

Lack of association of the G protein-coupled receptor for asthma susceptibility gene with atopic dermatitis

To the Editor:

The common genetic background of the allergic diseases is best reflected by the atopic march in which a susceptible child passes a characteristic sequence of transient or persistent disease stages that begins with atopic dermatitis in the young infant and continues with the development of asthma and allergic rhinitis later in life.^{1,2} The close familial and intraindividual association of these disease entities strongly suggests shared genetic determinants. Recently, an orphan G protein-coupled receptor named *G protein-coupled receptor for asthma susceptibility (GPRA)* was shown to be involved in the pathogenesis of atopy and asthma. *GPRA* undergoes alternative splicing, resulting in 2 main isoforms, A and B. Disease susceptibility is thought to be a result of the overexpression of the B isoform. The expression pattern of the disease-associated B isoform, including tissues commonly associated with allergic reactions such as bronchial smooth muscle cells and bronchial epithelium, skin keratinocytes, and intestinal epithelium, render the *GPRA* gene a prime candidate for the joint genetic origin of allergic diseases.³ The goal of this study was to evaluate the *GPRA* gene as a genetic risk factor for atopic dermatitis.

The *GPRA* gene (alternatively named *GPR154*) consists of 10 exons and is located on chromosome 7p14.3, which had earlier been linked to asthma or atopy in 3 Caucasian populations of Finnish and French-Canadian origin.⁴ Analysis of the associated region spanning from intron 2 to intron 5 in the *GPRA* gene revealed a conserved haplotype structure in the 3 different study populations, including 4 closely related risk haplotypes and 3 nonrisk haplotypes. The risk haplotypes were tagged by 1 single nucleotide polymorphism (SNP; rs323922) in *GPRA* that was significantly associated with asthma or atopy in all 3 study populations.³

We evaluated this risk haplotype tagging SNP for evidence of association with infantile atopic dermatitis in a large study group including 826 affected children. All families were of European descent and were composed of at least 1 affected child and 2 parents. The study sample consisted of 283 multiplex and 222 simplex families. The 283 multiplex families (1213 individuals) were recruited through an affected sibling pair with moderate to severe atopic dermatitis and an age of onset of ≤ 2 years. The diagnosis of atopic dermatitis was made according to standard criteria, as previously described.⁵ Total IgE levels and levels of specific IgE against 12 common environmental allergens were determined using CAP-RAST-FEIA (Pharmacia, Freiburg, Germany). The majority of the families originated from Germany, with others from Italy, Sweden, and The Netherlands. A subgroup of the multiplex families was also investigated for the

TABLE I. Association analysis of the tagging SNP rs323922 in the *GPRA* gene with atopic dermatitis, elevated IgE levels, and asthma

Cohort	Phenotype	Number of pedigrees	Number of affected children	Transmissions (T:NT)	P value
Multiplex	AD	283	604	286:275	.67
Simplex	AD	222	222	106:121	.34
Total	AD	505	826	399:398	1.0
Multiplex	IgE*	250	461	219:208	.63
Multiplex	Asthma	115	154	82:66	.25

AD, Atopic dermatitis; T:NT, number of transmitted alleles vs number of nontransmitted alleles.

*Either elevated total IgE or specific sensitization.

phenotype allergic sensitization (250 families) and asthma (115 families). In addition, 222 simplex families (666 individuals) from The Netherlands, the United Kingdom, Italy, Sweden, France, the Czech Republic, Belgium, and Germany were included from the Early Treatment of the Atopic Child (ETAC) cohort. In the ETAC trial, 817 infants diagnosed with atopic dermatitis in their first 2 years of life were enrolled in a randomized, double-blind, placebo-controlled trial of the efficacy of cetirizine treatment in the prevention of asthma. All children had a positive family history of atopy.⁶ All DNA samples were collected after informed consent was obtained. Allergic sensitization was defined as either the presence of specific IgE to at least 1 allergen (detection limit 0.35 kU/L) or an elevation of the total serum IgE above the age-specific norm.

Genotyping was performed using TaqMan allelic discrimination with probes and primers designed and synthesized by the supplier (Applied Biosystems, Foster City, Calif). The genotyping success rate was 98.7%. Parental genotype frequencies were found to be consistent with Hardy-Weinberg equilibrium. The transmission disequilibrium test⁷ was performed using ASPEX.^{8,9} The allele frequency for the risk haplotype tagging allele C among the founders of the study group was 0.44. Assuming an allele frequency of 0.44 for the risk allele and a relative risk of 1.7, the current sample would provide >95% power to detect an effect of this variant on the risk of atopic dermatitis at $P < .05$.

No association with atopic dermatitis was observed for the asthma-associated risk haplotype tagging SNP (rs323922) in the *GPRA* gene. Analysis of the subgroup of children with asthma or allergic sensitization likewise did not reveal evidence of association of this polymorphism (Table I). However, although the current sample provided excellent power to detect association with atopic dermatitis, the power to replicate association with asthma (0.33) or allergic sensitization (0.75) was limited, because the number of children with these traits was considerably lower. Because children were selected

on the basis of early-onset atopic dermatitis, many may not yet have manifested asthma or atopy at the time of recruitment.

In the original study, phylogenetic analysis revealed conserved boundaries of the haplotype blocks across the associated region of the *GPRA* gene in 3 diverse Caucasian populations. This allowed us to use a single tagging SNP, rs323922, for association analysis in our study group, which was extracted from the same Caucasian European background. The close correspondence of the risk allele frequency of rs323922 (0.44) in our data set with that detected in the original study of 0.43 in the French-Canadian population and 0.46 in the Finnish population³ is consistent with the similar ethnic background and haplotype structure. Other variants in the *GPRA* gene that are not in linkage disequilibrium with rs323922 were not assessed in our study. We used a robust study design (family-based association) to avoid potential sources of error caused by population admixture or stratification in the association analysis. Furthermore, the investigation of complete families allowed us to check genotypes for mendelian inconsistencies, which were not detected.

We conclude that the *GPRA* gene is not associated with atopic dermatitis in the populations studied. Our results demonstrate that this gene does not contribute to the shared genetic predisposition of atopic dermatitis and allergic airways disease. Our findings do not exclude a role of the *GPRA* metabolic pathway or its yet unidentified ligand in the pathogenesis of atopic dermatitis. Thus, further studies of this pathway may be warranted to elucidate its role in atopic dermatitis and the atopic march.

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Association of *COX-2* gene haplotypes with prostaglandins production in bronchial asthma

To the Editor:

Common single nucleotide polymorphisms (SNPs) are linked within a gene locus and are inherited as several gene variants of different functional properties. The haplotype analysis emerged from genomic studies as an important clinical tool. In the lung, inflammation leads to induction of *COX-2* gene and to enhanced production of both proinflammatory and anti-inflammatory eicosanoids. Recently, we reported on a possible functional importance of the promoter polymorphism of *COX-2* G₋₇₆₅C in subjects with asthma.¹ Peripheral blood monocytes' capacity for biosynthesis of prostaglandin (PG) E₂ and PGD₂ was increased 10-fold in CC homozygotes compared with GG ones. Similar genotype frequencies of *COX-2* G₋₇₆₅C