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ASSOCIATION BETWEEN THE RS2280091 POLYMORPHISM IN THE ADAM33 GENE AND CHILDHOOD ASTHMA

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ABSTRACT

Background: Asthma is a non-communicable disease that remains a global health concern, influenced by both environmental and genetic factors. The disintegrin and metalloproteinase 33 (ADAM33) gene has been identified as a susceptibility gene for asthma. This study aimed to investigate the association between the rs2280091 polymorphism in the *ADAM33* gene and the risk of asthma in Indonesian children.
Methods:A total of 60 buccal swab samples were collected from children aged 4–18 years, consisting of 13 asthma cases and 47 healthy controls. Genotyping of rs2280091 was performed using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis.
Results: The AA and GG genotypes were more frequent among asthma cases (69.23% and 7.69%, respectively) compared to controls (63.83% and 6.38%). Similarly, the A allele was detected more frequently in asthma cases (80.77%) than in controls (78.72%). However, no significant association was observed between the rs2280091 polymorphism and asthma risk (P = 1.0, OR [95% CI] = 0.8 [0.0779–8.592]). In addition, asthma was more commonly observed in boys (84.62%) than in girls (15.38%).
Conclusion: This study did not find a significant association between the rs2280091 polymorphism in the ADAM33 gene and childhood asthma. Further research involving larger sample sizes and additional ADAM33 polymorphisms is warranted to better understand the genetic susceptibility to asthma in children.
Keywords : *Asthma., ADAM33., rs2280091., Genetic Susceptibility., PCR-RFLP*

INTRODUCTION

Asthma is a major non-communicable disease and remains a significant global health concern, affecting approximately 358 million individuals worldwide.[1] According to the 2018 Indonesian Basic Health Research (Riskesdas), the national prevalence of asthma in Indonesia was reported at 2.4%.[2] In children, asthma is among the most commonly encountered chronic diseases, with increasing incidence over the past two decades. The incidence of asthma in Indonesian children aged 0–14 years is reported to be 9.2%.[3]

Asthma is a multifactorial disease influenced by both environmental and genetic factors.[4] The disintegrin and metalloproteinase 33 (ADAM33) gene was the first genetic factor identified as being associated with asthma susceptibility and remains one of the most extensively studied.[5] Located on chromosome 20p13, ADAM33 is expressed primarily in mesenchymal cells, including fibroblasts and smooth muscle cells. The ADAM family of proteins, which possess disintegrin and metalloprotease domains, plays critical roles in cell–cell and cell–matrix interactions, cell migration, adhesion, and signal transduction.[5] ,[6]

Several polymorphisms in the ADAM33 gene have demonstrated significant associations with asthma,[6–9] indicating its important role in asthma susceptibility. However, studies examining ADAM33 polymorphisms in Indonesia remain limited. A previous study in Indonesia found no significant association between the rs2787094 polymorphism and asthma in individuals with a family history of the disease.[10] In this study, we

aimed to investigate the association of the ADAM33 rs2280091 polymorphism with asthma in Indonesian children.

MATERIAL & METHODS

Sample Collection

In this case control study, a total of 13 case samples and 47 control samples from children aged 4–18 years were collected from Pulmonologist and Pediatric Doctor in Cibitung Regional General Hospital and EMC Pulomas Hospital, all of whom had a family history of asthma. Participants were suspected to have asthma if at least one of the following criteria was met: (a) a diagnosis of asthma by a pediatrician; or (b) a history of being prescribed asthma medication. Asthma diagnoses were then confirmed based on the National Guidelines for Pediatric Asthma by the Indonesian Pediatric Society (IDAI).[3]

This study received ethical approval from the Ethics Committee of YARSI University (No. 156/KEP-UY/EA.10/VII/2023). All participants provided informed consent prior to participation, and their legal guardians signed the consent forms on their behalf.

DNA Extraction and rs2280091 Genotyping

Buccal swab samples were collected from all children participant and stored in 3 mL of storage buffer. DNA was extracted using the gSYNC™ DNA Extraction Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan) according to the manufacturer's instructions. The quality and quantity of DNA were assessed using a Tecan Infinite Pro 200 spectrophotometer (Tecan, Männedorf, Switzerland).

Genotyping of rs2280091 was conducted using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. PCR was performed on a thermal cycler (T100, Bio-Rad, California, USA) in a 25 µL reaction containing 20 ng of DNA, 1× GoTaq Green Master Mix (Promega, Wisconsin, USA), and 0.1 µM of both forward and reverse primers (IDT, Singapore). The primer sequences used were:Forward: 5’-ACTCAAGGTGACTGGGTGCT-3’Reverse: 5’-GAGGGCATGAGGCTCACTTG-3’PCR conditions were as follows: initial denaturation at 95°C for 3 minutes; followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute; with a final extension at 72°C for 5 minutes and a hold at 4°C.

RFLP analysis was conducted using the NcoI restriction enzyme (NEB, Ipswich, Massachusetts, USA). The digestion was incubated at 37°C for 5 hours and visualized by gel electrophoresis under ultraviolet light.

The resulting fragments indicated the genotype:

1. GG genotype: 400 bp
2. AA genotype: 140 bp and 260 bp
3. AG genotype: 140 bp, 260 bp, and 400 bp

These fragments appeared as DNA bands on the electrophoresis gel.

Data Analysis

Comparisons between case and control groups were analyzed using the Chi-square (χ^2) test. A p-value < 0.05 was considered statistically significant. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to estimate relative risk. Statistical analyses were performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 presents the characteristics of the study samples. All case samples were individuals with a family history of asthma. Asthma was more commonly observed in male children (84.62%) than in females (15.38%).

Table 1. Sample Characteristics

Characteristics	Cases (n=13) / Frequency (%)	control (n=47) / Frequency (%)
Sex		
Female	2 (15.38)	37 (78.72)
Male	11 (84.62)	10 (21.28)
Age (year Mean, ± SD)	8.2 ± 4.09	17.9 ± 0.24

n = number of samples M, 100 bp DNA marker; lanes 1 and 3, AA genotype; lane 2, GG genotype; lane 4, AG genotype.

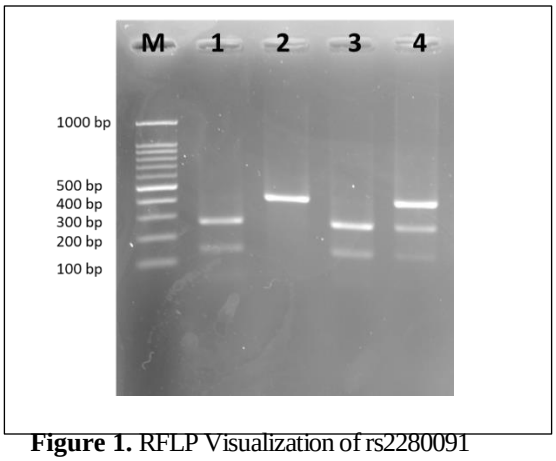

Figure 1. RFLP Visualization of rs2280091

As shown in Figure 1, the RFLP method successfully identified AA, AG, and GG genotypes. The gel image included a 100 bp DNA marker, covering a range from 100 bp to 1000 bp. Table 2 shows the genotype and allele frequencies of rs2280091. Genotypes AA and GG were more common in case samples (69.23% and 7.69%, respectively) than in control samples (63.83% and 6.38%, respectively). The A allele was also more frequent in case samples (80.77%) than in controls (78.72%). However, no significant association was found between rs2280091 genotype or allele and asthma (P = 1, OR [95% CI] = 0.8 [0.0779 – 8.592]).

Table 2. Distribution of rs2280091 Polymorphism

SNP	Genotype / Allele	Cases (n = 13) / Frequency (%)	Control (n = 47) / Frequency (%)
rs2280091	AA	9 (69.23)	30 (63.83)
	AG	3 (23.08)	14 (29.79)
	GG	1 (7.69)	3 (6.38)
	A	21 (80.77)	74 (78.72)
	G	5 (19.23)	20 (21.28)

SNP = single nucleotide polymorphism; n = number of samples

DISCUSSION

This study aimed to evaluate the association between the rs2280091 polymorphism and asthma in children aged 4–18 years. Our results indicated that asthma was more prevalent among male children compared to females. Epidemiological studies of asthma have shown marked sex-related differences in prevalence and severity, closely tied to age. Interestingly, these trends align with transitional phases of the female reproductive cycle.[11]

During childhood, boys have a higher prevalence of asthma and are twice as likely to be hospitalized for asthma exacerbations than girls.[11,12] In adulthood, however, asthma becomes more prevalent in females (9.6%) than in males (6.3%), with females being three times more likely to be hospitalized due to asthma-related events.[13,14] This increased prevalence in women persists until menopause, after which asthma rates decline.[15] These gender shifts in prevalence coincide with sex hormone changes, suggesting that sex hormones modulate pathways involved in asthma pathogenesis.[12]

Meta-analyses have shown that rs2280091 is significantly associated with asthma risk in Asian pediatric populations.[16] A recent meta-analysis also found significant associations of rs2280091 with asthma in Chinese children.[17] Similarly, a study in Hispanic-American children reported a significant association of rs2280091 with asthma susceptibility.[18]

In the present study, no significant association between rs2280091 and childhood asthma was observed (P = 1, OR [95% CI] = 0.8 [0.0779 – 8.592]), likely due to the small sample size. Larger studies are necessary to further explore the potential role of rs2280091 in asthma risk among Indonesian children. Nonetheless, this is the first study in Indonesia investigating the association between the ADAM33 rs2280091 polymorphism and asthma in children. Further research involving other polymorphisms within the ADAM33 gene is also warranted, considering the rising global prevalence of childhood asthma, particularly in low- and middle-income countries.[19] ,[20]

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

This study investigated the association between the ADAM33 rs2280091 polymorphism and asthma in Indonesian

children aged 4–18 years. Although the A allele and AA genotype were more frequent in asthmatic children, no statistically significant association was observed between the rs2280091 polymorphism and asthma susceptibility (P = 1.0, OR [95% CI] = 0.8 [0.0779–8.592]). The findings suggest that rs2280091 may not play a major role in asthma development among this specific population. However, the small sample size limits the strength and generalizability of this conclusion.

RECOMMENDATIONS

Further studies are recommended to clarify the potential role of ADAM33 gene polymorphisms in asthma susceptibility, particularly:

1. Larger-scale studies with a more balanced distribution of cases and controls to increase statistical power.
2. Inclusion of additional ADAM33 SNPs, such as rs2280089, rs2280090, or rs44707, which have shown associations in other populations.
3. Haplotype-based analyses that examine interactions between multiple SNPs within ADAM33.
4. Integration of environmental exposure data (e.g., air pollution, allergens) to assess gene–environment interactions.
5. Expanding the study to diverse ethnic groups within Indonesia to explore population-specific genetic contributions to asthma.

These efforts will contribute to a better understanding of asthma pathogenesis in Indonesian children and support the development of precision medicine approaches for asthma prevention and treatment.

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