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Association between rs2787094 Genetic Variants in ADAM33 Gene and Asthma in Indonesian Population: Preliminary Study

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Abstract

Background: Asthma is a multifactorial disease that encompasses a multitude of genetic and environmental factors. One such factor is the disintegrin and metalloprotein-33 (ADAM33) gene, which is correlated with asthma and bronchial hyperresponsiveness. Previous studies conducted on Asian populations have reported a significant association between rs2787094 polymorphism in the ADAM33 gene and asthma.

Methods: Our study involved 153 Indonesian participants. TaqMan genotyping assay was used to analyze rs2787094 polymorphism in the ADAM33 gene.

Results: No significant association was detected between the allele and genotype frequencies of rs2787094 and asthma in the case and control subjects (p = 1.00). The distribution of rs2787094 genotypes in healthy controls was CC (12.1%), CG (42.1%), and GG (45.8%). The genotype distribution in Indonesians was similar to East Asians in 1,000 genomes dataset.

Conclusions: This is the first study to investigate the association between rs2787094 polymorphism in the ADAM33 gene and asthma in the Indonesian population and concluded that it is not associated. Future studies with larger sample sizes and more single nucleotide polymorphisms in the ADAM33 gene are needed to validate these results.

Keywords: ADAM33 gene, asthma, gene polymorphism, Indonesia, rs2787094

INTRODUCTION

Current estimates suggest that asthma is the most prevalent chronic respiratory condition worldwide, directly impacting approximately 358 million individuals.[1] Despite successful therapies and new management paradigms, asthma significantly affects patient's lives. Furthermore, >45% of sufferers are estimated to have a poorly managed condition.[2] Despite global efforts, the mortality rate from asthma remained constant at 0.19 deaths per 100,000 individuals from 2006 to 2012.[3] This disease is among the top 10 causes of morbidity and mortality in Indonesia.[4] Based on 2018 data from the Ministry of Health Indonesia, asthma had a prevalence of 2.4%, accompanied by a recurrence rate of 57.5%.[5]

The clinical presentation of asthma is strongly associated with airway inflammation, a defining feature of the disease, along with bronchial hyperresponsiveness (BHP) and reversible airflow obstruction.[6] The pathogenesis of asthma is multifaceted, involving genetic and environmental factors. Thus, genetic factors are a promising clinical and basic research area.[6–8] Among such genetic factors are the disintegrin and metalloprotein-33 (ADAM33) gene, linked to susceptibility to asthma and BHP.[9] This highly polymorphic gene is located on human chromosome 20p13 and encompasses over 300 single nucleotide polymorphisms (SNPs) associated with asthma and other allergic conditions.[10–13] Among these SNPs, rs2280090, rs2787094, rs511898, and rs2280089 have a significant relationship with asthma, especially in Asian populations.[14] Particularly, rs2787094 polymorphism has revealed consistent results in several studies on Asian populations.[6,11,15,16]

Few genetic data are available on the association between ADAM33 polymorphism and asthma in Indonesia. rs2787094 polymorphism is essential for the functional activity and transcriptional regulation of ADAM33 expression, such as modifying signaling activity or binding specific microRNAs.[17] rs2787094 polymorphism has been studied in asthmatic patients from different ethnic populations.[14] Therefore, this preliminary study examined the association between rs2787094 polymorphism in the

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ADAM33 gene and asthma among YARSI University students.

METHODS

Subjects

This study involved 46 individuals diagnosed with asthma and 107 healthy controls. We collected the samples in January–March 2016 at the Faculty of Medicine from students of YARSI University Jakarta, Indonesia. A clinical immunology and allergy specialist assessed participants based on clinical examinations and family history, as well as guidelines established by the global initiative for asthma,[18] and the international study of asthma and allergies in childhood.[19] We only enrolled participants with hereditary asthma and did not assess their lung capacity. Normal individuals had no symptoms of asthma, allergy, autoimmunity, or inflammatory disease. The study groups were negative for parasitic diseases. This study was approved by YARSI University Ethics Committee (No. 023/KEP-UY-BIA/III/2016), and the participants signed informed consent.

DNA extraction and SNP genotyping

About 3 mL of peripheral blood was collected from the participants into an EDTA tube. About 500 µL of blood was drawn for automated DNA isolation using Maxwell Automated DNA instrument (Promega, Madison, WI, USA). Tecan Infinite 200 Pro nano spectrophotometer (Tecan, Mannedorf, Switzerland) was used to evaluate the quality and quantity of the extracted DNA. An analysis of quality and integrity was performed by gel electrophoresis.

rs2787094 polymorphism in ADAM33 gene was genotyped using 10 ng of DNA mixed with the TaqMan GT Express (Thermo Fisher Scientific, Waltham, MA, USA). TaqMan SNP rs2787094 genotyping assay (Thermo Fisher Scientific) was conducted following the manufacturer's instructions, SNP was genotyped using the real-time polymerase chain reaction (PCR) thermal cycler LightCycler 480 (Roche, Basel, Switzerland). PCR program included the following cycling conditions: 95 °C for 10 min, 40 cycles at 92 °C for 15 s, and 60 °C for 1 min. The amplification analysis was performed utilizing allelic discrimination, and the automated allele calling settings for Light Cycler Software 4.0 (Roche, Basel, Switzerland) were employed.

Data analysis

Comparisons between the groups were evaluated by χ^{2} test. A two-tailed p-value < 0.05 was considered significant. Odd ratios were used to estimate relative risk with 95% confidence intervals. The distribution of the alleles and genotypes was determined, and the genotype frequency data were compared to 1,000 genomes dataset (https://asia.ensembl.org/Homo_sapiens/Variation/). We used rs2787094 genotype frequency data from South Asian, European, East Asian, Ad Mixed American, and African populations and compared them with our study. All statistical analyses were conducted utilizing SPSS software version 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 153 participants, comprised of 46 participants with asthma (8 men, 38 women, and 22.37 years mean age) and 107 healthy control subjects (59 men, 41 women, and 25.70 years mean age), were included in this study. Table 1 shows the allele and genotype frequencies of rs2787094 polymorphism in ADAM33 gene. The allele and genotype frequencies were relatively similar between individuals with and without asthma (healthy controls). The Hardy–Weinberg equilibrium test revealed that the control group population adhered to Hardy–Weinberg proportions (p = 0.594). We observed no significant difference between the frequencies of the cases and controls (Table 1).

Figure 1 is a genotype discrimination cluster plot. The diagram was analyzed using EndPoint Genotyping Analysis on Light Cycler Software 4.0 software. The results demonstrated that the non-template control (NTC) was distant from all clusters, and three clusters were separated. Figure 2 compares the genotype distributions between Indonesian and other populations based on 1,000 genomes dataset. The results indicated similarity to the populations studied in East Asian countries, including Han Chinese in Beijing, China, Japanese in Tokyo, Japan, Southern Han Chinese Population, Chinese Dai in Xishuanaqbanna, China, and Kinh in Ho Chi Minh City, Vietnam, according to the Ensembl information data (https://asia.ensembl.org/Homo_sapiens/Variation/). However, no significant differences were observed between Indonesian and other populations.

TABLE 1. Allele and genotype frequencies of rs2787094

SNP	Position	Allele	Asthma (N = 46)		Non-Asthma (N = 107)		*p*	OR (95% CI)
			N	%	N	%		
rs2787094	3649161	Allele						
		C	31	33.7	71	33.2	Reference	
		G	61	66.3	143	66.8	1	1.02 (0.61–1.72)
		Genotype						
		CC	5	10.9	13	12.1	Reference	
		CG	21	45.6	45	42.1	0.78	0.82 (0.26–2.61)
		GG	20	43.5	49	45.8	1	0.94 (0.30–3.00)

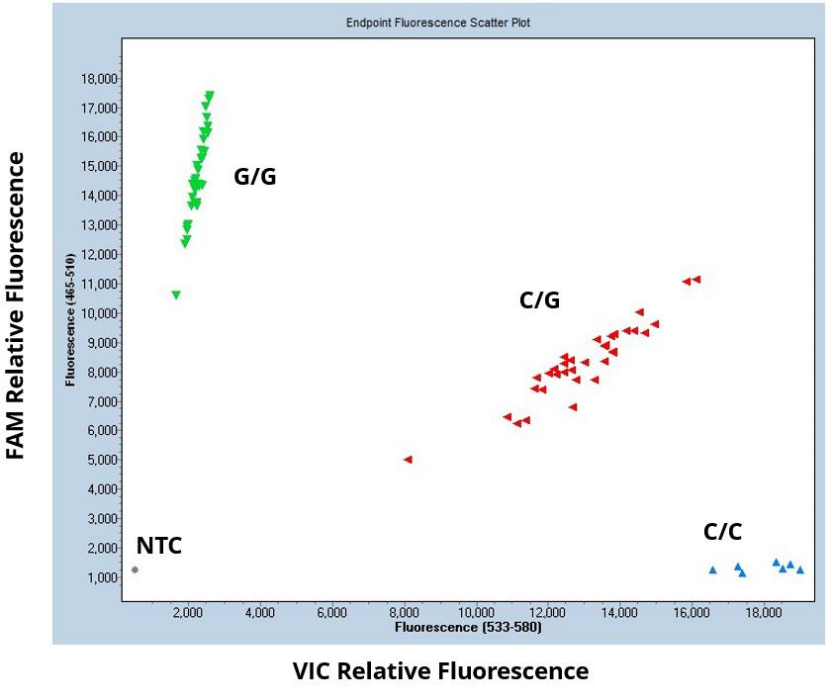

FIGURE 1. Distribution of rs2787094 polymorphism using LightCycler® 480 software; single dots represent genotyped individuals; green represents the GG genotype; red represents CG genotype; blue represents CC genotype

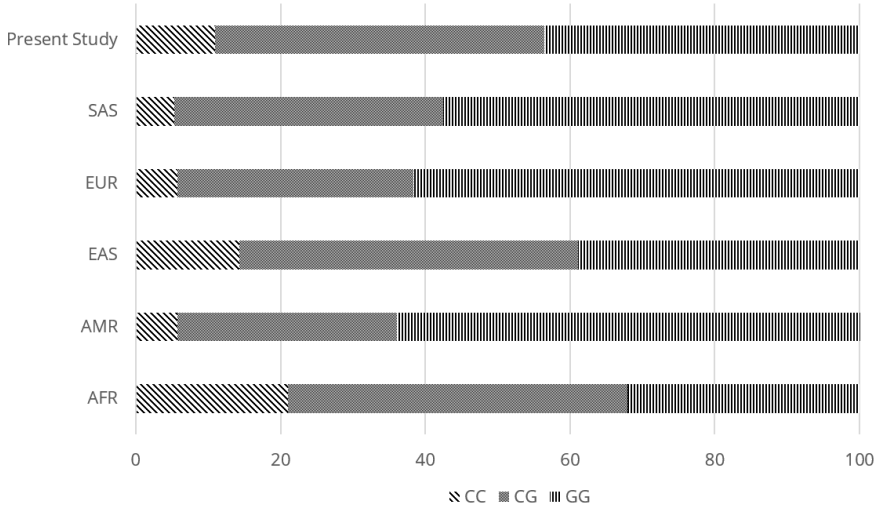

FIGURE 2. Distribution of rs2787094 polymorphism in Indonesian population compared to other populations using 1,000 genomes dataset; SAS: South Asian; EUR: European; EAS: East Asian; AMR: Ad Mixed American; AFR: African

DISCUSSION

Asthma is a multifactorial disorder arising from a complex interplay between genetic and environmental factors. Polymorphisms in ADAM33 gene have been associated with asthma in diverse populations.[11] ADAM33 is expressed specifically by mesenchymal cells, and changes in its activity alter the function of bronchial smooth muscle cells and fibroblasts, leading to airway remodeling.[9] The objective of this investigation was to examine the correlation between rs2787094 polymorphism and asthma. To our knowledge, no study has assessed the association between ADAM33 polymorphisms and asthma in an Indonesian population.

Our results indicated no association between the allele and genotype of rs2787094 in ADAM33 gene and the risk of asthma. This contradicts a previous study in the Asian population;[14,16,20] however, Karimi *et al*. reported an association between moderate asthma and G rs2787094

allele but no association was shown in mild and severe asthma.[20] Meta-analysis also showed that rs2787094 only has a positive association in the Asian population, whereas no significant association was observed in European or Latin American populations.[14]

This study used TaqMan SNP genotyping assay for rs2787094 genotyping, which is one of the most reliable methods for SNP genotyping due to cost-effectiveness and ease of handling. This assay allows genotyping of individuals for a specific SNP, making it a valuable tool for genetic association studies.[21] Our results revealed a good separation cluster; however, the cluster included a trailing cluster. This trailing cluster, which appears to spread across an imaginary line that extends from NTC, was attributed to variations in the concentration of genomic DNA in the samples.[22] Although we normalized the samples to the same concentration, we continued to get this trailing cluster, possibly because we used absorbance at 260 nm to quantify DNA concentration. To obtain a more accurate measurement of DNA concentration, a fluorescent method should be used rather than measuring absorbance at 260 nm.[23]

The distribution of rs2787094 polymorphism varies across different ethnicities globally. Our findings revealed that the genotype distribution of rs2787094 was comparable to the East Asian population studied. According to Ensemble (www.ensembl.org), EAS population study consists of Han Chinese from Beijing, China, Japanese from Tokyo, Japan, the Southern Han Chinese Population, Chinese Dai from Xishuanaqbanna, China, and Kinh from Ho Chi Minh City, Vietnam. However, no significant difference was found with the other populations in this study due to the small sample size and lack of ethnic specification.

Our study had limitations due to the small sample size. Future studies with larger sample sizes should be conducted to confirm our findings. Additionally, we recruited participants with hereditary asthma; therefore, further studies need to collect non-hereditary asthma samples, their lung capacity data, and environmental factors that cause asthma to strengthen the study. Additionally, further investigations of more ADAM33 gene SNPs to detect an association with asthma in the Indonesian population are needed. Understanding the genetics of asthma would support the development of a powerful predictive marker for preventing and managing asthma.

We also did not characterize asthma patients as mild, moderate, or severe asthma, which could potentially affect the results. Additionally, we did not specify the ethnicity of the participants. However, in future studies, it may be important to specify ethnicity, given that Indonesia has over 300 indigenous languages and associated ethnic groups.[24] Multiple genes have been identified and mapped in various ethnicities in Indonesia, including but not limited to human leukocyte antigen,[25] N-acetyltransferase 2,[26–28] and the GSTM1/GSTT1 null genotype.[29]

CONCLUSIONS

In conclusion, this study demonstrated no association between rs2787094 polymorphism and asthma in the Indonesian population. However, this may be the first study in Indonesia that investigated the role of ADAM33 polymorphism in this population. We observed a similarity in rs2787094 genotype distribution with EAS population. To obtain more reliable results, future studies with larger sample sizes are recommended, and screening of all ADAM33 gene SNPs may provide a better avenue for further research.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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