

Association of IgE-mediated allergen sensitivity and promoter polymorphisms of chemokine (C–C motif) ligand 5 gene in Han Chinese patients with allergic skin diseases

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Abstract Two single nucleotide polymorphisms (SNPs), rs2280788 (–28C>G) and rs2107538 (–403G>A), in the promoter region of chemokine (C–C motif) ligand 5 (*CCL5*) was reported to be involved in the immunoglobulin E (IgE) expression and IgE-mediated allergic reactions. This study was to investigate the characteristics of total serum IgE level, specific allergen sensitivities and the two SNPs in the allergic skin disease (ASD) patients. ASD patients visiting the dermatological outpatient department of a local hospital were included with certain criteria, and the fasting venous blood was sampled for analysis. Total serum IgE was assayed with an ELISA kit, and 14 kinds of allergen-specific IgE were tested with an allergen screening system. The polymerase chain reaction–restriction fragment length polymorphism method was used to analyze the two SNPs. Among the finally included 437 patients aged from 16 to 85 years, 68.2 % was positive for the total serum IgE, 49.2 % was positive for at least one of the assayed allergen-specific IgE, and 35.0 % was sensitive to house dust mite. In the SNPs analysis, the GG/(GA+AA) ratio and G/A ratio for the –403G>A locus in the male and/or female ≥ 45 years subgroup were significantly lower in the total serum IgE positive patients than in the negative patients ($P < 0.05$). Weak linkage disequilibrium was

found between –403A and –28C alleles in male subgroups adjusted by age. Conclusively, house dust mite was the most common allergen in ASD patients, and –403A allele of *CCL5* promoter was a risk factor for IgE-mediated sensitization.

Keywords Allergic skin disease · Chemokine (C–C motif) ligand 5 · Single nucleotide polymorphism · Immunoglobulin E

Introduction

When the body is exposed to the same specific allergen or antigen again after its first sensitization, abnormal or pathological immune responses may occur in the skin. This group of inflammatory diseases is called allergic skin disease (ASD), including atopic dermatitis, contact dermatitis, urticaria, angioedema, psoriasis, and autoimmune blistering disorders. The prevalence of ASD is getting increased year by year (Okada et al. 2010; Pawankar et al. 2011). For example, the lifetime prevalence of urticaria is more than 20 % (Pawankar et al. 2011), and atopic dermatitis affects adults and children with worldwide prevalence rates of 1–20 % (DaVeiga 2012). ASD is mainly mediated by immunoglobulin E (IgE) synthesized and secreted by B cells, and partly by non-IgE (Nichols and Cook-Bolden 2009; Jenerowicz et al. 2012; Rosenwasser 2011). The skin inflammation is generally caused by allergens via inhalation, ingestion, and local contact, and may lead to specific IgE secretion. In the IgE-mediated ASD, IgE is a critical factor of allergic inflammation of ASD, involved in both early-phase and late-phase of hypersensitivity reaction (Rosenwasser 2011). Thus, total serum IgE test is thought to be valuable for diagnosis and treatment of ASD.

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In addition to allergen exposure, genetic characteristics of some genes also affect the susceptibility to the IgE-mediated ASD. Chemokine (C–C motif) ligand 5 (*CCL5*, in italics for its gene), or regulated upon activation, normal T cell expressed and secreted (RANTES) is one of the most correlative chemokines in the IgE-mediated allergic reactions (Kimata et al. 1996; Steinke and Borish 2006). *CCL5* gene, located in 17q11.2–q12 (Lira and Furtado 2012), has two transcript variants encoding protein of 91 or 154 amino acids, and was grouped into the CC chemokine subfamily which had a pair of consecutive cysteine residues in the N-terminal. By interacting with chemokine receptors on the cell surface and triggering a couple of signaling events, *CCL5* played roles in recruiting lymphocytes to the inflammatory location, induction and activation of immune cell differentiation, and so on (Marques et al. 2013). In the early years, *CCL5* was only found to be expressed in limited cell types including T cells but not in B cells, and the regulation of *CCL5* on B cell-related phenomena (including IgE synthesis) was considered to be indirectly exerted through its regulation on T cells (Bacon et al. 1995; Appay et al. 2000). But recently, B cells were demonstrated to not only express *CCL5* (Mueller et al. 2007), but have potential *CCL5* receptor (Hartmann et al. 2008), and *CCL5* was able to activate helper B cells via autocrine and other pathways (Sullivan et al. 2011).

There are different binding domains in *CCL5* promoter region for many types of trans acting elements, and *CCL5* gene expression is regulated by these different kinds of promoting mechanisms depending on physiological conditions and tissue types (Nelson et al. 1993). In the *CCL5* promoter region, rs2280788 (–28C>G) and rs2107538 (–403G>A) are two important single nucleotide polymorphisms (SNPs), for allelic genes of –28G and –403A were able to improve the transcriptional activity (Kiyohara et al. 2008), correspondingly resulted in higher serum *CCL5* concentration (Tian et al. 2009), and may affect the IgE secretion. However affected by varieties of genetic background, environment, and other reasons, some studies did not get completely consistent results on the association of *CCL5* SNPs and allergic diseases.

In this study, the characteristics of allergens causing ASD to the Han Chinese outpatients was described, and the differences of the two SNPs (rs2280788 and rs2107538) in *CCL5* promoter between the IgE-mediated sensitization and non-IgE-mediated sensitization were analyzed, which provided a clue to explore the relationship of *CCL5* and IgE-mediated sensitization in this population.

Materials and methods

Subjects and sample processing

From July 2011 to July 2013, 437 ASD outpatients visiting the dermatological outpatient department of a hospital

meeting the following criterion were included: (1) caught by rash, itching, hives and other allergic skin symptoms without exposure to known chemical and physical factors; (2) not diagnosed to have organic diseases during the past one year; (3) not diagnosed to have inflammatory or immune-related diseases during the past four months; (4) living in Shenzhen City and not smoking for more than one year; and (5) with Han nationality aged ≥ 16 years (excluding those aged < 16 years due to the normal reference IgE concentrations varied greatly in different age periods and limited number of outpatients for epidemiological analysis).

Two aliquots of about 3 mL fasting blood was drawn from elbow vein and collected with tubes pretreated with coagulant or EDTA anti-coagulant. Serum sample was prepared after centrifugation at $3000 \times g$ for 10 min at room temperature, and allergen-specific IgE were analyzed within 2 days. The anti-coagulating blood samples were stored at -76°C for later use.

Allergy testing and subjective grouping

Total serum IgE levels were assayed by an ELISA kit (Dr. FOOKE Laboratorien GmbH, Neuss, Germany) on a microplate reader (Multiskan FC, Thermo Fisher Scientific Inc., USA). Allergen-specific serum IgE induced by inhalant and food allergens were tested with immunoblotting kit (AllergyScreenTM, Mediawiss Analytic GmbH, Moers, Germany) on its matching device, RAPID READER. Fourteen kinds of common inhalant and food allergens were (1) house dust mite (HDM), (2) short ragweed wormwood, (3) animal dander, (4) cockroach, (5) polyvalent fungus, (6) multivalent pollen, (7) *Humulus*, (8) egg, (9) milk, (10) fish, shrimp, and crab, (11) beef and mutton, (12) cashew nuts, peanuts, and soybeans, (13) wheat, and (14) mango. Suggested by the manual of the ELISA kit, the patients aged ≥ 16 years with total serum IgE level ≥ 100 IU/mL were grouped as positive (IgE sensitive) group [IgE(+) group], and those otherwise as negative (non-IgE sensitive) group [IgE(–) group].

SNPs analysis with PCR–RFLP

Primer design

The two SNPs of *CCL5* (GenBank ID no.: NG_015990.1), –28C>G (rs2280788) and –403G>A (rs2107538) were analyzed with polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. Primers with a base substitution to introduce a *Hinc* II site to analyze the present –28C>G was previously reported by Yao et al. (Yao et al. 2003; Yao et al. 2009), while primers with a base substitution to introduce an *Afa* I site for –403G>A analysis (sense primer: 5'-CAAGAGGACTCATTCCAACATCATAT-3',

and antisense primer: 5'-TCCTGCTTATTCATTACAGATC $\overline{G}$ TA-3', $\overline{G}$ replacing the wildtype T) was designed by the SiteFind online software (<http://www.utmb.edu/sccc/b/software/sitefind.html>) (Evans and Liu 2005) and Primer Premier 5 (Premier Biosoft International, Palo Alto, California, USA). Primers were synthesized by Invitrogen Corporation (Shanghai, China).

PCR-RFLP experiment and analysis

DNA was extracted from the anti-coagulated blood sample with commercial kit (Cat no.: DP318-03, TIANGEN BIOTECH Co. Ltd., Beijing, China). In the 25 μ L PCR system, about 100 ng of blood DNA acted as template, each primer concentration was 0.4 μ mol/L, and *Taq* DNA polymerase, dNTP, and buffer were added according to the instruction of a commercial kit (TaKaRa #DR001AM, Dalian, China). PCR program was 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, and followed by 72 °C for 10 min. The PCR products were digested with *Hinc* II (TaKaRa #D1059) for -28C>G and *Afa* I (TaKaRa #D1116) for -403G>A, respectively. About 5 μ L of digested DNA was loaded into 2.5 % (v/w) agarose gel for electrophoresis at 120 V for 40 min, and then analyzed with an imaging system (ChemIDoc XRS+Systems, BIO-RAD Laboratories Ltd., Shanghai, China). Three genotypes (major and minor homozygotes, and heterozygote) of each of the SNPs were sequenced by Invitrogen Corporation (Shanghai, China) to demonstrate the accuracy of the RFLP analyses.

Statistical analysis

Data were analyzed with Excel (Microsoft office), SPSS 17.0 for Windows (SPSS Inc., Chicago, USA), and SNPStats online software (<http://bioinfo.iconcologia.net/snpstats/start.htm>) (Sole et al. 2006). Age was divided into 4 groups (16–29, 30–44, 45–59, and ≥ 60 years) for descriptive analysis, and the latter two age groups were merged for SNPs analysis. Hardy–Weinberg equilibrium, genotype frequency, and genetic model comparisons were evaluated by χ^2 test. The genotype or haplotype relative risks were expressed with odds ratio (OR) and 95 % confidence interval (95 % CI). The level for statistical significance was set at 0.05 bilaterally.

Results

General profiles and allergen characteristics of subjects

Among the 437 subjects (aged from 16 to 85 years) with ASD included for the study, males accounted for 40.7 %

(178 cases), and females 59.3 % (259 cases). Figure 1 illustrated the counts of both sex of patients at different age periods. For the total serum IgE test, 31.8 % of them (139 cases) were negative, and 68.2 % (298 cases) were positive. For the specific IgE test, 49.2 % of the total 437 subjects were sensitive to at least one of the 14 kinds of allergens. Among the inhalant allergens, HDM ranked highest to cause specific IgE in 35.0 % of all the ASD subjects; while among the food allergens, fish, shrimp, and crab was the top one to induce specific IgE, and 9.6 % of the ASD subjects were sensitive to it (Table 1).

Qualification of PCR-RFLP analyses with sequencing

For -28C>G and -403G>A SNPs, the genotypes of major homozygote, minor homozygote, and heterozygote were analyzed with RFLP experiment (Figs. 2a, 3a). The *Hinc* II enzyme cut the -28C allelic site, while the *Afa* I enzyme cut the -403G allelic site. Accordingly, two bands were generated for -28CC or -403GG, three bands for -28CG or -403GA, and one band for -28GG or -403AA. These results were consistent with the sequencing results (Figs. 2b–d, 3b–d), respectively.

Association of IgE-mediated sensitization and SNPs of CCL5 promoter

Genotypes of the two *CCL5* SNPs, -28C>G and -403G>A, were in Hardy–Weinberg equilibrium ($P > 0.05$) in IgE(+) group, IgE(-) group, or combined either for all ages (≥ 16 years) or for each of the three age groups (16–29, 30–44, and ≥ 45 years), which indicated that the included subjects were representative. By controlling age and sex simultaneously, each of the two SNPs between IgE(+) and IgE(-) groups was compared with genotypic frequency, dominant model, recessive model, and multiplicative model (allelic frequency) (Lewis 2002). The P values for the statistic results were listed in Table 2, and the ORs (95 % CIs) for the P values < 0.05 were additionally presented in the

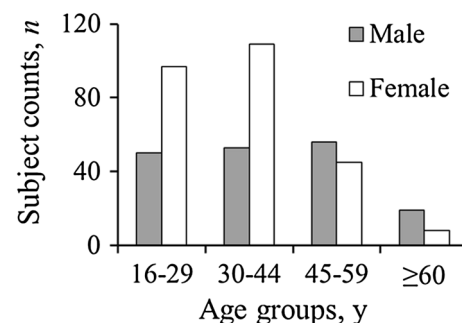


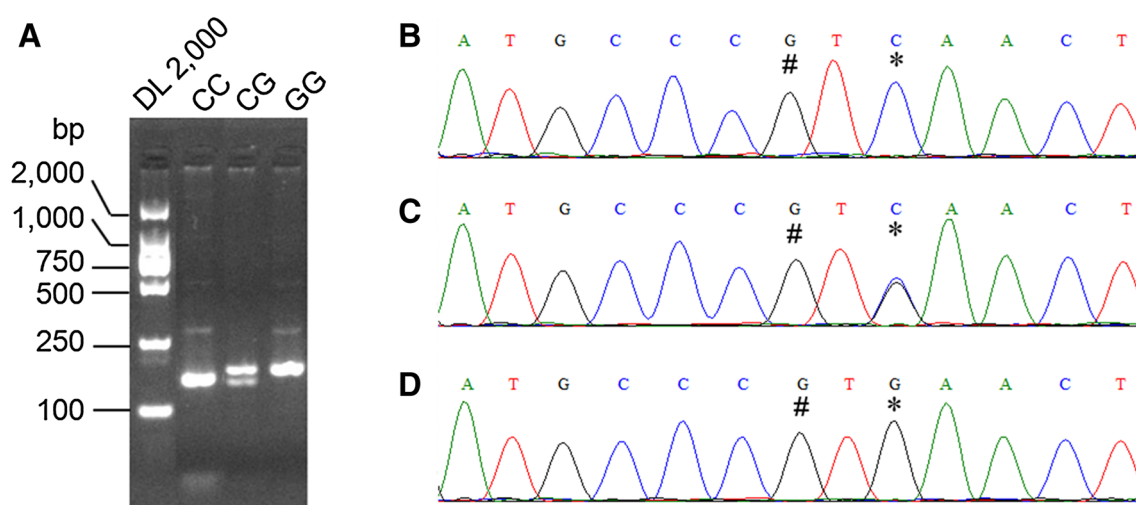
Fig. 1 Age and sex compositions of all the included 437 patients with allergic skin disease

Table 1 Sensitive rate of allergen-specific serum IgE in patients with allergic skin disease

Allergens	Sensitive rate, %	
	All patients, <i>n</i> = 437	IgE(+) patients, <i>n</i> = 298
Inhalant allergens		
House dust mite	35.0	43.6
Cockroach	10.3	12.8
Animal dander	9.6	12.8
Polyvalent fungus ^a	7.8	7.0
Multivalent pollen ^b	4.8	6.4
Short ragweed wormwood	3.9	5.0
<i>Humulus</i>	0.2	0.3
Food allergens		
Fish, shrimp, and crab	9.6	11.4
Milk	7.8	9.7
Cashew nuts, peanuts, and soybeans	4.8	6.7
Beef and mutton	2.5	3.7
Mango	2.5	3.7
Wheat	1.6	2.0
Egg	1.4	1.7

^a Including penicillium notatum, branching mortierella, aspergillus, alternaria mold

^b Including cypress, elm, firmiana, willow, and cottonwood

**Fig. 2** Genotyping of the *CCL5* -28C>G site (rs2280788). **a**: Electrophoresis of DNA marker (DL 2000) and PCR-RFLP products with CC, CG, or GG genotypes. **b–d**: the three genotypes were

demonstrated by sequencing as indicated with asterisk, respectively. # indicated the mutation introduced with primer

table notes. Between the IgE(+) and IgE(−) groups, the genotypic frequencies of the both SNPs were not significantly different ($P > 0.05$). When the -28C>G genotype was further examined with dominant model, recessive model, and multiplicative model, not any significant difference between the IgE(+) and IgE(−) groups was found ($P > 0.05$). The dominant model revealed that in the male ≥ 45 years subgroup for -403G>A locus, the IgE(+) group had significant lower GG/(GA+AA) ratio than the IgE(−) group had [$P = 0.032$, OR (95 % CI) = 0.33 (0.12–0.93)]. Also in this age subgroup, the multiplicative model found the

significant lower G allelic frequency or G/A ratio (namely higher A allelic frequency) in the IgE(+) group not only existed in the males [$P = 0.040$, OR (95 % CI) = 0.46 (0.22–0.97)], but also in the females [$P = 0.047$, OR (95 % CI) = 0.43 (0.18–1.00)].

To analyze the linkage disequilibrium or allelic association of the two SNPs of -28C>G and -403G>A, statistic results generated by SNPStats reported $D' = 0.765$, and $R = 0.392$ ($R^2 = 0.153$). If the threshold of R^2 was stringently set at 0.5 as recommended by Carlson et al. (Carlson et al. 2004), the linkage between the two

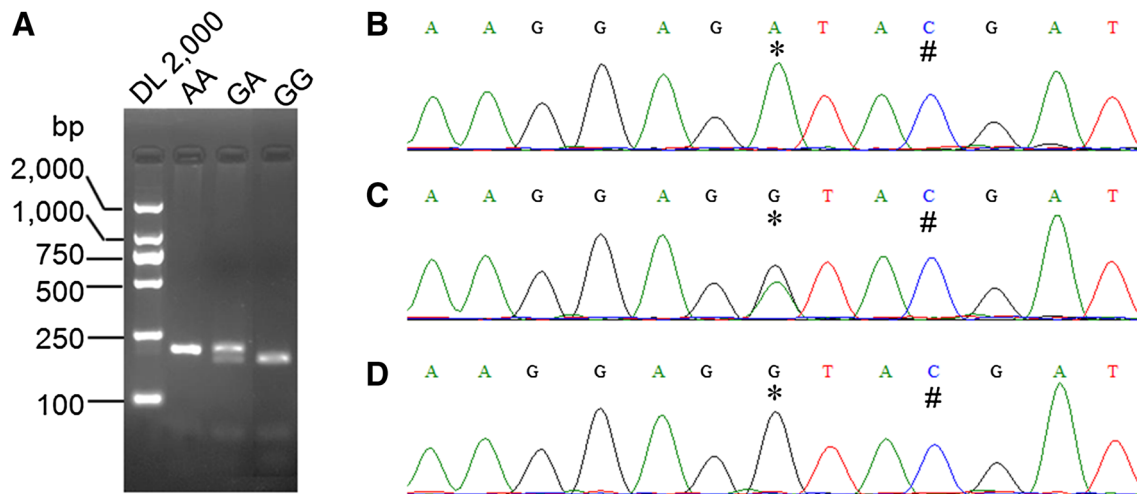


Fig. 3 Genotyping of the *CCL5* -403G>A site (rs2107538). **a**: Electrophoresis of DNA marker (DL 2000) and PCR-RFLP products with AA, GA, or GG genotypes. **b–d**: the three genotypes were demonstrated by sequencing as indicated with *asterisk*, respectively. # indicated the mutation introduced with primer

Table 2 Statistical comparisons (*P* values) of rs2280788 (–28C>G) or rs2107538 (–403G>A) between the IgE(+) group (*n* = 298) and the IgE(–) group (*n* = 139) analyzed with different genetic models by controlling sex and age simultaneously

Genetic model ^a	SNPs	Sex	16–29 years	30–44 years	≥45 years ^b	CMH ^c
Genotypic frequency	–28C>G	Female	0.390	0.195	0.322	– ^c
		Male	0.585	0.305	0.788	
	–403G>A	Female	0.725	0.065	0.202	– ^c
		Male	0.894	0.783	0.070	
Dominant model	–28C>G	Female	0.174	0.090	0.672	0.061
		Male	0.440	0.305	0.760	
	–403G>A	Female	0.686	0.234	0.160	0.656
		Male	0.659	0.942	0.032^b	
Recessive model	–28C>G	Female	0.605	0.902	0.213	0.797
		Male	0.614	– ^d	0.503	
	–403G>A	Female	0.443	0.127	0.102	0.055
		Male	0.794	0.499	0.175	
Multiplicative model (or Allelic frequency)	–28C>G	Female	0.172	0.145	0.980	0.080
		Male	0.585	0.335	0.660	
	–403G>A	Female	0.546	0.863	0.047^b	0.217
		Male	0.677	0.692	0.040^b	

^a The dominant model is used for major homozygotes versus combination of minor homo- and heterozygotes. The recessive model is used for minor homozygotes versus major homo- and heterozygotes. (Lewis 2002)

^b The *P* values in bold fonts were <0.05, and their corresponding ORs (95 % CIs) were 0.33 (0.12–0.93), 0.43 (0.18–1.00), and 0.46 (0.22–0.97) from top to bottom in this column

^c CMH: Cochran's and Mantel–Haenszel analysis. The *P* value was not available for the 2 × 3 contingency table in SPSS crosstabs analysis

^d The *P* value was not available due to the minor homozygote counts in both groups being zero

locus was relatively weak. When adjusted by sex and age, the *P* value of the global –28C>G/–403G>A haplotype association with the IgE(+)/(–) groups was 0.05 (Online

supplement Table 1). Interaction analysis of the haplotype and sex cross-classification indicated that the –28C/–403A haplotype in the male IgE(+) group had higher frequency

[$P < 0.05$, OR (95 % CI) = 2.19 (1.17–4.09), Table 3] than in the male IgE(−) group and female IgE(+) or IgE(−) groups.

Discussion

As IgE is one of the central players in allergic diseases (Galli and Tsai 2012), total and specific serum IgE assays were thought to be valuable for the diagnosis and therapy of ASD, though the total serum IgE concentration was affected by various factors, such as age, ethnicity, living environment, sex, smoking, and so on (De Amici and Ciprandi 2013; Jarvis et al. 1995; Carosso et al. 2007). For the reference values of total serum IgE concentrations in the patients at different age period <16 years varied greatly and the sample size of these patients visiting the dermatological outpatient department was small, the patients aged <16 years were not included in the present study. In addition, specified criteria were considered to including 437 ASD patients to reduce statistic complications derived from various affecting factors.

Among all the studied ASD patients, more than two-third of them was positive in the total serum IgE assay, which suggested the important participation of IgE in this kind of skin disorders. From the genetic aspect, being able to enhance the IgE production demonstrated by in vitro and in vivo experiments (Kimata et al. 1996), *CCL5* was one of the genes having promoter SNPs closely related to allergic diseases observed in human studies. In HIV infected subjects, −403A and −28G frequency were significantly increased (Ahlenstiel et al. 2005). The −403A allele frequency was increased with asthma (Fryer et al. 2000), wheezing (Tian et al. 2009), allergic rhinitis (Melake and Salama 2013), tuberculosis (Areeshi et al. 2013), atopic dermatitis (Tanaka et al. 2006) and its coupled high IgE level (Nickel et al. 2000). The −28G allele was a risk factor for asthma (Yao et al. 2003) and atopic dermatitis (Tanaka et al. 2006), and −28C>G polymorphism was associated with juvenile rheumatoid arthritis (Yao et al.

2009) and pneumonia (Doudouliaki et al. 2013). This locus was also related to the asthma types (Hizawa et al. 2002; Kaneko et al. 2013). For it was beyond our present scope, the previously reported relationship of these SNPs to more than allergic diseases (Tahara et al. 2009; Navratilova 2006) was not discussed herein. By consulting these previous reports and analyzing the linkage disequilibrium of the SNPs of *CCL5* with the Haploview software, the two SNPs in the promoter region of *CCL5*, −28C>G and −403G>A, were thus focused in our study. Their differences of genotype frequency and allele frequency (multiplicative model) were compared in 298 subjects with positive total serum IgE reaction and 139 subjects with negative reaction. The genotypic ratio of GG/(GA+AA) and allelic ration of G/A for the −403G>A locus was reduced in male and(or) female ≥45 years subgroup of IgE(+) group than of IgE(−) group, while −28C>G locus was irrelevant to IgE reaction. Very weak linkage existed between the two SNPs (consistent with the result in Han population analyzed with the Haploview software, see Online supplement Fig. 1), and −403A/−28C frequency in IgE(+) group was relatively higher. It was once reported that the presence of the A allele of −403G>A created a GATA transcription factor binding site and increased the basal promoter activity (Nickel et al. 2000), and the G carrier of −28C>G had higher *CCL5* transcription than the C carrier (Liu et al. 1999; Hizawa et al. 2002). Our findings supported the concept that the contribution of the two SNPs to the promoter activity varied in multiple ethnically diverse populations as pointed out by Nickel et al. (Nickel et al. 2000), and −403G allele probably dominated the transcription activity in the Han Chinese ASD patients.

We also noticed that *CCL5* was not listed in the hundreds of genes associated with total serum IgE level in healthy population and non-ASD patients reported recently by some of the genome-wide association studies (Weidinger et al. 2008; Granada et al. 2012; Liao et al. 2013; Yatagai et al. 2013; Kim et al. 2013b; Levin et al. 2013), and the inconsistent genes prevailed over the consistent ones among these studies. This phenomenon was probably

Table 3 Haplotype of *CCL5* and sex cross-classification interaction adjusted by age group analyzed with the SNPStats online software (Sole et al. 2006) ($n = 437$, interaction $P = 0.43$)

Haplotype	Haplotype frequencies estimation, %		OR (95 % CI)	
	IgE(+) group	IgE(−) group	Female	Male
CG	61.04	63.12	1.00	1.32 (0.69–2.53)
CA	27.21	21.06	1.20 (0.77–1.88)	2.19 (1.17–4.09)^a
GA	10.54	12.40	0.85 (0.46–1.57)	1.50 (0.65–3.45)
GG	1.21	3.43	0.13 (0.02–1.19)	1.09 (0.24–4.97)

Haplotype of rs2280788 (−28C>G) and rs2107538 (−403G>A)

Age groups were 16–29, 30–44, and ≥45 years

^a The OR (95 % CI) in bold fonts had $P < 0.05$

caused by various genetic and environment factors. Thus, further studies on the mechanisms of CCL5's regulation on IgE secretion in ASD patients were needed to understand complicating factors contributing to the disparities among those findings.

Besides the total serum IgE assay, specific IgE discrimination is valuable to direct ASD patients to avoid their sensitive allergen(s). Since HDM is the most common allergen globally (Thomas 2010; Takagi et al. 2005; Wan et al. 2010; Wang and Zhang 2012; An et al. 2013; Zhang et al. 2012; Kim et al. 2013a), it helps to interpret its first-ranking in the 14 kinds of serum allergen-specific IgE assay in our ASD patients. The species of mites in house dust were close to 40 (Frankland 1972), and the *Blomia tropicalis* was reported to be the most abundant species in HDM in many Asia-Pacific regions (Thomas 2010). Though this species of HDM was reported to account for 17 % of HDM in Shenzhen (Wang et al. 2009), the predominant mite species for our ASD patients awaited further analysis with specific antibodies. Shellfish allergy was recently noticed to be one of the major allergic problems in Asia-Pacific regions (Klaewsongkram 2012), and it was postulated to be associated with high prevalence of HDM allergy since the cross-reactivity probably existed between mites and shrimps (Arlian et al. 2009), and a significant correlation between shrimp, cockroach, and dust mite IgE levels in allergic children was observed (Wang et al. 2011). Thus, it was reasonable for us to find out that being sensitive to fish, shrimp, and crab had the highest prevalence among all the food allergy when HDM was the top one in all the allergens.

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Conflict of interest The authors had no conflict of interests to declare.

Ethical statement There were no new therapeutic technologies and medicines applied in the study. The protocol was approved by the Ethics Committee of Shenzhen Center for Chronic Disease Control, and got written consents of the participants.

References

- Ahlenstiel G, Iwan A, Nattermann J, Bueren K, Rockstroh JK, Brackmann HH, Kupfer B, Landt O, Peled A, Sauerbruch T et al (2005) Distribution and effects of polymorphic RANTES gene alleles in HIV/HCV coinfection—a prospective cross-sectional study. *World J Gastroenterol* 11:7631–7638
- An S, Chen L, Long C, Liu X, Xu X, Lu X, Rong M, Liu Z, Lai R (2013) Dermatophagoides farinae allergens diversity identification by proteomics. *Mol Cell Proteomics* 12:1818–1828
- Appay V, Dunbar PR, Cerundolo V, McMichael A, Czaplewski L, Rowland-Jones S (2000) RANTES activates antigen-specific cytotoxic T lymphocytes in a mitogen-like manner through cell surface aggregation. *Int Immunol* 12:1173–1182
- Areeshi MY, Mandal RK, Panda AK, Haque S (2013) A meta-analysis of the association between the CC chemokine ligand 5 (CCL5) -403 G > A gene polymorphism and tuberculosis susceptibility. *PLoS ONE* 8:e72139
- Arlian LG, Morgan MS, Vyszynski-Moher DL, Sharra D (2009) Cross-reactivity between storage and dust mites and between mites and shrimp. *Exp Appl Acarol* 47:159–172
- Bacon KB, Premack BA, Gardner P, Schall TJ (1995) Activation of dual T cell signaling pathways by the chemokine RANTES. *Science* 269:1727–1730
- Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA (2004) Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 74:106–120
- Carosso A, Bugiani M, Migliore E, Anto JM, DeMarco R (2007) Reference values of total serum IgE and their significance in the diagnosis of allergy in young European adults. *Int Arch Allergy Immunol* 142:230–238
- DaVeiga SP (2012) Epidemiology of atopic dermatitis: a review. *Allergy Asthma Proc* 33:227–234
- De Amici M, Ciprandi G (2013) The age impact on serum total and allergen-specific IgE. *Allergy Asthma Immunol Res* 5:170–174
- Doudouliaki T, Haidopoulou K, Pappa S, Sakellaropoulou A, Tsakiridis P, Emboriadou M, Hatzistilianou M (2013) Association of the RANTES gene promoter polymorphisms -28C/G and -403G/A with pneumonia in Greek children. *Int J Immunopathol Pharmacol* 26:681–690
- Evans PM, Liu C (2005) SiteFind: a software tool for introducing a restriction site as a marker for successful site-directed mutagenesis. *BMC Mol Biol* 6:22
- Frankland AW (1972) House dust mites and allergy. *Arch Dis Child* 47:327–329
- Fryer AA, Spiteri MA, Bianco A, Hepple M, Jones PW, Strange RC, Makki R, Tavernier G, Smilie FI, Custovic A et al (2000) The -403 G > A promoter polymorphism in the RANTES gene is associated with atopy and asthma. *Genes Immun* 1:509–514
- Galli SJ, Tsai M (2012) IgE and mast cells in allergic disease. *Nat Med* 18:693–704
- Granada M, Wilk JB, Tuzova M, Strachan DP, Weidinger S, Albrecht E, Gieger C, Heinrich J, Himes BE, Hunninghake GM et al (2012) A genome-wide association study of plasma total IgE concentrations in the Framingham heart study. *J Allergy Clin Immunol* 129(840–845):e821
- Hartmann TN, Leick M, Ewers S, Diefenbacher A, Schraufstatter I, Honczarenko M, Burger M (2008) Human B cells express the orphan chemokine receptor CCR4-1 in a maturation-stage-dependent and CCL5-modulated manner. *Immunology* 125:252–262
- Hizawa N, Yamaguchi E, Konno S, Tanino Y, Jinushi E, Nishimura M (2002) A functional polymorphism in the RANTES gene promoter is associated with the development of late-onset asthma. *Am J Respir Crit Care Med* 166:686–690
- Jarvis D, Luczynska C, Chinn S, Burney P (1995) The association of age, gender and smoking with total IgE and specific IgE. *Clin Exp Allergy* 25:1083–1091
- Jenerowicz D, Silny W, Danczak-Pazdrowska A, Polanska A, Osmola-Mankowska A, Olek-Hrab K (2012) Environmental factors and allergic diseases. *Ann Agric Environ Med* 19:475–481

- Kaneko Y, Masuko H, Sakamoto T, Iijima H, Naito T, Yatagai Y, Yamada H, Konno S, Nishimura M, Noguchi E et al (2013) Asthma phenotypes in Japanese adults—their associations with the CCL5 and ADRB2 genotypes. *Allergol Int* 62:113–121
- Kim EJ, Kwon JW, Lim YM, Yoon D, Seo JH, Chang WS, Kim HY, Park JW, Cho SH, Hong SJ et al (2013a) Assessment of total/specific IgE levels against 7 inhalant allergens in children aged 3–6 years in Seoul, Korea. *Allergy Asthma Immunol Res* 5:162–169
- Kim JH, Cheong HS, Park JS, Jang AS, Uh ST, Kim YH, Kim MK, Choi IS, Cho SH, Choi BW et al (2013b) A genome-wide association study of total serum and mite-specific IgEs in asthma patients. *PLoS ONE* 8:e71958
- Kimata H, Yoshida A, Ishioka C, Fujimoto M, Lindley I, Furusho K (1996) RANTES and macrophage inflammatory protein 1 alpha selectively enhance immunoglobulin (IgE) and IgG4 production by human B cells. *J Exp Med* 183:2397–2402
- Kiyohara C, Tanaka K, Miyake Y (2008) Genetic susceptibility to atopic dermatitis. *Allergol Int* 57:39–56
- Klaewsongkram J (2012) High prevalence of shellfish and house dust mite allergies in Asia-Pacific: probably not just a coincidence. *Asian Pac J Allergy Immunol* 30:247–248
- Levin AM, Mathias RA, Huang L, Roth LA, Daley D, Myers RA, Himes BE, Romieu I, Yang M, Eng C et al (2013) A meta-analysis of genome-wide association studies for serum total IgE in diverse study populations. *J Allergy Clin Immunol* 131:1176–1184
- Lewis CM (2002) Genetic association studies: design, analysis and interpretation. *Brief Bioinform* 3:146–153
- Liao M, Shi D, Wang Y, Zhang K, Chen X, Gao Y, Tan A, Xuan Q, Yang X, Hu Y et al (2013) Genome-wide scan on total serum IgE levels identifies no common variants in a healthy Chinese male population. *Immunogenetics* 65:561–568
- Lira SA, Furtado GC (2012) The biology of chemokines and their receptors. *Immunol Res* 54:111–120
- Liu H, Chao D, Nakayama EE, Taguchi H, Goto M, Xin X, Takamatsu JK, Saito H, Ishikawa Y, Akaza T et al (1999) Polymorphism in RANTES chemokine promoter affects HIV-1 disease progression. *Proc Natl Acad Sci USA* 96:4581–4585
- Marques RE, Guabiraba R, Russo RC, Teixeira MM (2013) Targeting CCL5 in inflammation. *Expert Opin Ther Targets* 17:1439–1460
- Melake NA, Salama MA (2013) Association of RANTES gene –403 G/A polymorphism with allergic rhinitis patients. *World J of Med Sci* 8:150–156
- Mueller CG, Boix C, Kwan WH, Daussy C, Fournier E, Fridman WH, Molina TJ (2007) Critical role of monocytes to support normal B cell and diffuse large B cell lymphoma survival and proliferation. *J Leukoc Biol* 82:567–575
- Navratilova Z (2006) Polymorphisms in CCL2&CCL5 chemokines/chemokine receptors genes and their association with diseases. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 150:191–204
- Nelson PJ, Kim HT, Manning WC, Goralski TJ, Krensky AM (1993) Genomic organization and transcriptional regulation of the RANTES chemokine gene. *J Immunol* 151:2601–2612
- Nichols KM, Cook-Bolden FE (2009) Allergic skin disease: major highlights and recent advances. *Med Clin North Am* 93:1211–1224
- Nickel RG, Casolaro V, Wahn U, Beyer K, Barnes KC, Plunkett BS, Freidhoff LR, Sengler C, Plitt JR, Schleimer RP et al (2000) Atopic dermatitis is associated with a functional mutation in the promoter of the C–C chemokine RANTES. *J Immunol* 164:1612–1616
- Okada H, Kuhn C, Feillet H, Bach JF (2010) The ‘hygiene hypothesis’ for autoimmune and allergic diseases: an update. *Clin Exp Immunol* 160:1–9
- Pawankar R, Canonica GW, Holgate ST, Lockey RF (2011) WAO White Book on Allergy 2011–2012: Executive summary. world allergy organization (WAO) http://www.worldallergy.org/UserFiles/file/WAO-White-Book-on-Allergy_web.pdf
- Rosenwasser LJ (2011) Mechanisms of IgE inflammation. *Curr Allergy Asthma Rep* 11:178–183
- Sole X, Guino E, Valls J, Iniesta R, Moreno V (2006) SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 22:1928–1929
- Steinke JW, Borish L (2006) Genetics of allergic disease. *Med Clin North Am* 90:1–15
- Sullivan NL, Eickhoff CS, Zhang X, Giddings OK, Lane TE, Hoft DF (2011) Importance of the CCR5-CCL5 axis for mucosal *Trypanosoma cruzi* protection and B cell activation. *J Immunol* 187:1358–1368
- Tahara T, Shibata T, Yamashita H, Hirata I, Arisawa T (2009) The role of RANTES promoter polymorphism in functional dyspepsia. *J Clin Biochem Nutr* 45:235–240
- Takagi K, Teshima R, Okunuki H, Hachisuka A, Sawada J-i, Kojima K, Takahashi K, Ohsawa M, Yoshida T (2005) Survey of food and airborne allergen-specific IgE levels in a general population of 3-year-old Japanese children. *Allergol Int* 54:581–587
- Tanaka K, Roberts MH, Yamamoto N, Sugiura H, Uehara M, Hopkin JM (2006) Upregulating promoter polymorphisms of RANTES relate to atopic dermatitis. *Int J Immunogenet* 33:423–428
- Thomas WR (2010) Geography of house dust mite allergens. *Asian Pac J Allergy Immunol* 28:211–224
- Tian M, Liu F, Wen GY, Shi SY, Chen RH, Zhao DY (2009) Effect of variation in RANTES promoter on serum RANTES levels and risk of recurrent wheezing after RSV bronchiolitis in children from Han, Southern China. *Eur J Pediatr* 168:963–967
- Wan KS, Yang W, Wu WF (2010) A survey of serum specific-IgE to common allergens in primary school children of Taipei City. *Asian Pac J Allergy Immunol* 28:1–6
- Wang RQ, Zhang HY (2012) Two hundred thousands results of allergen specific IgE detection. *Chin J Allergy Clin Immunol* 06:18–23
- Wang B, Wu J, Liu ZG, Ran PX, Gao Q, Luo CH, Ai M (2009) Mites in mattress dust and relevant environmental factors in student dormitories in Shenzhen. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 27(89–90):93
- Wang J, Calatroni A, Visness CM, Sampson HA (2011) Correlation of specific IgE to shrimp with cockroach and dust mite exposure and sensitization in an inner-city population. *J Allergy Clin Immunol* 128:834–837
- Weidinger S, Gieger C, Rodriguez E, Baurecht H, Mempel M, Klopp N, Gohlke H, Wagenpfeil S, Ollert M, Ring J et al (2008) Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. *PLoS Genet* 4:e1000166
- Yao TC, Kuo ML, See LC, Chen LC, Yan DC, Ou LS, Shaw CK, Huang JL (2003) The RANTES promoter polymorphism: a genetic risk factor for near-fatal asthma in Chinese children. *J Allergy Clin Immunol* 111:1285–1292
- Yao TC, Tsai YC, Huang JL (2009) Association of RANTES promoter polymorphism with juvenile rheumatoid arthritis. *Arthritis Rheum* 60:1173–1178
- Yatagai Y, Sakamoto T, Masuko H, Kaneko Y, Yamada H, Iijima H, Naito T, Noguchi E, Hirota T, Tamari M et al (2013) Genome-wide association study for levels of total serum IgE identifies HLA-C in a Japanese population. *PLoS ONE* 8:e80941
- Zhang C, Li J, Lai X, Zheng Y, Gjessing B, Spangfort MD, Zhong N (2012) House dust mite and storage mite IgE reactivity in allergic patients from Guangzhou, China. *Asian Pac J Allergy Immunol* 30:294–300

Online supplement materials: 1 table and 1 figure.

RESEARCH ARTICLE

**Association of IgE mediated Allergen Sensitivity and Promoter Polymorphisms of Chemokine (C-C motif)
ligand 5 Gene in Han Chinese Patients with Allergic Skin Diseases**

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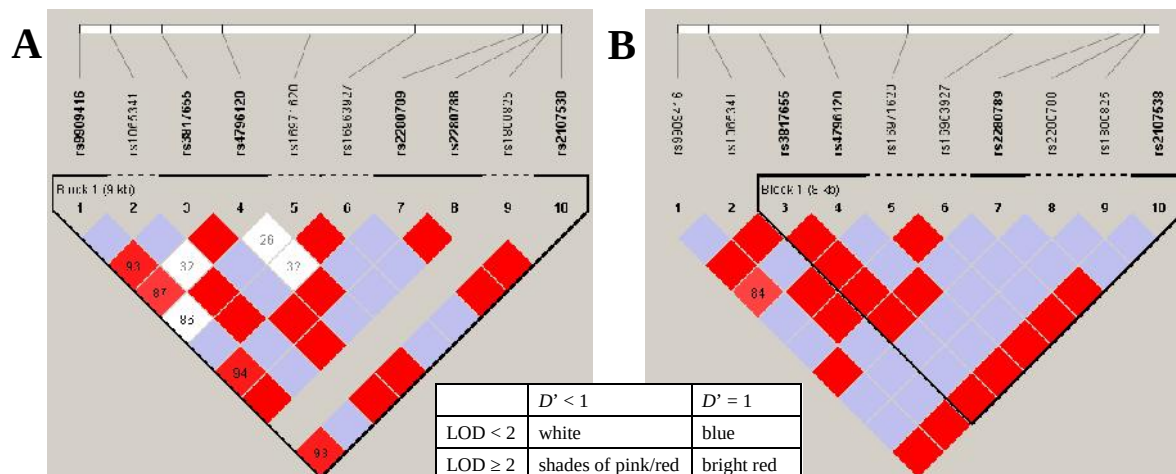
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Short running head: CCL5 SNPs and IgE-mediated allergic skin diseases



Online supplement figure 1 SNPs association analysis on chemokine (C-C motif) ligand 5 (*CCL5*) gene ($\pm$ 1kbp) in Han Chinese in Beijing, China (**A**) and Utah residents with ancestry from northern and western Europe (**B**). Data were downloaded from International HapMap project (<http://hapmap.ncbi.nlm.nih.gov/>), and the diagrams were generated with Haploview software with default parameter settings. In the linkage disequilibrium analysis of rs2280788 (-28C>G) and rs2107538 (-403G>A), $LOD = 3.48$, $D' = 1.0$, and $R^2 = 0.304$ in Han population (data of rs1800825 were not available), while $LOD = 0$, $D' = 1$, and $R^2 = 0$ in Utah population.

Online supplemental table1 Haplotype association of rs2280788 (-28C>G) and rs2107538 (-403G>A) with group classification adjusted by age and sex ($n = 437$, global haplotype association $P = 0.05$)^a

-28C>G	-403G>A	Frequency, %	OR (95% CI)	P value
C	G	61.64	1.00	—
C	A	25.31	1.34 (0.94-1.92)	0.11
G	A	11.07	0.89 (0.55-1.45)	0.65
G	G	1.97	0.38 (0.13-1.13)	0.083

^a Age groups were 16-29 y, 30-44 y, and ≥ 45 y.