Polymorphic markers of several immune regulatory genes modulate the susceptibility for eczema and related phenotypes in children

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Background: Eczema is associated with multiple genes regulating epidermal barrier functions and immunological pathways. However, their epistatic interactions are not well studied. This cross-sectional study investigated the relationship between childhood eczema phenotypes and single-nucleotide polymorphisms (SNPs) of immune regulatory genes.

Methods: One thousand three hundred and twenty-nine Chinese eczematous children and 1,179 non-allergic controls were recruited. Nine SNPs of immune regulatory genes signal transducer and activator of transcription 3 (*STAT3*), interleukin-10 (*IL10*), transforming growth factor-beta 1 (*TGFB1*), and IL-6 receptor (*IL6R*) were genotyped by TaqMan genotyping assays. Logistic regression was used to analyze the associations between SNPs and eczema phenotypes. Generalized multifactor dimensionality reduction (GMDR) was used to examine epistatic interactions among these SNPs as well as those reported by our group [filaggrin (*FLG*) and 11q13] for eczema phenotypes.

Results: *TGFB1*_rs1800469 was found to be associated with eczema [odds ratio (OR), 0.82; 95% confidence interval (CI): 0.73–0.92; P=0.001], atopic eczema (OR, 0.83; 95% CI: 0.72–0.95; P=0.009) and allergic rhinitis (OR, 0.84; 95% CI: 0.74–0.95; P=0.005). We also found a trend between *IL10*_rs1800872 and increased total immunoglobulin E (IgE) levels (P=0.009). Epistatic interaction among *IL10*_rs3021094, *TGFB1*_rs1800469, *IL6R*_rs2228145, and *STAT3*_rs4796793 were found for total IgE [testing accuracy (TA), 0.551; cross-validation consistency (CVC), 10; P=0.014]. Mean log-transformed total IgE (logIgE) levels in high-risk cases, low-risk cases, high-risk controls, and low-risk controls were 2.75, 2.60, 1.90, and 1.81 respectively (P=0.019 for trend).

Conclusions: Functional *TGFB1* polymorphism is associated with both eczema and allergic rhinitis, suggesting the role of TGF-β1 in allergy susceptibility. *IL10* may be associated with increased total IgE levels. Interaction among immune regulatory genes modulates total IgE levels.

Keywords: Eczema; genetics; regulatory cytokine; rhinitis; transforming growth factor-beta (TGF-β)

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Introduction

According to the Global Burden of Disease Study in 2019, eczema has an age-standardized prevalence rate of 2.28%, with peak prevalence in children aged between five and nine (1). It accounts for 36.17% of immune-mediated inflammatory disease cases and is more commonly observed in individuals with higher socio-demographic status (1,2). The treatment modality of choice is individualized, primarily depending on patients' signs and symptoms (3). Damiani *et al.* established an Italian guideline to maintain a standard of care for eczema control and guide practitioners in adopting the novel biologic—dupilumab for eczema (3).

The pathogenesis of eczema is multifactorial with environmental factors and genetic predisposition; these patients are thought to have skin barrier defects accompanied by skin microbial dysbiosis (4). Studies from the coronavirus disease 2019 (COVID-19) pandemic suggested mask-wearing may be associated with exacerbated eczema (5), possibly owing to the increased abundance of *Malassezia fungi* on skin (6,7). The composition of cutaneous microbiome not only varies between healthy and eczematous individuals, but also with age among the latter group (8). Genome-wide association study (GWAS) identified complex network of genes for eczema and allergic diseases. Elevated immunoglobulin E (IgE), a common laboratory finding in patients with atopic dermatitis, positively correlated with eczema severity and other allergic symptoms. IgE is produced by plasma cells as part of the adaptive immune response; this pathway is promoted by type 2 helper T (Th2) cell-mediated cytokines interleukin (IL)-4 and IL-13 (9). The abovementioned dupilumab acts against IL-4 receptors, thus hindering receptor-ligand binding and its downstream pathway (10).

Despite recent success of GWAS, previously studied populations only shared a limited number of candidate genes for the association with different eczema phenotypes; few were tested for epistatic interactions along the gene network. The main eczema predisposition genes included in the studies were those encoding epidermal barrier protein filaggrin (*FLG*) and Th2 pathway (11,12). *FLG* on chromosome 1q21 was the most studied and replicated gene for eczema in Caucasian children, yet many loss-of-function mutations of *FLG* that were identified in Europeans are less frequently detected in Asians.

Our group screened five published *FLG* mutations in southern Chinese children, and all of them were rare in our population (13). Besides genetics, the laboratory findings in eczema patients, such as blood IgE and eosinophil percentage (eos%), were different among eczematous patients of similar disease severity between Asian and Caucasian populations (14). Since corresponding data for the Asian population is lacking, our study serves to fill this knowledge gap.

Transforming growth factor-beta 1 (TGF-β1) interacts with multiple cell types to inhibit cell proliferation and apoptosis (15). IL-10, produced by B1 lymphocytes and found in normal skin (16), serves two main functions: (I) inhibiting synthesis of pro-inflammatory cytokines; and (II) inducing class switching in B cells (17). IL-6 is crucial for skin barrier repair and for forming complexes with IL-6 receptor (*IL6R*), which then migrate to the damaged epidermal layers for repairing permeability barriers through increased signal transducer and activator of transcription 3 (*STAT3*) phosphorylation (18). STAT3 is a key transcription regulator with multiple roles in inflammation and immune responses; mutations of this gene are associated with immunological diseases such as hyper-IgE syndrome. STAT3 activation is also involved in IL-10 and IL-6 anti-inflammatory signaling.

Highlight box

Key findings
- Transforming growth factor-beta 1 (*TGFB1*)_rs1800469 is associated with childhood eczema (including atopic eczema) and allergic rhinitis.
- Interleukin-10 (*IL10*) may be associated with increased total immunoglobulin E (IgE) levels.
- Individuals' total IgE levels may be modulated by particular single-nucleotide polymorphisms (SNPs) of immune regulatory genes (*IL10*_rs3021094, *TGFB1*_rs1800469, IL-6 receptor_rs2228145, and signal transducer and activator of transcription 3_rs4796793).

What is known and what is new?
- Certain gene mutations are associated with eczema susceptibility in the Caucasian population, but the previously identified eczema susceptibility gene polymorphisms in Caucasians have limited generalizability in Asians due to lower prevalences.
- This study serves to provide data on genetic associations between eczema and its related subphenotypes with certain immune regulatory gene SNPs particularly of the southern Chinese population, which was previously lacking in literature.

What is the implication, and what should change now?
- This genetic association study supports the importance of immune regulatory genes in the pathogenesis of eczema in children.
- Testing for polymorphic markers of *TGFB1* and other immune regulatory genes facilitates the risk prediction for childhood eczema.

This study investigated the genetic associations between eczema and related subphenotypes with single-nucleotide polymorphisms (SNPs) of four major immune regulatory genes *TGFB1*, *IL10*, *IL6R*, and *STAT3* in southern Chinese children. We present this article in accordance with the MDAR and STROBE reporting checklists (available at https://tp.amegroups.com/article/view/10.21037/tp-23-474/rc).

Methods

Subjects

This study recruited unrelated children, including those without siblings, with physician-diagnosed eczema and non-allergic controls from both pediatric clinics of our university-affiliated teaching hospital and several community-based studies in Hong Kong [19-22]. The latter community-based studies were conducted in local schoolchildren primarily for elucidating the epidemiology of childhood obesity and metabolic syndrome [23-26]. The participating subjects who suffered from physician-diagnosed eczema and those free from any allergic disease were also recruited into this study. Their blood samples were subjected to our SNP genotyping and specific IgE (sIgE) measurement. All subjects were aged 18 years or younger, whose parents reported themselves as Chinese. Patients' eczema severity was evaluated by SCORing Atopic Dermatitis (SCORAD). Controls did not have history of eczema, asthma, and allergic rhinitis. Demographics, early-life events and environmental exposures were recorded by the validated Chinese questionnaire of the International Study of Asthma and Allergies in Childhood. The Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee approved this study (Nos. 2008.123 and 2016.171). Subjects and/or their parents gave informed written consent to participate in this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Eczema subphenotypes

The subphenotypes of eczema refer to factors which predict progression to eczema and its severity [27]. Eos%, expressed as the percentage of total peripheral blood leukocytes, positively correlates with serum total IgE concentration, early eczema onset, and persistence of eczematous lesions [28]. Allergen sensitization, measured by skin prick test (SPT) or IgE levels, is often present in eczematous children (19,20,29,30).

Serum total IgE concentration was measured by microparticle immunoassay (IMx Analyzer; Abbott Laboratories, Abbott Park, IL, USA), then log-transformed (logIgE) before analysis. Peripheral blood eosinophils and other leukocytes were counted by Coulter STKS Counter (Beckman Coulter, Brea, CA, USA). Allergen sensitization was assessed by either SPT with standardized crude extracts of *Dermatophagoides pteronyssinus*, cat dander, and mixed cockroaches (ALK Abelló, Round Rock, TX, USA) or by plasma allergen-sIgE levels to the same allergens by fluorescent enzyme immunoassay (AutoCAP, Phadia AB, Uppsala, Sweden) as decided by the individual studies. In general, SPT was used in studies involving subjects recruited in hospitals while blood IgE assays were used in community-based studies. SPT results with wheal ≥3 mm larger than negative controls and sIgE ≥0.35 kIU/L were considered as positive. Subjects were defined as "atopic" if they had ≥ one positive SPT or sIgE result.

SNP selection and genotyping

Table 1 describes nine SNPs of the four genes being genotyped in this study. The tagging strategy was applied for SNPs with minor allele frequency ≥0.05 and pairwise $r^2 \geq 0.8$ that were within 5-kb both upstream and downstream from the top eczema-associated SNP in the respective target gene. SNPs published to have significant associations with eczema were all included in SNP selection. Nine SNPs selected (four for *IL10*, one for *TGFB1*, three for *IL6R*, and one for *STAT3*) were genotyped by TaqMan SNP Genotyping assays (Applied Biosystems, Waltham, MA, USA) using a 12-K Quant Studio thermocycler (Applied Biosystems).

Statistical analysis

Allele frequencies of SNPs were estimated by gene counting method, and χ^2 or Fisher exact test was used to determine Hardy-Weinberg equilibrium (HWE). Pairwise linkage disequilibrium (LD) coefficient was calculated for each SNP pair by HaploView software (Daly Lab, Boston, MA, USA). Associations between SNPs with dichotomous eczema and allergy outcomes were analyzed by logistic regression and those with eczema subphenotypes by linear regression, adjusting for age and sex as covariates.

Epistatic interactions between SNPs for eczema and its subphenotypes were evaluated using generalized multifactor

Table 1 Details of nine SNPs selected in this study

Gene	SNP	Position	Major/minor allele	Genotyping efficiency (%)	HWE in controls	HWE in cases
IL10	rs1800872	1q32.1	T/G	98.0	0.05	0.75
	rs1800896		T/C	99.1	0.32	0.65
	rs3790622		G/A	99.2	0.27	0.76
	rs3021094		G/T	98.6	0.66	0.43
TGFB1	rs1800469	19q13.2	A/G	97.6	0.52	0.29
IL6R	rs2228145	1q21.3	A/C	97.5	0.55	0.95
	rs6689393		A/G	98.9	0.36	0.80
	rs4845374		A/T	98.4	0.51	0.99
STAT3	rs4796793	17q21.2	C/G	98.6	0.81	0.82

SNP, single-nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; *IL10*, interleukin-10; *TGFB1*, transforming growth factor-beta 1; *IL6R*, IL-6 receptor; *STAT3*, signal transducer and activator of transcription 3.

dimensionality reduction (GMDR) beta version 0.9. (http://ibi.zju.edu.cn/software/GMDR/download.html); GMDR computed the maximum-likelihood estimates and the score values of all individuals under the null hypothesis. In the evaluation of the interactive models, outcome parameters including (I) testing accuracy (TA); (II) cross-validation consistency (CVC); and (III) statistical significance of the model were considered. TA, which measures the ability to classify individuals with respect to their score values, was used to choose the best model; a TA score of 0.5 indicated that the model is equal to 50% chance of correct prediction, whereas a score of 1.0 indicates a perfect prediction. CVC evaluated the consistency with which the selected interaction was classified as the best model among all possible combinations. The statistical significance of a model was determined by comparing the average prediction error from the observed data with the distribution of average prediction errors under the null hypothesis of the absence of association, derived empirically from 5,000 permutations. The null hypothesis was rejected when the P value derived from permutation was <0.05.

Subjects were classified by GMDR into low-risk and high-risk groups; their genotypes predicted the respective risks for having eczematous phenotypes and subphenotypes such as high eos% and raised total IgE levels. The results were stratified by subjects' eczema status. Ten-fold cross-validation was performed, and the possible number of loci was set to include our nine chosen SNPs, as well as additional *FLG* SNPs and SNPs on the chromosome 11q13 locus (19,22) that our group previously published for

eczema phenotypes. One-way analysis of variance (ANOVA) and Kruskal-Wallis test with post-hoc tests were used to compare logIgE level and eos% among different risk groups classified by GMDR. P<0.006 (0.05/9) was considered statistically significant after Bonferroni correction to adjust for multiple statistical testing.

Results

A total of 1,329 eczematous children and 1,179 non-allergic controls were recruited; data for logIgE level and eos% were available in 1,487 and 1,332 subjects, respectively. *Table 2* summarises the clinical features of subjects. Control group subjects were significantly older than those with eczema (mean, 13.6 *vs.* 11.2 years; P<0.001). Subjects with eczema had significantly higher eos%, logIgE level, and atopy values than controls (P<0.001).

All nine SNPs were successfully genotyped in over 97.5% of samples, and they met HWE (*Table 1*). *TGFB1_*rs1800469 was associated with both eczema [odds ratio (OR), 0.82; 95% confidence interval (CI): 0.73–0.92; P=0.001] (*Table 3*) and atopic eczema (OR, 0.83; 95% CI: 0.72–0.95; P=0.009), with the former association still significant after adjusting for coexisting asthma (P=0.005). This SNP was also associated with allergic rhinitis (OR, 0.84; 95% CI: 0.74–0.95; P=0.005) (*Table 4*). *TGFB1_*rs1800469 was not associated with other subphenotypes of eczema (Tables S1,S2), while *STAT3_*rs4796793 was inversely associated with SCORAD for patients' eczema severity when analysed by the dominant model (β, –8.35;

Table 2 Clinical characteristics of study participants

Variable	Eczema	Control	P
Sex, n (%)	n=1,329	n=1,179	0.001
Male	676 (50.9)	519 (44.0)	
Female	653 (49.1)	660 (56.0)	
Age (years), mean ± SD	11.2±4.3	13.6±4.5	<0.001
Eos%	n=725	n=607	
Mean ± SD	6.9±4.9	2.7±2.4	<0.001
LogIgE level	n=861	n=626	
Mean ± SD	2.7±0.8	1.8±0.7	<0.001
Atopy[†]	n=946	n=553	
Mean ± SD	759 (80.2)	296 (53.5)	<0.001
SCORAD	n=398	n=0	
Mean ± SD	30.8±19.4	NA	NA
With moderate-to-severe eczema (objective SCORAD ≥15) (%)	57.3	NA	NA
History of allergic rhinitis (%)	66.3	NA	NA
History of asthma (%)	31.8	NA	NA

[†], defined as ≥ one positive result by SPT or allergen-sIgE to house dust mite, cat and cockroach. SD, standard deviation; eos%, eosinophil percentage; logIgE, log-transformed total IgE; IgE, immunoglobulin E; SCORAD, SCORing Atopic Dermatitis; NA, not applicable; sIgE, specific IgE.

Table 3 Logistic regression analysis between eczema and SNPs of immune regulatory genes

Gene	SNP	Position	Alleles (major/minor)	MAF (%)		Eczema		Atopic eczema	
				Control	Eczema	OR (95% CI)	P[†]	OR (95% CI)	P[†]
IL10	rs1800872	1q32.1	T/G	29.8	28.9	0.95 (0.84–1.08)	0.409	0.94 (0.81–1.09)	0.380
	rs1800896	1q32.1	T/C	4.7	4.8	1.04 (0.79–1.36)	0.802	1.15 (0.84–1.57)	0.379
	rs3790622	1q32.1	G/A	5.2	5.3	0.94 (0.72–1.22)	0.628	1.00 (0.74–1.35)	0.976
	rs3021094	1q32.1	T/G	46.9	47.6	1.02 (0.91–1.14)	0.750	1.02 (0.89–1.16)	0.814
TGFB1	rs1800469	19q13.2	A/G	44.3	39.6	0.82 (0.73–0.92)	0.001	0.83 (0.72–0.95)	0.009
IL6R	rs2228145	1q21.3	A/C	33.2	36.4	1.13 (1.00–1.28)	0.053	1.15 (0.99–1.33)	0.062
	rs6689393	1q21.3	A/G	45.4	48.2	1.10 (0.98–1.23)	0.124	1.05 (0.92–1.21)	0.456
	rs4845374	1q21.3	A/T	12.8	11.9	0.94 (0.79–1.12)	0.485	0.81 (0.65–1.00)	0.052
STAT3	rs4796793	17q21.2	C/G	41.9	43.2	1.09 (0.97–1.23)	0.149	1.04 (0.91–1.20)	0.572

[†], adjusted for age and sex as covariates. SNP, single-nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; IL10, interleukin-10; TGFB1, transforming growth factor-beta 1; IL6R, IL-6 receptor; STAT3, signal transducer and activator of transcription 3.

95% CI: –14.10 to –2.60; P=0.005) (Table S2). The IL6R GT haplotype from rs6689393 and rs4845374 was associated with eczema but not atopic eczema (P=0.047) (Table S3). This association, however, became insignificant after Bonferroni correction.

GMDR analysis revealed significant epistatic interaction among four SNPs rs3021094, rs1800469, rs2228145 and rs4796793 for logIgE level after adjustment for age and sex

Table 4 Logistic regression for the association between SNPs and comorbid eczema and allergic rhinitis (n=2,449)

Gene	SNP	MAF (%)		Adjusted OR (95% CI)[†]	P
		Control	Allergic rhinitis		
IL10	rs1800872	30.4	28.0	0.87 (0.77–0.99)	0.041
	rs1800896	4.5	5.3	1.11 (0.84–1.47)	0.448
	rs3790622	5.1	5.1	0.98 (0.75–1.28)	0.870
	rs3021094	47.6	46.5	0.95 (0.85–1.07)	0.396
TGFB1	rs1800469	43.2	39.1	0.84 (0.74–0.95)	0.005
IL6R	rs2228145	34.1	36.0	1.07 (0.95–1.22)	0.277
	rs6689393	46.4	47.5	1.03 (0.91–1.16)	0.613
	rs4845374	12.6	11.8	0.95 (0.79–1.14)	0.591
STAT3	rs4796793	42.7	42.5	1.02 (0.90–1.15)	0.770

[†], analysed by additive model and adjusted for age and sex as covariates. SNP, single-nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; *IL10*, interleukin-10; *TGFB1*, transforming growth factor-beta 1; *IL6R*, IL-6 receptor; *STAT3*, signal transducer and activator of transcription 3.

Table 5 GMDR analysis for logIgE levels

Model formed by *IL10*, *TGFB1*, *IL6R*, and *STAT3*	CVC	TA	Cutoff TA 0.05	Cutoff TA 0.01	P
rs2228145	9	0.523	0.534	0.543	0.149
rs3021094_rs2228145	10	0.523	0.537	0.552	0.178
rs3021094_rs1800469_rs2228145	5	0.518	0.538	0.554	0.236
rs3021094_rs1800469_rs2228145_rs4796793[†]	10[†]	0.551[†]	0.538[†]	0.554[†]	0.014[†]
rs1800872_rs3021094_rs1800469_rs2228145_rs4796793	5	0.518	0.540	0.555	0.226
rs1800872_rs3021094_rs1800469_rs2228145_rs6689393_rs4796793	9	0.492	0.540	0.555	0.640
rs1800872_rs1800896_rs3021094_rs1800469_rs2228145_rs6689393_rs4796793	8	0.530	0.539	0.558	0.113
rs1800872_rs1800896_rs3790622_rs3021094_rs1800469_rs2228145_rs6689393_rs4796793	9	0.526	0.541	0.556	0.149
rs1800872_rs1800896_rs3790622_rs3021094_rs1800469_rs2228145_rs6689393_rs4845374_rs4796793	10	0.522	0.541	0.556	0.181

[†], significant 4-locus model for epistatic interaction. GMDR, generalized multifactor dimensionality reduction; logIgE, log-transformed total IgE; IgE, immunoglobulin E; *IL10*, interleukin-10; *TGFB1*, transforming growth factor-beta 1; *IL6R*, IL-6 receptor; *STAT3*, signal transducer and activator of transcription 3; CVC, cross-validation consistency; TA, testing accuracy.

(TA, 0.551; CVC, 10/10; P=0.014) (*Table 5*). The mean ± standard deviation logIgE levels in high-risk cases, low-risk cases, high-risk controls, and low-risk controls as assigned by GMDR were 2.75±0.79, 2.60±0.80, 1.90±0.66, and 1.81±0.71 respectively (P=0.019 by ANOVA) (*Figure 1*). The addition of *FLG* SNPs and 11q13 SNPs did not yield any significant finding for total IgE (Table S4). We did not detect any epistatic interaction for eczema (Table S5), atopic

eczema (Table S6), eos%, and SCORAD subphenotypes.

Discussion

Immune regulation is mostly mediated by two immunosuppressive cytokines, TGF-β1 and IL-10, that inhibit the activity of both Th1 and Th2 lymphocytes. Similar studies suggested epistatic interactions between
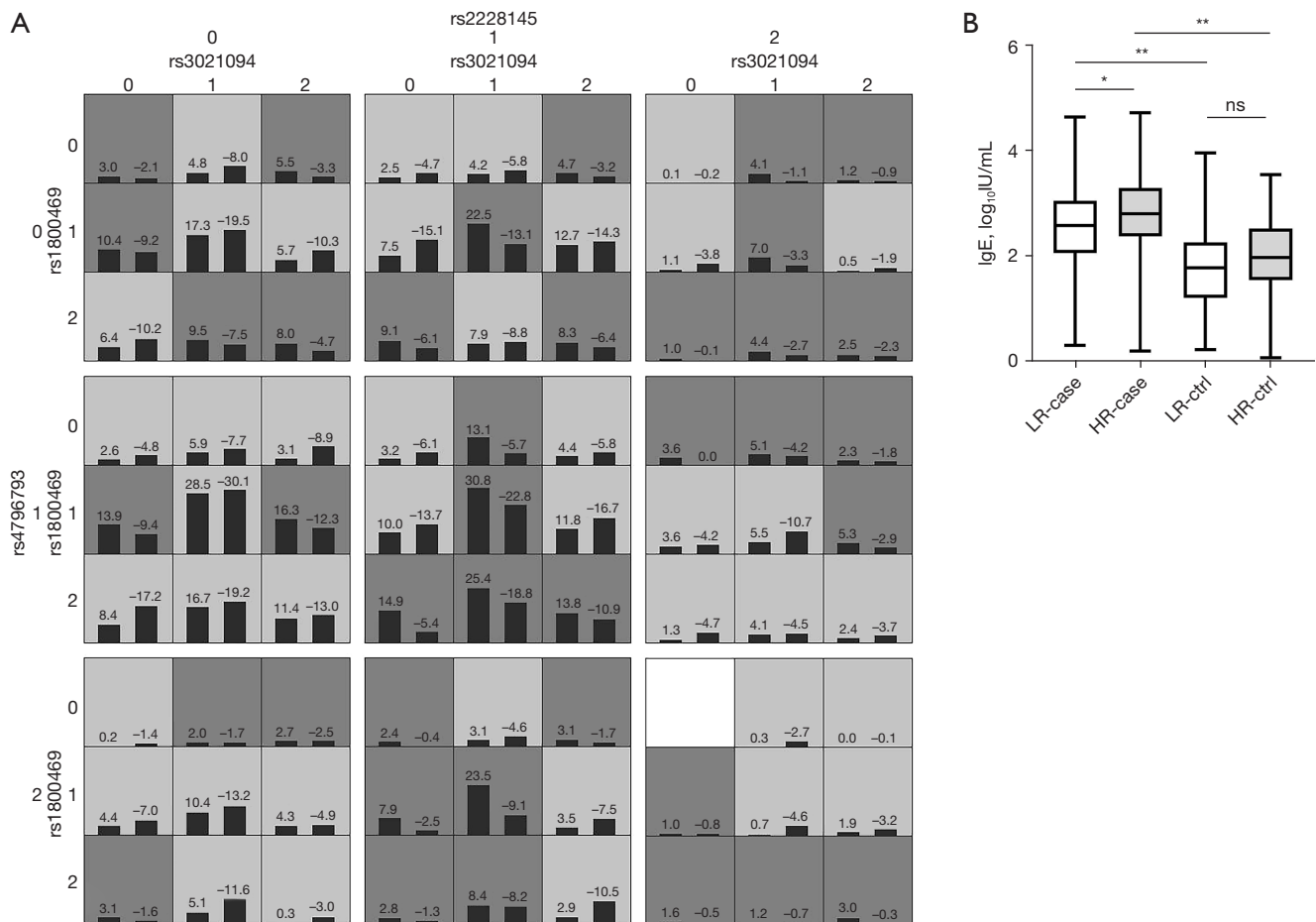
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Figure 1 GMDR analysis for logIgE levels, with the best four-locus model of rs3021094, rs1800469, rs2228145, and rs4796793 being identified after adjustment for age and sex. (A) High-risk genotypes are in dark grey, low-risk genotypes are in light grey. Left bars represent eczema patients who have high IgE while right bars are those with low IgE. The figures above the bars in each cell are the scores from GMDR analysis. (B) Distribution of logIgE level in cases and controls with high-risk and low-risk genotypes. The mean ± standard deviation of logIgE level among high-risk cases, low-risk cases, high-risk controls, and low-risk controls were 2.75±0.79, 2.60±0.80, 1.90±0.66, and 1.81±0.71 respectively (P=0.019 by ANOVA). *, P<0.05; **, P<0.001; ns, not significant. IgE, immunoglobulin E; LR, low-risk; HR, high-risk; Ctrl, control; GMDR, generalized multifactor dimensionality reduction; logIgE, log-transformed total IgE; ANOVA, analysis of variance.

immunoregulatory genes may contribute to allergic conditions, such as that among *IL2RA*, *TLR2*, *TGFBR2*, and *FOXP3* for asthma in Europeans (31).

This genetic association study focused on four important immune regulatory genes for eczema and related allergies in 2,508 southern Chinese children. We found the functional SNP rs1800469 of *TGFB1* to be associated with both eczema and allergic rhinitis, apart from observing epistatic interaction among *IL10*_rs3021094, *TGFB1*_rs1800469, *IL6R*_rs2228145, and *STAT3*_rs4796793 for modulating total IgE, which is an important eczema subphenotype

(*Table 5*), through GMDR analysis. In particular, the contribution of *IL6R* polymorphism in atopic dermatitis aligns with a recently published study involving Asian children (32). These results supported the importance of immune regulatory genes in conferring susceptibility for eczema and related subphenotypes in children. The addition of SNPs of *FLG* and 11q13 locus (17,22) did not yield additional significant results.

Previous trials investigated any association between *TGFB1* and eczema. In a mouse model with eczema, subcutaneous injection of recombinant TGF-β1 suppressed

skin lesions and reduced IgE levels (33), suggesting negative correlation between TGF-β1 and eczema severity. Arkwright *et al.* examined functional SNPs of these two cytokines in children with moderate-to-severe chronic eczema (34). They found that G915C polymorphism of *TGFB1*, being a low producer genotype, was associated with increased eczema risk.

Our SNP of interest, rs1800469, is located at position -1347 of *TGFB1* promoter—two of its alleles were linked to a nearly two-fold difference in plasma TGF-β1 levels, due to competitive binding with activator protein 1 (AP1) and hypoxia-inducible factor 1 (35). The binding of AP1 resulted in transcriptional suppression of *TGFB1* and downregulation of other genes. While TGF-β1 itself serves a general immunomodulatory role in preventing allergic diseases, *TGFB1* polymorphism and mutations predict increased risks for asthma and increased IgE (36-38).

In our study, *TGFB1*_rs1800469 was associated with eczema (OR, 0.82; 95% CI: 0.73–0.92), atopic eczema (OR, 0.83; 95% CI: 0.72–0.95) and allergic rhinitis (OR, 0.84; 95% CI: 0.74–0.95), with statistical significance. These observations supported the postulation that *TGFB1* polymorphism was associated with allergy outcomes.

According to a previous study, IL-10 was associated with asthma and serum IgE levels (39). This study, however, did not detect significant association between *IL10* and childhood eczema by single SNP and haplotype analyses although we identified a trend towards an association with total IgE levels (i.e., did not pass Bonferroni correction). Our finding of low minor allele frequency (0.05) of *IL10*_rs1800896 (-1082 A/G) was consistent with a Chinese report which found low frequency of 0.29% for GG genotype of this SNP (40). The frequencies of minor alleles for rs1800896 and rs3790622 of *IL10* as well as its AG haplotype were around 5%. At this allele frequency, our sample size of 1,329 cases and 1,179 controls had 84% power to detect OR 3 for any association between *IL10* allele and eczema with 95% confidence, which would dramatically decrease to 26% power to detect OR of 2 for eczema. Future studies must recruit sufficient sample size to examine the genetic associations for *IL10*.

Single SNPs of *IL6R* were not associated with eczema or allergic rhinitis in this study, whereas the GT haplotype from rs6689393 and rs4845374 was marginally associated with eczema but did not survive Bonferroni correction (Table S3). Similar *IL6R* haplotypes have been reported in other complex diseases such as rheumatoid arthritis and coronary heart disease (41,42). One of our studied *IL6R*

SNPs (rs2228145; Asp358Ala) was associated with persistent eczema, where carriers of the risk allele have increased serum levels of soluble IL-6R (43). The IL-6 cytokine system is involved in skin barrier repair (44), where IL-6 and IL-6R complex migrate to damaged epidermal layers and promote permeability repair through increased STAT3 phosphorylation.

Interestingly, our single SNP analysis revealed significant association between *STAT3*_rs4796793 and eczema severity as measured by SCORAD (Table S2). This *STAT3* SNP was also associated with eczema in Japanese (45).

This study was limited by the lack of functional data of our SNPs and haplotypes to delineate the possible mechanisms that linked different immune regulatory genes. Besides, the eczema subphenotypes of total IgE levels, eos%, and SCORAD were available or recorded only in a subgroup of recruited patients (*Table 2*). For example, atopy could only be assessed in 946 cases and 553 controls in total. Among the latter, 97 controls were detected by SPT and 456 controls were identified by allergen-sIgE assays. This study found high rate of atopy among the controls who were free from any asthma, rhinitis, and eczema. Although this atopy prevalence was high in our controls, this finding was consistent with our earlier genetic studies of Hong Kong children with different allergic diseases (19,20,22,46,47). The reasons accounting for this high rate of atopy are unclear, but such may be related to high indoor exposure to house dust mites and other inhalant allergens (48,49). Besides, our controls were significantly older (difference in mean of 2.4 years) than the cases (*Table 2*), suggesting that their clinical status as controls—freed from allergic diseases—was more reliable at an older age. This study was also limited to examining the SNPs of *TGFB1*, *IL10*, *IL6R*, and *STAT3*. Future research may investigate the associations between SNPs of the *IL4* gene and variations in efficacy and adverse drug reactions of dupilumab efficacy (50) as well as between SNPs of *STAT6* gene and the usefulness of topical nanoparticle cream (51).

Conclusions

The present study identified a functional SNP of *TGFB1* to be associated with eczema susceptibility in southern Chinese children. We also found epistatic interactions among multiple immune regulatory genes for the eczema subphenotype total IgE levels, supporting the complex nature of eczema that involves dysregulated T cell immunity in children. This may be further studied in

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children of other ethnicities.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee approved this study (Nos. 2008.123 and 2016.171). Subjects and/or their parents gave informed written consent to participate in this study.

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