J Microbiol Immunol Infect
2005;38:158-163

Asthma severity and genetics in Taiwan

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Received: May 19, 2004 Revised: May 24, 2004 Accepted: May 26, 2004

The prevalence of childhood asthma in Taiwan has increased dramatically during the last 2 to 3 decades. In Taipei city, the prevalence of asthma in schoolchildren has increased from 1.3% in 1974 to 19.0% in 2003. Genetic mapping and candidate gene analyses have revealed suggestive evidence for linkage of asthma to a number of different chromosomal regions and for association with several candidate genes. Over 70 variants in candidate genes have been reported to be associated with these phenotypes. The main regions these variants have been found are on chromosomes 2q, 5q, 6p, 11q, 12q, 16q and 17q. Five potential asthma susceptibility genes or complexes have been identified using a positional approach. These are A desintegrin and metalloproteinase 33 (ADAM33), dipeptidyl peptidase 10 (DPP10), plant homeodomain zinc finger protein 11 (PHF11) and SET domain, bifurcated 2, G-protein related receptor for asthma (GPRA) and serine protease inhibitor Kazal type 5 (SPINK5). It is also evident that environmental factors will influence the expression of genes and the ultimate clinical phenotype of asthma and atopy. Evidence for a genetic contribution to risk for fatal or near-fatal asthma in Caucasians and Taiwanese has been suggested. We have revealed that the regulation upon activation, normal T cell expressed and secreted (RANTES)-28C/G polymorphism exacerbates asthma severity and represents a genetic risk factor for life-threatening asthma attacks in Chinese children. Moreover, in the Chinese children the frequency of the chemoattractant receptor-homologous molecule expressed on T helper 2 cells (CRTH2) 1651G allele in near-fatal asthmatics was significantly higher than in mild-to-moderate asthmatics and normal controls. The CRTH2 1651G allele of single nucleotide polymorphism re545659 was also associated with a higher degree of bronchial hyperresponsiveness.

Key words: Asthma, gene expression regulation, genetic markers, genetic polymorphism, genetic predisposition to disease

The prevalence and severity of asthma have been increasing in many countries, the trends being most pronounced for children and adolescents [1]. For example, the prevalence of childhood asthma in Taiwan has increased dramatically during the last 2 to 3 decades. A series of surveys has been conducted in Taiwan over a 30-year period (1974-2003), with current asthma prevalence assessed for children of the same age at the same schools through use of the same method [2,3]. The results showed that the prevalence of asthma in schoolchildren has increased from 1.3% in 1974 to 19.0% in 2003 in Taipei city. Our recent work also indicates that the severity of asthma seems to have increased, as reflected by a significant upward trend in admission rates for cases of childhood asthma in 1 medical center [3]. Thus, childhood asthma is currently a major health problem, not only in western countries but also in Taiwan.

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The majority of bronchial asthma in childhood is atopic, with manifestations of allergic diathesis including asthma, rhinitis and eczema and sensitized to inhalant or food allergens. There is also strong evidence for a genetic component in asthma [4-6]. However, multiple environmental factors are also known to modulate the clinical expression of asthma, as well as the asthma-associated phenotypes — airway hyperresponsiveness (AHR), atopy and elevated immunoglobulin E (IgE) [7-9]. It is a commonly held view that asthma is caused by multiple interacting genes, some having a protective effect and others contributing to the disease pathogenesis, with each gene having its own tendency to interact with and be influenced by the environment [7-11]. Thus, the complex nature of the asthma phenotype, together with substantial locus heterogeneity and environmental influence, have made it difficult to uncover the genetic factors that underlie asthma.

The genetics of atopy and asthma are now being characterized using gene discovery and functional genetic methodologies. Susceptibility genes for asthma

remain to be defined. Genetic mapping and candidate gene analyses have revealed suggestive evidence for linkage to a number of different chromosomal regions and for association with several candidate genes. Because of the complexity of these conditions, and confounding gene-gene and gene-environmental interactions, characterization of candidate asthma and atopy genes and their products, either in terms of their expression (using single nucleotide polymorphisms [SNPs] and microarrays) or protein function, has been successful to only a limited extent [12,13].

There is evidence that genetic liability for asthma, AHR and allergic traits are regulated through distinct loci, although there is likely some shared overlap as well [14]. The candidate and genome screen approaches have both been used to clarify the roles of genetic factors in the pathogenesis of asthma. A variety of genetic susceptibility loci have been suggested using the candidate gene approach. Many regions of the genome have been found to have linkage with the phenotypes of asthma and atopy. Over 70 variants in candidate genes have been reported to be associated with these phenotypes. The main regions these variants have been found are on chromosomes 2q, 5q, 6p, 11q, 12q, 16q and 17q. Specifically, these include: (1) chromosome 5 in the area of the cytokine gene cluster; (2) chromosome 6 in the area of human leucocyte antigen and tumor necrosis factor genes; (3) chromosome 11 in the area of the high-affinity receptor (FcγRI) gene; (4) chromosome 12 in the areas of interferon-γ, insulin-like growth factor-1, β-subunit nuclear factor, and mast cell growth factor genes; (5) chromosome 14 in the area of T cell receptor gene; and (6) chromosome 16 in the area of the interkeukin (IL)-4R gene [12]. The severity of asthma and response to treatment have also been suggested to be dependent on genetic modulators, such as the polymorphism of the β_2-receptor (found on chromosome 5), which is involved in the bronchodilator response to β-agonists [15,16].

Five potential asthma susceptibility genes or complexes have been identified using a positional approach. These are A desintegrin and metalloproteinase 33 (ADAM33), dipeptidyl peptidase 10 (DPP10), plant homeodomain zinc finger protein 11 (PHF11) and SET domain, bifurcated 2, G-protein related receptor for asthma (GPRA) and serine protease inhibitor Kazal type 5 (SPINK5). It is evident that environmental factors will influence the expression of genes and the ultimate clinical phenotype of asthma and atopy. In 2002, Van Eerdewegh et al reported the fine mapping of the ADAM33 as an asthma and AHR gene on chromosome 20p13 [17], using genome-wide scan from 460 Caucasian families.

A survey of 35 SNPs in 23 genes showed that the ADAM33 variants might directly impact lung architecture and function [17,18]. Although subsequent to the report of Van Eerdewegh et al, other groups have tested ADAM33 SNPs in their asthma cohorts with mixed results. Raby et al [19] did not demonstrate the association of ADAM33 SNPs with asthma or airway responsiveness, and only weak associations were observed with IgE levels and total eosinophilia. Further work needs to be performed both in terms of replication of genetic association and determination of gene function before any firm conclusions can be made regarding the importance of ADAM33 in asthma.

Genetic linkage of asthma and atopy to chromosome 13q14 is one of the most consistent linkage findings and has been replicated in many different populations [9,20,21]. Zhang and coworkers [22] assessed linkage disequilibrium patterns around the marker using densely spaced SNPs, and in parallel studied the gene content of the segment. Evidence for association with total IgE was identified with variants in 2 adjacent blocks, and centered on 1 gene — PHF11 gene. Furthermore, using a stepwise analytic procedure, some studies revealed that 3 polymorphisms localized to PHF11 carried the bulk of the association with IgE: 2 were intronic, and 1 was in the 3'-untranslated region of PHF11 [22-25]. The precise function of this gene has not been determined but the presence of 2 zinc finger motifs in the translated protein suggests a role in the regulation of lymphocyte activation and immunoglobulin synthesis [26].

Repeated genome scans for asthma have implicated a broad peak of linkage on chromosome 2q14-q32 [9, 10]. Allen et al [27] studied the localization of the putative gene by genetic association method, and found association of the microsatellite D2S308 with asthma. The gene, named DPP10, belongs to the conserved family of dipeptidyl peptidases that can cleave off terminal dipeptides from chemokines and cytokines, and might regulate their activities. The functional properties of DPP10 still need further clarification.

There has been recognition of a group of asthmatic patients who suffer from severe and life-threatening asthmatic attacks and in whom high doses of steroids, frequent hospitalization and occasional intubation and ventilation are required. Evidence for a genetic contribution to risk for fatal or near-fatal asthma in Caucasians and Taiwanese has been suggested [28-44].

Table 1. Genotype and allele frequencies of regulation upon activation, normal T cell expressed and secreted (RANTES)-28C/G for asthmatics, atopics and normal controls of Chinese children[a]

Group	n	Genotype			Allele frequency (%)	OR (95% CI)	*p*
		C/C	C/G	G/G	G		
Near-fatal asthma	48	26 (54.2%)	18 (37.5%)	4 (8.3%)	27.1	2.926 (1.414-6.055)	0.006
Mild-to-moderate asthma	134	108 (80.6%)	21 (15.7%)	5 (3.7%)	11.6	0.833 (0.446-1.554)	0.677
Atopic non-asthmatic	69	55 (79.7%)	14 (20.3%)	0 (0%)	10.1	0.880 (0.419-1.849)	0.881
Control	107	83 (77.6%)	23 (21.5%)	1 (0.9%)	11.7	1.0	-

Abbreviations: OR = odds ratio; CI = confidence interval
[a]Modified from Yao et al [32].

The RANTES (regulated upon activation, normal T cell expressed and secreted) gene lies on chromosome 17q11.2-q12, a region for which linkage to asthma or atopy has been demonstrated in several studies [24,29, 30]. RANTES can produce chemotaxis and activation of inflammatory cells (eosinophils, monocytes, basophils and T cells) that are central to the airway inflammation characteristic of asthma [31]. After evaluation of RANTES SNPs of 48 patients with near-fatal asthma and 134 children with milder asthma, we reported that the RANTES-28C/G polymorphism exacerbates asthma severity and represents a genetic risk factor for life-threatening asthma attacks in Chinese children [32]. The odds ratio for the risk of asthma characterized by near-fatal incidents rather than the mild-to-moderate variant was 3.52 for children with the RANTES-28G allele versus those with the frequently occurring -28C/C genotype (Table 1). The RANTES-28G allele was also associated with an increased eosinophil level and a higher degree of AHR.

Monocyte chemoattractant protein 1 (MCP-1), one of the CC chemokines, appears to play a significant role in asthma pathogenesis because of its ability to attract monocytes, eosinophils, and activate mast cells and basophils, inducing leukotriene C4 release into the airway, thus directly inducing AHR [33,34]. It has been demonstrated that the levels of MCP-1 are increased in the bronchoalveolar lavage fluid of ventilated subjects with status asthmaticus compared with mild-to-moderate asthmatics [35], suggesting that this chemokine may be an important factor in life-threatening asthma attacks.

It has been suggested, however, that there are significant interethnic differences in the allele frequencies for asthma-predisposition genotypes between Asian and Caucasian populations [36]. A polymorphism in the MCP-1 gene regulatory region has been associated with asthma. The MCP-1 gene also lies on chromosome 17q11.2 [37], a region for which linkage to asthma or atopy has been demonstrated in several Caucasian studies [11, 24,30]. On the contrary, our study demonstrated that the presence of the MCP-1-2518G allele is not a risk factor for near-fatal asthma in the Taiwanese population [38]. Additionally, this study found no association of the -2518A/G polymorphism in the distal gene regulatory region of MCP-1 with asthma susceptibility or severity. This variation may contribute to the pathogenesis of asthma and must be considered in gene-association studies in different ethnic populations.

The chemoattractant receptor-homologous molecule expressed on T helper 2 cells (CRTH2) gene, located within the peak linkage region for asthma on chromosome 11q, encodes a G protein-coupled chemoattractant receptor selectively on Th2 cells, basophils and eosinophils [39-41], and is a novel receptor for prostaglandin, an important mediator in the regulation of inflammatory responses. In addition, CRTH2 has been implicated in the regulation of allergic inflammation [42]. Recently, we also identified a SNPs of the CRTH2 gene by sequencing analyses of genomic DNA samples from a Chinese population [43]. Two SNPs (SNP#1 and #2) with a G to C and an A to G substitution at position 1544 and 1651, respectively, were identified in the 3'-region.

Furthermore, our study suggested significant association of CRTH2 1544G-1561G haplotype with asthma under population-based case-control analyses which were conducted in Chinese and Caucasian populations (Table 2). Moreover, in the Chinese children the frequency of the 1651G allele in near-fatal asthmatics was significantly higher than in mild-to-moderate asthmatics (p=0.0001) and normal controls (p<0.0001). The 1651G allele of SNP re545659 was also associated with a higher degree of bronchial hyperresponsiveness (p<0.027). Moreover, transcriptional pulsing experiments showed that the 1544G-1651G haplotype confers a significantly higher level of reporter mRNA stability when compared with a non-transmitted haplotype,

Table 2. Association analysis of chemoattractant receptor-homologous molecule expressed on T helper 2 cells alleles in the Chinese case-control data[a]

SNP	Allele/genotype	Cases n (%)	Controls n (%)	OR (95% CI)	*p*
1544	G	245 (90.7)	115 (96.6)		
	C	25 (9.3)	4 (3.4)	0.340 (0.116-1.002)	0.043
	GG	113 (83.7)	55 (93.2)		
	GC	19 (14.1)	4 (6.8)		
	CC	3 (2.2)	0	0.374 (0.123-1.137)	0.074
1651	G	214 (79.3)	58 (50.9)		
	A	56 (20.7)	56 (49.1)	3.690 (2.305-5.906)	<0.001
	GG	88 (65.2)	18 (31.6)		
	GA	38 (28.1)	22 (38.6)		
	AA	9 (6.7)	17 (29.8)	4.057 (2.094-7.859)	<0.001
	Haplotype	Cases (%)	Controls (%)	*p*	
1544-1651	G-A	12.5	45.6	<0.001	
	C-A	8.7	3.5	0.076	
	G-G	78.4	50.9	<0.001	
	C-G	0.4	0	1.000	
Omnibus LR test				<0.001	

Abbreviations: SNP = single nucleotide polymorphism; OR = odds ratio; CI = confidence interval; LR = likelihood ratio
[a]Modified from Huang et al [44].

suggesting that the CRTH2 gene is a strong candidate gene for asthma [44].

It is noteworthy that the field of asthma genetics has evolved in a relatively short time into a research field. If successful, the genetic approach is likely to provide a new level of understanding on the pathophysiology of the disease. It is, however, clear that there is no single major genetic risk factor for the development of asthma, and the development of the disease in an individual will depend on the interaction of several genes of moderate effect with environmental factors. Identification of specific genetic polymorphisms that influence asthma and asthma-associated phenotypes will shed light on the molecular pathways involved in these complex disorders and provide a better understanding of the pathophysiology of asthma.

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