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Tzu Chi Medical Journal

ELSEVIER

Original Article

Polymorphisms of Interleukin 7 Receptor are Associated With Mite-Sensitive Allergic Asthma in Children in Taiwan

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Article info

Article history:
Received: September 17, 2009
Revised: December 21, 2009
Accepted: February 23, 2010

Keywords:
Allergic asthma
Der f-sensitive
Der p-sensitive
Interleukin 7 receptor
Single nucleotide polymorphism

Abstract

Objective: Single nucleotide polymorphisms (SNPs) in the coding region of interleukin 7 receptor (*IL7R*) are associated with severe combined immunodeficiency and multiple sclerosis. Based on the known mechanisms involved, *IL7R* has a high potential for being a candidate gene conferring allergy and asthma susceptibility. The purpose of this study was to investigate the possible genetic association between SNPs in the *IL7R* coding region and mite-sensitized asthma in Taiwanese children.

Materials and Methods: We conducted a case-control study with 82 normal controls and 200 allergic asthma patients sensitized to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. Twelve SNPs of *IL7R* from a total of 282 subjects were obtained by direct sequencing and TaqMan genotyping.

Results: We identified two specific genotypes, rs969129 GG and rs1494555 AA, which were present at higher frequencies in the patient group than in the control group. Moreover, the normal controls that carried these two specific genotypes appeared to have higher serum immunoglobulin E levels than those with other genotypes.

Conclusion: Our findings indicate that the SNPs rs969129 (in intron 2) and rs1494555 (in exon 4) in the *IL7R* gene may be associated with mite-sensitive allergic asthma in Taiwanese children. (*Tzu Chi Med J* 2010;22(1):18–23)

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1. Introduction

Allergies are the most common immune disorders in children. Allergic bronchial asthma, allergic rhinitis, and atopic eczema have steadily increased in many Western and developing countries (1). Although there are slight variations in allergic diseases in different geographic locations in Taiwan, the prevalence of these diseases is also rising in this region (1–3).

More than 90% of asthmatic children have allergic asthma. These patients have elevated serum immunoglobulin E (IgE) levels and allergen-specific IgE

antibodies against a wide range of environmental allergens [4]. In Taiwan, most asthmatic children are sensitized to two dust mites, *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f). The Mackay Memorial Hospital in Taipei reported that 75% of patients had sensitization to Der p and 74% had sensitization to Der f [5]. National Cheng-Kung University in Taiwan independently reported even higher sensitization rates of 82% to Der p and 82.5% to Der f [6]. It has been suggested that the decline of lung function in asthmatic children is associated with serum levels of Der p-specific and Der f-specific IgE [5].

IL7R (interleukin 7 receptor), a subunit of IL7 receptor and the receptor for thymic stromal-derived lymphopoietin (TSLP), has been implicated in the onset of severe combined immunodeficiency (SCID) [7]. Single nucleotide polymorphisms (SNPs) of *IL7R* have been shown to be associated with multiple sclerosis [8]. In addition, IL7, one of the IL7R ligands, is an important growth factor in T- and B-cell proliferation [9,10], and in memory CD8+ T-cell development and survival [11]. TSLP, another ligand of IL7R, is a potential key player in the induction of allergic inflammation [12]. The aim of this study was to evaluate the genetic associations of *IL7R* gene SNPs and mite-sensitized allergic asthma in Taiwanese children. Our results suggest that *IL7R* could be a susceptible gene for mite-sensitized allergic asthma.

2. Materials and methods

2.1. Subjects

The study subjects included asthmatic and control children aged between 3 and 12 years. The study protocol was approved by the Ethical and Clinical Trial Committee of National Cheng-Kung University Hospital. All participants and their guardians were informed about the study protocol and signed consent forms. They completed a modified British Medical Society respiratory questionnaire, which is the same as the European Community Respiratory Health Survey. These surveys have validity similar to the International Study of Asthma and Allergies in Childhood and are pertinent to the diagnosis and assessment of asthma [13,14]. Pulmonary function was tested using standard methods that included spirometry before and after the administration of two puffs of inhaled salbutamol (200 μg/puff). Patients were defined as having asthma if they had all of the following: (A) a history of wheezing and shortness of breath during or without concurrent respiratory infections; (B) chronic cough for more than 1 month and a previous diagnosis of wheezing; and (C) a bronchodilator test showing a positive response with a 15% increase in the forced expiratory volume in 1 second. Other evaluations included skin prick tests for responsiveness to six common aeroallergens, a differential blood count (including total eosinophil count), and measurement of total serum IgE, and IgE specific to house dust and mixed pollens using the Unicap system (Pharmacia Diagnostics AB, Uppsala, Sweden). A positive skin test was defined as the presence of ≥1 reaction with a wheal diameter ≥5 mm. Total serum IgE levels were measured by solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics AB). Eighty-two randomly chosen children were recruited as controls. All participants including controls were enrolled from National Cheng-Kung University Hospital. The children in the control group had a negative skin prick test and no history of asthma. Information on the study subjects is shown in Table 1.

Table 1 — Demographic data of study subjects

	Allergic asthma	Control
Total sample size (*n*)	218	82
First-treated/enrolled age (yr)	7.0±3.4	6.3±3.3
Average ln(IgE)	6.78±1.30	3.26±1.17

2.2. DNA preparation

Genomic DNA samples were prepared from 282 (82 controls and 200 with allergic asthma) unrelated Taiwanese children. DNA was isolated from blood samples using the QIAamp DNA Blood kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The quality of the isolated genomic DNA was first checked by agarose gel electrophoresis analysis followed by quantitative determination with spectrophotometry. The isolated DNA was stored at –80°C until use.

2.3. Genotyping

DNA fragments from the regions of the *IL7R* gene including exon 2, exon 6 and exon 8 were amplified on an ABI 2700 thermal cycler (Applied Biosystems Inc., Foster City, CA, USA) using two pairs of forward and reverse primers. The sequences of the primers and related information are listed in Table 2. The fragments of the PCR products were sequenced by an ABI 3700 automatic sequencer (Applied Biosystems Inc.) according to the manufacturer's protocol. The sequence data were analyzed by PolyPhred software to identify the potential SNP candidates. The potential SNPs were manually checked to ensure the presence of true SNPs and only confirmed SNP data were subjected to subsequent statistical analysis.

Table 2 — PCR primers used to detect polymorphisms of the *IL7R* gene

Primer sequence (5′ to 3′)	Region of interest	Polymorphism site	Annealing temperature (°C)	Product size (bp)
Forward: GCCTCTGGTTCTTTAACTCAGAAA Reverse: ATGAAGTTGGGAGGGATGTG	Exon 2	rs1494559 rs1494558* rs969128 rs969129	62	700
Forward: CACCCAAGTCAATGCCTTTT Reverse: TTACTTTGGGGACAGCGTTT	Exon 6	rs6897932*	60	500
Forward: CACAGCCAGTGGTCACTTCA Reverse: TTGATTTGATCCCAGGGAAG	Exon 8	rs3194051* rs1803814ND* rs2229232*	62	649

*Single nucleotide polymorphism. ND=no detection.

SNP genotyping at some polymorphism sites (such as rs1389832, rs1494555, rs2228141, and rs987106) was performed with TaqMan SNP genotyping assays (Applied Biosystems Inc.). The primers and probes of the above SNPs were purchased from ABI's AOD (assay on demand) kit. Reactions were performed according to the manufacturer's protocols. Probe fluorescence signal detection was performed using the ABI Prism 7900 Real-time PCR System, following the manufacturer's specifications.

2.4. Statistical analysis

The primary dichotomous outcome variable of association analysis was control or mite-sensitive allergic asthma (asthma with Der p and Der f sensitization). The principal explanatory variables were the genotyped polymorphisms. To reveal the relationship between genotypes and IgE levels, ANOVA was applied on the genotype and natural logarithm of the IgE levels. All statistical analyses, including χ^2 tests and analysis of variance, were performed with SAS software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of study subjects

We screened and recruited a total of 282 subjects including 82 healthy controls who were Der p and Der f negative, and 200 children with allergic asthma who were Der p and Der f positive. There was no significant difference in the mean age between the two groups (Table 1; *t* test). There was no significant difference in age between sexes (data not shown). Blood cells were collected from all subjects and DNA samples were extracted for the genotyping analysis.

3.2. Genotype frequencies of the SNPs in the coding region of the IL7R gene

Twelve SNPs including a newly discovered VGV3287 (Vita Genomics Variation No. 3287) were identified in the region of the *IL7R* gene in our study subjects. The locations of these SNPs are shown in Table 3. No significant differences were observed between the controls and children with asthma in the tested genotypes (Table 3) in the *IL7R* gene region. The newly identified VGV3287 was located at intron 2, 60 bp downstream from exon 2.

3.3. Association of SNP genotypes with different IgE levels in the control subjects

We found that the different genotypes of rs1389832, rs969129, and rs1494558 were correlated with IgE levels in the control group (Table 3). Our results showed that the genotypes of the SNPs rs1389832 GG, rs1494558 CC, and rs969129 GG were associated with the highest IgE levels; however, we did not find this association in the asthmatic patient group.

3.4. Genotypes associated with high IgE levels in the control group were more frequent in the patient group

The genotypes associated with high IgE levels in the control group were selected and their frequencies were compared with other genotypes, regardless of whether the ANOVA results were significant. We found that the genotypes rs969129 GG and rs1494555 AA were more frequent in the patient group than those in the control group (Table 4). The SNPs rs1494558, VGV3287 and rs969129 were located at exon 2 and intron 2 in the same PCR fragment. The SNP rs1494555

Table 3 — Genotype frequencies of SNPs in the *IL7R* gene and the association of SNPs with IgE levels

SNP ID	Location	Genotype	Subject count				ln(IgE) (SD)	
			Control	As-Ms	χ^2 p		Control	As-Ms
rs1389832	intron 1	AA	29	57	0.289		3.58 (1.10)	7.16 (1.17)
		AG	43	105			3.07 (1.28)	6.94 (1.26)
		GG	10	38			3.99 (1.42)	7.32 (1.03)
						ANOVA p	0.047	0.221
rs1494559	intron 1	CC	0	6	0.263		–	6.76 (1.23)
		CT	23	58			3.27 (1.53)	6.71 (1.27)
		TT	59	136			3.36 (1.17)	6.85 (1.34)
						ANOVA p	0.771	0.792
rs1494558	exon 2	CC	10	38	0.118		4.09 (1.45)	7.04 (1.20)
		CT	42	111			2.99 (1.25)	6.64 (1.35)
		TT	30	51			3.57 (1.12)	7.03 (1.27)
						ANOVA p	0.02	0.105
VGV3287	intron 2	CC	54	107	0.133		3.36 (1.26)	6.77 (1.28)
		CG	24	84			3.07 (1.15)	6.84 (1.38)
		GG	4	9			4.54 (1.76)	6.99 (0.90)
						ANOVA p	0.098	0.858
rs969128	intron 2	AA	59	137	0.338		3.36 (1.17)	6.86 (1.33)
		AG	23	58			3.27 (1.53)	6.71 (1.26)
		GG	0	5			–	6.73 (1.37)
						ANOVA p	0.771	0.760
rs969129	intron 2	GG	11	50	0.073		4.06 (1.38)	6.96 (1.23)
		GT	48	109			3.08 (1.25)	6.69 (1.32)
		TT	23	41			3.52 (1.15)	6.97 (1.33)
						ANOVA p	0.048	0.343
rs1494555	exon 4	AA	11	48	0.134		3.97 (1.36)	7.27 (1.01)
		AG	49	104			3.14 (1.27)	6.97 (1.24)
		GG	22	47			3.53 (1.13)	7.01 (1.28)
						ANOVA p	0.091	0.372
rs2228141	exon 4	CC	60	140	0.185		3.37 (1.16)	7.09 (1.21)
		CT	22	52			3.33 (1.55)	7.00 (1.19)
		TT	0	8			–	6.89 (1.12)
						ANOVA p	0.906	0.839
rs6897932	exon 6	AA	1	9	0.337		3.40 (–)	6.70 (0.88)
		AG	25	52			3.11 (1.28)	6.76 (1.38)
		GG	57	139			3.41 (1.28)	6.81 (1.30)
						ANOVA p	0.62	0.882
rs987106	intron 6	AA	47	100	0.451		3.45 (1.29)	7.01 (1.20)
		AT	29	87			3.16 (1.32)	7.02 (1.24)
		TT	6	13			3.63 (0.48)	7.64 (90.73)
						ANOVA p	0.528	0.212
rs3194051	exon 8	CC	1	3	0.774		3.97 (–)	8.04 (0.52)
		CT	9	28			3.51 (1.18)	6.83 (1.23)
		TT	72	169			3.28 (1.31)	6.79 (1.30)
						ANOVA p	0.781	0.249
rs2229232	exon 8	CC	78	187	0.758		3.30 (1.30)	6.80 (1.26)
		CT	4	12			3.62 (0.73)	6.69 (1.41)
		TT	0	1			–	8.03 (–)
						ANOVA p	0.629	0.599

SNP = single nucleotide polymorphism; As-Ms = asthma with mite sensitized; χ^2 p = p value of χ^2 test; ANOVA p = p value for analysis of variance test.

was located at exon 4. We could not define the genotypes associated with the highest IgE level in some of the SNPs because the mean±SD of the natural logarithm IgE levels were not available for one of the three genotypes (Table 4).

4. Discussion

Our results suggest that the SNPs rs969129 and rs1494555 of the *IL7R* gene are significantly associated with mite-sensitive allergic asthma. The rs1389832

Table 4 — Frequencies of genotypes associated with high IgE values in the control group and other genotypes by Der p and Der f sensitized asthma and control subjects

SNP ID	Genotype	As-Ms *vs.* control
rs1389832	GG *vs.* AG+GG	0.167
rs1494559	CC *vs.* CT+TT	0.110
rs1494558	CC *vs.* CT+TT	0.167
VGV3287	GG *vs.* CG+CC	0.057
rs969128	NA	
rs969129	GG *vs.* GT+TT	0.032*
rs1494555	AA *vs.* AG+GG	0.045*
rs2228141	NA	
rs6897932	NA	
rs987106	AA *vs.* AT+TT	0.803
rs3194051	NA	
rs2229232	NA	

*$p<0.05$. SNP=single nucleotide polymorphism; As-Ms=asthma with mite sensitized; NA=the genotype was not available due to being unable to define which genotype was associated with the highest IgE value in the control group.

GG, rs1494558 CC, and rs969129 GG carriers showed an association with high IgE levels in the control group and the rs969129 GG and rs1494555 AA carriers were more frequent in the patient group than those in the control group. We found that these two genotypes with a high frequency in the patient group were also associated with high IgE levels observed in the control group.

IL7R mutations have been reported in several patients with SCID [7,8]. However, the P132S mutation with polymorphism, which severely compromises affinity to IL7 resulting in defective signal transduction [8], was not found in our study population. The SNPs rs1494558 C/T and rs1494555 A/G that cause changes in amino acids from Thr66 to Ile and from Ile 138 to Val, respectively, were not rare mutations in our study population. As reported previously, homozygous rs1494558T and rs1494555G in SCID patients are correlated with the absence of *IL7R* mRNA but the disease is not caused by the changes in the two amino acid molecules [7]. Use of an *in vitro* reconstruction system revealed that the construction of homozygous rs1494558T and rs1494555G from SCID patients is modestly diminished in cell expression-altered IL7R compared with that in control subjects [7]. In our study, 18 cases in the patient group and no cases in the control group were homozygous for both rs1494558CC and rs1494555AA. These 18 patients were sensitized to both Der p and Der f. This observation suggests that a polymorphism correlated with "normal" IL7R expression confers susceptibility to mite-sensitized asthma.

IL7 is involved in the regulation of lymphopoiesis. The response of cells to IL7 is dependent on the presence of IL7R. Watanabe et al reported that human intestinal epithelial cells and epithelial goblet cells produce IL7, and that locally produced IL7 may serve as a potent regulatory factor for intestinal mucosal lymphocytes [15]. Moreover, the expression of TSLP receptor and IL7R, together but not alone, induces a proliferative response to TSLP, but not to IL7, indicating that the functional TSLP receptor consists of these two subunits [16].

The human TSLP receptor is a heterodimer composed of the IL7 receptor α chain and a novel receptor chain called TSLP receptor δ2. Both chains are required to respond to TSLP and form a high-affinity complex upon dimerization [16]. Expression of human TSLP receptor mRNA is restricted to dendritic cells, monocytes and some T cell clones. TSLP, which is a novel IL7-like cytokine, might represent an early event triggering the allergic immune cascade [13]. TSLP-activated human dendritic cells produce Th2-attracting chemokines but not IL12, and induce naïve CD4+ and CD8+ T cell differentiation into effector cells with a typical pro-allergic phenotype. TSLP is produced by human epithelial, stromal, and mast cells [12].

In conclusion, our study suggests that *IL7R* is a potential candidate gene that confers susceptibility to mite-sensitized asthma. Although there was no direct evidence for a relationship between the genetic polymorphisms of *IL7R* and the immunological function of IL7 and/or TSLP, it is reasonable to speculate that this polymorphism of IL7R may cause different responses to epithelial cell-derived TSLP. These responses might act as early events triggering the allergic immune cascade when in contact with mite allergens. The function of associated SNPs in mite allergies and asthma mechanisms remains to be elucidated.

Acknowledgments

We would like to express our thanks to Dr Ying-Jye Wu for his assistance in preparing this manuscript, and Ms Felicia Lin for data management.

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