

Original article

Asthma and atopy are associated with chromosome 17q21 markers in Chinese children

Background: Single-nucleotide polymorphism (SNP)-based genome-wide association study revealed that markers on chromosome 17q21 were linked to childhood asthma but not atopy in Caucasians, with the strongest signal being detected for the SNP rs7216389 in the *ORMDL3* gene. Such association was unknown in Chinese. This study delineated the allele and genotype frequencies of 10 SNPs at chromosome 17q21, and investigated the relationship between these SNPs and asthma and plasma IgE in southern Chinese children.

Methods: Asthmatic children and non-allergic controls were recruited from pediatric clinics. Their plasma total and aeroallergen-specific IgE concentrations were measured by immunoassay. Ten SNPs on 17q21 region were genotyped by multiplex SNaPshot™, and their genotype associations with asthma traits analyzed using multivariate regression.

Results: 315 patients and 192 controls were enrolled. The allele frequency for C allele of rs7216389 varied significantly from 0.232 in our controls, 0.389 in Han Chinese to 0.536 in Caucasians. Asthma diagnosis was associated with rs11650680 and five other SNPs including rs7216389 ($P = 0.019$ – 0.034), whereas atopy was associated only with rs11650680 ($P = 0.0004$). Linear regression revealed the covariates for plasma total IgE to be significant for rs11650680 ($P = 0.008$ – 0.0002). Haplotypic associations were found with atopy and increased plasma total IgE, with the respective odds ratios and 95% confidence intervals for TTTCCGTT haplotype to be 0.21 and 0.09–0.52 ($P = 0.0002$) and 0.41 and 0.18–0.90 ($P = 0.025$).

Conclusion: Childhood asthma and atopy are associated with chromosome 17q21 in Chinese, but such association may involve genes other than *ORMDL3* in this region.

T. F. Leung^{1*}, H. Y. Sy^{1*}, M. C. Y. Ng²,
I. H. S. Chan³, G. W. K. Wong¹,
N. L. S. Tang³, M. M. Y. Waye^{4,5},
C. W. K. Lam³

Departments of ¹Paediatrics, ²Medicine and Therapeutics, ³Chemical Pathology and ⁴Biochemistry, ⁵Croucher Laboratory for Human Genomics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

Key words: asthma; children; Chinese; genome-wide association; single-nucleotide polymorphism.

Dr Ting Fan Leung
Department of Paediatrics
6/F, Clinical Sciences Building
Prince of Wales Hospital, Shatin, N.T.
Hong Kong

*Both the authors contributed equally to this article.

Accepted for publication 11 July 2008

Asthma is one of the most common childhood diseases in developed countries. About one-tenth of Hong Kong schoolchildren suffer from asthma (1). Most patients have enhanced production of immunoglobulin E (IgE) to a variety of allergens. Asthma is a complex disease involving interactions between many genetic and environmental factors (2, 3). Over the past decade, genetic linkage and association studies have identified more than 25 asthma or allergy susceptibility loci. Nonetheless, fewer than half of these associations have been successfully replicated in multiple populations. Recently, Moffatt et al. published the first genome-wide association (GWA) study for childhood asthma in a cohort of 994 European patients and 1243 controls (4). They identified highly significant associations between asthma and single-nucleotide polymorphisms (SNPs) on chromosome 17q21, and replicated these results in two independent Caucasian cohorts. In particular, the strongest signal was detected for SNP rs7216389 in the gene *ORM1*-like Protein 3 (*ORMDL3*).

Following additional experiments, they identified *ORMDL3* to be a novel candidate gene for asthma.

Subsequently, several groups tried to replicate these genetic associations. In view of the great interethnic variations of asthma burden in the United States, Galanter et al. studied the association between asthma and *ORMDL3* in three independent cohorts of Mexicans, Puerto Ricans, and African-Americans (5). They observed significant associations between two SNPs within *ORMDL3* (rs4378650 and rs12603332) and asthma in Mexicans and African-Americans. None of the eight tested SNPs was associated with baseline lung function or bronchodilator responsiveness. Another Japanese study of the SNP rs7216389 supported the importance of *ORMDL3* for asthma (6). Very recently, Tavendale et al. reported that the SNP rs7216389 in *ORMDL3* was also associated with the risks for asthma susceptibility and exacerbations among Scottish children and young adults (7). These consistent findings suggest

that *ORMDL3* or adjacent genes on chromosome 17q21 is a strong risk factor for asthma in ethnically diverse populations. Nonetheless, inconsistent results at the SNP level call for more replication studies to define the genetic associations with markers at this chromosomal region.

We hypothesized that asthma and plasma IgE are associated with polymorphic markers at chromosome 17q21 region in Chinese, but that these traits may be linked with *ORMDL3* or its adjacent gene(s). The objectives of this study were to delineate the genetic epidemiology of SNPs at chromosome 17q21 in Chinese children, and to investigate the associations between asthma traits and these SNPs in our population.

Patients and methods

Study population

Unrelated Chinese children with physician-diagnosed asthma and aged 5 to 18 years were enrolled from pediatric clinics of a university teaching hospital. The diagnosis of asthma was made according to the American Thoracic Society (ATS) guidelines (8). Non-allergic controls were recruited among children attending our hospital for minor non-respiratory complaints, and all of them did not report any allergic disease using the validated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (1). All cases and controls were free of any infectious symptom for 4 weeks before study. The Clinical Research Ethics Committee of our University approved these studies, and subjects and their parents gave written informed consent.

Plasma IgE measurements

Total IgE concentration in plasma was measured by micro-particle immunoassay (IMx analyser; Abbott Laboratories, Abbott Park, IL, USA) and specific IgE antibodies to *Dermatophagoides pteronyssinus*, cat and cockroaches were measured by fluorescent enzyme immunoassay (AutoCAP; Phadia AB, Uppsala, Sweden). Data on total IgE concentrations were compared with our local references (upper limits of normal: 120 kIU/l for 5 to 6 years; 160 kIU/l for 6 to 7 years; 180 kIU/l for ≥ 8 years) (9). Specific IgE concentration ≥ 0.35 kIU/l was considered positive, and subjects with one or more allergen-specific IgE were considered atopic.

Spirometric measurements

All asthmatics underwent spirometry (COMPACT II; Vitalograph, Buckingham, UK) to measure their forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC). Results were compared with local references (10).

Selection and genotyping of SNPs at chromosome 17q21

The published SNPs at chromosome 17q21 were reviewed from HapMap data (11), and seven tagging SNPs were selected from those with minor allele frequency (MAF) ≥ 0.05 for Han Chinese and according to $r^2 \geq 0.90$. The three SNPs with the most significant *P*-values from Moffatt et al. (4) were added to our panel, i.e. total 10 SNPs, that is the maximum number of SNPs that can be genotyped by the SNaPshot assay. Table 1 summarizes the locations and

Table 1. Details of our 10 single-nucleotide polymorphisms on chromosome 17q21

SNP	Gene	Role (12)	rs number (11)	Probe length (bp)
T+34578C	<i>ZNFN1A3</i>	Intron 2	3816470	19
T+10507C/T +3620C	<i>ORMDL3/GSDML</i>	Downstream/ Intron 1	7216389	23
T+3033C/T-3854C	<i>ORMDL3/GSDML</i>	3' UTR/Promoter	2278894	26
T-2357C/T-9262C	<i>ORMDL3/GSDML</i>	Intron 1/Promoter	17608925	40
C-3894T	<i>ORMDL3</i>	Promoter	3744246	56
C-4929A	<i>ORMDL3</i>	Promoter	4795402	35
—	—	—	6503525	38
G-10587A	<i>GSDM1</i>	Promoter	1007654	42
C+6707T/C-8576T	<i>GSDM1/PSMD3</i>	Intron 6/Promoter	3859192	46
C-5193T	<i>TOP2A</i>	Promoter	11650680	50

SNP, single-nucleotide polymorphism; UTR, untranslated region.

gene functions of these SNPs, and Fig. 1 describes their distribution with respect to different genes at the 17q21 region.

Genomic DNA was extracted from peripheral blood leukocytes. The genomic region of interest at chromosome 17q21 was amplified by multiplex polymerase chain reactions (PCRs), the details of which are summarized in Table S1. These PCR reactions were performed in a total volume of 18 μ l containing 120 ng DNA, 200 μ M dNTPs, 1X PCR Gold buffer with 2.5 mM MgCl₂, 5–10 pmol of each primer and 0.5 unit of AmpliTaq Gold DNA polymerase (Roche Molecular Systems, Branchburg, NJ, USA). Samples were denatured at 95°C for 10 min followed by 30 cycles of 95°C for 60 s, 58°C for 60 s, followed by 72°C for 75 s, and a final extension at 72°C for 10 min.

Our 10 SNPs were genotyped by SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA, USA) as previously described (13). In brief, 5 μ l of mixed PCR products from the above reactions were then incubated with 2 μ l of exonuclease I/shrimp alkaline phosphatase (ExoSAP-IT; USB, Cleveland, OH, USA) at 37°C overnight, followed by heating at 80°C for 15 min to inactivate ExoSAP-IT. Following this, 2.5 μ l of SNaPshot Multiplex kit, 1.5 μ l of ExoSAP-IT treated PCR products and 1 μ l of mixed probes (Table S2) were incubated for 25 cycles at 96°C for 10 s, 54°C for 5 s, and 60°C for 30 s. Finally, 0.6 unit of SAP (USB) was added to the reaction product and incubated at 37°C for an hour to remove the unused primers and unwanted PCR products. SAP was then denatured by heating at 75°C for 15 min. The final reaction mix containing 9.25 μ l of Hi-Di Formamide, 0.5 μ l of sample and 0.25 μ l of GeneScan-120 LIZ internal size standard (Applied Biosystems) was denatured at 95°C 5 min, and genotypes of our 10 SNPs were identified by different fluorescent signals by ABI-3130 Genetic Analyzer (Applied Biosystems). Finally, the multiplex SNaPshot results from 40 randomly selected subjects were confirmed by direct sequencing of the target chromosome 17q21 region using BigDye Terminator Cycle sequencing kit (Applied Biosystems).

Statistical analysis

Results were expressed as proportions or mean and standard deviation (SD). Patient characteristics were compared using the Student *t*-test or ANOVA for numerical data, and chi-squared or Fisher's exact test for categorical variables. Plasma total IgE was logarithm-transformed to base 10 before analysis. Hardy–Weinberg equilibrium (HWE) for the 10 SNPs in cases and controls was evaluated by the exact test. To investigate the pattern of linkage disequilibrium (LD) in our SNPs on chromosome 17q21, pairwise LD coefficient

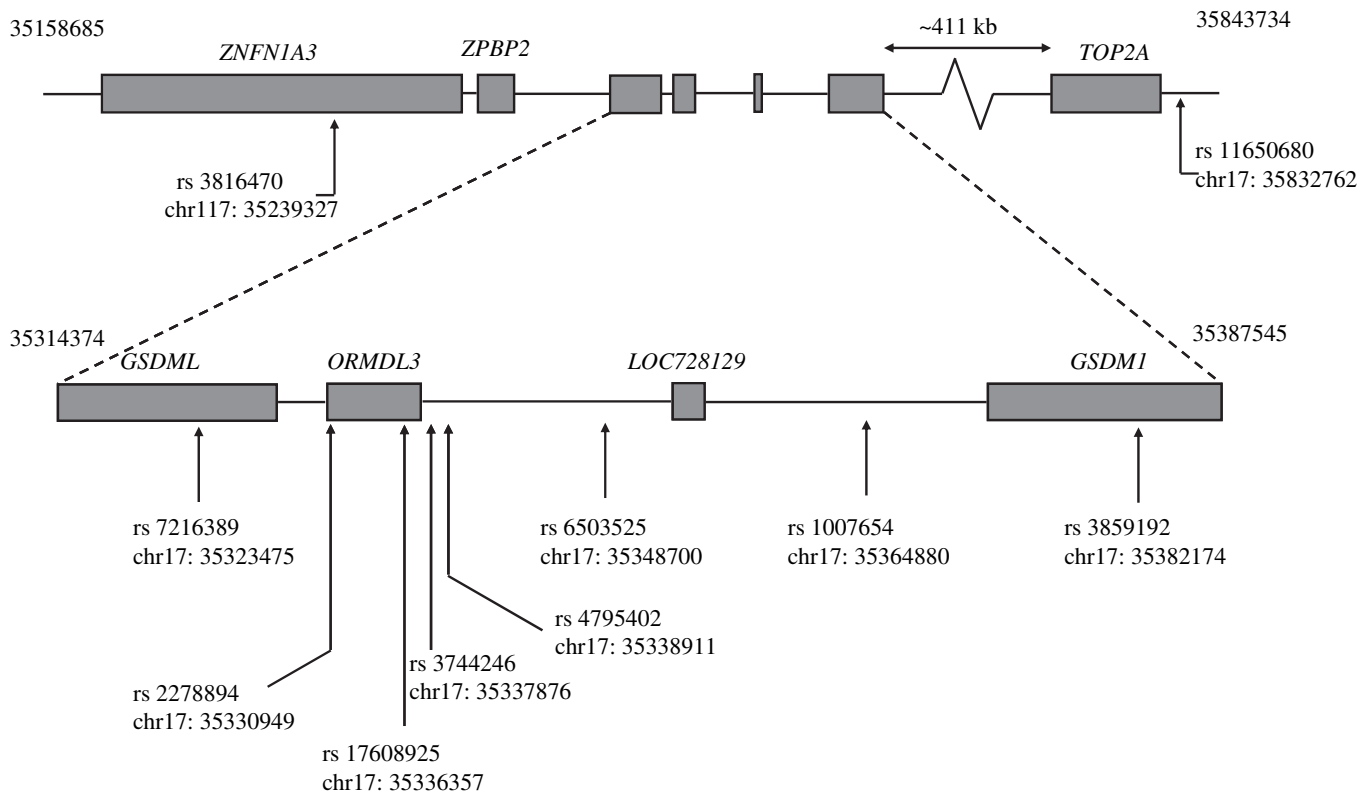


Figure 1. The distribution of our SNPs along the chromosome 17q21 region.

was calculated for each SNP pair by Haploview (Daly Lab, Cambridge, MA, USA). The associations between our SNPs and dichotomous phenotypes were analyzed using multivariate logistic regression, and with quantitative traits using linear regression. Two alternative models (recessive and co-dominant) were fitted for each outcome, adjusting for age and gender as covariates. Haplotypes were assigned as maximum likelihood estimates using EH program, and their associations with asthma traits were analyzed after 1000 permutations using WHAP (14). All comparisons were made two-tailed, and P -values less than 0.05 were considered significant.

Results

Study population

Three hundred and fifteen patients and 192 controls were recruited. Table S3 summarizes their demographic and clinical characteristics. The mean (SD) age of patients and controls was 11.1 (3.9) years and 11.8 (4.1) years, respectively ($P = 0.074$). Patients had higher log-transformed plasma total IgE than controls [mean (SD): 2.60 (0.61) vs 1.86 (0.70); $P < 0.0001$]. Atopy was also more common among our cases (86.6%) than the controls (43.2%; $P < 0.0001$).

Characterization of SNPs at chromosome 17q21

The overall genotyping efficiency was 99.6%. Table 2 summarizes MAF for our 10 SNPs. Two SNPs did not

follow HWE: rs2278894 among the cases ($P = 0.0002$) and rs4795402 among the controls ($P = 0.019$), and they were excluded from genotype and haplotype analyses. The LD scores for these SNPs among our subjects were generally medium to high for SNPs around the *ORMDL3* gene (Fig. S1), but only one haplotype block with rs17608925, rs3744246 and rs4795402 could be assigned to our SNP panel. Two SNPs distant from *ORMDL3* (rs3859192 in *GSDM1* and rs11650680 in *TOP2A*) were in generally low LD with the other SNPs.

Asthma traits and individual polymorphisms

The relation between dichotomous asthma-related traits and SNPs in chromosome 17q21 was analyzed by logistic regression (Tables 3 and 4). Adjusted for age and gender as covariates, asthma diagnosis was significantly associated with rs3816470, rs7216389, rs11650680 and rs3744246 under the co-dominant model, and with rs6503525 and rs11650680 under the recessive model. Atopy was significantly associated only with rs11650680 under both co-dominant ($P = 0.0004$) and recessive ($P = 0.0004$) models. Plasma total IgE level was analyzed as a continuous variable. The covariate for this variable was significant for rs11650680 under both co-dominant and recessive models ($P = 0.008$ and

Table 2. Interethnic comparisons of minor allele frequencies for polymorphic markers at chromosome 17q21 in our subjects with HapMap and published data for non-allergic controls

SNP	Minor allele	Present study		HapMap data (12)				Published data (4–7)		
		Asthmatics	Controls	Han Chinese	Japanese	European	African	Caucasian	Japanese	African-American
rs3816470	C	0.196	0.221	0.389	0.273	0.525	0.583	–	–	–
rs7216389	C	0.197	0.232	0.389	0.289	0.517	0.125	0.503–0.536	0.287	0.216
rs2278894	C	0.025	0.021	0.089	0	0	0	–	–	–
rs4795402	A	0.186	0.227	0.356	0.267	0.317	0.483	–	–	–
rs6503525	C	0.629	0.570	0.433	0.511	0.475	0.517	–	–	–
rs17608925	C	0.056	0.073	0.089	0.056	0.158	0.033	–	–	–
rs1007654	A	0.240	0.284	0.433	0.344	0.308	0.241	–	–	–
rs3859192	T	0.543	0.508	0.422	0.611	0.500	0.350	0.409–0.464	–	0.321
rs11650680	T	0.206	0.258	0.200	0.133	0.233	0	0.199–0.212	–	0.002
rs3744246	T	0.201	0.230	0.344	0.267	0.267	0.292	0.197–0.207	–	0.307

SNP, single-nucleotide polymorphism.

Table 3. Association between physician-diagnosed asthma and SNP genotypes in our subjects

Genotype	Asthmatics (<i>n</i> = 315)	Controls (<i>n</i> = 192)	Recessive model*		Co-dominant model*	
	<i>n</i> (%)	<i>n</i> (%)	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
rs3816470						
TT	203 (65.1)	110 (57.3)	1.00	Ref	1.00	Ref
TC	96 (30.8)	79 (41.1)			0.67 (0.44–0.95)	0.025
CC	13 (4.2)	3 (1.6)	3.04 (0.84–10.91)	0.089	2.60 (0.72–9.42)	0.146
rs7216389						
TT	203 (64.4)	107 (55.7)	1.00	Ref	1.00	Ref
TC	100 (31.7)	81 (42.2)			0.64 (0.44–0.94)	0.023
CC	12 (3.8)	4 (2.1)	2.05 (0.64–6.52)	0.225	1.74 (0.54–5.58)	0.354
rs6503525						
GG	50 (15.9)	33 (17.2)	1.00	Ref	1.00	Ref
GC	134 (42.5)	99 (51.6)			0.86 (0.51–1.44)	0.567
CC	131 (41.6)	60 (31.3)	1.55 (1.06–2.27)	0.024	1.39 (0.81–2.38)	0.234
rs17608925						
TT	280 (89.5)	163 (85.3)	1.000	Ref	1.00	Ref
TC	31 (9.9)	28 (14.7)			0.62 (0.36–1.08)	0.092
CC	2 (0.6)	0 (0)	NA	0.999	NA	0.999
rs1007654						
GG	182 (58.0)	96 (50.5)	1.00	Ref	1.00	Ref
GA	113 (36.0)	80 (42.1)			0.76 (0.52–1.11)	0.160
AA	19 (6.1)	14 (7.4)	0.87 (0.42–1.78)	0.696	0.77 (0.37–1.62)	0.492
rs3859192						
CC	69 (22.0)	43 (22.6)	1.000	Ref	1.00	Ref
CT	149 (47.5)	101 (53.2)			0.92 (0.58–1.45)	0.712
TT	96 (30.6)	46 (24.2)	1.32 (0.87–1.99)	0.191	1.24 (0.74–2.09)	0.418
rs11650680						
CC	196 (62.2)	108 (56.8)	1.00	Ref	1.00	Ref
CT	108 (34.3)	66 (34.7)			0.92 (0.62–1.36)	0.676
TT	11 (3.5)	16 (8.4)	0.42 (0.19–0.94)	0.034	0.41 (0.18–0.92)	0.031
rs3744246						
CC	202 (64.3)	106 (56.1)	1.00	Ref	1.00	Ref
CT	98 (31.2)	79 (41.8)			0.63 (0.43–0.93)	0.019
TT	14 (4.5)	4 (2.1)	2.36 (0.76–7.36)	0.139	2.00 (0.63–6.29)	0.238

CI, confidence interval; OR, odds ratio; NA, not available; Ref, reference.

*Analyzed by logistic regression, adjusted for age and gender as covariates.

0.0002 respectively), suggesting that subjects homozygous for the T allele had lower plasma total IgE levels (Table 5). The SNP rs3859192 was again marginally associated with plasma total IgE.

Asthma traits and haplotypes at chromosome 17q21

Eight-locus haplotypes were assigned from SNPs that followed HWE and with MAF $\geq 5\%$. There were no

Table 4. Association between atopy † and SNP genotypes in our subjects

Genotype	Atopy (<i>n</i> = 340)	Non-atopic controls (<i>n</i> = 148)	Recessive model*		Co-dominant model*	
	<i>n</i> (%)	<i>n</i> (%)	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
rs3816470						
TT	213 (63.2)	87 (58.8)	1.00	Ref	1.00	Ref
TC	112 (33.2)	58 (39.2)			0.80 (0.53–1.21)	0.298
CC	12 (3.6)	3 (2.0)	1.53 (0.42–5.59)	0.519	1.41 (0.38–5.20)	0.604
rs7216389						
TT	215 (63.2)	84 (56.8)	1.00	Ref	1.00	Ref
TC	114 (33.5)	60 (40.5)			0.74 (0.49–1.12)	0.153
CC	11 (3.2)	4 (2.7)	1.10 (0.34–3.56)	0.870	0.98 (0.30–3.21)	0.979
rs6503525						
GG	52 (15.3)	30 (20.3)	1.00	Ref	1.00	Ref
GC	152 (44.7)	73 (49.3)			1.24 (0.72–2.12)	0.439
CC	136 (40.0)	45 (30.4)	1.50 (0.99–2.28)	0.059	1.75 (0.99–3.10)	0.055
rs17608925						
TT	298 (88.2)	127 (86.4)	1.00	ref	1.00	ref
TC	39 (11.5)	19 (12.9)			0.85 (0.47–1.54)	0.588
CC	1 (0.3)	1 (0.7)	0.48 (0.03–7.80)	0.608	0.47 (0.03–7.66)	0.598
rs1007654						
GG	194 (57.2)	73 (50.0)	1.00	Ref	1.00	Ref
GA	123 (36.3)	63 (43.2)			0.75 (0.49–1.13)	0.165
AA	22 (6.5)	10 (6.8)	0.92 (0.42–2.01)	0.833	0.81 (0.36–1.81)	0.607
rs3859192						
CC	69 (20.4)	39 (26.7)	1.00	Ref	1.00	Ref
CT	174 (51.3)	69 (47.3)			1.45 (0.89–2.37)	0.136
TT	96 (28.3)	38 (26.0)	1.12 (0.71–1.75)	0.634	1.44 (0.83–2.50)	0.201
rs11650680						
CC	213 (62.6)	82 (56.2)	1.00	Ref	1.00	Ref
CT	118 (34.7)	48 (32.9)			0.95 (0.62–1.46)	0.820
TT	9 (2.6)	16 (11.0)	0.21 (0.09–0.50)	0.0004	0.20 (0.09–0.49)	0.0004
rs3744246						
CC	216 (63.1)	82 (56.6)	1.00	Ref	1.00	Ref
CT	113 (33.3)	58 (40.0)			0.74 (0.49–1.13)	0.161
TT	12 (3.5)	5 (3.4)	0.95 (0.33–2.77)	0.922	0.85 (0.29–2.51)	0.765

CI, confidence interval; OR, odds ratio; Ref, reference.

*Analyzed by logistic regression, adjusted for age and gender as covariates.

†Defined as $\geq$ one allergen-specific IgE in plasma.

significant association of haplotypes with asthma diagnosis ($P = 0.453$). On the other hand, atopy was significantly associated with our eight-locus haplotypes ($P = 0.048$). There was also a marginally significant haplotypic association ($P = 0.053$) for increased plasma total IgE levels. Table 6 summarizes the *post hoc* analyses for individual haplotypes. The haplotype TTTCCGTT was significantly protective against both atopy and increased plasma total IgE, with the respective OR and 95% CI for these phenotypes being 0.21 and 0.09–0.52 ($P = 0.0002$) and 0.41 and 0.18–0.90 ($P = 0.025$).

Discussion

Using case-control test of genetic associations, this study found significant associations between asthma and atopy traits and a number of SNPs on chromosome 17q21 in southern Chinese children. Interestingly, the SNP rs7216389 most significantly associated with

asthma in the Caucasian and Japanese populations was only marginally associated with asthma in this study. On the other hand, we observe that asthma, atopy and plasma total IgE were consistently associated with rs11650680 in *TOP2A* located upstream of *ORMDL3*. In this replication study, the associations between polymorphic markers at chromosome 17q21 and asthma-related traits are consistently significant or close to significant at 5% alpha level. These findings are clearly not a case of 'chance significant finding' coming out of a bunch of statistical comparisons (15), indicating a substantial evidence for the effects of chromosome 17q21 markers on asthma and atopy. We consider that adjusting for multiple comparisons is not needed. Besides, the majority of genetic associations at least for rs11650680 would remain significant after such corrections. On haplotypic analysis, atopy and increased plasma total IgE also showed significant associations with an eight-locus haplotype in this chromosomal region. These findings strongly support the importance

Table 5. Association between SNP genotypes and plasma total IgE as a quantitative trait

Genotype	All subjects (n = 498)		P-values		
	n (%)	Plasma log ₁₀ IgE*	ANOVA	RM†	CDM†
rs3816470					
TT	308 (62.2)	2.36 ± 0.72	0.130	0.390	0.325
TC	172 (34.7)	2.23 ± 0.77			
CC	15 (3.0)	2.52 ± 0.71			
rs7216389					
TT	305 (61.2)	2.35 ± 0.72	0.377	0.771	0.313
TC	178 (35.7)	2.26 ± 0.77			
CC	15 (3.0)	2.39 ± 0.73			
rs6503525					
GG	82 (1.16)	2.20 ± 0.80	0.237	0.099	0.166
GC	228 (45.8)	2.33 ± 0.72			
CC	188 (37.8)	2.36 ± 0.72			
rs17608925					
TT	435 (87.9)	2.33 ± 0.74	0.738	0.609	0.630
TC	58 (11.7)	2.26 ± 0.71			
CC	2 (0.4)	2.55 ± 1.08			
rs1007654					
GG	273 (55.2)	2.34 ± 0.73	0.484	0.265	0.311
GA	190 (38.4)	2.31 ± 0.74			
AA	32 (6.5)	2.18 ± 0.80			
rs3859192					
CC	111 (22.4)	2.22 ± 0.80	0.043	0.027	0.017
CT	246 (49.7)	2.30 ± 0.73			
TT	138 (27.9)	2.44 ± 0.69			
rs11650680					
CC	301 (60.7)	2.36 ± 0.72	0.001	0.0002	0.008
CT	170 (34.3)	2.32 ± 0.76			
TT	25 (5.0)	1.79 ± 0.63			
rs3744246					
CC	303 (61.3)	2.36 ± 0.72	0.213	0.504	0.300
CT	174 (35.2)	2.25 ± 0.77			
TT	17 (3.4)	2.45 ± 0.73			

ANOVA, analysis of variance; CDM, co-dominant model; RM, recessive model.

*Expressed in mean ± standard deviation.

†Analyzed by linear regression, adjusted for age and gender as covariates.

of genes in chromosome 17q21 in conferring the risks of childhood asthma and atopy.

The initial publication of asthma GWA by Moffatt (4) identified the chromosome 17q21 as a candidate locus for childhood asthma in Caucasians. They went on to show that this genetic association was due to the functional SNP rs7216389 and others in *ORMDL3* on this chromosome region. Two of the three replication studies published to date focused mainly on rs7216389 (6, 7), which could not provide data on the SNPs of other genes on chromosome 17q21. The third study genotyped eight *ORMDL3* SNPs in three independent cohorts of Mexicans, Puerto Ricans, and African-Americans (5). They observed significant associations between two SNPs within *ORMDL3* (rs4378650 and rs12603332 but not rs7216389) and asthma in Mexicans and African-Americans. The present study also detected weak yet significant associations between asthma and two SNPs (rs7216389 and rs3744246) within *ORMDL3*. These consistent findings suggest that *OR-*

Table 6. Relationship between atopy and increased plasma total IgE levels and haplotypes* assigned from eight SNPs at chromosome 17q21 region

Phenotype	Haplotype	Frequency		OR	95% CI	P †
		Atopy	No atopy			
Atopic status	TTTCCGTC	0.388	0.252	1.00	—	Ref
	TTTCCGCC	0.114	0.091	0.78	0.36–1.69	0.620
	TTTCCGTT	0.038	0.116	0.21	0.09–0.52	0.0002
	CCTTGACC	0.048	0.082	0.37	0.15–0.93	0.032
	TTTCCGCT	0.042	0.066	0.39	0.15–1.04	0.063
	TTTCGGCC	0.041	0.067	0.39	0.15–1.04	0.063
	TTTCGACC	0.050	0.052	0.60	0.22–1.64	0.388
		Increased	Normal			
Plasma total IgE‡	TTTCCGTC	0.369	0.277	1.00	—	Ref
	TTTCCGCC	0.124	0.097	0.96	0.49–1.92	0.958
	TTTCCGTT	0.053	0.096	0.41	0.18–0.90	0.025
	CCTTGACC	0.048	0.078	0.48	0.20–1.13	0.102
	TTTCCGCT	0.040	0.063	0.48	0.19–1.24	0.147
	TTTCGGCC	0.036	0.067	0.41	0.16–1.05	0.065
	TTTCGACC	0.051	0.047	0.86	0.33–2.26	0.908
	TTTCGACT	0.028	0.050	0.39	0.13–1.14	0.091

CI, confidence interval; OR, odds ratio; Ref, reference haplotype used for comparison.

*Haplotypes were constructed from the following SNPs: rs3816470, rs7216389, rs17608925, rs3744246, rs6503525, rs1007654, rs3859192 and rs11650680. Only haplotypes with ≥ 0.05 frequencies in at least one group were listed.

†Yates-corrected P-values obtained by chi-squared or Fisher exact test.

‡Compared with the upper limits of local references (120 kIU/l for 5–6 years; 160 kIU/l for 6–7 years; 180 kIU/l for ≥8 years).

MDL3, or adjacent genes on chromosome 17q21, is a strong risk factor for asthma in ethnically diverse populations. Despite the fact that *ORMDL3* is expressed ubiquitously, its function or pathogenic role in human diseases remains unclear. Hjelmqvist et al. showed *ORMDL3* to have significant sequence conservation to homologues in *Drosophila* and *Saccharomyces cerevisiae* (16). The Japanese group demonstrated that *ORMDL3* expression in normal human lung fibroblasts was strongly induced by polyinosine-polycytidylic acid, one of the pathogen-associated microbial patterns, but not by lipopolysaccharide, lipopeptide 2 or Pam3CSK4. This finding suggests that *ORMDL3* may be important in defense against viral respiratory infections (6).

We observe from the above findings that asthma is associated with different SNPs within *ORMDL3* in different ethnic groups – with rs7216389 in Japanese, Scottish and our southern Chinese and with rs4378650 and rs12603332 in Mexicans and African-Americans. This discrepancy of the results at SNP level suggests that gene(s) adjacent to *ORMDL3* on chromosome 17q21 may also influence asthma susceptibility. For instance, our study as well as the asthma GWA (4) identified a strong association signal for rs11650680 of *TOP2A* that is about 500 kbp from rs7216389. As these two SNPs fell within a large LD block in the Caucasian population, Galanter et al. (5) argued that such genetic association implied that these SNPs were in LD with one or more of the causative

SNPs. However, our LD pattern (Fig. 1) showed that rs11650680 and rs7216389 are not linked in Chinese in contrast to what Moffatt et al. found in their Caucasian samples. rs11650680 is located in the promoter region of *TOP2A* (DNA topoisomerase II, alpha isozyme; OMIM# 126430) (12). This gene is mapped to chromosome 17q21.2, and encodes a DNA topoisomerase of 29 327 base pairs with 35 exons (17). Topoisomerase II from eukaryotic cells catalyzes the relaxation of supercoiled DNA molecules, catenation, decatenation, knotting, and unknotting of circular DNA, implying its importance in cell cycle and DNA repair. *TOP2A* is increasingly being recognized to be an important predictor of treatment responses for many cancers (18, 19). Nonetheless, there has not been any study on the relevance of *TOP2A* in asthma and atopy pathogenesis. Together with our consistent findings of rs11650680 with a number of asthma traits, we suggest that *TOP2A* may be an additional candidate gene for asthma and plasma IgE. Further genetic studies with larger sample size and dense SNP mapping of the locus around rs11650680 are needed to confirm our observation. As in our published findings between *IL13*, *IL4RA* and *TARC* (20), it would also be interesting to investigate the epigenetic interactions for asthma and atopy between genes on chromosome 17q21 and other known candidate genes.

This study provides further data to support the interethnic variations of MAF for polymorphic markers on chromosome 17q21 (Table 2). The minor C allele of rs7216389 in our southern Chinese (0.232) was similar to that of Japanese (0.287) but much lower than Han Chinese (0.389) and Caucasians (0.503 to 0.536). In contrast, MAF for rs11650680 and its adjacent SNP (rs3859192) were similar to the published data for Caucasians (4, 11). Such interethnic differences possibly also affect the haplotype structure at chromosome 17q21: our initial HapMap search assigns rs3744246 and rs4795402 to the adjacent haplotype blocks for Caucasians, but these two SNPs are clustered into a single haplotype block in our southern Chinese (Fig. S1). These differences in MAF also affected the power of this study to detect significant genetic associations. For example, C allele of rs7216389 is the 'wild-type' allele in Caucasians but becomes a minor allele in our Chinese subjects. This finding is similar to what we found for *CD14*, *DEFB1*, and *PTGDR* in Chinese children (13, 21, 22). With MAF that is 30% lower than that found by Moffatt (4), this study in Chinese would expectedly yield less impressive *P*-values for rs7216389 with asthma traits. Barnes reviewed that the genetic epidemiology of asthma candidate loci showed marked variations among different racial and ethnic groups (23). As the global burden of asthma and allergy also varied widely (24), it would be crucial to delineate the influence of chromosome 17q21 markers for asthma traits in different populations.

One limitation of this study relates to the use of different criteria for defining cases and controls. Asthma

in our cases was diagnosed by the standard ATS criteria (8), i.e. presence of suggestive asthma symptoms together with BHR or reversibility of airflow limitation. On the other hand, our controls were ascertained by the Chinese translated ISAAC questionnaire (1). They gave negative returns for all four items 'current wheeze', 'asthma ever', 'rhinoconjunctivitis ever' and 'eczema ever'. de Meer et al. found that 66 of 470 asymptomatic schoolchildren had BHR to hypertonic saline according to the ISAAC protocol (25). Without measuring lung functions for the non-allergic controls, we agree that some of them may have asymptomatic BHR. This limitation would thus reduce the differences in genotype frequencies of our studied SNPs between cases and controls for the outcomes. Nonetheless, we argue that the findings of genetic associations with our SNPs would suggest that they are more likely to be genuine. Another limitation is that the number of controls was much lower than that of our cases, and this issue may weaken the power of this study in detecting any significant genetic association. Despite this, the existing sample size has 80% power to detect with 95% confidence an odds ratio of 1.8 for asthma for any SNP with MAF 0.2, which was applicable for eight of our studied SNPs (i.e. MAF for controls in Table 2). In addition, our controls were recruited from non-allergic children who attended our hospital outpatient clinics for minor, non-respiratory complaints. Although we cannot exclude any selection bias or population stratification, the pattern of allergen sensitizations of these controls (see Table S3) are comparable with those of local schoolchildren (26). Despite this, this study may be improved by recruiting control subjects from our general population.

In conclusion, this case-control study supports the genetic associations between asthma and related traits and chromosome 17q21 region. However, these clinical phenotypes were most strongly linked to rs11650680 of the *TOP2A* gene that is distant from rs7216389, the SNP in *ORMDL3* that is published among the Caucasian population. Future studies should replicate our findings and also investigate the possible interactions between genes on chromosome 17q21 in determining the susceptibility for asthma and atopy.

Acknowledgments

We thank E. Yung and Y. S. Wong for performing spirometry and processing DNA samples in our subjects. This study was supported by the Research Committee Group Research Scheme (no. 3110034) and Direct Grant for Research, The Chinese University of Hong Kong.

Conflicts of interest

The sponsors of the study had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing

of the report; and in the decision to submit the paper for publication.

Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1. Linkage disequilibrium (LD) pattern among our 10 SNPs by Haploview analysis.

Table S1. Primers for the three multiplex PCR reactions.

Table S2. Mixed probes for multiplex genotyping using SNaPshot kit.

Table S3. Clinical and laboratory characteristics of our subject.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1. Wong GW, Leung TF, Ko FW, Lee KK, Lam P, Hui DS et al. Declining asthma prevalence in Hong Kong Chinese schoolchildren. *Clin Exp Allergy* 2004;**34**:1550–1555.
2. Newman-Taylor A. Environmental determinants of asthma. *Lancet* 1995;**345**:296–299.
3. Ober C. Perspectives on the past decade of asthma genetics. *J Allergy Clin Immunol* 2005;**116**:274–278.
4. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S et al. Genetic variants regulating *ORMDL3* expression contribute to the risk of childhood asthma. *Nature* 2007;**448**:470–473.
5. Galanter J, Choudhry S, Eng C, Nazario S, Rodriguez-Santana JR, Casal J et al. *ORMDL3* gene is associated with asthma in three ethnically diverse populations. *Am J Respir Crit Care Med* 2008;**177**:1194–1200.
6. Hirota T, Harada M, Sakashita M, Doi S, Miyatake A, Fujita K et al. Genetic polymorphism regulating *ORM1-like 3* (*Saccharomyces cerevisiae*) expression is associated with childhood atopic asthma in a Japanese population. *J Allergy Clin Immunol* 2008;**121**:769–770.
7. Tavendale R, Macgregor DF, Mukhopadhyay S, Palmer CN. A polymorphism controlling *ORMDL3* expression is associated with asthma that is poorly controlled by current medications. *J Allergy Clin Immunol* 2008;**121**:860–863.
8. American Thoracic Society. Medical section of the American Lung Association Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**:225–244.
9. Leung TF, Tang NL, Li CY, Lam CW, Wong GW, Fok TF. Association between *TARC* C-431T and atopy and asthma in children. *J Allergy Clin Immunol* 2004;**114**:199–202.
10. Ip MS, Karlberg EM, Karlberg JP, Luk KD, Leong JC. Lung function reference values in Chinese children and adolescents in Hong Kong I. Spirometric values and comparison with other populations. *Am J Respir Crit Care Med* 2000;**162**:424–429.
11. SNP Home, National Center for Biotechnology Information (NCBI); (available at: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>; last accessed on 13 June 2008).
12. The Children's Hospital Informatics Program (CHIP), Children's Hospital Boston; (available at: <http://snpper.chip.org/>; last accessed on 13 June 2008).
13. Leung TF, Li CY, Kong AP, Chan IH, Ng MC, Chan MH, et al. *PTGDR* is not a major candidate gene for asthma and atopy in Chinese children. *Pediatr Allergy Immunol* (in press).
14. Purcell S, Daly MJ, Sham PC. WHAP: haplotype-based association analysis. *Bioinformatics* 2007;**23**:255–256.
15. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;**1**:43–46.
16. Hjelmqvist L, Tuson M, Marfany G, Herrero E, Balcels S, Gonzalez-Duarte R. *ORMDL* proteins are a conserved new family of endoplasmic reticulum membrane proteins. *Genome Biol* 2002;**3**:RESEARCH0027.
17. Lang AJ, Mirski SE, Cummings HJ, Yu Q, Gerlach JH, Cole SP. Structural organization of the human *TOP2A* and *TOP2B* genes. *Gene* 1998;**221**:255–266.
18. Murphy AJ, Hughes CA, Barrett C, Magee H, Loftus B, O'Leary JJ et al. Low-Level *TOP2A* amplification in prostate cancer is associated with *HER2* duplication, androgen resistance, and decreased survival. *Cancer Res* 2007;**67**:2893–2898.
19. Pritchard KI, Messersmith H, Elavathil L, Trudeau M, O'Malley F, Dhesy-Thind B. *HER-2* and topoisomerase II as predictors of response to chemotherapy. *J Clin Oncol* 2008;**26**:736–744.
20. Chan IH, Leung TF, Tang NL, Li CY, Sung YM, Wong GW et al. Gene-gene interactions for asthma and plasma total IgE concentration in Chinese children. *J Allergy Clin Immunol* 2006;**117**:127–133.
21. Leung TF, Li CY, Liu EK, Tang NL, Chan IH, Yung E et al. Asthma and atopy are associated with *DEFB1* polymorphisms in Chinese children. *Gene Immun* 2006;**7**:59–64.
22. Leung TF, Tang NL, Sung YM, Li AM, Wong GW, Chan IH et al. The C-159T polymorphism in the *CD14* promoter is associated with serum total IgE concentration in atopic Chinese children. *Pediatr Allergy Immunol* 2003;**14**:255–260.
23. Barnes KC. Genetic epidemiology of health disparities in allergy and clinical immunology. *J Allergy Clin Immunol* 2006;**117**:243–254.
24. Burchard EG, Ziv E, Coyle N, Gomez SL, Tang H, Karter AJ et al. The importance of race and ethnic background in biomedical research and clinical practice. *N Engl J Med* 2003;**348**:1170–1175.
25. de Meer G, Postma DS, Janssen NA, de Jongste JC, Brunekreef B. Bronchial hyper-responsiveness to hypertonic saline and blood eosinophilic markers in 8-13-year-old schoolchildren. *Clin Exp Allergy* 2004;**34**:1226–1231.
26. Wong GW, Li ST, Hui DS, Fok TF, Zhong NS, Chen YZ et al. Individual allergens as risk factors for asthma and bronchial hyperresponsiveness in Chinese children. *Eur Respir J* 2002;**19**:288–293.

Online Supplement

Asthma and atopy are associated with chromosome 17q21 markers in Chinese children

T. F. Leung¹, H. Y. Sy¹, M. C. Y. Ng², I. H. S. Chan³, G. W. K. Wong¹, N. L. S. Tang³, M. M. Y. Waye^{4,5}, C. W. K. Lam³

Departments of ¹Paediatrics, ²Medicine and Therapeutics, ³Chemical Pathology and ⁴Biochemistry, and ⁵Croucher Laboratory for Human Genomics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

Correspondence to:

Dr. Ting Fan Leung, Department of Paediatrics, 6/F, Clinical Sciences Building, Prince of Wales Hospital, Shatin, N.T., Hong Kong.

Tel: (852) 2632 2981. Fax: (852) 2636 0020. E-mail: tfleung@cuhk.edu.hk.

25 Table S1. Primers for the three multiplex PCR reactions

Polymorphism	Primer sequence	Product size (bp)
Group I PCR		
rs3816470	F: 5' AAC TGT AGT CAT TCA AAA CCG CTG 3' R: 5' TAT GTG TGA AGG GGT ACT AT 3'	163
rs11650680	F': 5' AAT GGG GAT AAT GGG AGT ACC TC 3' R': 5' AAA GAG CAG AAA GAA CGG AAG CAC 3'	376
rs1007654	F': 5' CAC ACA TGG GAG CTA ACC CC 3' R': 5' TAA GAA TCC GGA GAC CTA AG 3'	673
Group II PCR		
rs3744246	F': 5' TGA GGA GAC TCG GAC TTG TA 3' R': 5' CTT TAG ACG ATG CAG ATG TG 3'	452
rs3859192	F': 5' CTT CCC ACA TCT TCC GCC CTA 3' R': 5' CAA AGG TCT CAG GCG TCA GA 3'	631
rs4795402	F': 5' AGG GCT CAT TCC AAG ATC GA 3' R': 5' TAC ATG GGA TGA CTT CCT TA 3'	751
Group III PCR		
rs7216389	F': 5' AGC AGT TCT GTC GCT GTT GT 3'	185

	R': 5' AAG GCA CGC AGC CCC TTC GG 3'	
rs2278894	F': 5' ATG TTG CCC TTT TAT TTG CGT T 3'	244
	R': 5' CTC CCC TTC CCA GTT GGA CTA G 3'	
rs17608925	F': 5' AAA ACA ACC AAC TGG CCC CT 3'	485
	R': 5' GGG GGT GGG CGG AAT GGA CA 3'	
rs6503525	F': 5' TGA GTT TGG TAG GGG GTC AA 3'	878
	R': 5' CTC TGT GCA TGG GGT CTA GG 3'	

26 F, forward primer; R, reverse primer.

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41 Table S2. Mixed Probes for multiplex genotyping using SNaPshot™ kit

Polymorphism	Primer sequence	Length	Conc.
rs3816470	5' AAT GGG GGT CTA CTT AAT A 3'	19 mer	0.15 µM
rs7216389	5' GCA ACC CTG GAA AGT CAC AAA CA 3'	23 mer	0.03 µM
rs2278894	5' GCC TGT TTT CTC CCT CCT GAC CCT GC 3'	26 mer	0.03 µM
rs4795402	5' GAC TGA CTG CAC TTC GGA AAA AAC AGA GAC ACC TT 3'	35 mer	0.03 µM
rs6503525	5' TGA CTG ACT GAC AGA ATC ACT TGA ATC TGG GAA GTG AA 3'	38 mer	0.15 µM
rs17608925	5' GAC TGA CTG ACT GAC TGA CTT GCT TAG TGC TGG GCC CTG A 3'	40 mer	0.15 µM
rs1007654	5' ACT GAC TGA CTG ACT GAC TGA AAT AGG TGG AGC TGG AGA GGG 3'	42 mer	0.15 µM
rs3859192	5' GAC TGA CTG ACT GAC TGA CTA GAT ATA GAA ACT GAC AGA CAG AGA C 3'	46 mer	0.15 µM
rs11650680	5' ACT GAC TGA CTG ACT GAC TGA CTG TTC TCC AAA ACC AGC TCA AAA TTC TC 3'	50 mer	0.15 µM
rs3744246	5' TGA CTG ACT GAC TGA CTG ACT GAC TGA CTG ACT AGA GCG CTT TTC ACA ACA TTG GC 3'	56 mer	0.15 µM

42

43

44 Table S3. Clinical and laboratory characteristics of our subjects

Characteristics *	Asthmatics (n = 315)	Non-allergic controls (n = 192)
Age, years	11.1 ± 3.9	11.8 ± 4.1
Male (n, %)	201 (63.8)	106 (55.2)
Plasma log ₁₀ -transformed total IgE level	2.60 ± 0.61 †	1.86 ± 0.70
Increased plasma total IgE level (n, %) ‡	238 (77.3) †	65 (34.2)
Atopy (≥ one allergen-specific IgE) (n, %)	258 (86.6) †	82 (43.2)
IgE sensitization to aeroallergens		
<i>Dermatophagoides pternoyssinus</i> (n, %)	257 (86.2) †	80 (42.1)
Cockroaches (n, %)	11 (3.7)	6 (3.2)
Cat (n, %)	14 (4.7)	2 (1.1)
PB eosinophil percentage, %	7.4 ± 4.3 †	3.4 ± 2.9
Spirometric variables		
FEV ₁ , % predicted	94.1 ± 20.7	ND
FVC, % predicted	110.6 ± 26.8	ND
FEV ₁ /FVC	0.795 ± 0.108	ND

45 FEV₁, forced expiratory volume in 1-second; FVC, forced vital capacity; ND, not done; PB,
46 peripheral blood.

47 * Expressed in mean $\pm$ standard deviation unless stated otherwise; † $P < 0.0001$.

48 ‡ Compared with the upper limits of local references (120 kIU/l for 5-6 years; 160 kIU/l for 6-
49 7 years; 180 kIU/l for ≥ 8 years).

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

Figure Legend

Figure S1. Linkage disequilibrium (LD) pattern among our 10 SNPs by Haploview analysis. The legend summarizes **rs numbers** and locations of the markers along chromosome 17q21. Numbers inside the boxes represent D' values for LD.

