Significant Association of Adam 33 Polymorphism with COPD in Javanese Population of Indonesia

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Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD) is one of World health cases that is commonly known, which is triggered by the combination of environmental factors especially cigarette smoking and genetic factors. The association between A disintegrin and metalloprotease 33 (*ADAM33*) polymorphisms and COPD has been investigated and reported by other researchers. **Objective:** The main aim of this study is to identify the association between single nucleotide polymorphisms (SNPs) in *ADAM 33* gene with COPD in the Javanese population in Lampung, Indonesia. **Methods:** A randomized cross-sectional study was used in this research. PCR-Sequencing method was involved to analyze the polymorphic for three SNPs (T1, T2, and Q-1) of the *ADAM33* gene. Statistical analysis data was performed in descriptive and comparative as well as it was measured by parametric/non-parametric tests. **Results:** The results showed that the T2 GG, and T1AG genotypes in COPD group were significantly more frequent rather than in control group ($p < 0.05$). In case of allele, it was found that the T1G and T2G was higher in COPD group rather than in the control group ($p = 0.440$ and 0.131, respectively). **Conclusion:** The results clearly conclude that there was significant association between T1 and T2 polymorphisms of *ADAM33* gene and COPD in the Javanese population of Lampung, Indonesia.

Key Words: *ADAM 33 gene polymorphism, COPD, smoking, Javanese, SNP*

Introduction

A major disease which can cause morbidity and mortality in the world is chronic obstructive pulmonary disease (COPD). In addition, persistent airflow obstruction is identified as one of its characteristics. The COPD is correlated to an enhanced chronic inflammatory response in airways and lungs to noxious particles and gases, and it is also progressive[(1)]. The global burden of COPD estimates that affects 300 million people worldwide resulting in approximately 3 million deaths annually (2). The prevalence of COPD kept increasing globally. In a recent survey the estimated prevalence of COPD was 4.5% in Indonesia[(3)]. This is highly regarded in low tobacco control policies and large of smokers population[(4)]. In a recent health cost study suggested estimation of the smoking treatment cost in Indonesia attained 2.5% of the gross domestic product

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in 2015. Moreover, it became the biggest contributors of Indonesian state burden[5].

There are great numbers of genes which have been associated to the pathogenesis of COPD. The protease–antiprotease pathways, inflammatory response to cigarette smoke pathways, and oxidant–antioxidant pathways have been explored by case–control genetic association[6]. Nevertheless, COPD genetic profiling use in the clinic is current limited to find out Alpha-1 antitrypsin deficiency[7, 8]. Furthermore, the relationship between A disintegrin and metalloprotease 33 (*ADAM33*) polymorphisms and COPD has been reported by other researchers[9-11].

ADAM 33 is a complex molecule. It comes from a zinc-dependent metalloproteases ADAM family and is produced by mesenchymal cells, involving smooth muscle and fibroblast. It is important for cell adhesion, fusion, signaling and proteolysis by releasing various of growth factors as activator and Th2 cytokines[12]. *Its* gene, can be found in human chromosome 20p13. It was investigated as COPD susceptibility gene[13]. The first genetic study reported on the association ADAM 33 genetic polymorphisms and susceptibility done by Van-Dieman in a cohort of 1390 subjects in the general population. The research results highlighted that there was significant correlation between four SNPs (F+1, S1, S2, T2) and accelerated lung function decline[14]. Moreover, the reported research was strengthened by several similar studies in European and Asian regions such as India, China and Mongolia. Polymorphisms in ADAM33 to airway hyper-responsiveness have been correlated to airway inflammation in COPD, and accelerated lung function decline by other research[11, 13, 15]. As association between low lung function and high mortality risk, particularly on account of COPD, it is important to explore the genetic components that escalate susceptibility to lung function decrease and COPD. Later on, other researches, across different country studies were clearly explained the share of this gene in as COPD causation. COPD has become one of the common problems in Indonesia, especially for Javanese population. By considering smoking as their habit, they have high probability to get this disease[4].

Therefore, the current study was conducted to explore *ADAM 33* gene polymorphisms and COPD association in the Javanese population of Indonesia, which attained a high prevalence of this disease. This study might give an insight into the genetic basis of this disease and help in finding predictors for preventing COPD.

Method and Material

Setting

A randomized cross-sectional study was undertaken in this research. It included COPD male smoker patients who were randomly selected from Harum Melati Pulmonology Clinic and male healthy smokers who lived around Pringsewu District, Lampung Province, Indonesia. All researchers don't have any conflict of interest. The research permission was acquired from Ethics Committee of Medical Faculty, University of Lampung. Participants who were involved attained at least 40 years old, they had smoked (The Brinkman Index, number of years of smoking multiplied by the number of cigarettes smoked per day, more than 0) and they also had no symptoms of lung cancer, infections, and other lung diseases. All of them fulfilled admission of spirometry and a baseline which were standardized questionnaire involving smoking history and social and demographic information. Thirty-two healthy age-matched male smokers (control group) who had no family history of COPD were already involved. In addition, The Institutional Ethics Committee of Medical Faculty, Universitas Lampung approved this research by number 1914/UN26.18/PP.05.02.00/2018. All the participants were taken part to make written informed consent.

Furthermore, Pulmonary Function Tests (PFT) were performed by using spirometer according to the Pneumobile Indonesian Lung Health Survey utilizing a flow spirometer which was made by CHEST MI. INC, in Tokyo, Japan its brand namely CHESTGRAPH HI 101[16]. All of measurement values, (forced vital capacity

(FVC) values and Forced expiratory volume in the first second (FEV1)), were calculated and standardized in percent based on reference. The participants who had post bronchodilator FEV1/FVC attained airway obstruction if its ratio was less than 70%. Moreover, classification of airway limitation severity in COPD was categorized taken from the Global Initiative for Chronic Obstructive Lung Disease as source.

Genetic analysis

SNPS (T1, T2, and Q1) of the ADAM 33 gene were undertaken as genetic analysis (table 1). The genomic DNA extraction process was originated from 500 µL of blood using material from Promega's DNA Wizard® made in Madison, USA following the manufacturer's procedure. The results of DNA extraction were examined by using implant, a digital DNA nanophotometer, made in Germany. The DNA extracts were frozen at –20°C until they were analyzed.

Amplification of ADAM33 was performed using kit NEXpro^{TM} e PCR 2x Master Mix solution (Nex diagnostics, South Korea). Two primer pairs were used thereby designed in nearly located on SNPs. The first pair amplified the SNP at the locus rs2280090 (T2) and rs2280091 (T1), it was caused by the distance between SNP rs2280090 and rs2280091 only 29 nitrogen bases. The second pair amplified rs612179 (Q-1). We had confirmed the results of PCR using QIAxcel DNA High Resolution Kit digital electrophoresis equipment (Singapore). Next, the amplicons were sent to Indonesian Bioneer Ltd for two directional sequencing. It was analyzed by BioEdit software developed by Tom Hall. Thereafter, the researchers constructed a phylogenetic tree using Mega X which was developed by Kumar et al[17]. It was involved from several representative samples using maximum likelihood, boostrap method and 1000 times number of replications (Figure 1).

Statistical Analysis

The experimental data was analyzed by using parametric and non-parametric statistical tests. The data is showed as mean ± SD. The researchers performed data through SPSS type 11.0 which involved students't-test and chi square test. A significant different was indicated as p value of less than 0.05. Data analysis used IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA).

Result

There were three position of SNPs ADAM 33 gene that researchers focused on. They were T1, T2, and Q-1. The amplifications of gene sequence that contained of them were attained by using the primers in table 1. Primers were designed by using Gene Bank code access DQ995342.1 from the website https://www.ncbi.nlm.nih.gov. The research involved 42 COPD subjects who were matched for age and sex with 32 healthy smoker controls. All participants were Javanese and they had smoking history. The COPD subjects were in clinically stable condition. On the other hand, the healthy smokers did not have lung disease. Their characteristics were listed in Table 2. Related to COPD patient included in this study were generally similar to the control but slightly older and had lower Brinkman Index. Interestingly, there were significant pulmonary functions identified in the male COPD patients compare to the male healthy smokers as a control. The result found that there was more significant frequency T2GG genotype and T1AG genotype in COPD group if the researchers compared them with in the control group ($p < 0.05$). But, in the case of Q-1, there was no significant difference of genotype frequencies in the two groups (Table 3). Furthermore, there were no significant different allele frequencies in the two groups (Table 4). Based on phylogenetic tree the sequence of ADAM33 gene from Javanese ethnic in Lampung province was closely related to several populations especially Li and Han in China (Accession No: AF466288.1; DQ995342.1) with 99% and 86% respectively (Figure 1).

Table 1. The location of investigated ADAM33 SNPs and primer sequence

Chromosome position	Reference SNP ID	SNP Name	Alel	Primer Sequence
3590205	2280090	T2	A/G	F: 5'-TTCTCAGGGTCTGGGAGAAA-3' R: 5'-GCCAACCTCCTGGACTCTTA-3'
3590234	2280091	T1	A/G	F: 5'-TTCTCAGGGTCTGGGAGAAA-3' R: 5'-GCCAACCTCCTGGACTCTTA-3'
3592207	612709	Q1	A/G	F: 5'-GGATTCAAACGGCAAGGAG-3' R: 5'-GTTCACCTAGATGGCCAGGA-3'

Table 2. The clinical information of patient recruited

Variable	Case (n = 42)	Control (n = 32)	p
Age	64.5 ± 8.6	61.6 ± 9.5	0.79
Brikman Index	409.6 ± 199.22	426.14 ± 243.14	0.632
FEV1	45.40 ± 20.47	103.81 ± 20.07	< 0.001*
FVC	74.50 ± 19.49	94.64 ±16.18	< 0.001*
FEV1/FVC	60.89 ± 14.51	110.64 ± 18.64	< 0.001*

Note: *significant $p < 0.05$.

Table 3. Association between ADAM 33 genotypes and COPD

SNP	Case (n = 42)	Control (n = 32)	OR (CI)	p
rs2280090 (T2)				
GG	28 (66.66)	15 (46.87)	2.29 (0.35-0.81)	0.041*
AG	12 (28.57)	15 (46.87)	0.46 (0.15-0.58)	0.052
AA	2 (4.76)	2 (6.25)	0.82 (0.05-0.19)	0.420
rs2280091 (T1)				
AA	14 (33.33)	16 (50.00)	0.5 (0.18-0.62)	0.058
AG	26 (61.90)	13 (40.62)	2.45 (0.40-0.86)	0.024*
GG	2 (4.65)	3 (9.37)	0.53 (0.05-0.15)	0.236
rs612709 (Q-1)				
GG	33 (78.57)	27 (84.37)	0.82(0.67-0.95)	0.329
AG	8 (19.04)	5 (15.62)	1.07 (0.02-0.30)	0.444
AA	1 (2.38)	0 (0.00)	0.1 (0.02-0.06)	0.142

Note: *significant $p < 0.05$; SNP: Single Nucleotide Polymorphism; OR: Odds ratio; CI: Confidence Interval.

Table 4. Association between Allele frequency and COPD

SNP	Group	Genotype			Allele Frequency		p
		AA	AG	GG	A	G	
T1	Case Control	14 16	26 13	2 3	54 45	30 19	0.440
T2	Case Control	2 2	12 15	28 15	16 19	68 45	0.131
Q1	Case Control	1 0	8 5	33 27	10 5	74 59	0.414

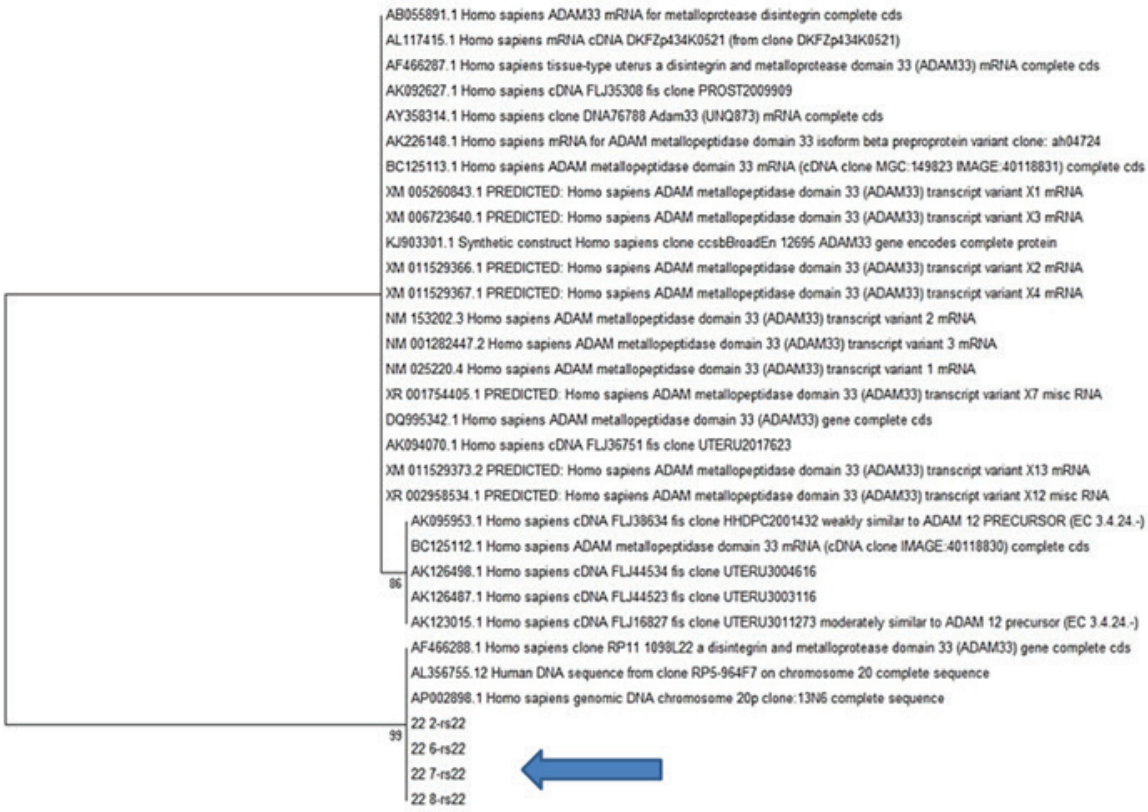

Figure.1. The phylogenetic tree of the ADAM33 gene sequence in Javanese smokers of Lampung. There are two big clades that are produced by phylogenetic analyzing ADAM33 gene. We can find ADAM33 gene sequence form Javanese which are located in same clade with ADAM33 gene sequence form Caucasian and China from them. It indicates that Javanese ADAM33 gene is similar with Caucasian and Chinese. ADAM33 Javanese sequence shown by the following arrow (by using code number start with 22).

Discussion

COPD is identified as a complex genetic disease. It also involves environmental risk factor such as tobacco smoking. COPD pathophysiology aspect is an imbalanced proteolysis theory. In line with this case, ADAMs is proteinases which strikingly associated with Matrix Metalloproteinases (MMPs). ADAMs proteins contain of metalloproteinase and disintegrin domains providing them with aspects of proteinases and adhesion

molecules. In addition, all kinds of physiological, pathological processes and display a wide spectrum of biological impacts comprising cell adhesion, cell fusion, cleavage of various substrates from the extracellular matrix, "shedding process", cytokines or growth factors are correlated to proteinases of the ADAM family[18].

The structure of ADAM33 consists of a signal sequence, pre-domain, catalytic domain, disintegrin domain, cysteine-rich domain, EGF domain, transmembrane domain, and cytoplasmic domain with a long 3′-untranslated region (UTR). The structures of ADAM 33 that has been explained above indicate that it is involved in many cellular activities, including cell activation, adhesion, fusion, proteolysis and intracellular signaling. Angiogenesis is promoted by soluble ADAM33. It is explained that as a gene of remodeling tissue which has potential to influence lung functions single-handedly of inflammation and airflow obstruction[19]. It can stimulate the release of growth factors and cytokines. It can trigger inflammatory cells to infiltrate into the airway[18]. Furthermore, there is an implication of soluble ADAM33 in forming of new vessels and angiogenesis. It may stimulate cytokines and inflammatory cells or growth factors in the airway. In the case of ADAM33, it has contribution in airway remodeling in consequence of its high expression in epithelium, fibroblasts, as well as the airway smooth muscle cells (ASMCs). Declining of proliferation and increasing the apoptosis of ASMCs in a rat model of allergic asthma can be caused by silencing of ADAM33[20]. The genetic susceptibility to patients who get chronic bronchitis caused by smoking or aerosols and industrial dust can be enhanced by ADAM33 gene polymorphisms[21].

In addition, this current research demonstrated that there was an association between the polymorphisms of ADAM 33 and COPD in Javanese. To the best of our knowledge, it is the first research report which conducted in Indonesia context. In this association study, we genotyped 42 well-characterized COPD cases and 32 healthy smoker controls, who were long-term tobacco smokers for ADAM33 gene of three SNPs (T2, T1and Q-1). The results revealed that SNPs T2 and T1 of this gene were significantly associated with COPD. Consistent with our result, Wang's research indicated the T2 was significantly associated with total cells in COPD patient's phlegm and SNP T1 was significantly associated with macrophage[9]. Our findings will give great contribution related to COPD genetic marker in Indonesia. It is caused by number of Javanese that attains 40% from of all Indonesian population and it is as the biggest tribe in Indonesia.

Furthermore, in East Asian population, the changes of pulmonary function and components of cells in sputum of COPD were obviously associated with T2, T1, and Q-1. Moreover, cytokines and mediators of inflammation in airway of COPD in recessive models were meaningfully associated with T1 and Q-1[9]. In the Kashmiri population of India, ADAM33 gene polymorphism in three SNPs (T1, T2, and Q-1) were obviously associated with COPD[11]. The results of present study showed that the similarities of findings in T1 and T2, but Q-1 was different. It was also supported from the study reported by Li et al found in a Chinese individual the polymorphism of T1 in ADAM33 would escalate patients COPD chance[10].

In other studies, another ADAM33 SNPs was also associated with COPD. Vijaya Laxmi et al reported that the polymorphism of ADAM33 gene which had SNPs name S1 and S2 become the main genetic factors and risk for COPD[22]. Aierken et al also reported that the inclined risk of COPD in Chinese but not in Caucasians is correlated to S1 (rs3918396) polymorphism of ADAM33[13]. In addition, Tan et al, found COPD in the Mongolian of China was associated with Seven SNPs in ADAM33[15]. Study of Reijmerink et al, who investigated the polymorphism V4 of ADAM33 is associated with COPD in Venezuelan patients. Interaction of *in utero* cigarette smoke exposure with *ADAM33* results in reduced lung function[23]. It was also supported by meta-analysis study of Zhou et al which clearly revealed that the ADAM33 polymorphisms which had SNPs name F+1, T2, T1, S1, ST+5 and Q-1, correlation to the risk of COPD. Moreover, T2, T1, S1, ST+5 and Q-1 pointed

of the risk of COPD in the Asian populations. T2, ST+5 and Q-1showed the risk of COPD in the European populations[24].

In this present study, the results clearly showed that when compared to the wild allele, heterozygous AG genotypes of T1 SNP, and homozygous GG genotype of T2 SNP were distributed significantly in higher ($p < 0.05$) frequency among the populations; suggesting that the increased susceptibility among carriers of these genotypes to develop COPD.

Genetically, the Javanese is closer to the ethnic groups in the Southeast Asia region, such as Thailand and Vietnam while tribes in eastern Indonesia are closer to people in the Pacific Ocean region. The Nias and Mentawai tribes are closer to the native tribes of Taiwan[25]. The phylogenetic analysis of this study has given the clear picture of the associations and the genetic similarity of the 33 ADAM gene sequences in this study with various populations in the world, thereby revealed that the Javanese tribe ADAM33 gene sequence showed similarities with Caucasian and Chinese populations.

Conclusions

The results demonstrated that T1 and T2 polymorphisms of *ADAM33* gene have shown an association of COPD. The results revealed that it might be risk factors for COPD susceptibility. The contribution of the disease-connected to SNPs must be clarified yet, specifically in the COPD pathophysiology context. The future studies are warranted to extend this preliminary research to conduct with large number of sample sizes, more SNP numbers to fully elucidate the potential candidate genes implicated in the genesis of COPD in Javanese population, and also further to develop screening procedures to identify patients at risk of developing COPD.

Abbreviation

ADAM33: A Disintegrin And Metalloprotease 33; ASMCs: Airway Smooth Muscle Cells; COPD: Chronic Obstructive Pulmonary Disease; FEV1: Forced Expiratory Volume in the First Second; FVC: Forced Vital Capacity; MMPs: Matrix Metalloproteinases; PFT: Pulmonary Function Tests; SNPs: Single Nucleotide Polymorphisms; UTR: Untranslated Region.

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