Tumor necrosis factor-α -308 G/A genetic polymorphism in patients with chronic obstructive pulmonary disease presenting with hyperactive airways

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Abstract. Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide. COPD is often diagnosed late in the disease leading to a delay in management. Notably, tumor necrosis factor-α (TNF-α) polymorphisms may serve an important role in the development of COPD. A single-center, case-control study was conducted to determine the presence of the TNF-α -308 G/A polymorphism among patients diagnosed with COPD presenting with hyperactive airways, patients without COPD presenting with hyperactive airways, and normal study participants without pulmonary comorbidities. Three genotypes: G/G (94%; 157/167), G/A (5%; 9/167) and A/A (1%; 1/167) were detected by quantitative PCR. The present study showed that the presence of the TNF-α -308 G/A polymorphism reduced the odds of having hyperactive airways with COPD by 29.3% and hyperactive airways without COPD by 26.3%. Multinomial logistic regression analysis showed that having the TNF-α -308 G/A polymorphism did not significantly reduce the odds of having hyperactive airways with COPD and without COPD compared to those with the G/G genotype. In conclusion, the presence of the TNF-α -308 G/A gene polymorphism showed no significant association with patients with COPD with or without hyperactive airways. The presence of the TNF-α -308 G/A polymorphism instead had a weak association with the reduction in the development of COPD regardless of the presence or absence of airway hyperactivity.

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Introduction

Chronic obstructive pulmonary disease (COPD) is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines as a persistent, progressive condition that causes airflow limitation due to exposure to noxious particles or gases (1). This leads to structural changes, such as the narrowing of small airways and the destruction of lung tissue (2). According to the GOLD, COPD is the fourth leading cause of death worldwide (1). In the Philippines, the incidence of mild cases is 13.8%, while that of moderate to severe cases is 12.5% (3). Individuals aged ≥40 years, cigarette smokers of >20 pack years, those with exposure to secondhand smoke or occupational exposure to cigarette smoke, those with a history of chronic cough or chronic bronchitis, or those with a family history of COPD are at a higher risk (3). COPD usually presents with airway hyperreactivity, such as coughing, wheezing, shortness of breath and chest tightness (4). Undiagnosed chronic airway obstruction also poses a higher risk for all-cause mortality (5).

Predicting the development of COPD may aid in appropriate management and improve patient outcomes (6). According to the GOLD 2020 report, genetics and environmental factors both contribute to the development of COPD. Researchers are exploring the use of molecular biomarkers for the early diagnosis of COPD. Biomarkers are measurable indicators of normal biological processes, pathogenic processes or pharmacological responses (1). Tumor necrosis factor-α (TNF-α) is a biomarker that serves a marked role in COPD development (7). TNF-α polymorphisms are the most studied proinflammatory cytokine polymorphisms amongst other variants in the TNF superfamily. Chung (8) reported that TNF-α concentrations were higher in the alveolar macrophages of patients with COPD and cigarette smokers compared with those in nonsmokers. TNF-α triggers an inflammatory cascade

by activating the transcription factor nuclear factor-κB, which transcribes and increases interleukin (IL)-6 and IL-8 release from the airway epithelium. IL-6 and IL-8 are glycoproteins produced by cells from the innate immune system, and are considered a marker of inflammation together with TNF-α (9). This is supported by findings that show higher levels of IL-6 and IL-8 in patients with COPD and hyperactive airways (10).

Several polymorphisms in the TNF-α gene are now being studied for their role in tissue destruction in autoimmune diseases, such as Crohn's disease (11) and rheumatoid arthritis (12). One of them, the TNF-α -308 G/A polymorphism, has been reported to increase the risk of COPD development compared with other variants, such as TNF-α +489G/A and -238G/A (13). This genotype has been shown to increase susceptibility to acute exacerbation of COPD and the risk of its progression (14). Studies have also found that this genotype increases the risk of COPD development in the Asian population (13,15). Moreover, Japanese patients with smoking-related COPD have a higher frequency of the TNF-α -308 G/A polymorphism compared with smokers without COPD (16). The A allele of the TNF-α -308 G/A genotype has been associated with higher transcriptional activity and is often implicated in the risk of COPD (11). Furthermore, Huang *et al* (17) reported that carrying the TNF-α -308 A allele is associated with a higher risk of developing COPD among the Taiwanese population.

By contrast, other studies have reported that the TNF-α -308 polymorphism does not exhibit an association with other pulmonary diseases, such as asthma. For example, a case-control study among Pakistani patients showed that patients with asthma did not present with the TNF-α genotype (18). Similarly, a case-control study among 194 European Caucasian patients found that the TNF-α -308 polymorphism did not confer susceptibility to the development of asthma nor grade its severity (19).

Understanding the incidence of TNF-α polymorphisms among various populations can provide useful information to improve the care of patients at risk of developing COPD. Moreover, the TNF-α polymorphism, particularly at region -308 G/A (rs1800629), may be utilized as a potential biomarker for predicting the development of COPD. This, in turn, could aid in addressing the delay in treatment strategies for COPD brought about by a late diagnosis (20).

Materials and methods

Study design, samples and participants. The present study was conducted at the Emergency Department (ED), St. Luke's Medical Center (Quezon City, Philippines) using a case-control design. All study participants were adult patients admitted to the ED from September 2021 to May 2022. A total of 167 participants were recruited from the initially targeted 186 participants since some patients tested positive for COVID-19 infection and were not included. The patients were divided into three patient sets with 62 participants in each of the two control groups (positive and negative controls) and 43 participants in the case group. A licensed medical technologist obtained peripheral blood samples. A single-blinded scheme was used. The primary investigator labeled the collected blood samples with a coding scheme, while the researchers handling the molecular tests were blinded to the clinical profiles.

Inclusion and exclusion criteria. The present study included adult patients aged between 19 and 65 years presenting to the ED, St. Luke's Medical Center. All participants enrolled in the study underwent COVID-19 testing and only those who were negative were recruited. Participants in the case group were either previously diagnosed with COPD by spirometry (forced expiratory volume in 1 sec/forced vital capacity <0.7 post-bronchodilation) or were patients maintained on medications for the control of COPD, such as β-agonist, anti-cholinergic or corticosteroid inhalers. The positive control group included those presenting with hyperactive airways, with symptoms including cough, wheezing, shortness of breath and chest pain, but not diagnosed with COPD. The negative control group included healthy participants with no pulmonary comorbidities, who were nonsmokers and did not present with symptoms of hyperactive airways. The present study excluded patients admitted to the ED who presented with hyperactive airways with concomitant cardiac disease or other pulmonary diseases aside from COPD.

Genotyping. A total of 167 whole blood samples submitted to the Research and Biotechnology Division of St. Luke's Medical Center were stored at 2-8˚C until use. Nucleic acid extraction from whole blood samples was performed using the QIAamp DNA Blood Mini Kit (Qiagen GmbH) according to the manufacturer's instructions. The purity and concentration of extracted genomic DNA were determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop; Thermo Fisher Scientific, Inc.). The DNA samples were stored at -20˚C until use. The single nucleotide polymorphism (SNP) genotype, TNF-α -308 G/A (rs1800629), was determined by quantitative PCR. The detection of the TNF-α -308 G/A (rs1800629) gene polymorphism was carried out using the QuantStudio™ 5 System (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following conditions: 60˚C for 30 sec, 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec and 60˚C for 1 min, and a final step at 60˚C for 30 sec. Genotyping of the TNF-α -308 G/A (rs1800629) polymorphism was conducted using a pre-designed TaqMan® SNP Genotyping Assay (cat. no. 4351379; Applied Biosystems; Thermo Fisher Scientific, Inc.). A qPCR thermocycler (QuantStudio 5 System) measured the intensity of fluorescence emitted by the probe at each cycle. One probe was labeled with VIC to detect the allele 1 sequence; the second probe was labeled with FAM to detect the allele 2 sequence. The context sequence (VIC/FAM) was: 5'-GAGGCAATAGGTTTTGAGGGGCATG[A/G]GGACGG GGTTCAGCCTCCAGGTCC-3' (20). Allelic discrimination analysis was performed using QuantStudio™ Design and Analysis Software v1.5.2 (Applied Biosystems; Thermo Fisher Scientific, Inc.). The laboratory technicians regularly maintained and validated the equipment and instruments to ensure accuracy and validity.

Statistical analysis. Demographic variables, such as age, are presented as the mean ± standard deviation, whereas categorical variables are presented as counts and percentages [including presence of TNF-α -308 G/A gene polymorphism,

Table I. Demographic data of the study participants in each study group.

Characteristic	All (n=167)	Case group (n=43)	Positive control group (n=62)	Negative control group (n=62)	P-value
Age, years	61.8±20.8	74.5±13.3	65.5±19.2	49.4±20.0	0.106
Male sex	99 (59.3%)	36 (83.7%)	27 (43.5%)	36 (58.1%)	<0.001
Smoking	47 (28.1%)	31 (72.1%)	8 (12.9%)	8 (12.9%)	<0.001
Hypertension	107 (64.1%)	37 (86.0%)	47 (75.8%)	23 (37.1%)	<0.001
Diabetes mellitus	61 (36.5%)	24 (55.8%)	28 (45.2%)	9 (14.5%)	<0.001

Data are presented as mean ± SD or counts (%).

Table II. Allelic and genotype frequencies of tumor necrosis factor-α -308 among the three groups.

	Genotype frequencies			Allele frequencies	
Study group	G/G	G/A	A/A	p (G allele)	q (A allele)
Negative control (n=62)	58 (93.5%)	4 (6.5)	0	0.97 (97%)	0.03 (3%)
Positive control (n=62)	59 (95.2%)	3 (4.8%)	0	0.98 (98%)	0.02 (2%)
Case group (n=43)	40 (93.0%)	2 (4.7%)	1 (2.3%)	0.95 (95%)	0.05 (5%)

sex, smoking, hypertension and diabetes mellitus (DM)]. One-way analysis of variance with the Bonferroni adjustment for post hoc analysis was applied to compare age, whereas the χ^2 test of independence, was used to compare sex, smoking, hypertension and DM among the three groups. Multinomial logistic regression was applied to determine if the TNF-α -308 G/A or G/G gene polymorphisms are predictors of having a hyperactive airway with and without COPD. Statistical tests were performed using Stata version 14.0 (StatCorp, LLC). Allele and genotype outcomes were assessed for deviation from the Hardy-Weinberg equilibrium using Pearson's χ^2 test. P<0.05 was considered to indicate a statistically significant difference.

Results

A total of 167 participants were recruited. The demographic characteristics of the study participants are presented in Table I. The mean ages of the patients in the positive control group, cases group and negative control group did not differ but there was a higher proportion of male patients in the case group compared with in the positive and negative control groups. There was a significantly higher proportion of smokers in the case group (31/43; 72.1%) vs. the positive (8/62; 12.9%) and negative control groups (8/62; 12.9%).

A summary of allele and genotype frequencies for TNF-α -308 among the study groups is shown in Table II. In the negative control group of 62 participants, 58 (93.5%) had the G/G polymorphism, 4 (6.5%) had the G/A polymorphism and 0 had the A/A polymorphism. Using the Hardy-Weinberg equilibrium, the G allele for the negative control group was 0.97 (97%), whereas the A allele was 0.03 (3%). For the positive control group of 62 participants, 59 (95.2%) presented with the G/G polymorphism, 3 (4.8%) presented with the G/A polymorphism, and none carried the A/A polymorphism. The computed allele frequencies for the positive control group were 0.98 (98%) for the G allele and 0.02 (2%) for the A allele. Finally, for the case group of 43 participants, 40 (93.0%) presented with the G/G polymorphism, 2 (4.7%) presented with the G/A polymorphism and 1 (2.3%) presented with the A/A polymorphism. Allele frequencies for the case group were 0.95 (95%) for the G allele and 0.05 (5%) for the A allele.

Based on the present data, the presence of the G/A genotype reduced the odds of having a hyperactive airway with COPD by 29.3% [odds ratio (OR)=0.707; 95% CI: 0.124-4.046] and reduced the odds of having a hyperactive airway without COPD by 26.3% (OR=0.737; 95% CI: 0.158-3.440).

As shown in Table III, the presence of the TNF-α -308 G/A gene polymorphism reduced the odds of having hyperactive airways with COPD by 33.3% [adjusted OR (AOR)=0.667; 95% CI: 0.117-3.813) and also reduced the odds of having hyperactive airways without COPD by 30% (AOR=0.700; 95% CI: 0.150-3.267), as compared with the G/G gene polymorphism. Furthermore, a 1-year increase in age increased the odds of having hyperactive airways with COPD by 5.7% (AOR=1.057; 95% CI: 1.02-1.09), while it increased the odds of having hyperactive airways without COPD by a factor of only 3% (AOR=1.030; 95% CI: 1.01-1.05). The odds of developing hyperactive airways with COPD were increased by 3.84 times (CI: 1.15-12.8) for patients with DM compared to those without DM. The odds were increased 3.15 times (95% CI: 1.14 to 8.71) for diabetic patients with hyperactive airways without COPD compared to patients without DM. Moreover, the odds of having hyperactive airways with COPD in patients who smoked were 8.09 times (95% CI: 2.43-26.93) as compared to patients who did not smoke. Smoking did not play a role as a predictor of having a hyperactive airway for those who did not have COPD. Finally, neither sex nor hypertension

Table III. Multinomial logistic regression.

Characteristic	Negative control group (n=62)	Positive control group (n=62)	AOR (positive vs. negative controls)	P-value	Case group (n=43)	AOR (control vs. case group)	P-value
G/A gene polymorphism	4 (6.5%)	3 (4.8%)	0.700	0.650	2 (4.7%)	0.667	0.649
Age, years	49.4±20.0	65.5±19.2	1.030	0.015	74.5±13.3	1.057	0.002
Male sex	36 (58.1%)	27 (43.5%)	0.525	0.130	36 (83.7%)	1.833	0.328
Smoking	8 (12.9%)	8 (12.9%)	0.762	0.658	31 (72.1%)	8.089	0.001
Hypertension	23 (37.1%)	47 (75.8%)	1.892	0.222	37 (86.0%)	2.047	0.306
Diabetes mellitus	9 (14.5%)	28 (45.2%)	3.146	0.027	24 (55.8%)	3.843	0.028

Data are presented as mean ± SD or counts (%). AOR, adjusted odds ratio.

was identified as a predictor of hyperactive airways, with or without COPD.

The genotypic frequencies observed were plotted on the Hardy-Weinberg equilibrium tester developed by Rodriguez *et al* in 2009 (21). Based on Pearson's χ^2 test, the computed value for the negative control group was 0.069 and the positive control group was 0.038, which was significantly smaller than the tabulated χ^2 value (df=1) of 3.84 for both the control groups indicating that they satisfy the Hardy-Weinberg equilibrium. By contrast, for the case group, the computed value based on Pearson's χ^2 test was noted to be 9.73, which is higher than tabulated value (df=1) of 3.84. This indicates that the alleles are not in equilibrium according to the Hardy-Weinberg principle.

Discussion

Research has focused on how molecular biomarkers can help identify COPD at an early stage (1). Among the pro-inflammatory cytokines, TNF-α can reduce glutathione levels in the lungs, which can lead to the production of reactive oxygen species. Upregulation of TNF-α has been associated with COPD-related changes in the lungs, such as pleural thickening, loss of small airspaces, and increased chest and lung cavity volumes (12). Previous studies have examined the role of the TNF-α -308 G/A gene polymorphism in the development of inflammatory diseases (11,12,22). However, only a few studies have established an association between the TNF-α -308 G/A polymorphism and pulmonary diseases, such as asthma (18,22). Research has shown that the TNF-α -308 G/A polymorphism is associated with a higher prevalence of COPD in Asian populations, an increased risk of smoking-related COPD and a higher frequency of COPD in general; however, the association is still unclear due to insufficient study designs and small group sizes (8,15,17).

The present study aimed to investigate whether the TNF-α -308 G/A gene polymorphism could serve as a biomarker for COPD by examining its presence in individuals diagnosed with COPD who have hyperactive airways. Data were obtained from a total of 167 out of the initially targeted 186 participants. Patient demographics were analyzed, and the results showed that an increase in age by 1 year and diabetes mellitus increased the odds of developing hyperactive airways with or without COPD. In addition, smoking increased the odds of developing COPD with hyperactive airways. However, hypertension and sex did not affect the development of hyperactive airways with or without COPD.

The present study found that the G/G genotype had a significantly higher frequency in individuals with COPD with hyperactive airways, those with undiagnosed COPD with hyperactive airways and the negative control group (93, 95.1 and 93.5%, respectively), compared with the G/A genotype (4.6, 4.3 and 6.4%, respectively) and the A/A genotype (2.3% for case group, none for both control groups). Based on allele frequency for all populations, the G allele was significantly more prevalent than the A allele (95 vs. 5% for those with COPD with hyperactive airways, 98 vs. 2% for undiagnosed COPD cases with hyperactive airways, and 97 vs. 3% for the negative control).

The TNF-α -308 G/A gene polymorphism did not significantly reduce the odds of having COPD with hyperactive airways nor the odds of hyperactive airways in individuals undiagnosed with COPD. The Hardy-Weinberg principle was also applied to determine if the genetic polymorphism was significantly associated with the possible development of COPD. Based on the findings, the negative control group and those without COPD but that presented with hyperactive airways satisfied the Hardy-Weinberg equilibrium principle. This implies that allele and genotype frequencies in the positive and negative control groups either did not change significantly or there was no mutation seen in the allele or no migration (21). However, for those with COPD presenting with hyperactive airways, there was a deviation of the A/A genotype, which caused the deviation from the Hardy-Weinberg equilibrium.

Further studies are needed to replicate the present findings in a larger, independent population. The TNF-α -308 G/A (rs1800629) genetic variant has been studied in relation to the risk of developing COPD. Individuals who carry the A allele of this variant are known to have a higher likelihood of activating the TNF-α promoter region, leading to upregulation of TNF-α (23). It has been observed that the A allele is strongly associated with COPD risk in the Asian population, particularly in the Taiwanese and Japanese populations, compared to that in Caucasian populations. However, the frequency of the A allele is relatively low in the Asian population (17,24,25).

Two meta-analyses were previously conducted to investigate the association between the TNF-α -308 G/A (rs1800629) variant and COPD risk. One study revealed that this variant was significantly associated with COPD risk in the Asian population, but this association was no longer significant after adjusting for smoking (26). Another study showed that individuals with the G/G and G/A genotypes have a lower risk of developing COPD compared with A/A genotype carriers (27).

In the present study, 94% (157/167) of participants carried the 'G' allele, only 5% (9/167) had the G/A genotype and 1 participant carried the risk 'A' allele. Since SNPs identified in association studies are not the cause of disease but are merely markers of correlated causal variants, it is necessary to replicate the findings in independent cohorts to determine the true association. Future studies should also consider investigating the combined effects of other genes and environmental factors on COPD risk. Increasing the sample size may also help validate if the lower frequency of the A allele in the Philippines population also predicts a lower burden or risk of COPD development compared with that in Caucasian populations.

The present study had some limitations. First, it was conducted in a single center. Due to the low incidence of COPD in the urban population, there were difficulties in performing randomization, which led to purposive sampling instead. Second, the study was planned before the COVID-19 pandemic, and approval and initiation of the study occurred during the pandemic. As a result, revisions were made to the criteria of the study participants, particularly the requirement of a negative COVID-19 reverse transcription-PCR result before enrollment. This made recruitment of subjects difficult, especially for the case group, as most of the patients with COPD who presented with hyperactive airways also tested positive for COVID-19 and were not recruited for the study. This, in turn, limited the sample size for the case group and we could not achieve the targeted 186 participants. However, to the best of our knowledge, the present study is the first report on the allelic and genotype frequencies of TNF-α among individuals from the Philippines, which may serve as a basis for future studies to identify the association of other cytokines, such as IL-6 and IL-8, with COPD in patients with hyperactive airways.

In conclusion, the findings of the present study revealed that the presence of the TNF-α -308 G/A gene polymorphism had no significant association with patients with COPD, whether they had hyperactive airways or not. However, the presence of the TNF-α -308 G/A polymorphism showed a weak association in reducing the development of COPD, regardless of the presence or absence of airway hyperreactivity. Furthermore, patient factors, such as diabetes mellitus and an increase in age, increased the odds of COPD with or without airway hyperreactivity. Smoking was also associated with an increased likelihood of developing COPD with airway hyperactivity.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PMDH, PFQ, MPSD, and MOB designed the study. PMDH, PFQ and MOB analyzed the data. PMDH and MOB wrote the paper. VRL and MXY processed the samples and performed all the laboratory procedures. All authors confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Review Committee of St. Luke's Medical Center (Quezon City, Philippines; approval no. SL-20383). Written informed consent was obtained from the participants before performance of the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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