Role of interleukin-4 genetic polymorphisms and environmental factors in the risk of asthma in children

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Genet. Mol. Res. 15 (4): gmr15048873
Received June 10, 2016
Accepted September 15, 2016
Published November 3, 2016
DOI http://dx.doi.org/10.4238/gmr15048873

ABSTRACT. Asthma is an allergic disease characterized by hyperresponsiveness and chronic inflammation of the airway. The interleukin-4 (*IL-4*) gene and its single nucleotide polymorphisms are associated with asthma susceptibility in children. A case-control study was performed to evaluate the relationship between asthma risk and the *IL-4* rs2243250 (589 C/T) and rs2070874 (107 T/C), and IL-4 receptor (*IL-4R*) rs1801275 (576 Q/R) polymorphisms in 317 childhood asthma patients and 351 healthy children as controls. Polymerase chain reaction amplification and sequencing was performed. The effects of interactions between the genes of interest and environmental factors were also analyzed. *IL-4* rs2243250 and rs2070874 allele and genotype frequencies did not significantly differ between the asthma and control groups ($P > 0.05$), but those of *IL-4R* rs1801275 did ($P < 0.05$). The RR genotype and R allele of this *IL-4R* variant were significantly associated with asthma risk, with odds ratios (ORs; and 95% confidence intervals) of 2.97 (2.08-4.25) and 2.99 (2.32-3.85), respectively. Logistic

regression analysis showed that the *IL-4R* 576 Q/R RR genotype demonstrated a positive interaction with environments associated with smoking or pets in its effect on asthma risk, with ORs of 2.18 (P = 0.02) and 2.29 (P = 0.01), respectively. Our results suggest that the *IL-4R* rs1801275 polymorphism is associated with childhood asthma, and the RR genotype confers a high risk of developing this condition. This variant exhibited positive interactions with environments in which smoking or pets were present in increasing the risk of childhood asthma.

Key words: *IL-4*; Asthma; Environmental factors; Smoking; Pets

INTRODUCTION

Asthma is a chronic allergic inflammatory disease of the airway. It is estimated that there are currently 300 million asthma patients worldwide, and the associated morbidity and mortality rates have been steadily increasing (Masoli et al., 2004). The global economic burden of asthma exceeds that of tuberculosis, human immunodeficiency virus infection, and acquired immune deficiency syndrome combined (Wills-Karp et al., 1998), and this disease has become a major public health problem globally (Jaakkola and Jaakkola, 2012).

As a complex disease, the pathogenesis of asthma remains unclear (Cook and Saglani, 2016). However, several studies have shown that it involves inflammatory immune cells (Cook and Saglani, 2016), the secretion of inflammatory mediators, and cytokine interactions (Osei-Kumah et al., 2006), leading to immunoglobulin (Ig) E-mediated hypersensitivity and eosinophil infiltration of the airway, causing chronic inflammation (Wongratanacheewin, 2014). Several complex and diverse risk factors contribute to the development of asthma, involving environment, immunity, lifestyle, and psychology (Hovland et al., 2015; Toskala and Kennedy, 2015; Demirdag and Ramadan, 2016). Many studies concerning this condition have been carried out, mainly focusing on epidemiological and genetic risk factors. It is widely believed that the occurrence of asthma is determined by the compounded effects of numerous genes and environmental aspects, including gene-gene and gene-environment interactions (Gonzalez-Garcia et al., 2015). In recent decades, single nucleotide polymorphisms have become one of the most commonly studied types of sequence variation in investigations of the relationship between genetics and disease susceptibility (Sengler et al., 2002; Tulah et al., 2013).

Interleukin-4 (IL-4) is primarily produced by Th2 cells (Smallwood et al., 2016). The biological function of IL-4 principally comprises increasing B cell major histocompatibility complex class II molecule expression, promoting resting B cell FcεR expression, elevating secretion of IgE and IgG1 in the activation of B cells, and stimulating IgG to IgE class switching in an autocrine manner to facilitate the differentiation of Th2 cells, while inhibiting Th1 cell proliferation and the Th1 response (Antczak et al., 2016; Pattnaik et al., 2016).

To date, many investigations have tested the association between *IL-4* polymorphisms and asthma risk, but their results have not been consistent (Micheal et al., 2013; Tang et al., 2014; Huang et al., 2015; Bal et al., 2016). Therefore, we performed a case-control study to analyze the relationship between three IL-4-related loci [rs2243250 (589 C/T) and rs2070874 (107 T/C) in *IL-4*, and rs1801275 (576 Q/R) in the IL-4 receptor α-chain gene (*IL-4R*)] and risk of childhood asthma. Moreover, the effects of interactions between environmental factors and genotypes on risk were also assessed.

MATERIAL AND METHODS

Subjects

From March 2012 to March 2016, we continuously recruited 317 patients with asthma at the Weinan Central Hospital Department of Pediatrics. Asthma was diagnosed according to the Global Initiative for Asthma guidelines (Borish, 2010). Cases were graded by severity according to clinical features into intermittent, mild sustained, moderate continued, and severe sustained categories. Patients with other respiratory diseases, such as recent upper or lower respiratory tract infections, were excluded from our study. A standard questionnaire was filled out by patients or their parents, and pulmonary function, chest X-ray, and other necessary examinations were performed. Diagnosis of atopic disease was based on the skin prick test (SPT).

During the same period, a total of 351 healthy children were selected as a control group. This group comprised individuals having visited our hospital for the treatment of diseases other than asthma or atopic asthma. All subjects were unrelated Han Chinese individuals residing in the vicinity of the hospital, and their parents' consent and cooperation were obtained. This study was approved by the Ethics Committee of our hospital.

Blood samples and DNA extraction

Between 7:00 and 9:00 a.m., 5-mL blood samples were collected in sterile vacuum tubes containing ethylenediaminetetraacetic acid. Each blood sample was centrifuged at 1300 rpm for 10 min at 4°C, and the upper plasma layer was stored at -70°C, while the buffy coat was preserved at -20°C before extraction of genomic DNA using a QIAamp DNA Blood Mini kit (QIAGEN, Hilden, Germany) following the manufacturer protocol.

Genotyping

Polymerase chain reaction (PCR) amplification and DNA sequencing were used. Primers were designed with Autoprimer (http://www.autoprimer.com) and synthesized by Shanghai SangGong (Shanghai, China). Primer sequences are shown in Table 1. PCRs consisted of 50-µL mixtures containing 75 µM deoxynucleotides, 20 ng genomic DNA, 50 nM primers, 3.5 mM $MgCl_2$, and 0.5 U HotStarTaq polymerase. Using 96-well plates, PCRs were carried out as follows: 95°C for 15 min, then 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s, before a final step at 72°C for 7 min. PCR products were stored at 4°C and subsequently sequenced.

Table 1. Polymerase chain reaction primers used in this study.

SNP	Primer	Sequence (5'-3')
rs2243250	Forward	AAACTAGGCCTCACCTGATACG
(C/T)	Reverse	TGCATAGAGGCAGAATAACAGG
rs2070874	Forward	GAGGTGAGACCCATTAATAG
(T/C)	Reverse	ACGTTGGATGTGCATCGTTAGCTTCTCCTG
rs1801275	Forward	GAGGAAGTAGAACCCGAGATGC
(Q/R)	Reverse	GCAGCCAGGAATGAGGTCTT

SNP = single nucleotide polymorphism.

Statistical analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Demographic and clinical variables of the patients and controls were compared by the *t*-test or chi-square test. Hardy-Weinberg equilibrium (HWE) of genotype frequencies in the control group was assessed by the goodness-of-fit chi-square test. Differences in genotype, allele, and haplotype frequencies between the patient and control groups were analyzed by chi-square tests, and multiple logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). We also tested interactions between genotypes and environmental influences, such as smoking, pets, humidity, respiratory infection, and other factors. $P < 0.05$ was considered statistically significant.

RESULTS

The general characteristics of the patients and control subjects are shown in Table 2. No statistically significant differences in average age or sex were observed between the two groups. Of the 317 asthma patients, 237 (75.00%) had positive SPT results. Concerning disease severity, 70 patients (22.01%) presented with intermittent, 50 (15.72%) with mild sustained, 87 (27.36%) with moderate continued, and 110 (34.91%) with severe sustained asthma.

Table 2. Comparison of the general characteristics of each study group.

	N	Average age (years)	Gender (%)		SPT-positive (%)	Asthma classification (%)			
			Male	Female		Intermittent	Mild sustained	Moderate continued	Severe sustained
Patients	317	5.80 ± 4.24	134 (42.45)	183 (57.55)	237 (75.00)	70 (22.01)	50 (15.72)	87 (27.36)	110 (34.91)
Controls	351	6.26 ± 3.31	151 (43.18)	200 (56.82)	0	-	-	-	-
P value		>0.05	>0.05						

SPT = skin prick test.

IL-4 rs2243250 and rs2070874 and *IL-4R* rs1801275 allele frequencies were consistent with HWE ($P > 0.05$). Comparing the asthma and healthy control groups, no differences in the distribution of *IL-4* rs2243250 or rs2070874 alleles were evident. However, *IL-4R* rs1801275 allele frequencies did significantly differ between these groups ($P < 0.05$). The *IL-4R* 576 Q/R RR genotype and R allele were significantly more common among asthma patients, with ORs (and 95%CIs) of 2.97 (2.08-4.25) and 2.99 (2.32-3.85), respectively, as shown in Table 3.

Table 3. Allele and genotype frequencies of the three loci considered in this study.

Locus	Allele or genotype	Patients [N (%)]	Controls [N (%)]	Chi-square	P	HWE	OR (95%CI)
rs2243250	T	452 (71.29)	518 (73.79)			0.819	1
	C	182 (28.71)	184 (26.21)	1.04	0.31		0.88 (0.69-1.12)
	TT	205 (64.67)	213 (60.68)				1
	CC	112 (35.33)	138 (39.32)	1.13	0.29		1.19 (0.87-1.62)
rs2070874	C	358 (56.47)	416 (59.26)			0.12	1
	T	276 (43.53)	286 (40.74)	1.07	0.30		0.89 (0.72-1.11)
	CC	147 (46.37)	170 (48.43)				1
	TT	170 (53.63)	181 (51.57)	0.28	0.59		0.92 (0.68-1.25)
rs1801275	Q	386 (71.90)	578 (85.00)			0.302	1
	R	248 (28.10)	124 (15.00)	119.39	<0.05		2.99 (2.32-3.85)
	QQ	195 (61.50)	290 (82.62)				1
	RR	122 (38.50)	61 (17.38)	37.31	<0.05		2.97 (2.08-4.25)

HWE = Hardy-Weinberg equilibrium, OR = odds ratio, CI = confidence interval.

The *IL-4R* 576 Q/R polymorphism demonstrated a positive interaction with environments affected by smoking (principally those involving family members smoking; OR = 2.18, 95%CI = 1.15-4.13), pets (OR = 2.29, 95%CI = 1.22-4.28), and damp (OR = 3.54, 95%CI = 1.99-6.28), as displayed in Table 4.

Table 4. Interactions between the *IL-4R*576 Q/R polymorphism and environmental factors.

Factor		Genotype	B	SE	Wald	P	OR	95%CI
Smoking related-environment		QQ	0.29	0.29	1.01	0.32	1.34	0.76-2.38
		RR	0.78	0.33	5.67	0.02	2.18	1.15-4.13
Pets		QQ	0.11	0.28	0.15	0.7	1.12	0.65-1.93
		RR	0.83	0.32	6.69	0.01	2.29	1.22-4.28
Humidity	Damp	QQ	0.55	0.28	3.93	0.08	1.73	1.01-2.98
		RR	1.26	0.29	8.67	0.04	3.54	1.99-6.28
	Dry	QQ	0.13	0.22	1.15	0.42	1.02	0.67-1.83
		RR	0.27	0.23	1.09	0.35	1.14	0.78-2.08
Respiratory infections		QQ	0.33	0.23	1.17	0.22	1.52	0.77-1.86
		RR	0.17	0.29	1.39	0.55	1.64	0.88-2.02

SE = standard error, OR = odds ratio, CI = confidence interval.

DISCUSSION

Bronchial asthma is one of the most common chronic diseases, particularly among children. Approximately 30-50% of childhood asthma cases persist into adulthood, and an estimated 4.4 million children in the USA suffer from this condition (Eaton et al., 2008; Boulet et al., 2012), with 10.9% of high school students being affected (Akinbami and Schoendorf, 2002). Asthma may gradually evolve into chronic obstructive pulmonary disease or pulmonary heart disease.

A series of polymorphisms associated with a variety of asthma phenotypes have been identified in the *IL-4* gene. For instance, it has been suggested previously that the 589 C/T variant in the *IL-4* promoter region is connected to decreased FEV1 (Lee et al., 2004). Such relationships have been confirmed in populations in Japan and the USA (Nambu and Holgate, 2009; Zahran and Bailey, 2013), but not China and Arabia (Al-Mazam and Mohamed, 2001; Chen et al., 2013). The *IL-4* and *IL-13* genes are located on chromosome 5q31, a site potentially linked to atopic asthma. Other studies have reported that *IL-4R* plays an important role in the occurrence of asthma (Cui et al., 2003). The protein encoded by this gene is a key functional component of Th2 cells. IL-4R-deficient mice are unable to produce IgE and exhibit a defective Th2 response, implying that IL-4R is important in IgE regulation, and that corresponding sequence variations can affect signal transduction and increase asthma risk (Barner et al., 1998). *IL-4R* has been considered as an important candidate gene in atopic asthma susceptibility (Barner et al., 1998). It has been reported that three IL-4R alleles, Ile50, Pro478, and Arg551, are related to susceptibility to asthma. Andrews et al. (2001) analyzed the association between asthma risk and IL-4R 576 Q/R genotypes, alleles, and haplotypes, finding that these variants are in linkage disequilibrium and are associated with development of atopic asthma. Other studies have also identified Gln576Arg as a risk factor for this condition. For instance, Rosa-Rosa et al. (1999) examined 149 asthma patients and 57 healthy controls, and established a strong association between the IL-4R 576Arg allele and asthma severity, suggesting that *IL-4R* is implicated in allergic asthma and may be used as a marker to classify the clinical severity of this disease. In our study, the *IL-4* 589 C/T and 107 T/C polymorphisms demonstrated no relationship with asthma susceptibility, whereas *IL-4R* 576 Q/R did.

However, some previous findings are inconsistent with the results of the present study and those of the above studies. For example, Mak et al. (2007) performed an investigation of 292 asthma patients and 292 healthy controls in Hong Kong, concluding that the *IL-4* 589 C/T and *IL-4R* Gln576Arg variants do not significantly correlate with asthma risk in this population. Hosseini-Farahabadi et al. (2007) studied 30 asthma patients and 50 normal controls from an Iranian population, and demonstrated an association between *IL-4* 589 C/T and asthma. Kamali-Sarvestani et al. (2007) also confirmed this polymorphism to be associated with this disease in a southern Iranian population. In addition, Li et al. (2008) performed a meta-analysis showing that this sequence variation plays an important role in asthma pathogenesis, although the authors suggested that further work is needed to confirm the mechanism responsible.

In conclusion, we suggest that the RR genotype of the *IL-4R* 576 Q/R variant may increase risk of asthma, and in exerting this effect, demonstrates a significant interaction with environments associated with smoking and pets. Further studies with large sample sizes are greatly needed to confirm the relationship between *IL-4* genetic polymorphisms and asthma pathogenesis.

Conflicts of interest

The authors declare no conflict of interest.

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