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Lack of association between genetic variation in G-protein-coupled receptor for asthma susceptibility and childhood asthma and atopy

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Abstract

G-protein-coupled receptor for asthma susceptibility (GPRA or GPR154) was identified as an asthma and atopy candidate gene by positional cloning. Some subsequent studies suggest associations of GPRA single nucleotide polymorphisms (SNPs) and haplotypes with asthma or atopy susceptibility. However, the associated SNPs or haplotypes vary among studies. The role of GPRA genetic variation in asthma and atopy remains unsolved. Published data on GRPA variants and asthma come exclusively from Caucasian and Asian populations. We examined whether GPRA SNPs and haplotypes are associated with asthma and atopy in a Mexican population. We genotyped and analyzed 27 GPRA SNPs in 589 nuclear families consisting of asthmatic children aged 4–17 years of age and their parents in Mexico City. Atopy was determined by skin prick tests to 25 aeroallergens. The 27 SNPs examined provided excellent coverage of the GPRA gene. GPRA SNPs and haplotypes were not associated with childhood asthma and the degree of atopy to aeroallergens in a Mexican population. Our review of studies of GPRA variants in relation to asthma phenotypes shows considerable heterogeneity. Accordingly, our results suggest that GPRA variants are not an important contributor to childhood asthma and atopy susceptibility in a Mexican population.

Keywords

GPR154; allergy; asthma; genetic predisposition to disease; single nucleotide polymorphism

Introduction

Asthma is a complex disease caused by multiple genetic and environmental factors. G-protein-coupled receptor for asthma susceptibility (*GPRA*, also known as G-protein-coupled receptor

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154, *GPR154*) located on chromosome 7 was identified as an asthma candidate gene by positional cloning in Finnish and French Canadian populations.[1] GPRA has two main isoforms with alternatively spliced 3′ exons (371 amino acids for isoform A and 377 amino acids for isoform B) and distinct tissue distribution patterns.[1] Expression patterns of the GPRA-B isoform in the lung are different between asthma patients and healthy controls.[1] Levels of the GPRA-B isoform are increased in airway smooth muscle cells and epithelial cells in asthma patients compared to healthy controls.[1] Transcription levels of *GPRA* were upregulated in mouse lung after ovalbumin-induced inflammation.[1] These data suggest that GPRA plays a role in asthma pathogenesis.

GPRA single nucleotide polymorphisms (SNPs) and haplotypes have been associated with asthma or atopy in several studies,[1–5] but not in others.[6,7] In the studies in which associations have been found, however, the SNPs and haplotypes related to asthma and atopy are inconsistent across populations. Thus, the role of genetic variation in *GPRA* in asthma and atopy remains inconclusive.

We used the case–parent triad design to examine associations of *GPRA* SNPs and haplotypes with childhood asthma and atopy in asthmatic children from Mexico City. To the best of our knowledge, published data on *GPRA* come exclusively from Caucasian and Asian populations. Study of associations in different ethnic groups is important to increase confidence in initial findings because polymorphic patterns vary across ethnicities.[8] We also examined for possible effect modification by two environmental risk factors for childhood asthma—ambient ozone concentrations, which are especially high in Mexico City, and parental tobacco smoking.

Results

Characteristics of the asthmatic children with genotyping data are presented in Table 1. The mean age of cases was 9.0 years (range 4–17 years). Most had mild (72.3%) as opposed to moderate or severe asthma (27.7%). Nearly all cases (98.5%) had used medication for asthma in the past 12 months. Wheezing in the past 12 months was reported by 90% of the cases and chronic dry cough was reported by 65.4%. For 73.6% of the cases, asthma symptoms had interfered with daily activities or school attendance in the past 12 months. Among cases with spirometry data, the mean forced expiratory volume in 1 s (FEV_1) percent predicted was 96.0 (s.d. = 19.6). Atopy was present in 91.6% of cases. The highest rates of skin test positivity were seen for dust mite (69.6%) and cockroach (43.6%). Only 5.7% of mothers reported smoking during pregnancy, but 50.9% of children had a parent who currently smoked.

The genotype frequency distributions and minor allele frequencies are shown in Table 2 for the 7 tagging SNPs and in Supplementary Table E1 in the online supplement for the other 20 SNPs that we evaluated for association with the asthma phenotypes under study. The frequency distributions for all mating types for the seven tagging SNPs are presented in Supplementary Table E2 in the online supplement for all families. All SNPs were in Hardy–Weinberg equilibrium ($P>0.01$) in the parents. Pairwise linkage disequilibrium (LD) coefficients (r^2) between *GPRA* SNPs, calculated and displayed using VG2 program (http://pga.gs.washington.edu/VG2.html), are shown in Supplementary Figure E1 in the online supplement. Consistent with our SNP selection based on LD, most genotyped SNPs were in weak LD with each other. For example, only 1.4% of the pairwise r^2 values were higher than 0.8, 4% were 0.5–0.8, 21% were 0.10–0.49 and 73% were lower than 0.1.

Neither the 7 tagging SNPs (Table 3) nor the other 20 SNPs (Supplementary Table E3) were associated with asthma. Results did not differ appreciably by gender, parental smoking status or ozone level (data not shown). The common haplotypes (frequency >5%) composed by the seven tagging SNPs were not associated with asthma (Table 4). None of the *GPRA* SNPs were

associated with the degree of atopy to aeroallergens, assessed by the number of positive skin tests out of a battery of 25 tests (data not shown). We also considered allergy to seasonal and perennial allergens separately. When we did this, none of the SNPs were associated with the degree to atopy to either type of allergen. We also did not observe associations between the *GPRA* SNPs and percent predicted FEV_1 (data not shown).

Discussion

In this case–parent triad study in a Mexico City population, we found no evidence that *GPRA* SNPs and haplotypes were associated with childhood asthma or degree of atopy.

GPRA was initially identified as an asthma candidate gene by positional cloning in Finnish and French Canadian populations.[1] Laitinen *et al.*[1] found that the GPRA-B isoform was highly expressed in airway smooth muscle cells in asthma patients, but not in healthy controls. In mice, transcription levels of *GPRA* were upregulated in alveolar macrophages in lung tissue samples and in the BAL fluid after ovalbumin challenge.[1,9] However, there are controversies on the importance of GPRA in lung inflammation. For example, Allen and co-workers did not detect expression of *GPRA* in human lung tissue or in mice with ovalbumin-induced lung inflammation.[10] They also reported that *GPRA*-deficient mice showed the same allergic airway inflammation responses as the wild-type mice after ovalbumin challenge followed by aerosols of antigen exposure.[10]

SNPs in *GPRA* have been associated with asthma and atopy susceptibility in Caucasians and Asians. However, findings are inconsistent across populations. In the initial study, Laitinen *et al.*[1] found that rs323922, the tagging SNP for the risk haplotypes, was associated with asthma or high serum immunoglobulin E (IgE) in a Finnish and French Canadian population. However, six other studies did not detect associations of rs323922 with either asthma or atopy in Koreans, Chinese, Germans, Italians and a mixed population from five Western European countries.[2–7] We did not observe an association between rs323922 and asthma or atopy in Mexican population either. For other *GPRA* SNPs, findings also vary across populations (Table 5). For example, associations with asthma phenotypes have been found for SNP546333 and rs740347 in the German[2] and Italian[5] populations, for rs324396 in Germans[2] and the mixed population from five Western European countries,[3] for rs324384 in the mixed population from five Western European countries[3] and for rs324981 in the Chinese population[4] (Table 5). The 27 SNPs analyzed in our study, including the above SNPs or SNPs in high LD with above SNPs, were representative of the *GPRA* gene. We did not find associations of *GPRA* SNPs with asthma or atopy in Mexican population.

In keeping with the single SNP analyses, haplotype analyses of *GPRA* and asthma-related phenotypes also vary across studies (Table 5). The initial study showed that the H2 haplotype was associated with asthma risk in a French Canadian population.[1] However, this finding was not confirmed in other populations.[2–7] The H4 haplotype conferred the risk for high serum IgE in a Finnish population,[1] but played a protective role in an Italian population.[5] The H4 haplotype was also associated with asthma in a German population.[2] The H5 haplotype was associated with high IgE in the Finnish population,[1] with asthma risk in a mixed population from five Western European countries[3] and with protective effect of asthma in the Italian population.[5] The H4 and H5 haplotypes are rare in Mexican population (frequency <5%). In Europeans, the H1 haplotype was associated with asthma in a single study[3] and H6 was associated in another study.[5] *GPRA* haplotypes were not associated with asthma or atopy in a Chinese population[4] and Mexicans.

In addition to asthma-related phenotypes, the H2 haplotype has been associated with inflammatory bowel disease[11] and the H1, H4 and H5 haplotypes have been associated with

respiratory distress syndrome[12] in European populations. In a Northern European population, *GPRA* SNPs and haplotypes were not associated with adult atopic dermatitis.[13]

There is a lack of replication of effects of *GPRA* variation on asthma and atopy in multiple studies of Europeans looking at the same SNPs.[1–3,5] Of note, other asthma candidate genes identified by positional cloning, such as *ADAM33* and *SPINK5*, also show differences in asthma-associated SNPs among studies.[14–17] There are several possible explanations for the inconsistency across populations, such as underpowered sample sizes,[18] false positive findings due to ethnic admixture and population stratification,[18] heterogeneity in phenotypes or environmental risk factors[19] and ethnic-specific LD or genetic effect of asthma.[19]

There are no known functional SNPs in the *GPRA* gene, and the role of this gene in asthma is not clear. For studies with positive findings, a possible explanation is that asthma-associated *GPRA* SNPs may be in LD with unknown functional SNPs. The LD pattern in Mexicans is different from that in Europeans, which may explain our negative findings. In specific, the H4 and H5 haplotypes that were common in Europeans were rare in Mexicans. However, the fact that SNPs and haplotypes associated with asthma phenotypes are inconsistent, even among studies restricted to European populations, suggests that differences in LD patterns between Mexicans and Europeans may not explain our negative findings.

Publication bias has been well documented in the genetic association literature;[20] negative studies tend not to be submitted or if submitted, not to be published. Thus, the studies on *GPRA* in the literature are likely to be skewed toward those with positive associations. Report of well-conducted negative studies is crucial for systematic review of association studies and valuable for separating false positive results from true positive findings.[8]

Our study has several strengths. The triad design and analysis protects against population stratification.[21] In addition to SNPs associated with asthma or atopy in the literature, we genotyped a large number of SNPs to cover the whole gene using sound bioinformatics tools. The number of families examined in our study is relatively large compared to other studies. The demographic and clinical characteristics of Mexican asthmatic children and their parents are well characterized. The asthma cases were diagnosed by pediatric allergists at a pediatric allergy specialty clinic of a large public hospital. Referral to this pediatric allergy clinic is a tertiary referral, and thus the children in our study had already been seen by a generalist and a pediatrician over time for recurrent asthma symptoms. Diagnoses were made on clinical grounds according to previous guidelines.[22] We did not test for bronchial hyperreactivity. However, physician diagnosis of asthma is a valid outcome compared to objective measurements.[23] We had objective data on atopy; skin prick tests revealed the vast majority of these children with asthma (92%) to be atopic to aeroallergens.

On the basis of the published literature, the role of *GPRA* in asthma pathogenesis is unclear. In the current study, we found no evidence that genetic variations in *GPRA* were associated with asthma or atopy in a Mexican population.

Materials and methods

Study design and subject enrollment

We used the case–parent triad design.[21,24] The study population included 589 families consisting of asthmatic children and their parents with adequate DNA samples for genotyping of at least one SNP for the *GPRA* gene. The cases were children aged 4–17 years of age with asthma diagnosed by a pediatric allergist at the allergy referral clinic of a large public pediatric hospital in central Mexico City (Hospital Infantil de México, Federico Gómez). Children and parents provided blood samples as sources of DNA. A parent, nearly always the mother,

completed a questionnaire on the child's symptoms and risk factors for asthma, including current parental smoking, parental smoking during the first 2 years of the child's life, maternal smoking during pregnancy and residential history.

Mexico City has the highest ozone concentrations in North America and ozone has been related to development of asthma.[25] We obtained measurements of ambient ozone from the Mexican government's air monitoring stations. Ozone levels were measured via UV photometry (analyzer-model 400; API, San Diego, CA, USA). The residence of each child who participated in this study was located using a map and the closest monitoring station was assigned to that residence.[26] The ozone exposure data were collected for the year prior to the time of entry into the study. The parameter we used was the annual average of the daily maximum 8 h averages. We dichotomized this variable at the median of 67 p.p.b. for stratified analyses.

The protocol was reviewed and approved by the Institute Review Boards of the Mexican National Institute of Public Health, Hospital Infantil de México, Federico Gómez and the US National Institute of Environmental Health Sciences. Parents provided the written informed consent for the child's participation. Children also gave their informed assent.

Clinical evaluation

The diagnosis of asthma was based on clinical symptoms and response to treatment by a pediatric allergist.[22] The severity of asthma was rated by a pediatric allergist for 564 cases according to symptoms in the Global Initiative on Asthma schema as mild (intermittent or persistent), moderate or severe.[27] At a later date, for research purposes, pulmonary function was measured using the EasyOne spirometer (ndd Medical Technologies, Andover, MA, USA) for 435 cases according to American Thoracic Society specifications.[28] The best test out of three technically acceptable tests was selected. Spirometric prediction equations from a Mexico City childhood population were used to calculate the percent predicted forced expiratory volume in 1 s (FEV_1).[29] Children were asked to hold asthma medications on the morning of the test.

Atopy was determined using skin prick tests. The following battery of 25 aeroallergens (IPI ASAC, Mexico) common in Mexico City was used: *Aspergillus fumigatus*, *Alternaria* species, *Mucor* species, *Blattella germanica*, *Periplaneta americana*, *Penicillium* species, cat, dog, horse, *Dermatophagoides pteronyssinus*, *D. farinae*, *Ambrosia* species, *Artemisa ludoviciana*, *Cynodon dactylon*, *Chenopodium album*, *Quercus robur*, *Fraxinus* species, *Helianthus annus*, *Ligustrum vulgare*, *Lolium perenne*, *Plantago lanceolata*, *Rumex crispus*, *Schinus molle*, *Salsola* species and *Phleum pratense*. Histamine was used as a positive control and glycerin as a negative control. Children were considered atopic if the diameter of the skin reaction to at least one allergen exceeded 4 mm. The test was considered valid if the reaction to histamine was ⩾6 mm according to the grading of the skin prick test recommended by Aas and Belin.[30] Skin test data on all 25 aeroallergens were available on 539 cases.

SNP selection

SNPs for *GPRA* were selected using HapMap data analyzed with Haploview (http://www.hapmap.org). Our Gene coverage pipeline includes tag SNPs for the gene (20 Kb 5′ through 10 Kb 3′) with minor allele frequency >0.05 and selected at an r^2 of 0.8 for the Caucasian population. In addition to using LD criteria, we also added SNPs based on spacing across the gene (every 2–5 Kb). Nonsynonymous SNPs were also included. Once SNPs were selected they were genotyped on the Human Genome Diversity Panel (http://www.cephb.fr/HGDP-CEPH-Panel). The data from the Human Genome Diversity Panel was used to tag the Pima population and these SNPs were included in the SNPlex in

addition to reported significant SNPs and tags from the European populations. We selected 43 SNPs for genotyping in Mexican population.

There is a trade-off between SNP coverage and power in association studies.[31] Given the loss of statistical power from multiple testing of 43 SNPs, from among the genotyped SNPs, prior to epidemiologic analysis, we selected tagging SNPs of *a priori* interest. We inferred the common (frequency >5%) haplotypes using PHASE.[32] We identified LD bins among SNPs with minor allele frequencies higher than 10% using ldSelect.[33] In ldSelect, a bin is formed by a group of SNPs where a given SNP (the tagging SNP) is in high LD (r^2>0.8 in our analysis) with the maximum number of other SNPs. When nothing is known about function, selecting SNPs in bins that contain at least one other SNP improves efficiency.[31] We aimed to select a sufficient number of SNPs to cover multiple SNP bins and to distinguish all common haplotypes. We also selected nonsynonymous SNPs as well as SNPs associated with asthma in the literature with minor allele frequency higher than 5% in Mexican population. On the basis of the above criteria, we identified seven SNPs of *a priori* interest for analysis: rs1023556, rs324396, rs324981, rs727162, rs740347, rs1419780 and rs13246028.

Genotyping

DNA was extracted from peripheral blood lymphocyte using Gentra Puregene kits (Gentra System, Minneapolis, MN, USA). Forty-two SNPs were genotyped simultaneously using the SNPlex genotyping assay. Among these 42 SNPs, genotyping of the rs2040854, rs323922 and rs740347 SNPs were redone using the TaqMan SNP Genotyping Assay because of the low genotype call rates for the SNPlex assay. In addition, SNP546333 was genotyped using TaqMan SNP Genotyping Assay only. All genotyping assays were done by researchers who were blinded to parent or child status of samples. Assays were considered validated if the genotypes they produced with the HapMap CEU samples were >98% concordant with results for the same SNPs from HapMap. Each plate of 96 contained three control samples with known genotypes with >99% concordance for both the SNPlex and Taqman assays. We did not analyze 10 SNPs with call rates between 93 and 94%, 5 SNPs with call rates <93%, and 1 SNP with no variation, leaving 27 SNPs for the statistical analysis. Among the 16 SNPs with low call rates, 7 SNPs were rare (minor allele frequency <5%), 4 SNPs were in LD (r^2>0.8) with analyzed SNPs and 5 were in single SNP bins. The call rates for all TaqMan assays were >95%. The SNPlex genotyping method requires more DNA, and probably higher quality DNA, than TaqMan, explaining the lower call rate; 5% of the samples had too little DNA for SNPlex. A total of 589 families had genotyping data for at least one of the 27 SNPs.

Nonparentage was ascertained with a set of short-tandem repeats (AmpFLSTR Profiler Plus; Applied Bio-systems, Foster City, CA, USA) analyzed using Pedcheck software (University of Pittsburgh, Pittsburgh, PA, USA).[34]

Statistical analysis

We used a log-linear likelihood approach to analyze associations between asthma and individual SNPs.[21] The log-linear likelihood ratio test is a powerful and more flexible generalization of the transmission disequilibrium test (TDT) and has the advantage of providing estimates of the magnitude of associations rather than simply tests of significance.[21] Similar to TDT-based methods for the analysis of case–parent data, such as the family-based association test (FBAT),[35] the log-linear model tests the same null hypothesis of no within-family relationship between variant and the disease and achieves robustness against genetic population structure through stratification on the possible parental mating types.[21,36] The log-linear method thus gives comparable *P*-values to TDT-based methods.

We calculated relative risks for individual SNPs without restricting to a specific genetic model. The log-linear models of case–parent data are inherently immune to confounding by demographic and lifestyle factors such as parental smoking or environmental exposures. However, we examined effect modification by gender, parental smoking and level of ozone exposure. All SNP analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA) and STATA version 8.0 (Stata Press, College Station, TX, USA). We used the FBAT program to analyze associations between asthma and *GPRA* haplotypes[35,37] (http://biosun1.harvard.edu/~fbat/fbat.htm).

To evaluate whether *GPRA* polymorphisms influenced the degree of atopy, as assessed by the number of positive skin tests out of 25 performed, we used the polytomous logistic method of Kistner and Weinberg to estimate the linkage and association between *GPRA* polymorphisms and atopy.[38] *P*-values were calculated from likelihood ratio tests. We also used this method to analyze the relationship between *GPRA* SNPs and lung function, as assessed by percent predicted FEV_1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic and clinical characteristics of the 589 asthmatic children

Clinical characteristics	**%**
Mean age (s.d.) (year)	9.0 (2.4)
Sex (male)	60.8
Asthma severity[a]	
Mild	72.3
Moderate to severe	27.7
Asthma Medication in the past 12 months[b]	98.5
FEV_1, mean (s.d.), percent predicted[c]	96.0 (19.6)
Skin test positivity (out of 24 aeroallergens)[d]	
⩾1 allergen	91.6
⩾5 allergens	52.5
Parental smoking[e]	
Mother smoked during pregnancy	5.7
Current smoking parent	50.9

Abbreviations: FEV_1, forced expiratory volume in 1 s.

[a] Asthma severity data were available for 564 children.

[b] Asthma medication data were available for 584 children.

[c] Pulmonary function data were available for 435 children.

[d] Skin test data were available for 539 children.

[e] Parental smoking data were available for 585 children.

Table 2

Genotype distributions for seven *GPRA* tagging SNPs

SNP	Genotype	No. of cases	Minor allele frequency[a]
rs1023556	GG	185	0.37
	GA	232	
	AA	61	
rs324396	CC	167	0.42
	CT	227	
	TT	83	
rs740347	GG	498	0.05
	GC	48	
	CC	0	
rs324981	AA	234	0.30
	AT	197	
	TT	50	
rs1419780	CC	299	0.20
	CG	151	
	GG	24	
rs727162	CC	142	0.47
	CG	232	
	GG	110	
rs13246028	GG	354	0.15
	GT	117	
	TT	7	

See the online supplement for the frequency distributions for all mating types for seven *GPRA* tagging SNPs for all families.

[a]Minor allele frequency was calculated using parent genotyping data.

Table 3

Seven tagging SNPs in *GPRA* in relation to childhood asthma

SNPs	Genotype	RR (95% CI)[a]
rs1023556	GG	1.00
	GA	1.05 (0.82–1.34)
	AA	0.98 (0.66–1.45)
rs324396	CC	1.00
	CT	0.87 (0.67–1.12)
	TT	0.79 (0.55–1.15)
rs740347	GG	1.00
	GC	0.77 (0.52–1.15)
	CC	—[b]
rs324981	AA	1.00
	AT	1.19 (0.91–1.54)
	TT	1.10 (0.72–1.69)
rs1419780	CC	1.00
	CG	1.23 (0.93–1.62)
	GG	1.53 (0.85–2.77)
rs727162	CC	1.00
	CG	1.04 (0.80–1.36)
	GG	1.06 (0.74–1.52)
rs13246028	GG	1.00
	GT	0.89 (0.67–1.19)
	TT	0.79 (0.32–1.95)

Abbreviation: SNP, single nucleotide polymorphism.

[a] Homozygotes of major allele are the reference category.

[b] We did not calculated this relative risk because none of the cases were homozygous for the C allele.

Table 4

GPRA haplotypes in relation to childhood asthma

Haplotype[a]	Frequency	*P*-value
GCGACGG	0.167	0.48
ATGACCG	0.155	0.82
GTGACCG	0.133	0.13
GCGTGGG	0.121	0.35
ATGACGG	0.084	0.38

[a]The haplotypes formed by rs1023556G>A, rs324396C>T, rs740347G>C, rs324981A>T, rs1419780C>G, rs727162C>G and rs13246028G>T in order 5′–3′.

Table 5

Published studies examining associations of *GPRA* SNPs and haplotypes with asthma or asthma-related phenotypes

Reference	Study population	Number of cases	Associations			
			SNP or haplotype[a]	Phenotype	RR or OR (95% CI)	*P*-value
Laitinen *et al.*[1]	French Canadian and Finnish	514 asthmatic chromosomes and 75 high IgE-chromosomes	rs323922	Asthma or high IgE	NA	0.004
			H2	Asthma	2.5 (2.0–3.1)	0.0009
			H4, H5	High IgE	1.4 (1.1–1.9)	0.01
Shin *et al.*[6]	Korean	439 asthma patients	rs323922	Asthma	Not significant	
			Haplotypes	NA		
Soderhall *et al.*[7]	European	115 families with a child with atopic dermatitis and asthma	rs323922	Asthma and atopic dermatitis	Not significant	
			Haplotypes	NA		
Kormann *et al.*[2]	German	351 asthmatic children, including 44 children with asthma and BHR, and 112 children with high IgE	SNP546333	Asthma	1.40 (1.04–1.88)	0.025
				Asthma and BHR	2.38 (1.22–4.66)	0.009
			rs324396	Asthma	0.77 (0.61–0.97)	0.027
				High IgE	0.33 (0.12–0.93)	0.028
			rs740347	Asthma	1.34 (1.04–1.72)	0.022
				Asthma and BHR	2.71 (1.45–5.09)	0.001
				High IgE	1.63 (1.10–2.42)	0.015
			H4	Asthma	1.83 (1.08–3.08)	0.024
				Asthma and BHR	3.51 (1.08–11.4)	0.036
Melen *et al.*[3]	A mixed population from five Western European countries	176 asthmatic children including 68 children with atopic asthma	rs324384	Atopic asthma	0.81 (0.66–1.00)	NA
			rs324396	Asthma	0.83 (0.70–0.97)	0.02
				Atopic asthma	0.78 (0.62–0.98)	NA
			H1	Atopic asthma	0.78 (0.63–0.96)	0.045
			H5	Atopic asthma	1.47 (1.01–2.14)	0.023
Feng *et al.*[4]	Chinese	451 asthma patients	rs324981	BHR	0.58 (0.37–0.91)	0.02
			Haplotypes	BHR	Not significant	
Malerba *et al.*[5]	Italian	211 families with an allergic asthmatic child	rs740347	High IgE	NA	0.006
			SNP546333	High IgE	NA	0.005
			H4	High IgE	Protective	0.013
			H5	Asthma	Protective	0.026
			H6	Asthma	Risk	0.008

Abbreviations: BHR, bronchial hyperresponsiveness; IgE, immunoglobulin E; NA, not available; SNP, single nucleotide polymorphism.

[a]The haplotypes formed by rs323917C/G, rs323922G/C, rs324377C/A, SNP546333G>A, rs324384T/C, rs324396C/T, and rs740347G/C in order 5′–3′. H1, CGCGCTG; H2, CCAGTCG; H3, CGCGCCG; H4, CCAATCC; H5, CCAGTCC; H6, CGCGTCG.