Association of *CTLA-4* and *IL-13* Gene Polymorphisms with Graves' Disease and Ophthalmopathy in Chinese Children

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Purpose. The frequency of childhood Graves' disease (GD) in Hong Kong Chinese is among the highest in the world but childhood Graves' ophthalmopathy (GO) appears to have milder clinical severity than does the adult disease. This study was conducted to investigate *CTLA-4* and *IL-13* polymorphisms in Chinese pediatric patients with GD and GO.

Methods. Recruited for the study were 177 childhood patients with GD (age range, 2–16 years) and 151 unrelated control subjects (age range, 4–16 years) for genotype analysis of *IL-13* single-nucleotide polymorphisms (SNPs) (−1112C/T and 2044G/A), *CTLA-4* SNPs (−318C/T, 49A/G, and CT60A/G), and the repeat length of $(AT)_n$ in the 3′ untranslated region (UTR) of *CTLA-4*.

Results. The patients with GD revealed higher frequencies of *CTLA-4* 49 GG genotype and G alleles than did the control subjects ($P = 0.005$ and $P = 0.03$, respectively). The CT60 GG genotype and G alleles were more prevalent in GD ($P = 0.07$ and $P = 0.02$, respectively). The *CTLA-4* SNPs (−318C/T, 49A/G, and CT60A/G) were in the same haplotype block, and the CGG haplotype was associated with GD ($P = 0.0071$) but not GO. The shortest allele of $(AT)_n$ was protective against GD ($P = 8.4 \times 10^{-6}$). The *IL-13* SNPs did not affect GD or GO risk. *IL-13* −1112C/T was associated with IgE elevation ($P = 0.044$) and 2044G/A with proptosis ($P = 0.02$), but these associations became insignificant after Bonferroni correction ($P = 0.22$ and 0.10, respectively).

Conclusions. Three SNPs and the AT repeat length in *CTLA-4* conferred susceptibility to childhood GD, whereas *IL-13* polymorphisms did not. No association was found between *CTLA-4* and *IL-13* with GO. (*Invest Ophthalmol Vis Sci.* 2008;49: 2409–2415) DOI:10.1167/iovs.07-1433

Graves' disease (GD) is an organ-specific autoimmune disorder characterized by the presence of thyroid-stimulating hormone (TSH) receptor antibodies leading to hyperthyroidism and goiter.[1] It is the most common cause of childhood and juvenile thyrotoxicosis.[2] A previous epidemiologic study showed a rising incidence of childhood GD in the Chinese population in Hong Kong,[3] where the reported incidence of childhood GD was about eight times higher than that reported in Europe.

Graves' ophthalmopathy (GO) is an autoimmune process strongly linked to GD. It affects the orbital connective tissues and extraocular muscles.[4] Proptosis, eyelid retraction, restrictive strabismus, periorbital swelling, conjunctival chemosis, and injections cause both functional disabilities and cosmetic sequelae. In less than 5% of the affected individuals, irreversible visual loss may result from dysthyroid optic neuropathy, uncontrolled intraocular pressure, or exposure keratopathy secondary to malignant exophthalmos.[5] Hyperthyroidism can be treated with antithyroid drugs, radioactive iodine, or thyroidectomy, alone or in combination. It is often the ophthalmopathy that leads to disfigurement and long-term visual impairment.[6] A questionnaire survey estimated that the prevalence of GO among patients with GD who were younger than 18 years was ~33% in Europe.[7] It has been suggested that childhood/juvenile GO is less severe than the adult counterpart.[8] We combined the data in four reports on childhood GO[9–12] and found that it was present in 42 (23%) of 182 subjects with childhood GD, compared with 118 (18%) of 1050 adults with the disease.[13,14] Lid retraction, soft tissue congestion, and proptosis are the predominant findings in childhood GO, but not restrictive myopathy or dysthyroid optic neuropathy. We have reported that 63% of children with GD had positive ocular findings and 23% were NOSPECS (no change, only signs, soft tissue changes, proptosis, extraocular motility problems, corneal complications, and sight loss) class 3 or higher.[9] None of them had vision-threatening complications or debilitating strabismus. The disease course of GO is unpredictable and may worsen, independent of the endocrine status. As current treatment of GO is often unsatisfactory, we acknowledge the need to identify the predisposing genetic and environmental factors associated with development of GO in patients with GD, regardless of age.[15,16]

Although genetic predisposition to GD has been established by several twin studies and genetic linkage analysis,[17–20] the contribution of genetic factors to GO remains conflicting. GO has been shown to be associated with HLA alleles, tumor necrosis factor-α, and interferon-γ gene polymorphisms,[21–24] but not with interleukin (IL)-1α, IL-1β, IL-1 receptor antagonist,[25] IL-1 receptor 1, IL-4 receptor, IL-6, IL-10, or transforming growth factor-β.[26] Cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) is a key negative regulator of T-cell activation and proliferation during the immune response and thereby may influence T-cell-mediated autoimmune diseases such as GD and GO.[27] *CTLA-4* single nucleotide polymorphisms (SNPs) have been described in adult GD: −318C/T in the promoter region, +49A/G in exon 1, CT60A/G at position 8358, and a dinucleotide $(AT)_n$ repeat in the 3′ untranslated region (UTR) with variant lengths.[28,29] Positive associations between *CTLA-4* polymorphisms and GD have been reported in different ethnic populations[28–33] including our Chinese pediatric cohort.[30] For GO, however, no

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association with *CTLA-4* A49G was found in a study involving two populations: Polish-Caucasian and Japanese.[31] Also, another Caucasian study indicated environmental factors but not genes to be more influential on GO predisposition.[34] A more recent report on Chinese patients with GD added conflicting results. GO was unassociated with the +49A and CT60 polymorphisms, but weakly with the +49A-CT60G haplotype.[35]

IL-13 is an important immunoregulatory protein produced primarily by activated T helper 2 cells.[36] It upregulates CD23/FcεRII and major histocompatibility complex (MHC) class II expression and promotes immunoglobulin E (IgE) isotype switching in human B cells.[37] An early study of in situ immunostaining showed IgE positivity in leukocytes and muscle fibers of orbital tissues of patients with GO, but not in non-Graves tissues.[38] Serum IgE levels has been found to be elevated in 29% of hyperthyroid Graves' patients.[39] Levels of serum IgE and IL-13 may be indicators of remission or recurrence of GD.[40] Two SNPs in the IL-13 locus have been shown to affect biological functions. SNP −1112C/T is located in the 5′-flanking region, and it regulates gene transcription.[41] The other one is SNP 2044G/A located in exon 4, which causes an amino acid exchange from arginine to glutamine at codon 130 and possibly affects ligand–receptor interactions.[42]

We conducted a hospital-based, case–control study to investigate the association of polymorphisms in *CTLA-4* and *IL-13* with the development of GD and GO in Chinese subjects younger than 16 years.

METHODS

Patient Recruitment and Clinical Information

We invited 177 unrelated Chinese children with GD at the Pediatric Endocrine Clinic of the Prince of Wales Hospital, Hong Kong, to participate in our study. They were all younger than 16 years at diagnosis. Diagnosis of GD was based on the typical clinical features of hyperthyroidism, diffuse enlargement of the thyroid gland, increased free thyroxine or triiodothyronine levels, suppressed thyroid stimulating hormone levels, positive thyrotrophin-receptor autoantibodies, and the presence of antimicrosomal or antithyroglobulin antibodies. Transient neonatal hyperthyroidism was excluded. Information regarding sex, age at onset of GD, treatment of hyperthyroidism, personal history of cigarette smoking, systemic diseases, and family history of autoimmune thyroid disease was obtained. We also recruited 151 local Chinese children matched by age and sex as control subjects. They did not have a family history or biochemical evidence of autoimmune thyroid disease and had tested negative for thyroid autoantibodies (Serodia-ATG/AMC; Fujirebio, Tokyo, Japan).

GO was classified according to the NOSPECS system recommended by the American Thyroid Association.[43] Normal upper eyelid position was 1.5 mm below the superior limbus, and normal lower eyelid position at the level of the inferior limbus in primary gaze. Proptosis was measured by the Hertel exophthalmometer and defined as the anteroposterior protrusion of the globe >19 mm from the lateral orbital rim in either eye or any discrepancy in the degree of protrusion of the two eyes by >1 mm.[44] Keratopathy was examined by fluorescein staining under slit lamp microscopy. All patients were clinically euthyroid for more than 3 months at the time of ocular examination and were categorized according to the highest NOSPECS class ever recorded in them.

This study was approved by the Ethics Committee of the Chinese University of Hong Kong and was conducted in accordance with the Declaration of Helsinki. Written parental consent was obtained from each participating subject after explanation of the nature of the study.

Genotyping

Genomic DNA was extracted from peripheral venous blood collected from patients and control subjects. SNPs *IL-13* −1112C/T (rs1800925), *IL-13* 2044G/A (rs20541), *CTLA-4* −318C/T (rs5742909), and *CTLA-4* CT60A/G (rs3087243) were identified (*Taq*man SNP Genotyping assay; Applied Biosystems, Inc. [ABI], Foster City, CA). After polymerase chain reaction (PCR), the signals were analyzed by a sequence-detection system (Prism 7000 and SDS software ver. 1.1; ABI). SNP *CTLA-4* +49A/G (rs231775) was detected by PCR restriction fragment length polymorphism (PCR-RFLP), forward primer: 5′-GCTCTACTTCCTGAAGACCT-3′, reverse primer: 5′-AGTCTCACTCACCTTTGCAG-3′.[45] After restriction digestion with *Fnu*4HI (New England BioLabs, Beverly, MA), the genotype was revealed on 12% polyacrylamide gel by ethidium bromide staining and UV illumination. The dinucleotide $(AT)_n$ repeats in *CTLA-4* were genotyped by PCR: forward primer 5′-GATGCTAAAGGTTGTATTGC-3′ labeled with 6FAM at the 5′end and reverse primer 5′-TGGTGTATTAGTGTCCTG-3′.[28] The PCR products were analyzed (model 3130xl analyzer; ABI), and the fragment lengths determined (GeneMapper, ver. 4.0; ABI). Sequencing was performed on approximately 25% of randomly selected samples, to confirm the genotype results.

Statistics

Distribution of genotypes and alleles among groups were compared using χ^2 tests unless otherwise specified. Bonferroni corrections were made for multiple comparisons, and statistical significance was defined as corrected P (P_c) < 0.05. Odds ratios (ORs) in the different models were calculated to assess the genotype–phenotype correlation, and only those models with significant correlation are presented. In case of additive genetic model inheritance, the probability for Armitage's trend test was also computed (OR_{dom} = OR of the dominant model; OR_{rec} = OR of the recessive model; OR_{het} = OR of heterozygote compared with wild-type). Previous studies indicate that smoking, age, male sex, and radioactive iodine affect the development of or the progression of GO in adults.[46–49] These factors were controlled using multivariate logistic regression analyses. Interaction between *CTLA-4* and *IL-13* polymorphisms for the disease trait was assessed by logistic regression. Haplotype analyses were performed with Haploview 4.0, and the association between haplotypes and phenotypes were computed with permutation test (performed 1000 times). All statistical analyses were performed with commercial software (SPSS, ver. 15.0; SPSS Chicago, IL).

RESULTS

Patients' Demographic and Clinical Data

Among the 177 patients with GD, 111 had NOSPECS grades recorded, 92 (83%) were girls and 19 (17%) were boys. Median (mean ± SD) age at presentation was 11 (11.2 ± 2.8) years (range, 2–16). Mean ± SD duration between onset of GD and ocular assessment was 60.6 ± 42 months. All patients were followed up by a single pediatric endocrinologist. Antithyroid drug was used as the initial treatment of hyperthyroidism. The relapse after stopping drug treatment was treated with radioactive iodine in 17 (15%) patients, and all received timely thyroxine supplementation. Thyroidectomy was performed in five (4%) patients, and both radioactive iodine and thyroidectomy in one (1%) patient in whom follicular and papillary carcinoma developed. Nine (8%) patients were current smokers and three (3%) were ex-smokers. Forty-three (32%) had a positive family history of autoimmune thyroid disease. Except one who had a sibling with Hashimoto thyroiditis, all other affected family members had GD.

Patients were categorized according to NOSPECS into the following classes: 38 (34%) in class 0, 15 (14%) in class 1, 11 (10%) in class 2, 14 (13%) in class 3, 6 (5%) in class 4, and 27 (24%) in class 5. There was no case of dysthyroid optic neuropathy. Defining NOSPECS class 3 or above as clinically evident GO, we found that advancing age of GD onset (P = 0.945), male sex (P = 0.594), and family history of autoim-

TABLE 1. *CTLA-4* and *IL-13* Gene Polymorphisms in Patients with GD and Control Subjects

Polymorphism	GD (n = 177) No.	GD (n = 177) %	Control (n = 151) No.	Control (n = 151) %	P
CTLA-4 −318C/T					
Genotype frequencies					
CC	147	83.1	122	80.8	0.318
CT	28	15.8	29	19.2	
TT	2	1.1	0	0	
Allele frequencies					
C	322	91.0	273	90.4	0.805
T	32	9.0	29	9.6	
CTLA-4 +49A/G					
Genotype frequencies					
AA	7	4	24	15.9	0.001*
AG	73	41.2	56	37.1	
GG	97	54.8	71	47	
Allele frequencies					
A	87	24.6	104	34.4	0.006†
G	267	75.4	198	65.6	
CTLA-4 CT60A/G					
Genotype frequencies					
AA	4	2.3	12	7.9	0.014‡
AG	48	27.1	51	33.8	
GG	125	70.6	88	58.3	
Allele frequencies					
A	56	15.8	75	24.8	0.004§
G	298	84.2	227	75.2	
IL-13 −1112C/T					
Genotype frequencies					
CC	120	67.8	106	70.2	0.233
CT	51	28.8	44	29.1	
TT	6	3.4	1	0.7	
Allele frequencies					
C	291	82.2	256	84.8	0.379
T	63	17.8	46	15.2	
IL-13 2044G/A					
Genotype frequencies					
GG	68	38.4	66	43.7	0.478
GA	81	45.8	67	44.4	
AA	28	15.8	18	11.9	
Allele frequencies					
G	217	61.3	199	65.9	0.223
A	137	38.7	103	34.1	

* P_c = 0.005; OR_{dom} = 4.589; 95% CI = 1.917–10.985.
† P_c = 0.03; OR = 1.612; 95% CI = 1.149–2.262.
‡ P_c = 0.07; OR_{dom} = 3.734, 95% CI = 1.178–11.832; OR_{rec} = 1.721 (1.089–2.719); P for Armitage's trend test = 0.005.
§ P_c = 0.02; OR = 1.758; 95% CI = 1.194–2.589.

mune thyroid disease (P = 0.611) were not associated with risk for GO in our pediatric cohort. The use of radioactive iodine or not (61.1% vs. 38.7% treated, P = 0.078), and cigarette smokers or nonsmokers (66.7% vs. 39.4%, P = 0.079) were linked with GO but the differences did not reach statistical significance.

Genotyping

The observed genotype distributions of all SNPs *CTLA-4* −318C/T, *CTLA-4* +49A/G, *CTLA-4* CT60A/G, *IL-13* −1112C/T, and *IL-13* 2044G/A, but not the dinucleotide $(AT)_n$ repeats in the 3′UTR of *CTLA-4,* attained Hardy-Weinberg equilibrium in the control subjects (Table 1). None of the genotype frequencies showed a differences between the sexes. Patients with GD presented with a significantly higher portion of *CTLA-4* +49 GG genotypes and G allele compared with control (P_c = 0.005 and P_c = 0.03, respectively). The +49GG genotype significantly increased the risk of GD in a dominant model (OR = 4.59, 95% CI = 1.917–10.985). Likewise, for *CTLA-4* CT60A/G, GG genotypes and G alleles were more prevalent in patients with GD compared with the prevalence in control subjects (P_c = 0.07 and P_c= 0.02, respectively). The CT60G allele conferred susceptibility to GD in both dominant and recessive models (OR_{dom} = 3.734, 95% CI = 1.178–11.832; OR_{rec} = 1.721, 95% CI = 1.089–2.719). An allele dosage effect was observed with P for Armitage's trend test = 0.005, as the risk of GD increased significantly when both CT60G alleles were present.

The three SNPs in *CTLA-4* (−318C/T, +49A/G, and CT60A/G) were in the same haplotype block (with $D' = 1.0$; Fig. 1). Haplotype frequencies are shown in Table 2. The most common haplotype, CGG, was significantly increased in patients with GD when compared with its presence in control subjects (75.1% vs. 65.6%, P_c = 0.033, OR = 1.588, 95% CI = 1.132–2.227). The CAA haplotype decreased the susceptibility to GD significantly (15.5% vs. 24.8%, P_c = 0.017; OR = 0.557; 95% CI = 0.378–0.821).

We identified 14 allele lengths for $(AT)_n$ repeats in the 3′UTR in *CTLA-4* in our cohort (Table 3). The shortest allele (192 bp) conferred a protective effect against GD ($P = 8.4 \times 10^{-6}$; P_c = 0.00,012; OR = 0.436, 95% CI = 0.301–0.631). Although a 208-bp allele may link with GD (OR = 1.719; 95% CI = 1.183–2.498), such linkage was statistically insignificant after Bonferroni correction (P = 0.004; P_c = 0.056). No significant difference was detected in the distribution of the allele length when comparing GO with non-GO patients.

For the two SNPs in *IL-13*, the genotype and allele frequencies were not different between patients with GD and control subjects (Table 1). −1112C/T was associated with an increased serum IgE level (P = 0.044, P_c = 0.22; OR_{het} = 2.363, 95% CI = 1.104–5.059) and 2044G/A with proptosis (P = 0.02, P_c = 0.1). Both did not reach statistical significance after Bonferroni correction (Tables 4, 5). The AA homozygous genotype of 2044G/A occurred rarely in patients with proptosis, only 1 (4.5%) in 22, but a quarter (22/89, 24.7%) of patients without

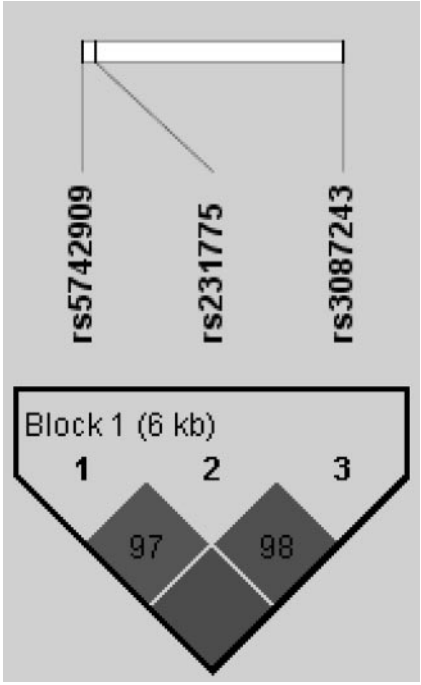

FIGURE 1. *CTLA-4* −318C/T (rs5742909), +49A/G (rs231775), and CT60A/G (rs3087243) are in the same LD block.

Table 2. Haplotype Frequencies of *CTLA-4* Gene Polymorphisms in Patients with GD and Control Subjects

CTLA-4 Polymorphism	GD (*n* = 354)		Control (*n* = 302)		*P*
	n	%	*n*	%	
−318C/T +49A/G CT60A/G haplotype frequencies					
CGG	266	75.1	198	65.6	0.0071*
CAA	55	15.5	75	24.8	0.0031†
TAG	32	9	29	9.6	0.7908

* Permutation $P = 0.033$; OR = 1.588; 95% CI = 1.132–2.227.
† Permutation $P = 0.017$; OR = 0.557; 95% CI = 0.378–0.821.

proptosis (Table 5). No association was found between the polymorphisms investigated herein with the serum level of antimicrosomal or antithyroglobulin antibodies (results not shown). Logistic regression showed the absence of gene–gene interaction between *IL-13* and *CTLA-4* ($P > 0.05$).

Discussion

Childhood/adolescent GD is rare in Caucasians but a higher prevalence is observed among the Chinese population in Hong Kong.[3] Therefore, we were able to follow-up a large cohort of pediatric Chinese patients with GD and to study the association of four *CTLA-4* and two *IL-13* gene polymorphisms in patients with GD and GO under the age of 16. Ours is the first genotyping study on this age group of patients. Although it has been argued that childhood GD and GO is clinically and/or phenotypically distinct from adult-onset GD and GO,[50] it still remains to be confirmed empirically and experimentally whether different times of disease onset are due to fundamental differences in genetic or immunologic mechanisms.

CTLA-4 genotype distribution and allele frequencies determined in Chinese children in this study were different from those in Caucasians but similar to those in Japanese.[50] In particular, the G allele at +49A/G and CT60A/G were the most prevalent among Chinese. Our results confirmed that the presence of at least one G allele (GG or AG) at either locus confers susceptibility to GD in Chinese children. Haplotype analysis also confirmed that +49G/CT60G confers a significant risk for GD, whereas +49A/CT60A correlates with a protective effect. Although located in the same haplotype block, −318C/T does not associate with an increased risk of GD, which may be due to its low allele frequency (~10%) in our study population.

A recent meta-analysis[16] suggested that, apart from the original and the extended study from Vaidya et al.,[51,52] there was no evidence of a significant association between *CTLA-4* +49G and GO. A study on five *CTLA-4* SNPs in Chinese adult patients with GD showed that the −318T allele played a protective role in GO, particularly among male patients.[53] Our results in children revealed no association of the −318T allele with GD/GO. The −318T allele frequency in our cohort was much lower (10% vs. 25%). The discrepancy may be due to population stratification: The study by Han et al.[53] was based in Chongqing in southwestern China, and ours in Hong Kong in the south. What should also be considered is the notion of

Table 4. Association of *IL-13* and *CTLA-4* SNPs with IgE Level in Patients with GD

	High ≥180 (*n* = 50)		Normal <180 (*n* = 83)		*P*
	n	%	*n*	%	
CTLA-4 −318C/T					
CC	41	82	72	86.7	0.385
CT	8	16	11	13.3	
TT	1	2	0	0	
CTLA-4 +49A/G					
AA	3	6	5	6	0.636
AG	24	48	33	39.8	
GG	23	46	45	54.2	
CTLA-4 CT60A/G					
AA	3	6	5	6	0.74
AG	17	34	23	27.7	
GG	30	60	55	66.3	
IL-13 −1112C/T					
CC	29	58	62	74.7	0.044*
CT	21	42	19	22.9	
TT	0	0	2	2.4	
IL-13 2044G/A					
GG	18	36	33	39.8	0.906
GA	24	48	38	45.8	
AA	8	16	12	14.5	

* $P_c = 0.22$; $OR_{het} = 2.363$; 95% CI = 1.104–5.059.

Table 3. Distribution of Variant Length of *CTLA-4* Dinucleotide $(AT)_n$ Repeats

	Size (bp)	GD (*n* = 354)		Control (*n* = 302)		Overall *P*	GO (*n* = 94)		Non GO (*n* = 128)		Overall *P*
		n	%	*n*	%		*n*	%	*n*	%	
1	192	59	16.7	95*	31.5*	0.002	15	16	20	15.6	0.566
2	206	5	1.4	1	0.3		1	1.1	3	2.3	
3	208	98	27.7	55†	18.2†		30	31.9	30	23.4	
4	210	137	38.7	98	32.5		32	34	56	43.8	
5	212	25	7.1	31	10.3		9	9.6	10	7.8	
6	214	17	4.8	14	4.6		4	4.3	4	3.1	
7	220	1	0.3	0	0		1	1.1	0	0	
8	222	0	0	2	0.7		0	0	0	0	
9	224	2	0.6	1	0.3		1	1.1	0	0	
10	226	1	0.3	1	0.3		0	0	0	0	
11	228	4	1.1	2	0.7		1	1.1	2	1.6	
12	230	2	0.6	1	0.3		0	0	1	0.8	
13	232	2	0.6	0	0		0	0	2	1.6	
14	234	1	0.3	1	0.3		0	0	0	0	

* $P = 8.4 \times 10^{-6}$; $P_c = 0.00012$; OR = 0.436; 95% CI = 0.301–0.631.
† $P = 0.004$; $P_c = 0.056$; OR = 1.719; 95% CI = 1.183–2.498.

TABLE 5. Association of *CTLA-4* and *IL-13* SNPs with Different Manifestation of GO (NOSPECS Class 2, 3, or 4)

	Class 2* (*n* = 22)		No Class 2 Finding (*n* = 89)			Class 3† (*n* = 22)		No Class 3 Finding (*n* = 89)			Class 4‡ (*n* = 12)		No Class 4 Finding (*n* = 99)		
Polymorphisms	***n***	**%**	***n***	**%**	***P***	***n***	**%**	***n***	**%**	***P***	***n***	**%**	***n***	**%**	***P***
CTLA-4 −318C/T															
CC	17	77.3	75	84.3	0.453	18	81.8	74	83.1	0.724	11	91.7	81	81.8	0.672
CT	5	22.7	12	13.5		4	18.2	13	14.6		1	8.3	16	16.2	
TT	0	0	2	2.2		0	0	2	2.2		0	0	2	2	
+49A/G															
AA	0	0	7	7.9	0.275	1	4.5	6	6.7	0.247	0	0	7	7.1	0.217
AG	8	36.4	38	42.7		6	27.3	40	44.9		3	25	43	43.4	
GG	14	63.6	44	49.4		15	68.2	43	48.3		9	75	49	49.5	
CT60A/G															
AA	0	0	6	6.7	0.111	0	0	6	6.7	0.239	0	0	6	6.1	0.444
AG	3	13.6	26	29.2		4	18.2	25	28.1		2	16.7	27	27.3	
GG	9	86.4	57	64		18	81.8	58	65.2		10	83.3	66	66.7	
IL-13 −1112C/T															
CC	14	63.6	57	64	0.571	15	68.2	56	62.9	0.586	10	83.3	61	61.6	0.133
CT	8	36.4	28	31.5		7	31.8	29	32.6		1	8.3	35	35.4	
TT	0	0	4	4.5		0	0	4	4.5		1	8.3	3	3	
2044G/A															
GG	7	31.8	37	41.6	0.535	7	31.8	37	41.6	0.02§	5	41.7	39	39.4	0.935
GA	11	50	33	37.1		14	63.6	30	33.7		5	41.7	39	39.4	
AA	4	18.2	19	21.3		1	4.5	22	24.7		2	16.7	21	21.2	

* Class 2: soft tissue involvement including chemosis, lid swelling, lagophthalmos, tearing, retrobulbar discomfort and photophobia.
† Class 3: proptosis.
‡ Class 4: extraocular muscles involvement, usually with diplopia.
§ $P_c = 0.1$.

dissimilar genetic or etiologic contribution to childhood GD in our study against adult onset GD in theirs. It is noted that the recent International HapMap project reported the T allele frequency in Han Chinese in Beijing to be 11% in Rel22/phase II April 2007.[54]

IL-13 gene polymorphisms were associated with GD but not GO in the Japanese population.[55] Such association could not be replicated in the Chinese subjects of this study. Several studies suggested that *IL-13* polymorphisms may affect serum IgE level, which in turn could be related to GO severity.[41,42,56] Presence of IL-13 mRNA and IgE had been detected in thyroid and orbital tissues of patients with GD.[38,57,58] In one study, a higher serum IgE level occurred in about a third of those with hyperthyroid GD, who had slower recovery in reduction in thyroid stimulating hormone receptor antibody levels during methimazole and/or prednisolone treatment.[39] These may suggest a difference in the autoimmune processes in a subgroup of patients with GD with elevation of IgE.[39] Still, our results may indicate an association of *IL-13* −1112C/T on IgE level and *IL-13* 2044G/A on development of proptosis (Table 5), the number of patients was small, and the associations were statistically insignificant after Bonferroni correction. Our preliminary observation may imply involvement of IL-13 in certain clinical manifestations of GO, provided that it is confirmed in a large cohort of patients and different ethnic origins. −1112C/T has been shown to regulate gene transcription and cause IgE elevation.[41,42] On the other hand, 2044G/A leads to R130Q, a substitution of a positively charged arginine with a neutral glutamine. Computer modeling suggests that this polymorphism affects ligand receptor interactions.[59] R130 is a moderately conserved amino acid residue in humans, monkeys, and cattle.[60] The R130Q variant has been shown to be more active in mediating activities than is wild-type IL-13.[60] It has also been shown to associate with allergic asthma, probably due to its enhancement of allergic inflammation.[61] Proptosis and diplopia have been reported as ophthalmic complications in patients with allergic fungal sinusitis.[62] Whether IL-13 2044G/A exerts any biological effects that ultimately contribute to the development of proptosis warrants further investigation.

Phenotypic definition of GO is a confounding factor in genetic studies of GO.[16] Although numerous classification schemes for GO have been proposed over the years,[63] new grading systems are still being sought, indicating inadequacies in the existing ones.[64] NOSPECS classification is the most widely used, but it lacks step-wise progression across the grading system. An individual with GO can have more than one grade, which may change with time or treatments. There can also be improvement with time, irrespective of treatment. For example, patients with severe soft tissue congestion and proptosis and requiring immunosuppressants or surgical decompression are given a lower grade (class 3) than are patients with lid retraction, lagophthalmos, and corneal punctate epitheliopathy requiring only topical lubricants (class 5). Patients with extraocular motility disturbance (class 4) may not experience proptosis (class 3) or soft tissue changes (class 2) during the whole disease course. Clinically evident GO in pediatric patients is often defined as class II (soft tissue involvement) or higher, whereas some diagnosed GO in the presence of upper lid retraction only, with or without lid lag (class 1).[11] Most genetic studies on adult GO use class 3 to define clinically evident GO.[16] At this moment, we believe that class 3 and higher should be uniformly used to define clinically evident ophthalmopathy, because milder forms are difficult to quantify objectively. In a Japanese study on HLA genotype,[50] only class 3 or 4 patients were included as clinically evident GO. Class 0 patients were assigned as no GO. Patients in all other classes were not included. No consensus has been reached as to whether pediatric GO should use the same classification criteria as adults.[16]

In conclusion, our results showed that *CTLA-4* −318C +49G CT60G haplotype conferred susceptibility to childhood

GD in Hong Kong Chinese, whereas *IL-13* was not associated with GD. No association between these gene polymorphisms and the development of GO was detected. Recent progress in the clinical management of GO, including the use of tumor necrosis factor-α blockage, anti-CD20 antibodies, and contraindication of PPAR-γ agonist, all stemmed directly from basic research. We should seek a better understanding of the underlying pathophysiology and predicting outcome of GD/GO at onset and to identify family members at risk. Such information should help to improve disease management and allow earliest intervention in GO, a distressing condition with long-term disability, even after achieving euthyroidism.

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