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Discovery of genetic difference between asthmatic children with high IgE level and normal IgE level by whole genome linkage disequilibrium mapping using 763 autosomal STR markers

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Abstract The genome-wide linkage disequilibrium screen for loci associated with genetic difference between allergic and nonallergic asthma was conducted with 763 autosomal STR markers and included 190 asthmatic children. Evidence for association with differences between the two forms of asthma was observed for 36 STR markers. Marker-to-marker synergetic effect and by simulation resampling tests revealed D5S2011, D6S305, and D9S286 were important loci in allergic asthma while D6S1574, D8S1769, and D19S226 were important in nonallergic asthma. Our results show strong genetic evidence that these markers play an important role in defining allergic and nonallergic asthma and provides important candidates of susceptible genes in these two categories of asthma. This study further shows that asthma is, indeed, a heterogeneous group of underlying diseases and, although with similar clinical phenotypes, may have different clinical severities, outcomes, and need more tailor-made management.

Keywords Allergic asthma · Nonallergic asthma · STR markers · Linkage disequilibrium mapping · Synergetic effect

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Introduction

Asthma is a chronic airway inflammatory disease, affects nearly 155 million individuals worldwide, and is a multifactorial complex disease and phenotypically heterogeneous (Cookson 1999). Children who suffered from asthmatic diseases are more prone to have sensitized allergens detected by skin prick tests and/or by total and allergen-specific IgE antibodies in the serum, hence the extrinsic or allergic asthma. The timing and degree of allergen sensitization and total serum IgE levels are also the major determinants for the clinical severity of allergic asthma. Nonallergic asthma, or intrinsic asthma, another group of asthmatic patients, is not demonstrably allergic to common allergens by skin prick test. Serum total IgE concentrations are usually within normal ranges. These patients are usually older than their allergic counterparts and have onset of symptoms in later life, often with a more severe clinical course. There is a predominance in the female gender, and association of nasal polyps and aspirin sensitivity occurs more frequently in the nonallergic asthmatic (Rackeman 1947; Bentley et al. 1992; Humbert et al. 1996a, 1999). From these distinctive clinical manifestations, although the pathological findings are similar, nonallergic asthma is viewed as a different entity to allergic asthma.

In contrast, some authors have argued that intrinsic asthmatics are allergic to an as yet undetected allergen and suggest a role for IgE and atopy in nonallergic asthma and they could, therefore, benefit from allergen avoidance, as previously demonstrated in atopic asthmatics (Dorward et al. 1988; Burrows et al. 1989). Analysis of bronchial mucosal expression of “pro-eosinophilic” and “pro-atopic” markers, i.e., IL-3, IL-4, IL-5, IL-13, GM-CSF, RANTES, MCP-3, IgE, and FcεRI, demonstrates that there are more similarities than differences in immunopathology between allergic

biopsies from nonallergic asthma patients compared with those from allergic asthmatics. This could represent macrophage dysfunction or simply be a reflection of age

Table 1 Summary and test results of the allele and/or genotype frequency of 35 STR markers, which were significantly different between IgE high (H) and IgE normal (N) asthmatics

Locus	Allele no.	<i>p</i> value*	Associated allele test		Meaningful genotype test	
			Allele-bp (IgE-H/IgE-N)	OR (95% CI)	Genotype (IgE-H/IgE-N)	OR (95% CI)
D1S450	14	0.042	Not found	Not found	Not found	Not found
D2S323	8	0.004	D-194 (117/117)	0.57 (0.37–0.87)	DD (29/41)	0.41 (0.22–0.75)
D2S2241	15	0.022	Not found	Not found	Not found	Not found
D3S1569	14	0.048	G-169 (55/57)	0.70 (0.45–1.10)	GG + GX (46/51)	0.52 (0.29–0.93)
D4S1592	11	0.043	H-137 (16/25)	0.48 (0.25–0.93)	Not found	Not found
D4S2994	13	0.025	A-96 (148/108)	Not found	Not found	Not found
D4S2971	11	0.033	Not found	Not found	Not found	Not found
D5S2011	12	0.003	E-143 (67/28)	2.38 (1.44–3.91)	EE + EX (56/24)	2.90 (1.58–5.34)
			J-153 (7/18)	0.29 (0.12–0.71)	JJ + JX (7/17)	0.29 (0.11–0.73)
D5S674	8	0.041	C-273 (19/32)	0.42 (0.23–0.79)	CC + CX (18/29)	0.40 (0.20–0.79)
D5S400	14	0.042	Not found	Not found	Not found	Not found
D6S434	12	0.025	E-213 (77/44)	1.66 (1.06–2.58)	Not found	Not found
D6S305	12	0.044	G-224 (93/53)	1.75 (1.15–2.68)	GG (19/6)	2.91 (1.11–7.65)
D6S462	6	0.015	Not found	Not found	Not found	Not found
D6S1573	12	0.002	C-287 (59/26)	2.16 (1.29–3.62)	CC + CX (50/25)	2.18 (1.19–3.99)
D6S1574	8	0.002	A-154 (72/85)	0.52 (0.37–0.79)	AA (10/22)	0.30 (0.13–0.68)
			C-158 (62/31)	1.88 (1.15–3.06)	CC + CX (50/27)	1.95 (1.08–3.54)
D8S520	11	0.035	Not found	Not found	Not found	Not found
D8S1827	6	0.046	Not found	Not found	Not found	Not found
D8S1769	10	0.030	C-249 (111/116)	0.52 (0.34–0.80)	CC (27/43)	0.34 (0.18–0.62)
			D-251 (34/14)	2.15 (1.11–4.16)	DD + DX (31/14)	2.12 (1.04–4.32)
D8S272	11	0.010	F-248 (56/62)	0.63 (0.41–0.98)	FF + FX (49/52)	0.56 (0.31–0.99)
			H-252 (28/11)	2.22 (1.07–4.61)	HH + HX (26/11)	2.21 (1.02–4.80)
D8S1743	7	0.043	Not found	Not found	Not found	Not found
D9S1677	16	0.010	H-240 (39/47)	0.60 (0.37–0.97)	Not found	Not found
D9S1843	11	0.047	Not found	Not found	Not found	Not found
D9S286	15	0.049	J-159 (69/36)	1.82 (1.14–2.91)	JJ + JX (61/28)	2.82 (1.56–5.12)
D10S591	7	0.002	C-332 (17/31)	0.39 (0.21–0.74)	CC + CX (16/28)	0.37 (0.18–0.74)
			E-336 (143/96)	1.65 (1.08–2.50)	EE (48/25)	2.02 (1.10–3.70)
D10S185	11	0.037	Not found	Not found	Not found	Not found
D10S212	8	0.035	Not found	Not found	Not found	Not found
D11S935	9	0.037	C-202 (36/44)	0.59 (0.36–0.97)	CC + CX (33/40)	0.52 (0.29–0.93)
D11S4175	19	0.035	Not found	Not found	Not found	Not found
D12S1613	7	0.034	Not found	Not found	Not found	Not found
D13S219	8	0.015	C-123 (62/34)	1.68 (1.04–2.70)	Not found	Not found
			G-131 (26/42)	0.43 (0.25–0.74)	GG + GX (26/37)	0.43 (0.23–0.79)
D15S118	8	0.014	D-230 (60/34)	1.60 (0.99–2.59)	DD + DX (51/27)	2.06 (1.12–3.68)
			F-234 (16/31)	0.37 (0.19–0.70)	FF + FX (16/29)	0.35 (0.17–0.70)
D16S3057	9	0.013	G-209 (45/52)	0.62 (0.39–0.98)	GG (4/11)	0.27 (0.08–0.87)
D18S468	8	0.036	Not found	Not found	Not found	Not found
D19S226	14	0.007	D-240 (37/15)	2.21 (1.17–4.18)	DD + DX (32/14)	2.22 (1.10–4.51)
			G-246 (20/33)	0.44 (0.24–0.79)	GG + GX (20/30)	0.43 (0.22–0.83)
D21S1252	12	0.013	G-162 (68/73)	0.64 (0.42–0.97)	GG + GX (55/59)	0.48 (0.27–0.88)

* Monte-Carlo estimates of exact *p* values

and length of disease rather than a basic difference in the pathological mechanism between these two forms of asthma (Humbert et al. 1999). But still, these findings cannot fully explain why nonallergic asthma, without elevation of total serum IgE, has a similar pattern of allergen-induced bronchial inflammation, either in the underlined etiology or in the disease development of this form of asthma.

Recently, Holgate and Davies (2001) postulated that epithelial damage and allergy-prone T_H2 cytokines can act in concert to cause a functional disturbance of the epithelial-mesenchymal trophic unit (EMTU), which leads to myofibroblast activation and induction of inflammatory and remodeling responses characteristic of chronic asthma (Holgate and Davies 2001). This model

differs from the traditional sequential view of asthma pathogenesis by setting the structural abnormalities in parallel with T_H2 cytokine-induced inflammation. Because allergic diseases, including asthma, rhinitis, and eczema, are caused by both environmental and genetic factors, the expression of allergic asthma is dependent on inheritance of local susceptibility genes, which have the potential to interact with the atopy predisposition, whereas nonallergic asthma may have a different set of responsible genes to the environmental stimuli that lead to EMTU damage and chronic airway inflammation in the absence of IgE elevation (Davies et al. 2003).

Based on this hypothesis, the aim of the present study was to investigate whether there is a different set(s) of susceptible genes between allergic and nonallergic

Table 2 Result of logistic regression (data adapted from SAS output) and discrimination equation^a

Parameter	Estimate	Standard error	Wald chi-square	<i>p</i> value	Odds ratio	95% Wald confidence limits	
Intercept	1.1918	0.4934	5.8337	0.0157			
D2S323-DD	-1.5489	0.4369	12.5703	0.0004	0.212	0.090	0.500
D5S2011-JJ + Jx	-1.9221	0.6604	8.4701	0.0036	0.146	0.040	0.534
D6S305-GG	2.1439	0.7196	8.8762	0.0029	8.532	2.082	34.961
D6S1573-CC + Cx	1.4265	0.4402	10.4993	0.0012	4.164	1.757	9.868
D6S1574-AA	-1.2502	0.5696	4.8182	0.0282	0.286	0.094	0.875
D8S1769-CC	-1.3587	0.4303	9.9715	0.0016	0.257	0.111	0.597
D9S286-JJ + Jx	1.6917	0.4299	15.4831	< 0.0001	5.429	2.337	12.609
D10S591-CC + Cx	-1.8324	0.5105	12.8851	0.0003	0.160	0.059	0.435
D15S118-DD + Dx	1.0592	0.4276	6.1361	0.0132	2.884	1.247	6.668
D19S226-GG + Gx	-1.3222	0.4791	7.6165	0.0058	0.267	0.104	0.682
D21S1252-GG + Gx	-1.0445	0.4201	6.1823	0.0129	0.352	0.154	0.802

^a Discrimination equation: $\text{logit } p = 1.1918 \text{ (intercept estimate)} + \sum \text{estimate} \times [1(\text{meaningful genotype existed}) \text{ or } 0 \text{ (meaningful genotype did not exist)}]$. $p = e^{\text{logit } p} / (e^{\text{logit } p} + 1)$

asthma in children based on total serum IgE levels and responsiveness to common aeroallergen. We conducted a genome scan using 763 autosomal STR markers to search the loci associated with these two types of asthma in Taiwanese, non-obviously related, children selected by narrow phenotype definitions. Our data revealed evidence of genetic differences between allergic and nonallergic asthma. The results implied chronic asthma initiated from allergy and/or nonallergy factors was determined by different susceptibility genes.

Materials and methods

Clinical sample collection

Our study population consisted of asthmatic children aged between 1 and 12 years old, and the Ethical and Clinical Trial Committee of the National Cheng-Kung University Hospital approved this study protocol. All participants or their guardian, after being well informed of the study protocol and signing the consent forms, answered a modified British Medical Society respiratory questionnaire, which is exactly the same as the European Community Respiratory Health Survey (ECHRS), which has similar validity as the ISAAC (Burney et al. 1994; Pearce et al. 2000), as well as additional questions pertinent to the diagnosis and assessment of asthma. Pulmonary function was tested using standard methods, which included spirometry before and after the administration of two puffs of inhaled salbutamol (200 µg/puff). The definition of asthma included: (1) History of wheezing and shortness of breath during or without concurrent respiratory infections, (2) chronic cough for more than 1 month and being diagnosed by a physician of the presence of wheezing episode(s), (3) bronchodilator test that confirmed the positive response of an increase of 15% in forced expiratory volume in 1 s (FEV1). Other evaluations included skin prick tests for responsiveness to six common aeroallergens, a differential blood count (including total

eosinophil count), and measures of total serum IgE, as well as IgE specific to house dust and mixed pollens using the Unicap system (Pharmacia Diagnostic, Sweden). A positive skin test was defined as the presence of ≥1 reaction with a wheal diameter ≥5 mm. Total serum IgE was measured by solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics). The confirmed asthmatic children were further divided by their serum IgE levels: the allergic group (105 subjects) had high IgE serum concentrations (≥1,000 IU/ml) and sensitized to at least one aeroallergen; in the nonallergic group (85 subjects), IgE serum levels were within normal range (≤ 200 IU/ml), and there was no detectable allergen sensitization.

DNA preparation

Genomic DNAs were extracted from 190 unrelated Taiwanese children. DNA was isolated from blood sample using QIAamp DNA blood kit according to the manufacture's instructions. The isolated genomic DNA was quality-checked by agarose gel electrophoresis analysis, the quantity was determined spectrophotometrically, and it was stored at -80°C.

Microsatellite genotyping

Genotyping was performed using the ABI PRISM Linkage Mapping Sets HD-5 (811 markers) to provide coverage of human genome at 5 cM average resolution. Each marker set included a fluorescence-labeled forward primer and a tailing reverse primer. The PCR amplifications were carried out according to the manufacturer's instructions. The PCR products were separated on ABI 3700 DNA analyzers. The use of GeneScan 500 LIZ as the internal size standard assisted polymorphic fragment-length calling and allowed more accurate allele calling and unambiguous comparison of data across experimental conditions.

Table 3 Contingency table testing results^a. Marker orders were dependent on their chromosome location and are rearranged here for effective visualization. The matrix is partitioned into two triangles indicated by bold type^b and one rectangle indicated by normal type

	D3S1569	D4S1592	D5S674	D6S1574	D8S1769	D9S1677	D11S935	D13S219	D15S118	D16S3057	D19S226	D21S1252	D5S2011	D6S434	D6S305	D6S1573	D8S272	D9S286	D10S591
D2S323	0.001	0.003	0.001	<0.001	<0.001	0.006	<0.001	0.001	<0.001	0.001	<0.001	<0.001	0.882	0.680	0.379	0.962	0.313	0.820	0.647
D3S1569		0.020	0.004	<0.001	<0.001	0.003	0.007	0.002	0.001	0.005	0.002	0.005	0.646	0.003	0.181	0.358	0.593	0.297	0.644
D4S1592			0.005	<0.001	<0.001	0.016	0.021	0.003	<0.001	0.008	0.001	0.005	0.264	0.425	0.667	0.661	0.535	0.077	0.333
D5S674				<0.001	<0.001	0.008	0.005	0.001	<0.001	0.001	0.002	0.002	0.329	0.583	0.450	1.000	0.202	0.529	1.000
D6S1574				<0.001	<0.001	<0.001	0.002	0.001	<0.001	0.001	<0.001	0.001	0.013	0.137	0.314	0.014	0.832	0.157	0.914
D8S1769					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.520	0.445	0.262	0.608	0.279	0.800	0.520
D9S1677					0.018	0.006	0.002	0.007	0.002	0.031	0.007	0.020	0.390	0.870	0.832	0.258	0.291	0.500	0.867
D11S935						0.003	0.001	0.002	0.001	0.002	0.006	0.004	0.623	0.944	0.839	0.199	0.006	0.659	0.783
D13S219							<0.001	0.001	<0.001	0.001	0.001	0.001	0.407	0.409	0.498	0.069	0.265	0.774	0.458
D15S118								0.001	<0.001	0.001	<0.001	0.001	0.543	0.796	0.538	0.470	0.658	0.114	0.564
D16S3057								0.001	0.007	<0.001	0.001	0.007	0.051	0.067	0.462	0.095	0.163	0.050	0.317
D19S226									0.003	0.003	0.003	0.003	0.063	0.719	0.553	0.721	0.455	0.505	0.932
D21S1252													0.355	0.923	0.112	0.682	0.224	0.739	0.311
D5S2011													0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D6S434														0.038	0.038	0.006	0.033	0.001	0.020
D6S305																0.001	0.018	<0.001	0.005
D6S1573																	0.006	<0.001	0.002
D8S272																		<0.001	0.001
D9S286																			<0.001

^a Fisher's exact test was performed to 2 (IgE high and IgE normal) $\times$ 3 (both risk genotypes, one of the two risk genotypes, and neither risk genotype) contingency tables, which were generated by every two markers
^b Bold type also indicates *p* values less than 0.05

Table 4 Odds ratios of combined and single markers^a. NA not analyzed due to at least one cell in 2 $\times$ 2 contingency table being equal to zero

	D3S1569	D4S1592	D5S674	D6S1574	D8S1769	D9S1677	D11S935	D13S219	D15S118	D16S3057	D19S226	D21S1252	OR of single marker
D2S323	0.178	0.250	0.191	0.142	0.165	0.257	0.280	0.231	0.191	0.178	0.104	0.176	0.412
D3S1569		0.271	0.231	0.043	0.198	0.365	0.263	0.218	0.193	0.089	0.220	0.232	0.522
D4S1592			0.240	NA	0.185	0.197	0.303	0.316	NA	NA	0.529	0.169	0.517
D5S674				0.062	0.128	0.273	0.316	0.124	0.049	NA	0.163	0.212	0.402
D6S1574					0.088	0.047	0.295	0.200	0.167	NA	0.200	0.160	0.303
D8S1769						0.234	0.132	0.131	0.159	NA	0.163	0.135	0.340
D9S1677							0.278	0.263	0.253	NA	0.195	0.314	0.589
D11S935								0.226	0.111	0.264	0.241	0.255	0.517
D13S219									0.172	0.178	0.148	0.169	0.429
D15S118										0.276	0.103	0.163	0.349
D16S3057											NA	0.167	0.268
D19S226												0.209	0.433
D21S1252													0.487

	D6S434	D6S305	D8S272	D9S286	D10S591	OR of single marker
D5S2011	5.234	3.185	10.065	7.955	5.758	2.888
D6S434		3.496	2.668	4.007	3.295	1.659
D6S305			7.130	13.448	12.145	2.894
D6S1573			5.564	6.430	5.036	2.173
D8S272				20.824	2.132	2.205
D9S286					5.320	2.806
D10S591						2.014

^a Bold indicates synergistic odds ratio smaller than 0.14 or larger than 7.1

Table 5 The results of odds ratio after being simulated 10,000 times^a. *NA* not analyzed due to at least one cell in 2×2 contingency table being equal to zero

	D3S1569	D4S1592	D5S674	D6S1574	D8S1769	D9S1677	D11S935	D13S219	D15S118	D16S3057	D19S226	D21S1252
D2S323	0.171	0.233	0.180	0.128	0.159	0.248	0.273	0.224	0.180	0.166	0.094	0.170
D3S1569		0.261	0.223	0.054	0.192	0.356	0.255	0.211	0.185	0.110	0.211	0.223
D4S1592			0.217	NA	0.169	0.179	0.287	0.295	NA	NA	0.507	0.157
D5S674				0.078	0.117	0.260	0.307	0.112	0.061	NA	0.150	0.205
D6S1574					0.083	0.059	0.278	0.183	0.158	NA	0.187	0.151
D8S1769						0.226	0.125	0.124	0.147	NA	0.154	0.130
D9S1677							0.269	0.254	0.238	0.166	0.181	0.306
D11S935								0.219	0.101	0.333	0.231	0.249
D13S219									0.157	0.222	0.136	0.162
D15S118										0.345	0.097	0.156
D16S3057											NA	0.150
D19S226												0.202
	D6S434	D6S305	D6S1573	D8S272	D9S286	D10S591						
D5S2011	5.127	2.998	6.957	9.774	7.772	5.624						
D6S434		3.401	4.435	2.606	3.939	3.235						
D6S305			NA	6.803	12.850	11.733						
D6S1573				5.423	6.270	4.912						
D8S272					20.368	2.008						
D9S286						5.197						

^a Bold indicates synergetic odds ratio smaller than 0.14 or larger than 7.1

Genotypes were initially scored using Genescan and Genotyper (ABI) software and were then verified independently by three individuals without prior knowledge of phenotype.

Statistical analysis

To evaluate STR markers associated with the difference between IgE-high and IgE-normal asthmatics, the STR markers with significantly different allele frequencies in two asthma subtypes were sought by χ^2 test, in which the significant level was estimated by Monte-Carlo simulation (Sham and Curtis 1995). Alleles in significant marker loci were tested for linkage disequilibrium with null causative or signature polymorphism by analysis with the contingency table. The significant alleles were tested by odds ratio as risk factors. Genotype contingency tables were constructed according to specific alleles with significant *p* values and odds ratio. The meaningful genotypes were defined by significant χ^2 and odds ratio. Information on the genotype of all significant markers was collected, and the data set was transformed into a binary category, with the meaningful genotype and the others represented by “1” and “0,” respectively. In other words, a genotype showing association (meaningful genotype) was coded 1, and a genotypes showing no association was coded 0 in the binary data set. Discrimination equation was performed by logistic regression using the transformed binary data set.

For elucidating marker–marker synergetic effect to asthma subtype, the binary information of each of the two markers was combined as a single factor. Three categories were generated by combining two markers: both were risk genotypes, one of the two was a risk genotype, and neither was a risk genotype. The associ-

ation between marker-to-marker synergetic effect and IgE status was tested by 2×3 contingency table using Fisher’s exact test. Odds ratios of two-marker synergetic effect were generated using both and neither risk genotypes. If the two genes were synergetic, the odds ratio was significantly higher than only one each of the gene’s odds ratio. It was shown that interaction between two genes caused multiplicative or additive risks (Szklo and Nieto 2000). According the Slutsky’s theorem, the distribution of odds ratio via natural logarithm transformed was converged to normal distribution (Agresti 2002). In our data, the standard deviation was less than 1. The critical values of 95% of standard normal distribution with mean 0 and standard deviation 1 are +1.96 and −1.96. The values 7.1 and 0.14, the natural exponential of +1.96 and −1.96, were significant thresholds of defining multiplicative risks. All statistical analyses were performed by SAS software (SAS 8.0, SAS Inc.)

Results

STR markers associated with significant difference between allergy and nonallergy asthma

Briefly described the Fig. 1 is the result of the whole autosomal genome region screened for loci associated with differences between allergic and nonallergic asthmatics. Normal and high IgE may be consistent with allergic and nonallergic asthma, as defined in the **Material and methods** section. Thirty-six STR markers were sought by allele and/or genotype frequency with significantly different IgE high and IgE normal groups (Table 1). The alleles of each locus were tested to odds ratio and 2×2 χ^2 (data not shown) with other alleles of

Table 6 List of candidate genes and related articles

Locus	Gene		Chromosome location	Reference
D1S450	PIK3CD	Phosphoinositide-3-kinase, catalytic, delta polypeptide	1q36.22	Condliffe et al. (2000)
D2S323	MGC3279	Hypothetical protein MGC3279 similar to collectins	2p25.3	Cheng et al. (2000)
D4S2994	HMX1	Homeo box (H6 family) 1	4p15.31	Stelinicki et al. (1999)
D4S2971	PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide	4q11	Chanez et al. (1995)
D5S674	ADAMTS12	A disintegrin-like and metalloprotease (repolysin type) with thrombospondin type 1 motif, 12	5p13.2	Santiago et al. (2001)
D5S2011	CD14	CD14 antigen	5q31	Baldini et al. (2002)
	FGF1	Fibroblast growth factor 1 (acidic)	5q31	Pendurthi et al. (1997)
D6S1574	LY86	Lymphocyte antigen 86	6p24.3	Miyake (2003)
D6S1573	IL17	Interleukin 17	6p12.1	Toda et al. (2003)
	IL17F	Interleukin 17F	6p12.1	Hurst et al. (2002)
D8S520	MSRA	Methionine sulfoxide reductase A	8p23	Mohri et al. (2002)
	CTSB	Cathepsin B	8p23	Layton et al. (2001)
D8S1769	GSR	Glutathione reductase	8p12	Smith et al. (1997)
D9S286	RLN2	Relaxin 2 (H2)	9p24	Bani (1997)
D9S1843	GNA14	Guanine nucleotide binding protein (G protein), alpha 14	9q21.31	Hotta et al. (1999)
	GNAQ	Guanine nucleotide binding protein (G protein), q polypeptide	9q21.31	Hotta et al. (1999)
D9S1677	TXN	Thioredoxin	9q32	Henderson et al. (2002)
D10S591	IL15RA	Interleukin 15 receptor, alpha	10p15.1	Wei et al. (2001)
	IL2RA	Interleukin 2 receptor, alpha	10p15.1	Brown et al. (1995)
D10S212	INPP5A	Inositol polyphosphate-5-phosphatase, 40kDa	10q26.3	Tao et al. (2000)
D11S935	CAT	Catalase	11p13	Tekin et al. (2000)
	ELF5	E74-like factor 5 (ets domain transcription factor)	11p13	Silverman et al. (2002)
	EHF	Ets homologous factor	11p13	Silverman et al. (2002)
	CD44	CD44 antigen	11p13	Sano et al. (1997)
	RAG1	Recombination activating gene 1	11p12	Messier et al. (2003)
	RAG2	Recombination activating gene 2	11p12	Messier et al. (2003)
D12S1613	SELPLG	Selectin P ligand	12q24.12	Wardlaw (2001)
D13S219	RFXAP	Regulatory factor X-associated protein	13q13.1	Dziembowska et al. (2002)
D15S118	CHRM5	Cholinergic receptor, muscarinic 5	15q13.1	Fryer and Jacoby (1998)
D16S3057	CCL22	Chemokine (C-C motif) ligand 22	16q12.1	Teran (2000)
	CX3CL1	Chemokine (C-X3-C motif) ligand 1	16q12.1	Teran (2000)
	CCL17	Chemokine (C-C motif) ligand 17	16q12.1	Teran (2000)
D18S468	PIK3C3	Phosphoinositide-3-kinase, class 3	18q12.3	Curnock and Ward (2003)
D19S266	CD97	CD97 antigen	19p13	Lin et al. (2000)
	EMR3	Egf-like module containing mucin-like receptor 3	19p13	Stacey et al. (2001)
	EMR2	Egf-like module containing mucin-like hormone receptor-like sequence 2	19p13	Stacey et al. (2001)

the same locus. In Table 1, the “Associated allele test” lists the alleles, which were statistically significant in the test. Eight loci—for example, D5S2011—have more than one associated allele. The genotypes of each associated allele were classified as homozygous and heterozygous then tested with odds ratio and 2×2 contingency table analysis (data not shown) with other genotypes of the same locus. The test results are shown in “Meaningful genotype test” in Table 1. Eighteen meaningful genotypes are shown in the dominant model (i.e., D5S2011) as having the same mean; the meaningful genotypes being homozygous and heterozygous was a risk factor for the two types of asthma compared with each other. Six meaningful genotypes were shown in the recessive model (i.e., D2S323), that is, the meaningful genotypes being homozygous was a risk factor for the

two types of asthma compared with each other. All odd ratios are either larger than or smaller than one.

Discrimination for two forms of asthma by genotype data

Twenty-one of 36 significant STR markers were found on at least one meaningful genotype. Transformed binary data set contained information on 21 markers reduced by data transformation. Discrimination equation using the transformed binary data set was performed by logistic regression (Table 2). The combination equation of 11 markers that was generated by logistic regression without pro-selection can divide patients into two groups: high IgE ($p > 0.5$) and normal IgE ($p < 0.5$). The

cut-off point of 0.5 was chosen, which showed 0.84 specificity and 0.23 sensitivity by receiver operating characteristic (ROC) curve analysis. Misclassification rate was 0.189. The result implied that two type of asthma have a different genetic polymorphism background. The misclassification rate may be due to some asthmatics belonging to mixed type, or to other unrevealed genetic factors.

Marker to marker synergetic effect

To find the marker-to-marker synergetic effect in two forms of asthma, the p values of Fisher's exact test for every marker-marker genotype combination associated with IgE status was used. Results are reported in Table 3. A p value less than 0.05 is presented in bold. The marker orders were dependent on their chromosome location and are rearranged here for effective visualization. The matrix is partitioned into two triangles indicated by bold type and one rectangle indicated by normal type. To make sure whether there was a correlation between every two markers, the odds ratio of marker-to-marker genotype combinations was applied. The odds ratios of combined and single markers are listed in Table 4. Simulation using the resampling method was performed to evaluate the odds ratio generated by chance or not. The real correlation between two markers was maintained after resampling. On the other hand, the real correlation did not disappear due to different subgroups of total samples. For resampling, 152 subjects were randomly chosen from the 190 subjects and repeated 10,000 times. Table 5 displays the results of odds ratios after simulation. Markers D5S674, D6S1574, and D19S226 in the upper-left triangle combined with more than two markers had an increased odds ratio ($OR < 0.14$) compared with those with a single marker. In the lower-right triangle, markers D5S2011, D6S1573, and D9S286 combined with more than one other marker and had a significantly decreased odds ratio ($OR > 7.1$) compared with those with a single marker.

Discussion

According our investigation, 36 STR markers were significantly different between allergic and nonallergic asthma. Twenty-one of these 36 STR markers were found to have at least one meaningful genotype. Using an 11-marker combination equation generated by logistic regression and divided by cutoff points of $p = 0.5$, we clearly show that these two forms of asthma have different genetic polymorphism backgrounds. Marker-to-marker synergetic effect and simulation resampling tests revealed D5S2011, D6D1573, and D9S286 were important loci in allergic asthma while D5S674, D6S1574, and D19S226 were important in nonallergic

asthma, with odds ratio < 0.14 and > 7.1 , respectively. The results implied several genes, not just one major gene, contribute asthmatic genotype and phenotype in our studied subjects.

The STR markers, as multiallelic markers, always have more power to detect LD than do diallelic markers (such as SNPs) under otherwise equivalent conditions (Ott and Rabinowitz 1997; Chapman and Wijsman 1998). Power equivalent to that achieved by a multiallelic screen can theoretically be achieved by use of a denser diallelic screen, but mapping panels of the necessary resolution are not currently available and may be difficult to achieve. Theoretically, existing sets of microsatellite data, if sufficiently dense, can make initial predictions about the level of short-range LD present in susceptibility regions identified by linkage studies. These predictions can be useful for planning the density of markers needed for fine-mapping experiments (Schulze et al. 2002).

When analysis is restricted to members of reasonably well-defined ethnic or political groups, evidence of LD can frequently be found, with markers spanning the disease loci at 0.5–1.4 cM intervals (Wijsman 1997). Examples are found in Japanese (Goddard et al. 1996), Italians (Pandolfo et al. 1990), British (Votruba et al. 1998), and Poles (Brzustowicz et al. 1993). According to the above literature, candidate genes mentioned by previous references were selected, surrounding significant markers within 1 Mb physical distance. They are listed in Table 6. Several markers had no known candidate genes nearby. Candidate genes were being divided into some functionally related categories as response to environmental factors (such as CD14), inflammation (cytokines, cytokine receptors, chemokines), remodeling (FGF1, ADAMTS12, PDGFRA and epidermal growth factor), and others (transcription related genes, kinase). From another view, those candidate genes may imply or indicate that there is some biological pathway or mechanism related to asthma development, such as IL2RA, IL15RA, RAG1, and RAG2 in cytokine and signaling pathways related to immunodeficiencies (Schluns and Lefrancois 2003; Candotti et al. 2002); CD14 and Ly86 in innate recognition of lipopolysaccharides (Miyake 2003); and CCL17 and CCL12 correlated with the recruitment of specific leukocyte subsets expressing the receptors for those chemokines in asthma and other pulmonary diseases (D'Ambrosio et al. 2003).

Our findings have clearly showed that, even in a very homogenous population, the susceptible genes for the concentration of serum IgE and responsive to environmental aeroallergens were quite different in asthmatic children. Although the difference of clinical manifestations between these two forms of asthma are not so prominent as in adult asthmatics, nonallergic asthmatic children tend to have a higher frequency of asthmatic attacks with more adherence to controlled medications and are also more prone to have airway remodeling than those with allergic asthma (Romanet-Manent et al. 2002; Inouye et al. 1985). Our results have demonstrated that

these two forms of asthma are governed by two different sets of susceptible genes that lead to common end results of eosinophilic bronchial inflammation found in all asthmatic patients. It is also strongly suggested that, in fact, allergic and nonallergic asthma are two different clinical entities that demand a different consideration and management in the prevention and treatment of childhood asthma.

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