The Diversity of type-2 Inflammatory Cytokine Polymorphisms Associated with Clinical Outcomes in Thai Asthmatic Patients

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Abstract

Background: Type 2 inflammation contributes to severe asthma pathogenesis via type-2 cytokines. IL4, IL13, and IL5 are vital in type 2 inflammation and airway smooth muscle proliferation. Hence, these cytokines' single nucleotide polymorphisms (SNPs) may affect the asthma phenotype in Thai asthmatic patients. This study investigated the association between type 2 cytokine polymorphisms and clinical outcomes of Thai asthmatic patients. **Subjects and methods:** A cross-sectional study was performed on adult Thai asthmatic patients. TaqMan SNPs genotyping assay was used to determine the genotypes of IL4 (C33T and C-590T), IL5 C-703T, and IL13 (A2044G and C-1112T). Spirometry was utilized to assess lung function. The specific IgE level was measured by Fluoro-enzyme immunoassay. Bead-based immunoassays were used to measure cytokine levels and ILC2 and Th2 cell count. **Results:** A total of 98 consecutive asthmatic patients were recruited. IL4 C33T polymorphism, the TT genotype was associated with FAO compared to CC and CT+CC genotypes (p=0.037, 0.039, respectively), and the T allele was associated with fixed airflow obstruction (FAO) (p = 0.021). IL13C-1112T polymorphism, the CT genotype was associated with FAO (p=0.006), and the CT+TT genotype was associated with FAO compared with the CC genotype (p=0.019). IL-5 C-703T, CT, and CT+TT genotype was associated with FAO compared with the CC genotype (p=0.017, 0.033, respectively). Neither IL-4 SNPs nor the IL-13 polymorphisms were associated with allergen sensitization, as confirmed by the positive specific IgE. **Conclusions:** FAO in asthma mediated via airway smooth muscle proliferation is associated with IL4 C33T and IL13C-1112T in Thai asthmatic patients.

***Keywords*:** *Asthma, Type 2 Inflammatory Cytokines, Gene Polymorphism, Phenotype*

1. Introduction

Asthma is a heterogeneous disease often characterized by chronic airway inflammation affecting the airways of the lungs, affecting approximately 300 million people worldwide, affecting approximately 7% of the Thai population in 2018. In addition, the asthma mortality rate in the Thai population was 8-9 per day, equivalent to 3,142 per year in 2018 (Global Asthma Network (2018); Thai Asthma Guideline in Adults (2018)). The pathophysiology of asthma is provoked by airway remodeling, and inflammation to promote airway inflammation, mucus overproduction, bronchial hyperresponsiveness (BHR), and immunoglobulin E (IgE) synthesis in the lungs. Also, asthma symptoms include shortness of breath, chest tightness, wheezing, and coughing (Gillissen, 2015). Inflammation is a significant part of the pathophysiology of asthma and is divided into type 2 high airway inflammation and type 2 low airway inflammation (Godar et al., 2018). The most common type in adult asthmatic patients is type 2 high airway inflammation. The incidence of type 2 high airway inflammation was found in up to 70% of asthma patients (Canonica et al., 2021).

Type 2 high inflammation is associated with th2 lymphocytes, including eosinophils, basophils, mast cells, and important cytokines that effectively induce inflammation comprising interleukin (IL) 4, IL5, and IL13, which are often produced by the adaptive immune system on recognition of allergens and activated by allergens, viruses, bacteria, pollution, and oxidants that stimulate the innate immune system via the production of Alarmin by epithelial cells. The Type 2 high inflammation phenotype can be defined by high blood eosinophils, elevated IgE, or fractional exhaled nitric oxide. IL4 and IL13 are crucial cytokines for allergic inflammation. IL5 is a vital cytokine that induces eosinophil inflammation. (GINA, 2022).

The asthmatic patients required maintenance and estimated American Thoracic Society/European Respiratory Society Severe Asthma Taskforce 2019 to determine whether severe asthma contained the asthmatic patients that have uncontrolled asthma assessed by ACT score, frequent asthma exacerbation, severe asthma exacerbation, or fixed airflow obstruction (Holguin (2020); Travers (2015)). Some asthma patients have severe and uncontrollable symptoms of asthma (Tonga et al., 2018) caused by type 2 inflammation that does not respond to corticosteroids. It requires treatment with biological agents that directly affect type 2 inflammation (Froidure et al., 2016).

Several studies have reported that genetic influence increases the risk of asthma. Imani, Danyal, et al. (2020) demonstrated that IL-4 C33T polymorphism potentially acts as a risk factor for asthma in Asians, Europeans, and Americans. Al-Ahmad, Mona, et al. (2023) reported that IL-4 C-590T polymorphism is associated with a higher chance of developing mild asthma in Asians. Nie, Wei, et al. (2013) reported that IL13 C-1112T and A2044G polymorphisms were risk factors for asthma in Asians and Caucasians. Furthermore, Utsumi, Yu et al. (2013) reported that IL13 A2044G was associated with airway hyperresponsiveness in Japanese adult asthmatics.

Currently, the diagnosis and severity assessment of asthma symptoms is quite delayed. It can only be assessed when the patient has clinical symptoms, a history of respiratory symptoms, a family history, physical examination, and pulmonary function tests, including wheezing, spirometry, and peak expiratory flow measurements in response to bronchodilators and bronchial sensitivity tests. The previous studies focus only on the risk of asthma; however, few studies focus on comparing the phenotypes of polymorphisms. Therefore, this study focuses on determining polymorphisms of type 2 inflammatory cytokines associated with clinical outcomes to enhance the ability to prognosis or monitor these conditions based on individual genetics.

2. Objectives

This study investigated the association between SNP in IL-4 C33T, IL-4 C-590T, IL-5 C-703T, IL-13 A2044G, and IL-13 C-1112T with clinical outcomes in Thai asthmatic patients.

3. Materials and Methods

3.1 Subject recruitment 98 adult asthma patients were diagnosed according to GINA guidelines and treated with ICS at the chest clinic in Ramathibodi Hospital to collect the EDTA blood (5 mL) and this study received approval from the Human Research Ethics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University (COA. MURA2023/435)

3.1.1 Sample size calculations were used to estimate population proportion to calculate each sample size on each SNPs, and they were calculated based on minor allele frequency (MAF) in the NCBI database and the expected frequencies of genotypes of each single nucleotide polymorphisms using the Hardy-Weinberg equilibrium by modifying the method of Hameed et al. (2019)

3.1.2 Inclusion criteria comprised patients who were diagnosed according to GINA guidelines and treated with inhaled corticosteroids at the chest clinic in Ramathibodi Hospital

3.1.3 Exclusion criteria comprised participants who declined or withdrew, participants who were unable to perform spirometry, patients with a history and clinical signs of any infection and exposure to allergen and pollution within eight weeks, patients who taking steroid medication within eight weeks, patients with a history of cytokine/receptor affinity studied were monoclonal antibody inhibitors of the following cytokines: IL-5, IL-4, and IL-13, and patients who have a history of tobacco smoking $\geq$ 10 pack-year, patients with parasitic infection, systemic autoimmune disease, and hematologic malignancy

3.2 Spirometry is used to assess lung function to assess forced expiratory volume - one second (FEV)1% predicted. After that, divide the patients and phenotypes comprise fixed airflow obstruction

3.2.1 Fixed airflow obstruction was estimated to be FEV1% predicted and divided into two groups comprised of patients with fixed airflow obstruction (FEV1% predicted <70%) and patients without fixed airflow obstruction (FEV1% predicted $\geq$70%) (GINA, 2022)

3.3 400µL of whole blood from an EDTA blood tube was utilized for DNA extraction via nucleic acid purification. The automated machine disrupts cells, digests proteins, binds nucleic acid with magnetic particles, removes debris using wash buffer, and finally elutes the nucleic acid. The resulting product is a nucleic acid prepared for genotyping (Roche Diagnostics, 2012)

3.4 4 µL of extracted and purified genomic DNA was utilized for genotyping using Taqman™ Real-Time PCR Assays targeting SNPs, including IL4 C33T, IL4 C-590T, IL13 A2044G, IL13 C-1112T, and IL5 C-703T (ThermoFisher, 2016)

3.5 Serum 50 µL was used to measure interleukin (IL) levels of T2 inflammation (IL-4, IL-5, IL-6, IL-10, IL-13) with bead-based immunoassays (BioLegend, 2023)

3.6 Blood 3 ml was used to measure sIgE level (EDTA tube) with ImmunoCAP™ by Fluoro-Enzyme Immunoassay (FEIA), automatic Phadia instrument (Thermo Fisher Scientific, Thailand), and Specific IgE levels were considered inhalation allergy 18 substances such as House dust mite, American cockroach, Timothy grass, Bahia grass, Candida albicans, Penicillium chrysogenum, Cat dander, Bermuda grass, Meadow grass, Acacia, Alternaria alternate, Cladosporium herbarum, Dog dander, Rye-grass, Johnson grass, Careless weed, Setomelanomma, Aspergillus fumigatus

3.6.1 Allergen sensitization was estimated to specific IgE levels and divided into two groups comprised asthma with allergen sensitization (sIgE $\geq$ 0.35 KUA/L) and asthma without allergen sensitization (sIgE < 0.35 KUA/L) (Zeng, G. et al., 2018)

3.7 Serum 50 µL was used to measure eosinophil count (EDTA blood tube) and was analyzed by flow cytometry (BD FACSLyric™ Flow Cytometry, BD Biosciences, Thailand)

4. Results and Discussion

4.1 Result

Ninety-eight consecutive asthmatic patients were recruited at the chest clinic in Ramathibodi Hospital, and the following results were demonstrated. **Table 1** shows the demographic and biochemical profile of asthmatic patients. According to statistics analysis, the results show mean ± SD and median (min-max).

Table 1: Demographic and biochemical profile of asthmatic patients.

Demographic Categories	**Asthmatic patients (n=98)**
Gender (n, %)	
Female	66 (67.35%)
Male	32 (36.65%)
Age (year, mean±SD)	63.61±13.36
Specific IgE levels (kIU/L; median, min-max)	0.61 (0.02-101)
Spirometry (mean ±SD; median, min-max)	
FEV_1 (%predicted)	64.33±23.48
Cytokines levels (pg/ml; median, min-max)	
Interleukin-4	0 (0-93.12)
Interleukin-5	0.54 (0-17.16)
Interleukin-6	0.06 (0-31.65)
Interleukin-10	0.05 (0-16.46)
Interleukin-13	0 (0-153.85)
TNF-α	0 (0-128.77)
Cell count (Cells/uL; mean ±SD; median, min-max)	
Absolute Eosinophil counts	260 (38-1635)
Absolute T helper cell counts (Th cell)	848.65±388.74
Absolute Innate lymphoid type-2 cell counts (ILC2)	121 (16-476)

Absolute T helper two cell counts (Th2 cell)	130 (0-1567)
%Th of Lymphocyte	37.342±7.984
%ILC2 of Th	10.12 (1.74-64.67)
%Th2 of Th	15.25 (0-99.71)

FEV_1: Forced expiratory volume in one second, FVC: Forced vital capacity.

Interleukin-13 A2044G (rs20541) polymorphism genotypes among asthmatic patients, and IL13 is the crucial cytokine on airway smooth muscle proliferation; the genotype distribution was as follows: AA genotype in 19 individuals (19.39%), AG genotype in 49 individuals (50%), and GG genotype in 30 individuals (30.61%). We applied the Kruskal-Wallis Test, as presented in **Table 2**, to analyze these results. Notably, we observed significant differences between genotypes in interleukin-4 levels (p=0.024), and the GG genotype has the highest IL-4 level compared to AA and AG genotypes. However, we did not observe statistically significant differences in various other factors.

Table 2: Comparison of clinical characteristics according to interleukin-13 A2044G genotypes in asthmatic patients

Parameter	IL-13 A2044G genotypes (median, min-max)			P-value
	AA N = 19 (19.39%)	AG N = 49 (50%)	GG N = 30 (30.61%)	
sIgE levels (kUA/l)	0.5 (0.03-101)	0.79 (0.02-50.5)	0.71 (0.02-20.4)	0.629
Cytokines levels (pg/ml)				
Interleukin-4	0 (0-36.34)	0 (0-78.07)	0.53 (0-93.12)	**0.024***
Interleukin-5	1.57 (0-6.99)	0 (0-16.43)	0.53 (0-17.16)	0.2
Interleukin-6	0 (0-28)	0 (0-31.65)	1.29 (0-22.28)	0.512
Interleukin-10	0.04 (0-1.72)	0.05 (0-16.46)	0.12 (0-4.23)	0.613
Interleukin-13	0 (0-42.48)	0 (0-66.87)	0 (0-153.85)	0.98
TNF-α	0 (0-32.74)	0 (0-128.77)	0.02 (0-90.04)	0.154
Cell counts (Cells/uL)				
Absolute Eosinophil counts	328.5 (114-1635)	245 (41-1561)	242.5 (38-1077)	0.352
Absolute Th cell counts	918.5 (303-1933)	761 (272-2752)	731.5 (374-1413)	0.148
Absolute ILC2 counts	151 (34-476)	107 (16-340)	133.5 (40-419)	0.147
Absolute Th2 cell counts	245.5 (0-1567)	104 (0-1311)	132.5 (0-1131)	0.404
%Th of Lymphocyte	36.98 (23.92-44.12)	37.45 (18.64-50.1)	39.68 (18.33-64.72)	0.548
%ILC2 of Th	11.53 (2.19-33.76)	9.05 (2.05-64.67)	11.71 (1.74-33.61)	0.218
%Th2 of Th	23.75 (0-99.71)	14.72 (0-99.51)	16.66 (0-99.08)	0.772

*Significant difference (P≤0.05). N: Number of patients, IL: Interleukin, Th: T helper cell, ILC2: Type 2 innate lymphoid cells, Th2: T helper two cells

Interleukin-13 C-1112T polymorphism genotypes among asthmatic patients (**Table 3**), the genotype distribution revealed that the CC genotype was present in 60 individuals (61.22%), the CT genotype in 31 individuals (31.63%), and the TT genotype in 7 individuals (7.14%). Utilizing the Kruskal-Wallis Test, statistically significant differences were detected among these genotypes regarding absolute Th2 cell counts (p=0.024) and %Th2 of Th (p=0.043). Specifically, the TT genotype showed the highest absolute Th2 cell counts and %Th2 of Th compared to the CC and CT genotypes. However, no statistically significant variations were observed in numerous other factors.

Table 3: Comparison of clinical characteristics according to interleukin-13 C-1112T genotypes in asthmatic patients

Parameter	IL-13 C-1112T genotypes (median, min-max)			P-value
	CC N = 60 (61.22%)	CT N = 31 (31.63%)	TT N = 7 (7.14%)	
sIgE levels (kUA/l)	0.91 (0.02-101)	0.42 (0.02-50.5)	0.55 (0.08-28.3)	0.853
Cytokines levels (pg/ml)				
Interleukin-4	0 (0-93.12)	0 (0-78.07)	0 (0-36.34)	0.629
Interleukin-5	0.33 (0-17.16)	0.4 (0-16.43)	1.73 (0-4.4)	0.659
Interleukin-6	0.17 (0-26.86)	0 (0-31.65)	3.88 (0-28)	0.165
Interleukin-10	0.11 (0-16.46)	0.01 (0-5.77)	0.09 (0-1.01)	0.384
Interleukin-13	0 (0-153.85)	0 (0-59.5)	0 (0-0)	0.843
TNF-α	0 (0-90.04)	0 (0-128.77)	0 (0-10.79)	0.478
Cell count (Cells/uL)				
Absolute Eosinophil counts	228.5 (38-1116)	345 (67-1561)	248.5 (155-1635)	0.102
Absolute Th cell counts	765 (319-1894)	797 (272-2752)	1215.5 (303-1933)	0.443
Absolute ILC2 counts	125 (20-419)	109 (16-340)	172.5 (34-476)	0.806
Absolute Th2 cell counts	106 (0-1131)	116 (0-1311)	895 (292-1567)	**0.016***
%Th of Lymphocyte	38.16 (18.33-64.72)	37.73 (18.64-50.1)	32.19 (31.35-37.93)	0.271
%ILC2 of Th	10.39 (1.74-33.61)	8.11 (2.19-64.67)	11.93 (6.47-14.88)	0.664
%Th2 of Th	10.46 (0-99.08)	14.72 (0-99.71)	69.96 (31.01-96.11)	**0.043***

*Significant difference (P≤0.05). N: Number of patients, IL: Interleukin, Th: T helper cell, ILC2: Type 2 innate lymphoid cells, Th2: T helper two cells

Analysis of interleukin polymorphisms revealed a significant association between IL-13 C-1112T and fixed airflow obstruction (p=0.015). The IL-13 C-1112T CT genotype had a 2.94 times greater chance of fixed airflow obstruction compared to the CC genotype (95% CI = 1.22-6.88). Similarly, the IL-5 C-703T CT genotype had a 1.81 times greater chance of fixed airflow obstruction compared to the CC genotype (95% CI = 1.06-3.12). Significant differences were also observed in homozygous and heterozygous genotypes, with IL-13 C-1112T and IL-5 C-703T CT+TT genotype had a 2.13- and 1.5-times greater chance of fixed airflow obstruction compared to CC genotype (95% CI = 1.07-4.14 and 1.01-2.22), respectively and IL-4 C33T TT genotype had a 1.87 times greater chance of fixed airflow obstruction compare to CT+CC genotype (95% CI = 0.99-3.53). Additionally, allele T of the IL-4 C33T genotype showed a 1.35 times greater chance of fixed airflow obstruction compared to allele C (95% CI = 1.03-1.78), as detailed in **Table 4**. These findings significantly enhance our understanding of the relationship between interleukin polymorphisms and the manifestation of fixed airflow obstruction

Table 4: Comparison between interleukin polymorphisms and fixed airflow obstruction in asthmatic patients

Gene	Genotypes	N	Fixed airflow obstruction (n, %)		PR (95% CI)	P-value		
			Patients with FAO (FEV_1 predicted < 70%)	Patients without FAO (FEV_1 predicted ≥ 70%)				
IL-4C33T						0.091		
	CC	10	3 (8.33%)	7 (21.87%)			Ref.	
	CT	30	14 (38.88%)	16 (50%)			0.355	Ref.
	TT	28	19 (52.79%)	9 (28.13%)	1.53 (0.96-2.43)		**0.037***	0.103
	CT+TT	58	33 (91.67%)	25 (78.13%)	1.17 (0.95-1.44)	0.115		
	CC	10	3 (8.33%)	7 (21.87%)				
	TT	28	19 (52.77%)	9 (28.12%)	1.87 (0.99-3.53)	**0.039***		
	CT+CC	40	17 (47.23%)	23 (71.88%)				
	T allele	86	52 (72.23%)	34 (53.13%)	1.35 (1.03-1.78)	**0.021***		
	C allele	50	20 (27.77%)	30 (46.87%)				
IL-4C-590T						0.221		
	CC	9	3 (8.33%)	6 (18.75%)			Ref.	
	CT	31	15 (41.66%)	16 (50%)			0.424	Ref.
	TT	28	18 (50.01%)	10 (31.25%)			0.103	0.219
	CT+TT	59	33 (91.67%)	26 (81.25%)	1.12 (0.92-1.37)	0.205		
	CC	9	3 (8.33%)	6 (18.75%)				
	TT	28	18 (50%)	10 (31.25%)	1.6 (0.87-2.94)	0.117		
	CT+CC	40	18 (50%)	22 (68.75%)				
	T allele	87	51 (70.84%)	36 (56.25%)	1.25 (0.96-1.63)	0.08		
	C allele	49	21 (29.16%)	28 (43.75%)				
IL-13A2044G						0.210		
	AA	13	9 (25%)	4 (12.5%)			Ref.	
	AG	34	19 (52.77%)	15 (46.87%)			0.404	Ref.
	GG	21	8 (22.23%)	13 (40.63%)			0.077	0.199

	AG+GG	55	27 (75%)	28 (87.5%)	0.85 (0.68-1.07)	0.191		
	AA	13	9 (25%)	4 (12.5%)				
	GG	21	8 (22.22%)	13 (40.62%)	0.54 (0.26-1.14)	0.101		
	AG+AA	47	28 (77.78%)	19 (59.38%)				
	G allele	76	35 (48.62%)	41 (64.07%)	0.75 (0.56-1.02)	0.07		
	A allele	60	37 (51.38%)	23 (35.93%)				
IL-13C-1112T						**0.015***		
	CC	41	17 (47.22%)	24 (75%)			Ref.	
	CT	22	17 (47.22%)	5 (15.62%)	2.94 (1.22-6.88)		**0.006***	Ref.
	TT	5	2 (5.56%)	3 (9.38%)			0.950	0.099
	CT+TT	27	19 (52.78%)	8 (25%)	2.13 (1.07-4.14)	**0.019***		
	CC	41	17 (47.22%)	24 (75%)				
	TT	5	2 (33.33%)	3 (9.37%)	0.59 (0.11-3.32)	0.547		
	CT+CC	63	34 (66.67%)	29 (90.63%)				
	T allele	32	21 (29.17%)	11 (17.19%)	1.69 (0.88-3.24)	0.100		
	C allele	104	51 (70.83%)	53 (82.81%)				
IL-5C-703T						0.057		
	CC	25	9 (25%)	16 (50%)			Ref.	
	CT	31	21 (58.33%)	10 (31.25%)	1.82 (1.06-3.12)		**0.017***	Ref.
	TT	12	6 (16.67%)	6 (18.75%)			0.416	0.280
	CT+TT	43	27 (75%)	16 (50%)	1.5 (1.01-2.22)	**0.033***		
	CC	25	9 (25%)	16 (50%)				
	TT	12	6 (16.66%)	6 (18.75%)	0.88 (0.31-2.48)	0.822		
	CT+CC	56	30 (83.34%)	26 (81.25%)				
	T allele	55	33 (45.84%)	22 (34.38%)	1.33 (0.87-2.03)	0.174		
	C allele	81	39 (54.16%)	42 (65.62%)				

* Significant difference (P≤0.05). N: Number of patients, PR: Prevalence ratio, FAO: Fixed airflow obstruction.

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The relationship between interleukin polymorphisms and allergic sensitization asthma was subjected to a chi-square test. This analysis revealed that no significant differences were observed in each interleukin polymorphism when compared to the allergic sensitization asthma, as indicated in **Table 5**

Table 5: Comparison between interleukin polymorphisms and allergic sensitization asthma in asthmatic patients

			Allergic sensitization (n, %)					
Gene	Genotypes	N	Patients without allergic sensitization (sIgE < 0.35)	Patients with allergic sensitization (sIgE ≥ 0.35)	PR (95% CI)	P-value		
IL-4 C33T						0.424		
	CC	11	3 (7.70%)	8 (13.55%)			Ref.	
	CT	49	18 (46.15%)	31 (52.54%)			0.552	Ref.
	TT	38	18 (46.15%)	20 (33.91%)			0.235	0.317
	CT+TT	87	36 (92.31%)	51 (86.45%)	1.06 (0.93-1.22)	0.368		
	CC	11	3 (7.69%)	8 (13.55%)				
	TT	38	18 (46.15%)	20 (33.89%)	1.36 (0.83-2.22)	0.223		
	CT+CC	60	21 (53.85%)	39 (66.11%)				
	T allele	95	24 (50%)	71 (60.17%)	0.83 (0.60-1.14)	0.229		
	C allele	71	24 (50%)	47 (39.83%)				
IL-4C-590T						0.691		
	CC	10	3 (7.69%)	7 (11.86%)			Ref.	
	CT	50	19 (48.71%)	31 (52.54%)			0.631	Ref.
	TT	38	17 (43.6%)	21 (35.6%)			0.400	0.524
	CT+TT	88	36 (92.31%)	52 (88.14%)	1.04 (0.92-1.19)	0.504		
	CC	10	3 (7.69%)	7 (11.86%)				
	TT	38	17 (43.58%)	21 (35.59%)	1.22 (0.74-2.01)	0.426		
	CT+CC	60	22 (56.42%)	38 (64.41%)				
	T allele	136	63 (71.59%)	73 (61.87%)	1.15 (0.95-1.40)	0.145		
	C allele	70	25 (28.41%)	45 (38.13%)				
IL-13 A2044G						0.765		
	AA	19	9 (23.07%)	10 (16.94%)			Ref.	
	AG	49	18 (46.15%)	31 (52.54%)			0.421	Ref.
	GG	30	12 (30.78%)	18 (30.52%)			0.611	0.771
	AG+GG	79	30 (76.93%)	49 (83.06%)	0.92 (0.75-1.13)	0.453		
	AA	19	9 (23.07%)	10 (16.94%)				
	GG	30	12 (30.76%)	18 (30.51%)	1.01 (0.54-1.85)	0.978		
	AG+AA	68	27 (69.24%)	41 (69.49%)				
	G allele	109	42 (53.85%)	67 (56.78%)	0.94 (0.73-1.22)	0.685		
	A allele	87	36 (46.15%)	51 (43.22%)				
IL-13C-1112T						0.457		
	CC	60	22 (56.41%)	38 (64.41%)			Ref.	
	CT	31	15 (38.46%)	16 (27.11%)			0.281	Ref.
	TT	7	2 (5.13%)	5 (8.48%)			0.672	0.341
	CT+TT	38	17 (43.59%)	21 (35.59%)	1.22 (0.74-2.01)	0.426		
	CC	60	22 (56.41%)	38 (64.41%)				
	TT	7	2 (5.31%)	5 (8.47%)	0.61 (0.12-2.96)	0.528		
	CT+CC	91	37 (94.69%)	54 (91.53%)				
	T allele	45	19 (24.36%)	26 (22.04%)	1.11 (0.65-1.85)			
	C allele	151	59 (75.64%)	92 (77.96%)				
IL-5C-703T						0.487		

CC	34	11 (28.21%)	23 (38.98%)			Ref.	
CT	48	22 (56.41%)	26 (44.06%)			0.220	Ref.
TT	16	6 (15.38%)	10 (16.96%)			0.720	0.561
CT+TT	64	28 (71.79%)	36 (61.02%)	1.17 (0.88-1.56)	0.273		
CC	34	11 (28.21%)	23 (38.98%)				
TT	16	6 (15.38%)	10 (16.94%)	0.91 (0.35-2.29)	0.837		
CT+CC	82	33 (84.62%)	49 (83.06%)				
T allele	80	34 (43.59%)	46 (38.99%)	1.11 (0.79-1.56)	0.521		
C allele	116	44 (56.41%)	72 (61.01%)				

* Significant difference (P≤0.05). N: Number of patients, PR: Prevalence ratio.

4.2 Discussion

Asthma is a chronic lung disease affecting people of all ages. It is caused by inflammation (a systematic analysis for the Global Burden of Disease Study, 2019). The inflammation consists of type-2 high asthma and type-2 low asthma. Type 2 high asthma is an important type because that causes about 70% of asthma (Canonica et al., 2021) and is the pivotal inflammation contributor to the pathogenesis of severe asthma, with type-2 cytokines such as IL-4, IL-13, and IL-5. In addition to type 2 cytokines being important that is initiated from epithelial cells in the lungs, triggers a cascade of responses, activating T-helper type 2 cells, mast cells, dendritic, eosinophils, and basophils. IL-4 and IL-13, primarily secreted by Th2 cells and type 2 innate lymphoid cells, are essential in the development and pathogenesis of asthma. They contribute to allergic inflammation by promoting isotype switching in B cells, leading to IgE-mediated mast cell degranulation (Mak, 2006). Additionally, IL-4 and IL-13 drive fixed airflow obstruction through airway smooth muscle proliferation. Moreover, the synergistic proliferative effect is significantly amplified in the presence of proinflammatory cytokines (Bossé et al., 2008). IL-5 is produced by Th2 cells and ILC2s induced by epithelial innate cytokines and plays a central role in the functions of eosinophils, and that can also result in the induction, maintenance, and amplification of eosinophilic inflammation (Pelaia et al., 2019).

Previous studies reported the polymorphism with asthma on IL-4, IL-13, and IL-5 influence to increase the risk of asthma in different ethnicities. In our study, we conducted a cross-sectional study to evaluate the correlation between genotypes and clinical outcomes in asthmatic patients.

In the meta-analysis on IL4C33T polymorphisms and asthma risk, Imani et al. (2020) reported that IL-4 C33T polymorphism might serve as a potential risk factor for asthma across various ethnicities and age groups. In the Asian population, there was an association between IL-4 C33T polymorphism and the risk of asthma under recessive and allelic models. Moreover, the IL-4 C33T polymorphism has been linked to enhanced IL-4 activity, as supported by previous research (Rosenwasser, 1995). The improvement in IL-4 activity is functionally significant, as it has been associated with the ability to reduce the proliferation of smooth muscle cells in the human airway, which is a crucial factor contributing to fixed airflow obstruction (Hawker et al., 1998). This finding adds a valuable feature to our study, highlighting the potential impact of genetic variations on IL-4 function and its associations with fixed airflow obstruction. Our study suggests that the T allele of the IL-4 C33T polymorphism in asthmatic patients may contribute to the observed association with fixed airflow obstruction. However, this study is associated with the accepted role of IL-4 in the proliferation of smooth muscle cells in the human airway. In contrast, Li, Zhi-Peng, et al. 2014 reported that the CC+CT genotype is a risk of allergic disease, but our study found that the TT genotype is a risk of fixed airflow obstruction compared to the CC+CT genotype. This finding prompts further investigation into the potential underlying mechanisms fundamental to this genetic association. It underscores the importance of understanding the role of IL-4 C33T polymorphism in developing fixed airflow obstruction in asthma.

A recent meta-analysis performed by Mei Q and Qu J demonstrates that IL13 A2044G polymorphisms were risk factors for asthma in the Asian population (109). IL-13 shared signaling pathways with IL-4, which involved autoactivation in producing IL-4 (Bao, 2015). In our study, we found that each genotype was significantly different for IL-4 levels, and the GG genotype found the highest levels of IL-4.

IL-13 C-1112T has been reported to be associated with the risk and susceptibility of asthma in meta-analysis (Nie et al., 2013). The baseline function of IL-13 is associated with the pathogenesis of asthma through type 2 inflammation, specifically via the Th2 cell pathway. Furthermore, IL-13 enhances the migration of dendritic cells, leading to increased stimulation of the Th2 cell, thus activating the signaling pathway associated with the Th2 cell differentiation (Walker and McKenzie, 2018). Our study identified an association between the IL-13 C-1112T polymorphism, the absolute Th2 cell count, and the percentage of T helper two cells among all T helper cells. The IL-13 C-1112T polymorphism occurs from a single nucleotide variation within the DNA promoter, possibly influencing the expression of IL-13. This variation may impact the functionality of IL-13 expression. Additionally, IL-13 was mediated via airway smooth muscle proliferation (Risse et al., 2011), contributing to its association with fixed airflow obstruction. The results suggest a significant association between IL-13 C-1112T polymorphism and fixed airflow obstruction. Specifically, asthmatic patients with the CT genotype demonstrate an increased susceptibility to fixed airflow obstruction compared to those with the CC genotype. In contrast, asthmatic patients with the CT+TT genotype demonstrate a similar association when contrasted with the CC genotype.

Hameed et al. (2019) mentioned polymorphism associated with the TT genotype in Iraqi asthmatic children. Additionally, the TT genotype of IL-5 C-703T polymorphism impacts IL-5 levels and eosinophil count but has no consequence on the IgE level. TT genotype is a risk factor for mild asthma. However, both previous studies demonstrated contradictory results regarding severity and genotype. In our study, the CT+TT genotype is associated with fixed airflow obstruction. However, previous studies have not investigated the CT+TT genotype of IL-5 C-703T polymorphism. Our study showed significant differences in IL-5 C-703T polymorphism on IL-10 and fixed airflow obstruction. IL-10 was produced by most cells of the innate and adaptive immune response (Howes et al., 2014). The specific mechanism by which IL-5-stimulated cells contribute to expression changes IL-10 driven by the IL-5 C-703T polymorphism remains unclear, and the details of the molecular pathways and gene variations involved have not been fully explained. Kay et al., (2004) reported that IL-5 is also associated with the induction of airway remodeling. In our study, we found that IL-5 C-703T polymorphism is a risk factor for fixed airflow obstruction that is the cause of genetic variation.

The current study's focus is on asthmatic patients with SNPs to assess and confirm the phenotype such as IL-4 C33T, IL-13 C-1112T, and IL-5 C-703T were associated with fixed airflow obstruction, IL13-A2044G was associated with cytokines levels when we know the genotype of the asthma patients that can enhance the prognosis. Hence, genetic variation is crucial in asthma prognosis, and the future we can apply to asthma management revolves around achieving optimal control of symptoms and minimizing the risk of exacerbations while mitigating potential adverse effects of medication.

5. Conclusion

Fixed airflow obstruction in asthma mediated via airway smooth muscle proliferation is associated with IL4 C33T and IL13C-1112T in Thai asthmatic patients. IL5 C-703T is associated with IL10 levels and has consequences for fixed airflow obstruction via airway remodeling and airway smooth muscle proliferation.

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