Journal of
Clinical Trials and Regulatory Affairs

RESEARCH ARTICLE

Association of a Single Nucleotide Polymorphism of IL-21 Gene with Asthma in a Chinese Han Population

Jing Lin[1]* and Guangre Xu[2]

[1]Department of Gastrology, Sichuan Academy of Medical Sciences &Sichuan Provincial People's Hospital, Chengdu 610041, PR China
[2]Department of Cardiology, Sichuan Academy of Medical Sciences &Sichuan Provincial People's Hospital, Chengdu 610041, PR China

Abstract

Background: Autoimmune abnormalities appear to be major predisposing factors for asthma. Rs12508721 and rs2055979 of interleukin-21 (*IL-21*) gene polymorphisms have been previously found to be associated with autoimmune diseases. This study aimed to assess the role of *IL-21* in asthma in a Chinese Han population.

Method: A total of 199 independent asthma patients and 249 unrelated healthy controls were recruited for this case-control association study. Two SNPs (rs12508721 and rs2055979) were genotyped by PCR-RFLP.

Results: The allele T frequency of rs12508721 was significantly higher in asthma patients than in controls (OR=1.58, 95% CI=1.18-2.11, P=0.0021). The effect of dominant model (CC versus CT + TT, OR=.83, 95CI=1.24-2.69, P=0.0021) was observed. Distributions of allele and genotype frequencies of the SNP rs2055979 showed no significant differences between asthma patients and controls.

Conclusion: Our findings suggest that polymorphism of *IL-21* gene plays an important role in susceptibility to asthma in a Chinese Han Population.

Keywords: Asthma; Interleukin-21; Single nucleotide polymorphism

Introduction

Recent decades have brought dramatic increases in the prevalence and severity of allergic asthma; and the worldwide incidence, morbidity, and mortality of allergic asthma are increasing [1]. Asthma control represents the main goal of asthma management and different strategies aim to avoid the long term downsides of inhaled corticosteroids [2]. Although different strategies are broadly administered to patients, a better understanding of pathogenesis is essential to prevent the prognosis.

It has been shown that multi-factorial parameters and many clinical conditions can cause allergic asthma: the pathophysiological features of allergic asthma are thought to result from the aberrant expansion of $CD4^+$ T cells [1,3]; immunosuppressive therapy was shown beneficial to the treatment of allergic asthma, but is not without risks [2,4,5]; immunity related genes such as *IL-2, IL-4, IL-13* were shown associated with asthma [6-10]. These findings collectively demonstrate that autoimmune mechanism-mediated damage may play an important role in the pathogenesis.

IL-21, an *IL-2* family multifunctional cytokine, is produced by activated $CD4^+$ T cells. *IL-21* is a multifunctional cytokine associated with multiple autoimmune diseases, including systemic lupus erythematosus, ulcerative colitis, and DCM [11-16]. We hypothesized that asthma susceptibility was associated with certain polymorphisms in the *IL-21* gene. In the present study, we investigated two single nucleotide polymorphisms (SNPs) of *IL-21* in asthma patients and controls: rs12508721 (promoter region) and rs2055979 (intron region). The findings allowed evaluating the contribution of these SNPs to asthma risk e using available genotyping data in a Chinese Han population.

Subjects and Methods

Study subjects

The present study was approved by the hospital ethics committee and all subjects gave written informed consent to participate. This case–control study enrolled 199 unrelated asthma patients (years, mean ± SD, 43 ± 35.17; gender, male/female, 121/78) from Sichuan Academy of Medical Sciences

Correspondence to: Lin J, Department of Gastrology, Sichuan Academy of Medical Sciences &Sichuan Provincial People's Hospital, Chengdu 610041, PR China, Tel: 8618782998965; E-mail: 582301352[AT]qq[DOT]com

Received: Nov 21, 2017; **Accepted:** Nov 23, 2017; **Published:** Nov 27, 2017

and Sichuan Provincial People's Hospital between 2012 and 2013. The diagnosis of asthma was made in accordance with the revised criteria: a positive skin prick test reaction to at least 1 aeroallergen (pollen) and a history of shortness of breath and wheezing due to chest tightness. A total of 249 healthy unrelated individuals (years, mean ± SD, 49.94 ± 20.17; gender, male/female, 128/121) from a routine health survey were enrolled as controls. All individuals were Han population living in Sichuan Province of southwestern China. All subjects involved were privy to the study and gave written informed consent.

PCR amplification and restriction enzyme digestion

Genomic DNA of each individual was extracted from 200 ul EDTA-anticoagulated peripheral blood samples by a DNA isolation kit from Bioteke (Peking, China), according to the manufactrer's instructions. Genotyping of the *IL-21* gene polymorphisms was carried out using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). PCR reaction was performed in a total volume of 25 ul, including 2.5 ul 10 × PCR buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTPs, 0.5 umol/L each primer, 100 ng of genomic DNA and 2 U of TagDNA polymerase. PCR products were digested with corresponding restriction enzyme for 8 hours and analyzed by 6% polyacrylamide gels with silver staining. About 10% of the samples were randomly selected to perform the repeated assays and the results were 100% concordant. Both of the primers and restriction enzymes in the genotyping analysis as well as the temperature were listed in Table 1.

Statistical analyses

Data were analyzed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Genotype frequencies of rs12508721 and rs2055979 were obtained by direct counting and Hardy–Weinberg equilibrium evaluated by chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) were utilized to assess the effects caused by any differences in alleles, genotypes. Genotypic association tests in a case-control study assuming co-dominant, dominant, recessive or over-dominant genetic models were performed using SNPstats [17].

Results

Genotype distribution of rs12508721 and rs2055979 SNPs were determined under the Hardy-Weinberg equilibrium in control and DCM subjects. As shown in Table 2, genotype and allele frequencies were differently distributed among patients and controls for the two SNPs. These data showed that association between the polymorphisms and asthma risk corresponded to co-dominant, dominant, recessive or over-dominant genetic model. The allele T frequency of SNP rs12508721 was 40 % and 31% in asthma patients and healthy controls, respectively (T versus C, OR=1.58, 95% CI=1.18-2.11, *P*=0.0021). In the co-dominant model, CC versus CT versus TT, OR=1.75, 2.23 respectively (*P*=0.0066). Moreover, the effect of dominant model (CC versus CT+TT, OR=1.83, 95CI=1.24-2.69, *P*=0.0021) was observed.

SNPs	Primers (5'- -3')	Annealing Temperature (°C)	PCR products (bp)	Enzyme	Digested PCR products (bp)
rs12508721	ggagctgttgtttcagaagtagag gttgttgtggaactgaaatcagggt gctctgaacccaaacactctc	64	158	HincII	123 + 25
rs2055979	aaggtctcaaaggaccgaca	56	213	HphI	162 + 51

Table 1: Primers and enzymes for genotyping *IL-21* SNPs.

		rs12508721				rs2055979				
		Cases n (%)	**Controls n (%)**	**OR (95% CI)**	***P* value**		**Cases n (%)**	**Controls n (%)**	**OR (95% CI)**	***P* value**
Model	Genotype					Genotype				
Co-dominant	CC	65 (34.7%)	117 (47%)	1.00	**0.0066**	GG	82 (41.2%)	116 46.6%)	1.00	0.52
	CT	108 54.3%)	111 (44.6%)	**1.75 (1.17-.62)**		GT	100 50.2%)	114 45.8%)	1.24 (0.84-.83)	
	TT	26 (13.1%)	21 (8.4%)	**2.23 (1.16-.17)**		TT	17 (8.5%)	19 (7.6%)	1.27 (0.62-.58)	
Dominant	CC	65 (34.7%)	117 (47%)	1.00	**0.0021**	GG	82 (41.2%)	116 46.6%)	1.00	0.25
	CT/TT	134 67.3%)	132 (53%)	**1.83 (1.24-.69)**		GT/TT	117 58.8%)	113 53.4%)	1.24 (0.85-.81)	
Recessive	CC/CT	173 86.9%)	228 91.6%)	1.00	0.11	GG/GT	182 (91.5%)	230 92.4%)	1.00	0.72
	TT	26 (13.1%)	21 (8.4%)	1.83 (1.42-.69)		TT	17 (8.5%)	19 (7.6%)	1.13 (0.57-.24)	
Overdominant	CC/TT	91 (45.7%)	138 55.4%)	1.00	**0.041**	GG/TT	99 (49.8%)	135 54.2%)	1.00	0.35
	CT	108 54.3%)	111 (44.6%)	**1.48 (1.01-.15)**		GT	100 50.2%)	114 45.8%)	1.20 (0.82-.74)	
	Allele					Allele				
	C	238 (60%)	345 (69%)	1.00	**0.0021**	G	264 (66%)	346 (69%)	1.00	0.29
	T	160 (40%)	153 (31%)	**1.58(1.18-2.11)**		T	132 (34%)	152 (31%)	1.17 (0.87-.58)	

Table 2: Distribution of the *IL-21* SNPs among cases and controls and their associations with asthma risk.

Discussion

IL-21 is a newly described cytokine that modulates B, T, and natural killer cell responses [18,19]. IL-21 displays multiple actions on a range of lymphohematopoietic lineages, including expansion and differentiation of T helper cell subsets [20]. $CD4^+$ T cells, one subset of T cells, upon activation and expansion, develop into different T helper cell subsets with different cytokine profiles and distinct effect functions [21]. The *IL-21* gene, located on chromosome 4q26-q27 approximately 180 kb from the *IL-2* gene, is highly expressed in activated $CD4^+$ T cells [22-24]. IL-21 plays a crucial role in immunoglobulin production and regulates B cell differentiation [25-27]. Moreover, it has also been shown to down-regulate IgE production from IL-4 stimulated B cells by the inhibition of germ line C (epsilon) transcription [28-30]. In addition, *IL-21* inhibits inducible regulatory T cells' (Tregs) differentiation and affects $CD4^+$ T cells [31,32]. The effect of *IL-21* on immune system makes this cytokine an attractive candidate protein for inflammatory disease studies. Most importantly, *IL-21* triggers an increased STAT3 activation without affecting the other STATs including STAT1/2 [33]. It has been shown that defective expression of *IL-21* in STAT3-deficient $CD4^+$ T cells resulted in diminished B-cell helper activity *in vitro* [34,35]. The effect of *IL-21* on immune system makes this cytokine an attractive candidate protein for inflammatory disease studies.

In this work, we analyzed allele and genotype frequencies at two SNPs (rs12508721and rs2055979) of *IL-21* gene in a Chinese Han population. Our data indicated that he allele T frequency of rs12508721 was significantly higher in asthma patients than in controls (OR=1.58, 95% CI=1.18-2.11, P=0.0021). The effect of dominant model (CC versus CT + TT, OR=0.83, 95CI=1.24-2.69, P=0.0021) was observed. However, distributions of allele and genotype frequencies of the SNP rs2055979 showed no differences between asthma patients and controls.

Overall, the present study suggests that *IL-21* gene polymorphisms play an important role in susceptibility to asthma in a Chinese Han population. Future studies are needed to further explore the molecular mechanisms involved in the susceptibility to asthma.

References

1. Sela B (1999) Interleukin IL-13: a central mediator in allergic asthma. Harefuah 137: 317-319. [View Article]
2. Marogna M, Braidi C, Bruno ME, Colombo C, Colombo F, et al. (2013) The contribution of sublingual immunotherapy to the achievement of control in birch-related mild persistent asthma: a real-life randomised trial. Allergologia et Immunopathologia 41: 216-224. [View Article]
3. Kannan AK, Sahu N, Mohanan S, Mohinta S, August A (2013) IL-2-inducible T-cell kinase modulates TH2-mediated allergic airway inflammation by suppressing IFN-gamma in naive $CD4^+$ T cells. J Allergy Clin Immunol 132: 811-820.e5. [View Article]
4. Vovolis V, Kalogiros L, Mitsias D, Sifnaios E (2013) Severe repeated anaphylactic reactions to sublingual immunotherapy. Allergol Immunopathol (Madr) 41: 279-281. [View Article]
5. Kannan JA, Epstein TG. Immunotherapy safety: what have we learned from surveillance surveys?. Curr Allergy Asthma Rep 13: 381-388. [View Article]
6. Corren J. Role of interleukin-13 in asthma. Curr Allergy Asthma Rep 13: 415-420. [View Article]
7. Wang ZD, Lian D, Shen JL, Sun R, Xu W, et al. (2013) Association between the interleukin-4, interleukin-13 polymorphisms and asthma: a meta-analysis. Mol Biol Rep 40: 1365-1376. [View Article]
8. Nie W, Zhu Z, Pan X, Xiu Q (2013) The interleukin-4 -589C/T polymorphism and the risk of asthma: a meta-analysis including 7,345 cases and 7,819 controls. Gene 520: 22-29. [View Article]
9. Kim YJ, Park SW, Kim TH, Park JS, Cheong HS, et al. (2013) Genome-wide methylation profiling of the bronchial mucosa of asthmatics: relationship to atopy. BMC Med Genet 14: 39. [View Article]
10. Ricciardolo FL, Sorbello V, Silvestri M, Giacomelli M, Debenedetti VMG, et al. (2013) TNF-alpha, IL-4R-alpha and IL-4 polymorphisms in mild to severe asthma from Italian Caucasians. Int J Immunopathol Pharmacol 26: 75-84. [View Article]
11. Zhernakova A, Alizadeh BZ, Bevova M, van Leeuwen MA, Coenen MJH, et al. (2007) Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. Am J Hum Genet 81: 1284-1288. [View Article]
12. Festen EA, Goyette P, Scott R, Annese V, Zhernakova A, et al. (2009) Genetic variants in the region harbouring IL2/IL21 associated with ulcerative colitis. Gut 58: 799-804. [View Article]
13. Webb R, Merrill JT, Kelly JA, Sestak A, Kaufman KM, et al. (2009) A polymorphism within IL21R confers risk for systemic lupus erythematosus. Arthritis Rheum 60: 2402-2407. [View Article]
14. Nakou M, Papadimitraki ED, Fanouriakis A, Bertsias GK, C. Choulaki C, et al. (2013) Interleukin-21 is increased in active systemic lupus erythematosus patients and contributes to the generation of plasma B cells. Clin Exp Rheumatol 31: 172-179. [View Article]
15. Marquez A, Davila-Fajardo CL, Robledo G, Rubio JL, de Ramón Garrido E, et al. (2013) IL2/IL21 region polymorphism influences response to rituximab in systemic lupus erythematosus patients. Mol Biol Rep 40: 4851-4856. [View Article]
16. Diaz-Gallo LM, Simeon CP, Broen JC, Ortego-Centeno N, Beretta L, et al. (2013) Implication of IL-2/IL-21 region in systemic sclerosis genetic susceptibility. Ann Rheum Dis 72: 1233-1238. [View Article]
17. 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. [View Article]
18. Chatterjee R, Batra J, Ghosh B (2009) A common exonic variant of interleukin21 confers susceptibility to atopic asthma. Int Arch Allergy Immunol 148: 137-146. [View Article]
19. Habib T, Nelson A, Kaushansky K (2003) IL-21: a novel IL-2-family lymphokine that modulates B, T, and natural killer cell responses. J Allergy Clin Immunol 112: 1033-1045. [View Article]
20. Diehl SA, Schmidlin H, Nagasawa M, Blom B, Spits H (2012) IL-6 triggers IL-21 production by human CD4+ T cells to drive STAT3-dependent plasma cell differentiation in B cells. Immunol Cell Biol 90: 802-811. [View Article]

21. Duhen T, Duhen R, Lanzavecchia A, Sallusto F, Campbell DJ (2012) Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. Blood 119: 4430-4440. [View Article]

22. Monteleone G, Pallone F, Macdonald TT (2009) Interleukin-21 as a new therapeutic target for immune-mediated diseases. Trends Pharmacol Sci 30: 441-447. [View Article]

23. Yuan FL, Hu W, Lu WG, Li X, Li JP, et al. (2011) Targeting interleukin-21 in rheumatoid arthritis. Mol Biol Rep 38: 1717-1721. [View Article]

24. Huang Z, van Velkinburgh JC, Ni B, Wu Y (2012) Pivotal roles of the interleukin-23/T helper 17 cell axis in hepatitis B. Liver Int 32: 894-901. [View Article]

25. Ozaki K, Spolski R, Feng CG, Qi CF, Cheng J, et al. (2002) A critical role for IL-21 in regulating immunoglobulin production. Science 298: 1630-1634. [View Article]

26. Ozaki K, Hishiya A, Hatanaka K, Nakajima H, Wang G, et al. (2006) Overexpression of interleukin 21 induces expansion of hematopoietic progenitor cells. Int J Hematol 84: 224-230. [View Article]

27. Ozaki K, Spolski R, Ettinger R, Kim HP, Wang G, et al. (2004) Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. J Immunol 173: 5361-5371. [View Article]

28. Suto A, Nakajima H, Hirose K, Suzuki K, Kagami S et al. (2002) Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. Blood. 100: 4565-4573. [View Article]

29. Shang XZ, Ma KY, Radewonuk J, Li J, Song XY, et al. (2006) IgE isotype switch and IgE production are enhanced in IL-21-deficient but not IFN-gamma-deficient mice in a Th2-biased response. Cell Immunol 241: 66-74. [View Article]

30. Wood N, Bourque K, Donaldson DD, Collins M, Vercelli D, et al. (2004) IL-21 effects on human IgE production in response to IL-4 or IL-13. Cell Immunol 231: 133-145. [View Article]

31. Piao WH, Jee YH, Liu RL, Coons SW, Kala M, et al. (2008) IL-21 modulates CD4+ CD25+ regulatory T-cell homeostasis in experimental autoimmune encephalomyelitis. Scand J Immunol 67: 37-46. [View Article]

32. Koenen HJ, Smeets RL, Vink PM, van Rijssen E, Boots AM, et al. (2008) Human CD25highFoxp3pos regulatory T cells differentiate into IL-17-producing cells. Blood 112: 2340-2352. [View Article]

33. Eriksen KW, Sondergaard H, Woetmann A, Krejsgaard T, Skak K, et al. (2009) The combination of IL-21 and IFN-alpha boosts STAT3 activation, cytotoxicity and experimental tumor therapy. Mol Immunol 46: 812-820. [View Article]

34. Battaglia A, Buzzonetti A, Baranello C, Fanelli M, Fossati M, et al. (2013) Interleukin-21 (IL-21) synergizes with IL-2 to enhance T-cell receptor-induced human T-cell proliferation and counteracts IL-2/transforming growth factor-beta-induced regulatory T-cell development. Immunology. 139: 109-20. [View Article]

35. Mazerolles F, Picard C, Kracker S, Fischer A, Durandy A (2013) Blood CD4+CD45RO+CXCR5+ T cells are decreased but partially functional in signal transducer and activator of transcription 3 deficiency. J Allergy Clin Immunol 131: 1146-1156.e1-5. [View Article]

Citation: Lin J, Xu G (2017) Association of a Single Nucleotide Polymorphism of *IL-21* Gene with Asthma in a Chinese Han Population. Journal of Clinical Trials and Regulatory Affairs 1: 001-004.