JOURNAL OF FUNCTIONAL VENTILATION AND PULMONOLOGY
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J Func Vent Pulm 2016; 20(7): 1-68
DOI: 10.12699/jfvp.7.20.2016.54

ORIGINAL ARTICLE

The frequency of FCER2 genotype distributions related to corticosteroid response in asthma patients treated at the National Hospital of Paediatrics

La fréquence de la distribution des génotypes FCER2 liée à la réponse aux corticostéroïdes chez les patients asthmatiques traités à l'Hôpital National de la Pédiatrie

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SUMMARY

Introduction. Recent studies showed that FCER2 is an important gene related to regulate IgE production which is a mediator associated with asthma.

Method. In this study, PCR-Sequencing was used to analyze FCER2 polymorphism in 130 patients at National Hospital of Paediatrics.

Results. The results of the present study showed that 17 SNPs in FCER2 were identified and had strong linkage. These 17 SNPs recombinated into 5 haplotypes, in which h1 and h2 had the most frequency; Rs28364072 polymorphism in FCER2 is well known to be associated with exacerbation of asthma, subjects who were homozygous for the CC mutant alleles was significantly associated with elevated IgE level, severe exacerbations, the risk of asthma-related hospitalization, and also associated with increased daily corticosteroid dose.

Conclusion. In this study, frequency of alleles C in rs28364072 in asthmatic children was similar with that in Kinh HoChiMinh city in the project genome 3, but significantly different from patient group without asthma ($p<0,05$).

KEYWORDS: rs28364072, FCER2, pediatric asthma

RÉSUMÉ

Introduction. Des études récentes ont montré que le FCER2 est un gène important lié à la régularisation de la production d'IgE, qui est un médiateur associé à l'asthme.

Méthode. Dans cette étude, la PCR-séquençage a été utilisé pour analyser FCER2 polymorphisme chez 130 patients à l' Hôpital National de la Pédiatrie.

Résultats. Les résultats de cette étude ont montré que 17 SNP dans FCER2 ont été identifiés et avaient liaison forte. Ces 17 SNPs recombinés en 5 haplotypes, où h1 et h2 avaient le plus de fréquence; Rs 28364072 polymorphisme dans FCER2 est bien connue pour être associée à une exacerbation de l'asthme, les sujets qui étaient homozygotes pour les allèles mutants CC était significativement associée à taux élevé d'IgE, les exacerbations sévères, le risque de l'hôpitalisation liée à l'asthme, et également associée à une augmentation la dose quotidienne de corticostéroïdes.

Conclusion. Dans cette étude, la fréquence des allèles C rs28364072 chez les enfants asthmatiques était semblable à celle dans la ville Kinh HoChiMinh dans le génome du projet 3, mais significativement différent du groupe des patients sans asthme ($p <0,05$).

MOTS CLÉS: rs28364072, FCER2, enfance asthmatique

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INTRODUCTION

Asthma is a common chronic inflammatory disease of respiratory tract in both adults and children. So far there are about 300 million of people with asthma in the world. It is estimated that in 2025, more than 100 million of other people will suffer from asthma worldwide [1]. To maintain, control and prevent asthma well, the selected drugs include inhaled corticosteroids (ICS), β_2 enhanced drugs, long-acting beta-agonist (LABA), leucotrien-antagonist drugs, etc ... [2,4]. In these drugs, ICS are most commonly used for the asthma control and prevention. However, every race, every individual has different response to ICS. There are many factors affecting the drug response at the level of individuals, in which genetic differences affecting corticoid response have been studied by many authors.

FCER2 is a protein-coding gene, which plays an important role in the regulation of IgE production, an important mediator of allergic asthma located on chromosome 19. The Single Nucleotide Polymorphism (SNP) at rs28364072 of FCER2 gene supposedly related to the severity of asthma [3,5], the risk of asthma-related hospital visits, and length of hospitalization [3,5,6]. Researches show that the mutations of replacing T by C in rs28364072 of FCER2 gene are associated with the increased IgE levels and increased risk of exacerbations in asthma patients using ICS [3,5-7].

The project of sequencing the human genome "1000 genome" in Phase 3 was conducted in a total of 2504 people in 26 countries worldwide, including Kinh people living in Ho Chi Minh city. Results showed that the rate of minor Alleles frequency (MAF) (C) of rs28364072 in Kinh people living in Ho Chi Minh City is 0.364 [8]. Does this ratio differ from that of patients with asthma? And does this affect treatment effectiveness? This study aims to partly answer the questions above.

SUBJECTS AND METHODS

Subjects

Patients checked-up and treated at the National Hospital of Paediatrics from July to August of 2015. This study was approved by the Ethical Board of National Hospital of Paediatrics (Hanoi, Vietnam).

Methods

Designed case-control study, in which patients are diagnosed asthma in the Department of Immunology, Allergology, and Rheumatology of National Hospital of Pediatrics and the control group chosen by the method of convenient sampling is children without asthma, going to Hospital for the check-up and surgery of the spermatic cord cyst, inguinal hernia, hidden testicle.

FCER2 genotype analysis was conducted at the Department of Basic Sciences in Medicine and Pharmacy, School of Medicine and Pharmacy, Vietnam National University-Hanoi with 5-step process as follows: arm vein blood sampling; separation of DNA from peripheral blood; designing the specific primers for gene cloning; amplification by PCR of genomic regions of interest; determining genotype by two-way sequencing method at First Base Company (Malaysia).

Statistical analysis

Determining the frequencies of genotype and alleles of determined gene segments, comparing those between the asthma group and the control group to find out the difference in frequency of genotype, and then comparing the results with the theoretical frequency of balanced populations, followed Hardy-Weinberg's rule with chi-square test.

Linkage disequibrium (LD) and haplotypes analysis: using the software of SNPAnalyzer 2. Expectation-maximization (EM algorithm) and Expectation-Partition-Ligation-maximization (PL-EM) are used to evaluate haplotypes frequencies; Gabriel's method is used to analyze the haplotypes structure [7].

RESULTS

Characteristics of study subjects

TABLE 1 Characteristics of study subjects

Characteristics of study subjects	Asthma group (n=107)	Control group (n=32)	p
Average age, *years*	9.2 ± 2.5	5.4±1.2	<0.05
Female (Male), *%*	32.7 (67.3)	86.9 (13.1)	<0.05
Average height, *cm*	132.2± 13.3	105.5±8.9	<0.05
Average weight, *kg*	39.9± 10.2	17.3±5.4	<0.05
Diagnosis results, %	Asthma (100%)	Inguinal hernia (30.6%) Spermatic cord cysts (21.7%) Hidden testicles (21.7%) Hydrocele testis (13.0%) Phimosis (13.0%)	

There are remarkable differences in age, gender, height and weight of the two groups of patients with and without asthma (*Table 1*). In the control group, patients mainly involve in the surgery of the reproductive system abnormalities such as inguinal hernia, spermatic cord cysts, hidden testicles, hydrocele testis, phimosis, which are common for boys.

With the gender difference, the patients' average height and weight of 2 groups are different (according to physiological characteristics). Due to the convenient research sampling, in order to compare the difference of rs28364072 in patients with and without asthma, we acknowledge the differences mentioned above.

The rs28364072 analysis on FCER2 gene

Sequencing FCER2 gene segment of 818bp long containing rs28364072 showed that beyond rs28364072, 16 different SNPs appeared with the frequency of the alleles distribution as shown in *Table 2.*

There are opposing distribution of allelic frequency of major alleles and minor alleles between the asthma group and control group, in which the major alleles occupy high percentage in the asthma group and low percentage in the control group and vice versa. Ratio of major alleles in the asthma group is similar to the frequency distribution of the whole population of study subjects.

There is a strong linkage among the SNPs in the FCER2 gene analyzed, showing the similarities in the proportion of major and minor alleles on all the SNPs. The proportion of the major alleles in the asthma group is 0.7; and 0.4 in the control group. In the SNPs detected, most alleles are distributed, following Hardy-Weinberg's law ($p>0.05$), except rs4996972 and rs4996973 ($p<0.05$).

To analyze the haplotype and Linkage disequibrium (LD) among the SNPs, we use the automatic analysis software of SNPAnalyzer2. The results show that: in the segment of FCER2 gene amplified, 5 haplotypes appeared with the frequencies and the SNPs sequence as shown in *Figure 1*.

TABLE 2 **The frequency of the alleles in the SNPs located on the FCER2 segment amplified in the asthma group, the control group and in both groups**

No	SNPs	The position in chromosome	Alleles	Alleles frequency			P value*
				Asthma group (n=107)	Control group (n=32)	Both groups (n=130)	
1	rs2277994	7690632	T/C	0.724/0.276	0.457/0.543	0.677/0.323	>0.05
2	rs76013233	7690599	C/T	0.724/0.276	0.457/0.543	0.677/0.323	>0.05
3	rs77121754	7690596	T/C	0.724/0.276	0.457/0.543	0.677/0.323	>0.05
4	rs78283814	7690593	C/T	0.724/0.276	0.457/0.543	0.677/0.323	>0.05
5	rs75584211	7690592	A/G	0.724/0.276	0.457/0.543	0.677/0.323	>0.05
6	rs74927160	7690586	C/A	0.724/0.276	0.457/0.543	0.677/0.323	>0.05
7	rs2277995	7690583	A/G	0.724/0.276	0.457/0.543	0.677/0.323	>0.05
8	rs28364072	7690399	T/C	0.724/0.276	0.457/0.543	0.677/0.323	>0.05
9	rs4996972	7690327	G/A	0.762/0.238	0.457/0.543	0.707/0.293	<0.05
10	rs4996973	7690273	T/C	0.762/0.238	0.457/0.543	0.707/0.293	<0.05
11	rs2228138	7690170	C/T	0.721/0.279	0.457/0.543	0.672/0.328	>0.05
12	rs4996975	7690085	G/A	0.721/0.279	0.457/0.543	0.672/0.328	>0.05
13	rs4996976	7690082	T/C	0.725/0.275	0.444/0.556	0.683/0.317	>0.05
14	rs4996977	7690078	A/G	0.721/0.279	0.457/0.543	0.672/0.328	>0.05
15	rs4996978	7690056	T/G	0.725/0.275	0.457/0.543	0.676/0.324	>0.05
16	rs4996979	7690031	A/G	0.721/0.279	0.455/0.545	0.673/0.327	>0.05
17	rs4996980	7690026	C/T	0.716/0.284	0.435/0.565	0.664/0.336	>0.05

*: *P-value for Hardy-Weinberg Equivalence of Alleles Distribution*

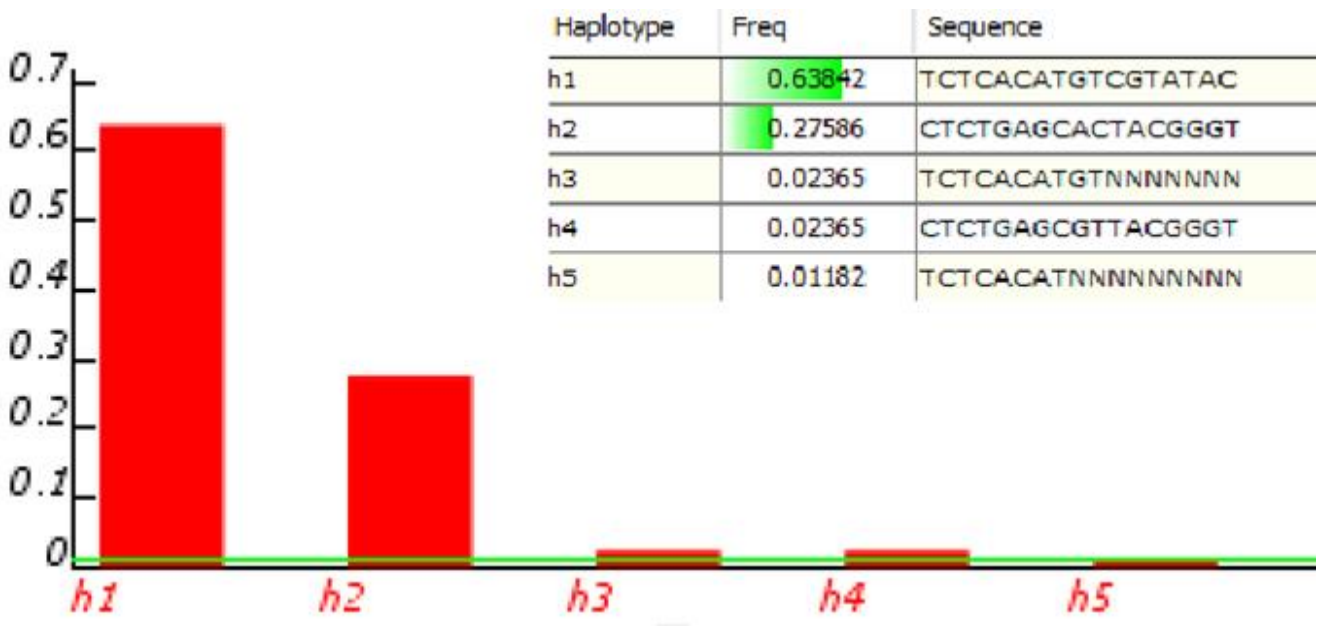

FIGURE 1. *Haplotype structure on FCER2 segment analyzed by SNPAnalyzer2 (Freq: Frequency).*

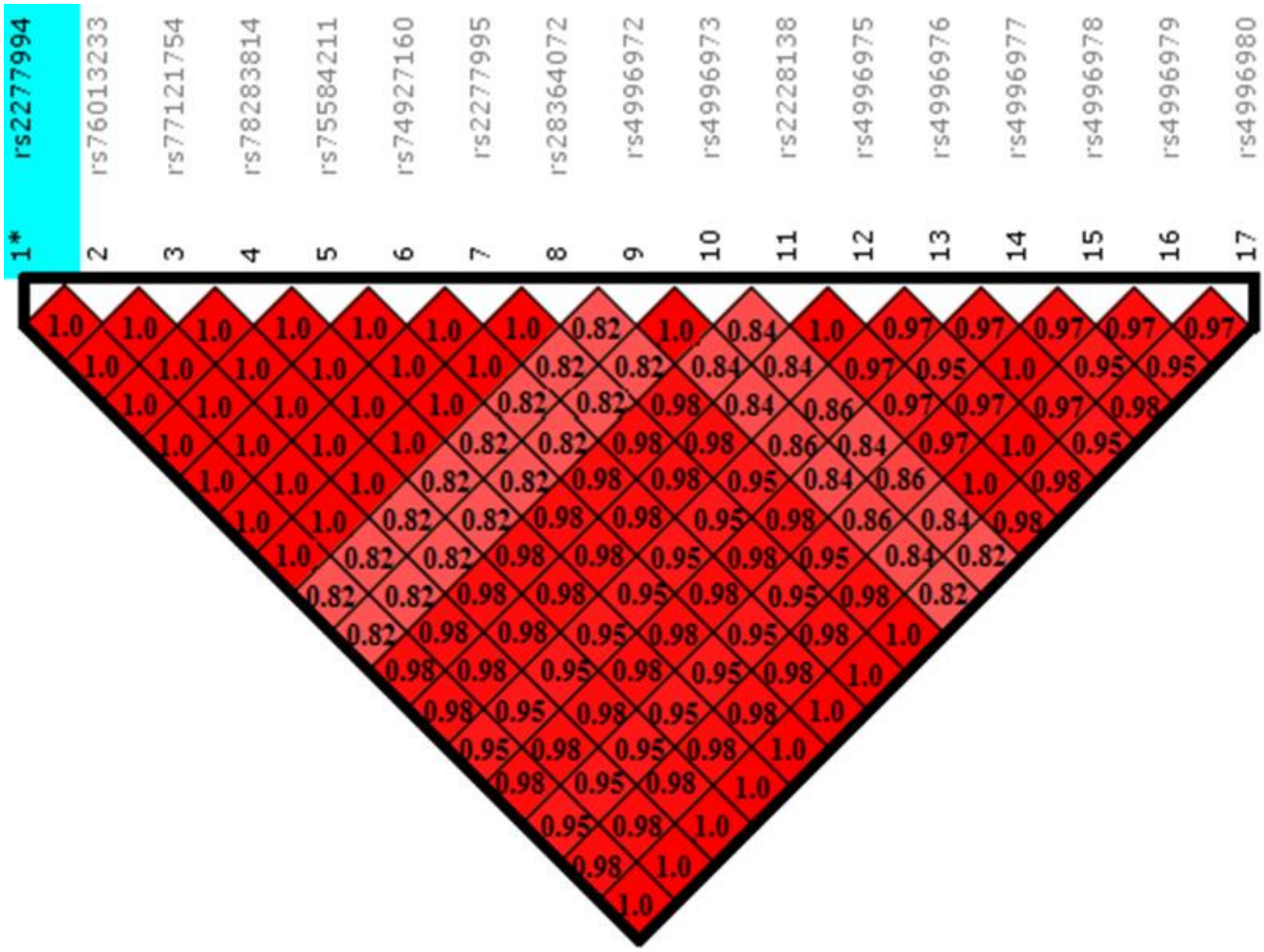

FIGURE 2. *The map of Linkage Disequibrium in 17 SNPs located on FCER2 segment. Each lozenge performed the coefficient of determination (r^2) between pairs of SNPs.*

In 5 haplotypes formed, haplotypes h1 and h2 occupied the highest proportion; corresponding to 17 major alleles (h1) or 17 minor alleles (h2) of the corresponding SNPs. This demonstrates that the SNPs have a very strong linkage and are often combined together. This is also shown in *Figure 2.*

The results in *Figure 2* show that the SNPs link closely together, with the coefficient of determination $r^2 > 0.8$. A lot of SNP couples have $r^2=1$, showing that they always go together.

Rs28364072 on FCER2 genes are thought to be related to the progression of asthma and increasing the risk of exacerbations in patients with asthma using ICS [3,5,6], so we focused on the analysis of SNP polymorphisms on study subject population. The results are shown in *Table 3.*

There is a statistically significant difference in the alleles frequency between the asthma group and control group (p = 0.004). The frequencies of Allen C are 0.276 in the asthma group and 0.543 in the control group respectively.

Compared to the genome project in Phase 3 conducted on 2504 people in 26 countries worldwide, including Kinh people living in Ho Chi Minh City [8], the results of the alleles frequency distribution of rs28364072 are presented in *Table 4*.

TABLE 3 **Frequency distribution of FCER2 rs28364072 genotypes and alleles on study subject populations**

Genotypes	Asthma group (n, %)	Control group (n, %)	p
TT	58 (54.2)	5 (21.7)	
TC	39 (36.4)	11 (47.8)	**0.004**
CC	10 (9.4)	7 (30.4)	
Total	*107*	*23*	
Alleles			
T	155 (0.724)	21 (0.457)	**0.004**
C	59 (0.276)	25 (0.543)	
Total	*214*	*46*	

TABLE 4 **Comparing the alleles C frequency of rs28364072 located on FCER2 segment in different research subject populations in the world**

	Research subject populations in the world								
	National Pediatric Hospital			Kinh People in HCMC	East Asia	South Asia	Africa	Europe	America
	Asthma (B)	Control (C)	Total (B+C)						
Sampling sizes	n=107	n=23	n=130	n=198	n=1008	n=978	n=1322	n=1006	n=694
Alleles C frequency	0.276	**0.543**	**0.323**	0.364	0.340	**0.428**	**0.570**	0.284	0.218
p compared to the total study subjects in the National Hospital of Pediatrics (B + C)	>0.05	**<0.05**	-	>0.05	>0.05	**<0.05**	**<0.05**	>0.05	>0.05

The results in *Table 4* show that, if calculated on a total of 130 study subjects (regardless the children with or without asthma), our results are similar to those of the research on 198 Kinh people in Ho Chi Minh city in the genome project in Phase 3 ($p > 0.05$). This rate is similar to the rate of occurrence in East Asian people in general, slightly higher than the European and American people, but this difference is not statistically significant. However, compared with South Asian and African people, the frequency of alleles C is lower with the statistically significant difference ($p<0.05$). Interestingly, comparing between the asthma group and control group (without asthma), the frequency of Alleles C in the control group is higher.

DISCUSSION

The personalization in treatment is one of the trends of modern medicine. So understanding the genetic structure and distribution of allelic frequencies can contribute significantly to the impact associated with the clinical and treatment response.

Rs28364072 on FCER2 gene has been proved to affect the severity of asthma [3,5], the number of emergency hospital admissions for asthma, and length of hospitalization [3,5,6]. Many studies have shown the mutations of replacing T by C of rs28364072 were associated with increased IgE levels and increased risk of exacerbations in patients with asthma using ICS [3,5,6]. This research has not shown the relevance of genetic variants of rs28364072 with clinical characteristics and treatment response of asthma.

CONCLUSION

The analysis of FCER2 gene segment on 130 pediatric patients at National Pediatrics Hospital shows that: identifying 17 SNPs with 5 haplotypes of FCER2 gene segments analyzed. SNPs closely interlink; the frequency of rs28364072 alleles C in patients with asthma is lower than that of the control group (with surgery of spermatic cord cyst, inguinal hernia, hidden testicles) with statistically significant difference.

However, it shows the differences in the frequency distribution of alleles C between the asthma group and control group, in which the frequency of allen C in the control group is higher than that that of the asthma group, with statistically significant difference. In our study, the control group were chosen by the convenient sampling method (the children going to the hospital for the surgery of cystic spermatic cord, inguinal hernia, hidden testicles). The question if there is any correlation between SNP rs28364072 polymorphism and the risk of genital disease of male children or not should be clarified in further studies.

Acknowledgement

The authors would like to thank the Vietnam National University-Hanoi, project QG.14.59 for funding this study.

CONFLIT OF INTERST

The authors declare no conflict of interest.

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