype tagging SNPs (98) of 11 genes *IL6R*, *IL10*, *HMOX1*, *TGFBR2*, *IL2*, *TLR2*, *IL6*, *TGFBR1*, *IL2RA*, *TGFB1*, and *FOXP3* were selected from the HapMap database[32] or from the Innate Immunity web site[33] on the basis of the largest number of SNPs with a minor allele frequency >0.1 available in each database. In addition, the biomedical literature was screened for SNPs within the candidate genes known to have functional impact or to be associated with atopy or asthma. Genomic DNA was extracted from buccal swabs or blood by using standard methods.[34] DNA was amplified by using REPLI-g UltraFast technology (Qiagen). Genotyping was performed by Competitive Allele-Specific PCR using KASPar genotyping chemistry, performed under contract by K-Biosciences (Hoddesdon Herts, UK). Quality of genotype data was guaranteed by standards of K-Biosciences and verified by comparing the genotyping results in genomic versus amplified DNA in a subset of children.

Statistical methods

All SNPs were analyzed for Hardy-Weinberg equilibrium by using the χ^2 statistic ($P > .01$). We used χ^2 tests to analyze whether genotypes in this pathway are associated with elevated serum IgE levels at 1 to 2 years and at 6 to 8 years (highest vs lowest tertile), sIgE for milk and egg (ages 1-2 years), sIgE for indoor allergens (ages 6-8 years), and the presence of asthma (ages 6-8 years) by using a codominant model. For SNPs with a $P < .10$, the Akaike information criterion was evaluated to determine the best fitting genetic model (additive, dominant, or recessive). Odds ratios (ORs) and 95% CIs were calculated by logistic regression analysis. *FOXP3* is located on the X-chromosome and was therefore analyzed for boys and girls separately. Calculations were performed by using SPSS 14.0 statistical software (Chicago, Ill) and considered significant if $P < .05$ (2-sided). Because the haplotypes-tagging SNP selection uses multimarker predictors to capture all information of the gene (ie, aggressive tagging), we also analyzed SNPs that were captured by multiple SNPs by constructing haplotypes. Single SNP analyses were corrected for multiple testing by using the false discovery rate (FDR) as described by Benjamini and Hochberg[35] with a P value of .05 as the cutoff value. For each gene, we corrected for the number of genetic tests (*ie*, the number of SNPs per gene) as well as the number of phenotypes (n = 6). Single SNP analyses on *FOXP3* and sensitization were reported in a separate article.[36]

Gene-gene interactions were analyzed by using MDR (version 1.0.0). The MDR approach has been described previously[37-39] and applied in this cohort.[40-42] The *TLR2* gene has been studied in relation to other TLRs and atopy development by MDR (Reijmerink et al, unpublished data, May 2010). A detailed description of the MDR method is included in the Online Repository at www.jacionline.org, "MDR" section. The significance of the average prediction error was calculated by using the MDR permutation test, and a P value <.05 was considered significant. Logistic regression analyses were performed to confirm significant 2-way interaction results from MDR analyses; if the interaction term was significant (in a multiplicative model), the best fitting genetic model—for example, dominant or recessive—was analyzed.

RESULTS

Study population

A total of 3062 children were genotyped, and 2927 Dutch children were selected for genetic analyses after exclusion of non-Dutch children (5.7%) to avoid effects resulting from population stratification. Characteristics of the children participating in the genetic study are presented in Table I.

Single SNP and haplotype analysis

Minor allele frequencies and level of linkage disequilibrium per gene are presented in this article's Tables E2 and E3,