

GENETICS

Multilocus Analysis of Candidate Genes Involved in Neurogenic Inflammation in Pediatric Asthma and Related Phenotypes: A Case–Control Study

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Objectives. Asthma is a heterogenous complex disorder caused by chronic inflammation of the airways. The key issue in genetic association studies of complex disorders is the identification of multiple low-risk genes that individually have little impact on the phenotype, but in combination account for the clinical manifestation of asthma. Since neurogenic inflammation is emerging as a candidate factor in the pathogenesis of asthma, the aim of the study was to investigate whether genetic variants of neurotrophin genes are associated with asthma disease severity or asthma-related phenotypes in a pediatric population. **Methods.** We genotyped 27 polymorphisms located in neurotrophin genes, using TaqMan SNP genotyping assays or Polymerase Chain Reaction - Restriction Fragments Lengths Polymorphism (PCR-RFLP) in 200 children diagnosed with asthma and 226 controls. Interactions between 27 polymorphic loci and asthma-related phenotypes were determined using the Multifactor Dimensionality Reduction (MDR) method. **Results.** In single marker analysis, we observed an association of *MAP3K1* gene polymorphisms (rs702689 and rs889312) with asthma. We also observed that four Single Nucleotide Polymorphisms (SNPs) were associated with severe asthma. Analysis stratified by asthma-related phenotype revealed an association between atopy and *NGFR* (rs3785931), while *BDNF* (rs7124442), *NTRK2* (rs1212171), *NGFR* (rs2072446), and *FYN* (rs3730353) variants were associated with increased exhaled nitric oxide (exNO). In addition, gene–gene interaction analysis revealed a significant epistatic interaction between *MAPK* (rs889312) and *NGF* (rs11102930) variants in asthma susceptibility. **Conclusions.** Our results suggest that genetic variants of *MAP3K1* and *NGF* genes involved in the regulation of neurogenic inflammation may contribute to asthma, possibly via enhanced NGF expression and MAPK signaling pathway activation.

Keywords atopy, children, epistatic interaction, genetic association, polymorphism

INTRODUCTION

Asthma is the most common chronic respiratory disease in childhood and is largely characterized by chronic airway inflammation. Although the etiology of asthma is complex, a large proportion of asthmatic children (80%) exhibit allergic background. It has been observed that allergic asthma is associated with changes in neuronal control of the airways (1). Allergic inflammation in asthma causes changes in neuronal networks of the lung, particularly in sensory hyperreactivity whereby enhanced signal transmission in local parasympathetic ganglia amplifies the inflammatory response. This direct potentiation of an inflammatory process following the release of enhanced neurotransmitters has been termed neurogenic inflammation (2). Neurogenic inflammation is mediated by neurotrophins (nerve growth

factor, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4), a family of growth factors that play an important role in the plasticity differentiation and survival of sensory and motor neurons (3). In addition, neurotrophins are homologous proteins that mediate inflammatory signals between neuronal, immune, and structural cells in the bronchi (3). Therefore, it is likely that neurogenic inflammation and, specifically, neurotrophins play an important role in both allergy and asthma.

The biological effects of neurotrophins are mediated by either high (TrkA for NGF, TrkB for BDNF and NT-4, TrkC for NT-3) or low (NGFR) affinity tyrosine kinase receptors which mediate signal transduction through activation of specific kinases (e.g., PLC γ , MAPK, FYN) (4). Neurotrophin receptors are widely expressed not only on neurons but also on nonneuronal cells involved in the asthma pathogenesis such as immune cells, muscle cells, and epithelial cells (5). Recently it was shown that mammalian monocytes/macrophages have the capacity to produce, store, and release their own neurotrophins (6–8), while eosinophils express receptors for the neurotrophins

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capable of eosinophil activation and patients' survival in severe allergic bronchial asthma (9). T cells also produce neurotrophins; however, the rate of synthesis is dependent on the level of T-cell activation (10, 11). Studies in animal models of allergic challenge have detected that increased expression of neurotrophin-positive cells enhanced levels of neurotrophins in serum and bronchoalveolar lavage fluid (1, 12). Conversely, administration of exogenous neurotrophins resulted in increased Th2-mediated inflammation and airway hyperresponsiveness (12, 13). In further support, several studies have demonstrated a clear association between altered neurotrophin function and asthma (14–19).

To date, only a few genetic association studies investigating the relationship between asthmatic symptoms and variation in neurotrophic pathway genes have been performed (20–23). These studies only consider the involvement of single genes and polymorphisms and therefore a comprehensive analysis of candidate genes involved in the regulation of neurogenic inflammation in pediatric asthma has yet to be performed.

In response, the aim of this study was to investigate whether genetic variants from neurotrophic signaling pathway genes are associated with pediatric asthma and whether the functional variants identified may be used as a biomarker to predict pediatric asthma and related clinical phenotypes.

METHODS

Study Design

In this case–control study, 200 children diagnosed with asthma and 220 healthy control subjects were included. All participants as well as their parents had given written informed consent. Local ethics committee accepted the project. The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Patients

The study was performed on a Polish sample of 200 asthmatic patients of Caucasian origin ranging from 6 to 18 years old. Patients were recruited from inpatients treated for asthma in the Department of Pediatric Pulmonology, Allergy and Clinical Immunology of Poznan University of Medical Sciences and in the Outpatient Allergy Clinics from Poznan and Rzeszow. Asthma diagnosis was made according to GINA recommendation, based on clinical asthma symptoms and lung function test (bronchodilator responsiveness, exercise-induced hyperresponsiveness). Spirometry was performed using a LungTest 1000 (MES) according to ERS/ATS guidelines (24). Decreased lung function was defined as FEV1% predicted value below the 5th percentile (FEV1 value adjusted for ethnicity, age, gender, height, and weight) as recommended according to ATS/ERS guidelines (25).

Exhaled nitric oxide (exNO) concentration (an indicator of airway inflammation) was assessed by

TABLE 1.—Characteristics of the analyzed population.

Characteristic	Asthmatic patients	Control subjects
Number of individuals (n)	200	226
Age (years), mean $\pm$ SD	11.4 $\pm$ 3.50	12.4 $\pm$ 3.42
Male gender (%)	62	46
Asthma severity		
Severe, n (%)	63 (31.5)	
Moderate, n (%)	110 (55.0)	
Mild, n (%)	27 (13.5)	
BMI	19.77 $\pm$ 5.70	19.45 $\pm$ 3.11
Baseline spirometry		
FEV1 to VC ratio	97.23 $\pm$ 10.76	99.20 $\pm$ 9.95
FEV1 (%)	101.82 $\pm$ 68.84	106.72 $\pm$ 12.10
FVC (%)	98.70 $\pm$ 15.27	100.67 $\pm$ 11.67
exNO	29.11 $\pm$ 35.75	16.82 $\pm$ 14.48
Age at onset (years)	5.25 $\pm$ 3.36	–
Early age at onset (<3 yrs) (%)	47.30	–
ETS (%)	39.50	47.40
Positive skin prick test (%)	67.32	–
Elevated total IgE level (%)	69.90	–
Other allergic diseases (%)	81.77	–

chemiluminescence using NIOX analyzer (Solna, Sweden) and following the ATS/ERS recommended procedures (26). Increased exNO concentration was regarded as >20 ppb (equivalent to nanoliters per liter).

Clinical diagnosis of atopy depended on current or past symptoms of atopic dermatitis, allergic rhinoconjunctivitis, or food allergy. Atopy was confirmed when children fulfilled the following criteria: total IgE level higher than the upper normal limits for age; positive skin prick test to at least one aero-allergen (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, *Alternaria alternata*, *Cladosporium herbarum*; pollen: grass mix, rye, birch pollen, alder, hazel—Allergopharma, Germany). Any reaction with mean wheal diameter at least 3 mm greater than negative control was regarded positive. Total serum IgE level was measured by a fluoroimmunoassay with Pharmacia UniCap 100 System® (Pharmacia, Uppsala, Sweden) following manufacturer's instruction. The upper limits of normal range for total IgE was age dependent (70 kU/l for 6-year-old children; 79 kU/l for 7-year-old children; 89 kU/l for 8-year-old children; 98 kU/l for 9-year-old children; 107.0 kU/l for children of 10 years and older). The clinical description of the group was given in Table 1.

Control Group

Control group consisted of 220 healthy subjects of Caucasian origin. Control subjects were recruited from the same geographic region from a group of carefully chosen volunteers without current and past asthma and allergy symptoms. Any allergic diseases or asthmatic episodes were excluded based on clinical examination, history, lung function test, and exhaled NO measurement. The description of the control group was given in Table 1.

Genotyping

We have genotyped 27 SNPs from 11 genes involved in the neurotrophic signaling cascade encoding both

TABLE 2.—Description of candidate gene polymorphisms included in this study.

Gene	SNP	Location in the gene	MAF	Genotyping method	Enzyme/assay
<i>BDNF</i>	rs6265	Val74Met, exon 1	0.19	RFLP	<i>Eco72I</i>
	rs12273363	Near 5'-gene	0.17	RFLP	<i>BseGI</i>
	rs203024	Intron 1	0.13	RFLP	<i>TaiI</i>
	rs7124442	Exon 2 (3'UTR)	0.32	TaqMan	C_27833027_10
<i>NGF</i>	rs63330	Ala35Val, exon 3	0.40	TaqMan	C_2525309_10
	Rs910330	Intron 2	0.42	RFLP	<i>HaeIII</i>
	Rs11102930	Near 5' gene	0.27	TaqMan	C_26680904_10
<i>NTRK1</i>	Rs6334	Gln558Gln, exon 4	0.22	RFLP	<i>TaiI</i>
	Rs2768755	Intron 3	0.25	TaqMan	C_27532280_10
<i>NTRK2</i>	Rs1936434	Intron 12	0.25	TaqMan	C_11930872_10
	Rs1212171	promoter region	0.40	TaqMan	C_7424025_10
<i>NTRK3</i>	Rs1991287	Intron 2	0.36	TaqMan	C_11442029_10
	Rs11073763	Intron 8	0.43	TaqMan	C_3041241_10
	Rs2059588	Intron 5	0.42	RFLP	<i>AcI</i>
<i>NTF3</i>	Rs1805149	Glu89Gly, exon 1	0.14	RFLP	<i>HaeIII</i>
	Rs6332	Pro84Pro, exon 1	0.45	TaqMan	C_170592_10
<i>NTF4</i>	Rs11669977	5'UTR	0.20	TaqMan	C_2884550_10
<i>MAP3K1</i>	Rs702689	Asn806Asp, exon 6	0.30	RFLP	<i>AseI</i>
	Rs889312	Intron	0.30	RFLP	<i>HinIII</i>
	Rs832582	Ile906Val, exon 6	0.17	TaqMan	C_8961435_10
<i>FYN</i>	Rs706895	Intron 1	0.31	RFLP	<i>HphI</i>
	Rs3730353	Intron 10	0.21	RFLP	<i>MboI</i>
	Rs6916861	3'UTR	0.2	RFLP	<i>ApoI</i>
<i>NGFR</i>	Rs3785931	Intron 1	0.25	RFLP	<i>BtsI</i>
	Rs2072446	Leu205Ser, exon 4	0.05	TaqMan	C_15870920_10
<i>PLCγ</i>	Rs2228246	Ser279Gly, exon 9	0.15	RFLP	<i>AluI</i>
	Rs753381	Ile813Thr, exon 13	0.45	TaqMan	C_2227239_10

neurotrophins (Brain-Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), NTF-3, NTF-4), their receptors (NTRK1, NTRK2, NTRK3, Nerve Growth Factor Receptor (NGFR)), and downstream intracellular kinases (PLC γ , FYN, MAP3K1). The SNP selection was based on at least two of the following criteria: functionality demonstrated in previous experimental studies, high frequency (Minor Allele Frequency (MAF) > 0.05), indication as tag SNP in HapMap Caucasian database (www.hapmap.org/), or previously reported associations for asthma and allergic diseases (both positive and negative findings). The chosen SNPs cover both coding regions as well as noncoding regions (promoters, introns, UTRs) possibly affecting gene regulation. A brief description of the polymorphisms included in this study is presented in Table 2.

DNA was extracted from 10 ml of EDTA anticoagulated whole blood using the salting out method of Miller (27). Genotyping of 27 polymorphisms was performed using PCR-RFLP or TaqMan SNP genotyping assays (Applied Biosystems, Carlsbad, California, USA). Primer and probe sequences, conditions for the 14 polymorphisms analyzed with PCR-RFLP method, and the assays' ID numbers for 13 polymorphisms analyzed using of 5' nuclease method are shown in Table 3. TaqMan SNP genotyping was performed using an ABI Prism® 7900HT Sequence Detection System. Data acquisition and analysis was performed using the allelic discrimination analysis module in SDS v2.1 software (Applied Biosystems). Reaction components and amplification parameters were based on manufacturer's instructions.

For each reaction plate genomic control DNA samples and nontemplate controls were included. To check for RFLP analysis and TaqMan SNP genotyping accuracy 25% of randomly chosen samples from both groups for each SNP were repeatedly analyzed. Identical genotypes were identified in all repeated samples. All genotyping was performed without knowing the clinical status of the subjects.

Statistical Analysis

Pearson's chi-square (χ^2) test and Fisher's exact test were used to test differences in the genotypic and allelic (respectively) distribution in case control. A selection data mining algorithm, using χ^2 statistics, was used for each predictor to identify the best genetic predictors of asthma status. Calculations were performed using Statistica version 9.0 software (Statsoft, Cracow, Poland). Odds ratios (ORs) with a 95% confidence interval (CI) were calculated using GraphPad InStat 3 program. Concordance with Hardy-Weinberg law was performed using a "Utility Programs For Analysis of Genetic Linkage" application (Copyright © 1988 J. Ott).

Higher-order gene-gene interactions among tested SNPs were analyzed using a nonparametric and genetic model-free Multifactor Dimensionality Reduction (MDR) approach (v.2.0 beta 8.3). A detailed description of the methodology can be found in Ritchie et al. (28). All interactions were tested using 10-fold cross-validation in an exhaustive search considering all possible SNP combinations. The model with the highest testing balance accuracy

TABLE 3.—The results of single marker association analysis in the comparison between the asthmatic patients and the control group for the analyzed polymorphisms.

Gene	SNP	P-value (genotypes)	P-value (alleles)	OR	95%CI	HWE <i>p</i> -value (control; cases)
<i>BDNF</i>	rs6265	.910	.850	0.954	0.659–1.381	.642; .326
	rs12273363	.790	.676	1.113	0.739–1.675	.841; .366
	rs203024	.322	.208	1.223	0.897–1.665	.460; .710
	rs7124442	.995	1.000	0.986	0.703–1.382	.626; .546
<i>NGF</i>	rs63330	.067	.252	1.196	0.887–1.614	.094; .251
	rs910330	.815	.812	0.962	0.705–1.314	.457; .901
	rs11102930	.352	.275	1.196	0.880–1.625	.515; .555
<i>NTRK1</i>	rs6334	.505	.398	1.185	0.818–1.716	.312; .970
	rs2768755	.672	.543	1.124	0.799–1.582	.638; .724
<i>NTRK2</i>	rs1936434	.885	.725	1.079	0.764–1.523	.715; .440
<i>NTRK3</i>	rs1212171	.818	.581	0.909	0.667–1.239	.543; .750
	rs1991287	.705	.449	1.131	0.840–1.522	.592; .507
<i>NTF3</i>	rs11073763	.507	.816	1.046	0.772–1.418	.845; .064
	rs2059588	.878	.692	1.070	0.783–1.460	.680; .417
	rs1805149	.350	.137	0.202	0.024–1.691	.966; .002
<i>NTF4</i>	rs6332	.199	.108	1.293	0.957–1.747	.357; .023
	rs11669977	.098	.128	0.746	0.513–1.086	.359; .211
<i>MAP3K1</i>	rs702689	.038*	.575	1.126	0.781–1.622	.587; .001
	rs889312	.050	.013*	1.591	1.103–2.297	.404; .535
	rs832582	.528	.512	1.137	0.787–1.641	.729; .375
<i>FYN</i>	rs706895	.672	.923	0.969	0.664–1.413	.092; .631
	rs3730353	.223	.069	0.659	0.421–1.031	.083; .055
	rs6916861	.002*	.021*	1.472	1.063–2.038	<.000 ; <.000
<i>NGFR</i>	rs3785931	.875	.773	1.065	0.729–1.554	.357; .621
	rs2072446	.573	.597	1.216	0.604–2.448	.483; .539
<i>PLCγ</i>	rs2228246	.433	.908	0.959	0.608–1.513	.578; .145
	rs2228245	.468	.537	1.106	0.816–1.497	.605; .326

Note: *Significant result of association analysis.

Significant deviation from Hardy–Weinberg equilibrium are highlighted in bold.

and cross-validation consistency of > 5 out of 10 was selected as “best model.” Statistical significance was determined using a 1000-fold permutation test (MDR permutation testing module v.1.0 beta 2). The software is available online (<http://www.epistasis.org>).

RESULTS

Hardy–Weinberg Equilibrium Test

All studied polymorphisms were tested for concordance with the Hardy–Weinberg equilibrium (HWE). The results are presented in Table 3. Rs6916861, a SNP in the *FYN* gene, showed significant deviation from HWE both in asthma patients and control subjects ($p < .0001$), and therefore was excluded from further analysis and interpretation. Deviation was also observed for rs70268 (*MAP3K1*), rs180514, and rs6332 (*NTF3*); however, this deviation was only observed in asthma patients, and therefore these SNPs were included in further analysis. Other analyzed polymorphisms were in concordance with HWE.

Single Marker Association Results

In an association analysis of 27 SNPs we found a significant difference in genotype and allele distributions for 2 polymorphisms localized in the *MAP3K1* gene (AG genotype of rs702689 and C allele of rs889312). Results of association analysis of individual markers with asthma

were shown in Table 3. For the other SNPs we did not observe significant differences in genotypic and allelic distribution between asthmatic patients and healthy children. Importantly, the association was also observed following application of data mining algorithms (Feature Selection module), which identified both *MAPK* polymorphisms ($\chi^2 = 8.543$, $p = .013$ and $\chi^2 = 5.465$, $p = .065$ for rs889312 and rs702689, respectively) as the best genetic predictors for asthma status from all SNPs analyzed.

In the analysis stratified by gender we observed a significant association between the following polymorphisms: *NGF* (rs6330) ($p = .011$), *MAP3K1* (rs702689) ($p = .0001$), and *PLCγ* (rs2228245) ($p = .048$), and asthma in boys. Moreover, we also observed that four polymorphisms were associated with severe asthma: rs11073763 (*NTRK*) ($p = .047$), rs702689 (*MAP3K1*) ($p = .004$), rs2228246 (*PLCγ*) ($p = .004$), and rs1805149 (*NTF3*).

The analysis was also stratified by asthma-related phenotypes, such as increased total IgE, decreased lung function, atopy, and increased exNO level. We observed an association between atopic asthma and *NGFR* polymorphisms (rs3785931) ($p = .010$ for genotypes; $p = .002$ for alleles; OR = 3.655). In the analysis of exNO level, we observed association with the following SNPs: *BDNF* (rs7124442) ($p = .021$); *NTRK2* (rs1212171) ($p = .029$); *NGFR* (rs2072446) ($p = .033$); and *FYN* (rs3730353) ($p = .017$) (data not shown). For decreased lung function

TABLE 4.—Multilocus interaction model for asthma and intermediate phenotypes by MDR method.

Model	Loci combination	Testing balanced accuracy (%)	Cross-validation consistency	P-value
Asthma				
2-locus	MAPK rs889312 and NGF rs11102930	62	7	.013*
3-locus	NTRK2 rs1212171, MAPK rs889312, and NGF rs11102930	59	4	.073
4-locus	MAPK rs702689, NGFR rs3785931, NGF rs11102930, and PLC γ rs2228245	51	3	.804
Increased IgE level				
2-locus	NGFR rs3785931 and NT-4 rs116699	59	6	.073
3-locus	PLC γ rs2228246, NT-3 rs6332, and NT-4 rs116699	48	2	.972
4-locus	BDNF rs2030324, NGFR rs3785931, NGFRs11102930, and PLC γ rs2228245	41	2	.999
Decreased FEV1 value				
2-locus	FYN rs706895 and NT-3 rs6332	40	2	1.000
3-locus	MAPK rs702689, FYN rs706895, and NT-3 rs6332	41	5	.999
4-locus	NTRK3 rs2059588, MAPK rs702689, FYN rs706895, and NT-3 rs6332	49	2	.934
Atopy				
2-locus	NGF rs11102930 and PLC γ rs2228245	65	7	.001*
3-locus	NTRK3 rs11073763, NGF rs11102930, and NGF rs910330	60	4	.038*
4-locus	BDNF rs2030324, NTRK3 rs1991287, MAPK rs702689, and NGF rs11102930	57	4	.190
5-locus	BDNF rs2030324, NTRK3 rs1991287, MAPK rs702689, NGF rs11102930, and NGF rs910330	55	5	.338
6-locus	BDNF rs2030324, NTRK3 rs11073763, MAPK rs889312, NGF rs11102930, NGF rs910330, and NT-3 rs6332	52	3	.694
Increased exNO level				
2-locus	BDNF rs7124442 and MAPK rs889312	56	7	.265
3-locus	BDNF rs7124442, NTRK3 rs2059588, and MAPK rs702689	45	2	.999
4-locus	NTRK3 rs2059588, MAPK rs702689 NGF, rs11102930, and NTF3 rs6332	57	10	.191

Note: *Significance of accuracy; the empirical *p*-value was based on 1000 permutations.

and increased IgE level, no significant associations were observed (data not shown).

Gene–Gene Interaction Analysis

Results of exhaustive MDR analysis evaluating combinations of all tested SNPs for each comparison are summarized in Table 4. The best combination of possibly interactive polymorphisms in predicting asthma was observed for rs889312 of *MAP3K1* gene and rs11102930 of *NGFR* gene. The testing-balanced accuracy for this 2-locus model was 62%, cross-validation consistency was 7/10 (70%), and an empirical *p*-value of .013 based on 1000-fold permutations was obtained. One of these polymorphisms (rs889312 of *MAP3K1* gene) was also significantly associated in single marker analysis with the risk of asthma. The best combination of interactive polymorphisms in predicting atopy included a 2-locus model (rs11102930 of *NGF* and rs2228245 of *PLC γ*) (with cross-validation consistency 7/10) and a 3-locus model (rs11073763 of *NTRK3*, rs11102930 of *NGF* and rs910330 of *NGF*) (with cross-validation consistency 4/10) (Table 4). None of those SNPs showed significant association with atopy in the single marker analysis. For the other analyzed intermediate phenotypes none of the combinations of polymorphisms reached statistical significance in predicting susceptibility to increased IgE level, decreased lung function, and increased exNO level.

DISCUSSION

This study has demonstrated for the first time that pediatric asthma susceptibility is significantly associated with *MAP3K1* genetic variants (rs702689 and rs889312) and an epistatic interaction between *NGF* (rs1102930) and *MAP3K1* (rs889312). In addition, several polymorphisms showed a significant association with phenotypes related to asthma, such as atopy (put SNP here), asthma severity (put SNP here), and ex level (put SNP here), both in single marker and gene–gene interaction analyses.

Our findings support a previously identified involvement of neurotrophins and their receptors in the pathogenesis of asthma and related phenotypes (29–35). However, only a limited number of association studies of genes related to neurogenic inflammation have been performed so far. Those were focused on the analysis of *BDNF* variants (20–23). To our knowledge, this is the first study describing both association analysis and gene–gene interactions for the candidate genes in relation to neurogenic inflammation. In particular our study has highlighted the role of *MAP3K1* polymorphisms in asthma susceptibility. The protein product of *MAP3K1* is a serine threonine kinase that forms part of the MAPK cell-signaling pathway implicated in cellular response to mitogenic stimuli such as growth factors (36, 37). Thus, an altered regulation of *MAP3K1* may influence asthma susceptibility. We also observed associations with asthma in male gender, asthma severity atopy, and increased exNO level. To our

knowledge, there are no previous reports on the involvement of the genetic variations analyzed here and susceptibility to asthma or related phenotypes, so it is difficult to compare our findings.

In this study, we applied multidimensionality reduction method (MDR) to identify significant gene–gene interactions. The use of this method facilitates the detection of multiple gene combinations that modify disease susceptibility. Essentially, MDR groups multilocus genotypes into either high or low risk. MDR includes no assumption regarding the mode of inheritance and minimizes false-positive results due to multiple testing. MDR has been shown to have reasonable power to identify multilocus interactions in case–control studies with relatively small samples (38) and has been applied so far in gene–gene interactions of complex disorders such as hypertension (39), type 2 diabetes mellitus (40), myocardial infarction (41), asthma (42, 43), and atopy (44). In our study, we found a significant gene–gene interaction for asthma between 2 loci (rs889312 of *MAPK* gene and rs11102930 of *NGF* gene) out of 27 different loci from multiple genes, which confirms the sensitivity of MDR method in detecting polygenic interactions. A possible explanation for the observed interaction between these polymorphisms is that the C allele *NGF* promoter polymorphism enhances NGF expression. Conversely, this may increase stimulation of the MAPK signaling pathway resulting in enhanced B lymphocytes proliferation (45), an amplified inflammatory response, and asthma disease progression. In addition, the polymorphism in the minor allele of *MAP3K1* is located within a linkage disequilibrium block that encodes part of the MAPK signaling pathway implicated in gene regulation. Taken together, an interaction of these alleles increases the risk of asthma susceptibility.

The main limitation of this association study is the relatively small sample size of asthmatic patients and control subjects (approximately 200 in each group). However, previous studies had successfully used MDR method to identify gene–gene interactions for complex traits with a total sample size between 177 and 686 (28, 39–41) and sufficient power to detect gene–gene interactions in the presence of 5% genotyping error, 5% missing data, or a combination of both (28, 46).

CONCLUSIONS

In summary, we demonstrate here for the first time that genetic variants of *MAP3K1* and *NGF* genes involved in the regulation of neurogenic inflammation are significantly associated with asthma pathogenesis, potentially acting through enhanced NGF expression and increased MAPK signaling.

Identification of the role of MAPK and NGF in asthma may serve to highlight novel therapeutic targets, as well as diagnostic and prognostic markers to help better classify the asthma phenotype. Although our results suggest that regulation on DNA level may be important in asthma development, further experimental studies are required to

investigate the exact mechanisms of neuronal dysfunction underlying asthma development.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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