International Journal of COPD

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ORIGINAL RESEARCH

# Effect of N-acetylcysteine in COPD patients with different microsomal epoxide hydrolase genotypes

This article was published in the following Dove Press journal:
International Journal of COPD
13 May 2015
Number of times this article has been viewed

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**Background:** The role of the antioxidant N-acetylcysteine (NAC) in the treatment of chronic obstructive pulmonary disease (COPD) has not been clarified as yet. In early studies, we found that the proportion of smokers with COPD having extremely slow/slow microsomal epoxide hydrolase (EPHX1) enzyme activity is significantly higher than that in healthy smokers. The purpose of this study was to evaluate whether different EPHX1 enzyme activity is related to differential therapeutic effects of treatment with NAC in COPD.

**Methods:** A total of 219 patients with COPD were randomly allocated to an extremely slow/slow EPHX1 enzyme activity group (n=157) or a fast/normal EPHX1 enzyme activity group (n=62) according to their EPHX1 enzyme activity. Both groups were treated with NAC 600 mg twice daily for one year. The main study parameters, including forced expiratory volume in one second ($FEV_1$), St George's Respiratory Questionnaire (SGRQ), and yearly exacerbation rate, were measured at baseline and at 6-month intervals for one year.

**Results:** Both $FEV_1$ and SGRQ symptom scores were improved after treatment with NAC in the slow activity group when compared with the fast activity group. Further, changes in $FEV_1$ and SGRQ symptom score in patients with mild-to-moderate COPD were more significant than those in patients with severe-to-very severe COPD. The yearly exacerbation rates were reduced in both groups, but the reduction in the slow activity group was significantly lower than in the fast activity group.

**Conclusion:** NAC treatment in COPD patients with extremely slow/slow EPHX1 enzyme activity improves $FEV_1$ and the SGRQ symptom score, especially in those with mild-to-moderate COPD, and polymorphism in the EPHX1 gene may have a significant role in differential responses to treatment with NAC in patients with COPD.

**Keywords:** N-acetylcysteine, chronic obstructive pulmonary disease, microsomal epoxide hydrolase, polymorphism

## Introduction

It is well known that chronic obstructive pulmonary disease (COPD) is strongly associated with genetic factors and that susceptibility to COPD depends in part on the genetic phenotypes and gene polymorphism of a variety of factors involved in the pathogenesis of COPD, such as inflammatory cytokines, proteases, antiproteases, oxidoreductases, and detoxifying enzymes. There is good evidence to suggest that increasing oxidative stress is a key factor in the pathogenesis of COPD.[1–3] The body has a perfect enzymatic and nonenzymatic antioxidation system to cope with oxidative stress and protect the body from attack by oxidants. The main known oxidation inhibition enzymes in the body including glutathione-S-transferase, microsomal epoxide hydrolase (EPHX1), and heme oxygenase, hydrolyze and inactivate oxygen metabolites, thus fighting against

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or neutralizing the oxidative damage caused by oxidative stress, and eventually maintaining the dynamic balance of oxidation/antioxidation in the body. When the production of oxidation inhibitors is decreased or their activity is diminished as a result of genetic variation, the dynamic balance of oxidation/antioxidation is lost, leading to oxidative damage.

In our early oxidation inhibition enzyme and antiprotease gene polymorphism studies, we found that there was no significant correlation between GSTP1 I105V polymorphism and COPD, and we did not find any association between polymorphisms in the serine protein inhibitor E2 gene and COPD in the Han population of southwest China.[4,5] However, we did find that the exon 3 heterozygous genotype of EPHX1 (Tyr113/His113) in smokers with COPD was significantly higher than in otherwise healthy smokers, and so was the proportion of subjects with extremely slow/slow EPHX1 enzyme activity.[6,7]

Being a representative antioxidant and mucus-modifying drug,[8–10] N-acetylcysteine (NAC) is the focus of a good deal of pharmaceutical research at present. Numerous researchers have demonstrated that NAC can reduce the number of acute exacerbations of COPD,[11–14] but the evidence for whether NAC can improve lung function or not remains equivocal. Stav and Raz conducted a double-blind, randomized, placebo-controlled study and found that treating COPD patients with NAC had a beneficial effect on physical performance, probably due to a reduction in air trapping.[15] The results of a large multicenter study conducted in Europe show that although NAC could improve symptoms in patients with COPD and reduce their average medical expenditure, the decline in forced expiratory volume in one second ($FEV_1$) and forced vital capacity (FVC) was not significantly different between the NAC group and the placebo group.[16] Another multicenter study followed 523 patients with COPD for 3 years and found that there was no difference in lung function or in prevention of exacerbations between NAC and placebo.[17]

What types of patients with COPD may benefit more from NAC treatment? Our hypothesis is that the equivocal effect of NAC as antioxidant therapy in patients with COPD may be associated with genetic phenotypes and gene polymorphism in oxidation inhibiting enzymes. This study was conducted to explore whether there was a difference in the curative effect when COPD patients with different EPFX1 genotypes were given the same NAC treatment. To our knowledge, no relevant reports have been published to date.

# Materials and methods

## Subjects

A total of 219 unselected smokers with stable COPD (diagnostic criteria in accordance with the 2011 Global Initiative for Chronic Obstructive Lung Disease guideline) were recruited from the Department of Respiratory Critical Care Medicine, First Affiliated Hospital of Kunming Medical University, Kunming, People's Republic of China, between June 2012 and December 2013. Two hundred and twenty-three otherwise healthy smokers without COPD and matched for age and sex were recruited as controls from the medical center at the First Affiliated Hospital of Kunming Medical University. All subjects were of Han nationality and from southwest China. Eligible patients with stable COPD and post-bronchodilator spirometry $FEV_1/FVC < 70\%$, aged 60–80 years, and with the duration of COPD of 5 years or more were included. Patients were excluded if they had asthma or other chronic bronchial lung or allergic disease, had a history of upper respiratory tract infection in the 6 weeks before enrolment or upper respiratory tract infection history in the first 2 weeks of the screening period, or had a history of lung surgery, malignancy, or other severe organ dysfunction. Patients with severe hypoxemia who failed to cooperate with lung function testing or had poor reliability or compliance were also excluded.

None of the patients needed to change their current medication (including inhaled long-acting beta-agonists and corticosteroid). The study was approved by the ethics committee of Kunming Medical University. All subjects provided their written informed consent.

## DNA genotyping

Genomic DNA was extracted from peripheral blood leukocytes in the 219 COPD patients and 223 healthy smokers by a phenol-chloroform method. We identified the EPHX1 polymorphisms using direct sequencing for EPHX1 exon 3 and polymerase chain reaction-restriction fragment length polymorphism for EPHX1 exon 4. Based on their EPHX1 exon 3 and exon 4 polymorphism status, according to the classification of Smith and Harrison,[18] the patients could be classified into four groups according to putative EPHX1 phenotype for enzyme activity, ie, normal, fast, slow, and extremely slow, as we have described previously.[6]

## Study design

Eligible patients were allocated to either an extremely slow/slow EPHX1 enzyme activity group or a fast/normal EPHX1 enzyme activity group based on their level of enzyme activity. All subjects were given NAC 600 mg twice daily (Zambon Pharma, Bresso, Italy) for one year. Study parameters, including age, sex, smoking status, body mass index, current medication, exacerbation rate during the previous year, lung function, SGRQ score, and 6MWD, were recorded at baseline before the beginning of

the trial. Symptoms, changes in current medication, and patient compliances with NAC therapy were recorded at each follow-up visit day (every 3 months). Lung function, SGRQ score, and 6MWD were measured at 6 and 12 months. An exacerbation episode was defined as an increase in cough, sputum, or dyspnea, making it necessary to use antibiotics and/or oral corticosteroids or to hospitalize the patient during the study. Adverse events were also recorded throughout the study.

## Lung function tests

Spirometry was performed by a trained specialist technologist using a Pony FX portable spirometer (Cosmed, Rome, Italy) according to the American Thoracic Society standards for lung function tests.[19] Spirometric data ($FEV_1$, FVC, $FEV_1$/FVC ratio, $FEV_1$% predicted) were measured at 0, 6, and 12 months during the trial.

## Statistical analysis

The sample size was estimated so that the genetic association would be adequately powered to detect in excess of 200 subjects using Quanto (assuming 90% power, $K_p$ 0.1, $R_G$ 2.6). Statistical analyses were performed using Statistical Package for the Social Sciences version 21.0 (IBM Corporation, Armonk, NY, USA). All hypothesis tests were two-sided, and $P<0.05$ was defined as being statistically significant. Data are expressed as the mean ± standard deviation unless indicated otherwise.

The frequencies of the EPHX1 genotypes between patients with COPD and controls were compared using the two-tailed chi-squared test. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated to assess the relative risk of COPD. The differences in enumeration data between the two groups at baseline (such as age, smoking index, $FEV_1$% predicted, $FEV_1$/FVC) were compared using the unpaired *t*-test, and measurement data (such as sex, composition, and frequency of EPHX1 genotype distribution) were compared using the chi-squared test. Changes in lung function parameters and other variables in the two groups were analyzed before and after treatment with NAC using the paired *t*-test.

# Results

We recruited 240 patients with COPD and 223 healthy smokers from June 2012 to December 2013, but 21 patients with COPD had dropped out by the end of the study. Six patients with COPD withdrew their consent, eight were lost to follow-up, four showed poor compliance, and three died during the study, leaving 219 patients who completed the study.

Table 1 shows the baseline characteristics of the 219 patients with COPD and the 223 healthy smokers; Table 2 shows the distribution of EPHX1 genotypes between these two groups. The proportion of heterozygous (Tyr113/His113) individuals was significantly higher in the COPD group than in the control group (63.9% versus 38.1%, respectively, $P<0.001$; OR 1.6, 95% CI 1.4–1.9). Conversely, the frequency of homozygous wild-type (Tyr113/Tyr113) subjects was significantly lower in the COPD group than in the control group (18.7% versus 41.3%, respectively, $P<0.001$; OR 0.4, 95% CI 0.3–0.6). However, there was no significant difference in distribution of exon 4 genotype polymorphism between the COPD group and control group. Table 3 shows the distribution of EPHX1 phenotypes in the COPD and healthy smokers. The frequency of the slow activity EPHX1 phenotype was significantly higher in the COPD group than in the control group (54.3% versus 32.3%, $P<0.001$, OR 1.4, 95% CI 1.2–1.6), and the frequency of the fast activity EPHX1 phenotype was lower in the COPD group than in the control group (4.1% versus 15.7%, $P<0.001$, OR 0.2, 95% CI 0.1–0.4).

All eligible patients with COPD were allocated to either a slow activity group (n=157) or fast activity group (n=62) based on EPHX1 enzyme activity. Baseline characteristics

**Table 1** Baseline characteristics of COPD patients and healthy smokers

| Characteristics | COPD patients (n=219) | Control subjects (n=223) | *P*-value |
|---|---|---|---|
| Males/females | 194/25 | 195/28 | 0.85 |
| Age, years | 70.2±7.2 | 69.1±6.2 | 0.89 |
| Smoking, pack-years | 28.1±7.2 | 27.2±6.6 | 0.83 |
| $FEV_1$% predicted | 51.8±15.9 | 93.1±8.2 | <0.01 |
| $FEV_1$/FVC | 53.2±10.9 | 85.6±6.4 | <0.01 |

**Note:** Data are presented as n or mean ± standard deviation, unless otherwise stated.
**Abbreviations:** COPD, chronic obstructive pulmonary disease; $FEV_1$, forced expiratory volume in one second; FVC, forced vital capacity.

**Table 2** Distribution of EPHX1 genotypes in COPD patients and healthy smokers

| Genotypes | COPD patients (n=219) | Control subjects (n=223) |
|---|---|---|
| Exon 3 polymorphism | | |
| Tyr113/Tyr113 (%) | 41 (18.7) | 92 (41.3)[#] |
| Tyr113/His113 (%) | 140 (63.9)[§] | 85 (38.1) |
| His113/His113 (%) | 38 (17.4) | 46 (20.6) |
| Exon 4 polymorphism | | |
| His139/His139 (%) | 173 (79) | 170 (76.2) |
| His139/Arg139 (%) | 36 (16.4) | 45 (20.2) |
| Arg139/Arg139 (%) | 10 (4.6) | 8 (3.6) |

**Notes:** Data are presented as n (%), unless otherwise stated. [#]Homozygous wild-type (Tyr113/Tyr113) in the COPD group versus the control group ($P<0.001$, OR 0.4, 95% CI 0.3–0.6). [§]Heterozygous (Tyr113/His113) in the COPD group versus the control group ($P<0.001$, OR 1.6, 95% CI 1.4–1.9).
**Abbreviations:** COPD, chronic obstructive pulmonary disease; EPHX1, microsomal epoxide hydrolase; CI, confidence interval; OR, odds ratio.

**Table 3** Distribution of EPHX1 phenotypes in COPD patients and healthy smokers

| Phenotypes | COPD patients (n=219) | Control subjects (n=223) |
|---|---|---|
| Fast (%) | 9 (4.1) | 35 (15.7)[#] |
| Normal (%) | 53 (24.2) | 74 (33.2) |
| Slow (%) | 119 (54.3)[§] | 72 (32.3) |
| Extremely slow (%) | 38 (17.4) | 42 (18.8) |

**Notes:** Data are presented as n (%), unless otherwise stated. [#]Fast activity EPHX1 phenotype in the COPD group versus the control group ($P<0.001$, OR 0.2, 95% CI 0.1–0.4). [§]Slow activity EPHX1 phenotype in the COPD group versus the control group ($P<0.001$, OR 1.4, 95% CI 1.2–1.6).
**Abbreviations:** COPD, chronic obstructive pulmonary disease; EPHX1, microsomal epoxide hydrolase; CI, confidence interval; OR, odds ratio.

and lung function parameters for patients with COPD did not differ between the slow and fast activity groups (Table 4).

There were significant improvements in $FEV_1$ (from 1.37±0.35 L to 1.46±0.39 L, $P$=0.041) and $FEV_1$% predicted (from 59.1±16.5 to 63.2±17.8, $P$=0.038) in the slow activity group after treatment with NAC for one year, but there were no significant differences with regard to changes in $FEV_1$ and $FEV_1$% predicted in the fast activity group after treatment with NAC for one year (Table 5 and Figure 1). When the patients in the slow activity group were classified as having either mild to moderate COPD (Global Initiative for Chronic Obstructive Lung Disease [GOLD] stage 1 and 2) or severe to very severe COPD (GOLD 3 and 4) based on disease severity, we noted that the changes in $FEV_1$ (1.55±0.30 L versus 1.65±0.35 L, $P$=0.030) and $FEV_1$% predicted (68.4±12.4 versus 73.1±13.2, $P$=0.01) were statistically significant in the mild to moderate subgroup (Figure 2). There were also improvements in $FEV_1$ (1.06±0.16 L versus 1.12±0.20 L, $P$=0.075) and $FEV_1$% predicted (42.4±7.2 versus 45.2±8.5, $P$=0.064) in the severe to extremely severe subgroup, but these did not reach statistical significance.

Exacerbation rates in both the slow activity and fast activity groups were significantly lower than at baseline

**Table 4** Baseline characteristics of patients with COPD in different EPHX1 enzyme activity groups

| Characteristics | Slow activity group (n=157) | Fast activity group (n=62) | *P*-value |
|---|---|---|---|
| Males/females | 138/19 | 56/6 | 0.10 |
| Age, years | 70.2±7.1 | 69.1±6.2 | 0.31 |
| Smoking, pack-years | 28.1±7.2 | 27.2±6.6 | 0.38 |
| Smoking status | | | |
| Ex-smoker | 105 | 45 | 0.12 |
| Current smoker | 52 | 17 | |
| GOLD stage | | | 0.14 |
| 1 | 31 (19.7%) | 10 (16.1%) | |
| 2 | 70 (44.6%) | 29 (46.8%) | |
| 3 | 49 (31.2%) | 21 (33.9%) | |
| 4 | 7 (4.5%) | 2 (3.2%) | |
| Pharmacological treatments | | | |
| SABA | 86 (54.8%) | 30 (48.4%) | 0.13 |
| LABA | 9 (5.7%) | 4 (6.5%) | 0.08 |
| ICS | 7 (4.5%) | 3 (4.8%) | 0.11 |
| SAMA | 18 (11.5%) | 8 (12.9%) | 0.21 |
| LAMA | 32 (20.4%) | 11 (17.7%) | 0.09 |
| LABA + ICS | 98 (62.4%) | 40 (64.5%) | 0.17 |
| Theophylline | 115 (73.2%) | 46 (74.2%) | 0.10 |
| Post-BD $FEV_1$, L | 1.37±0.35 | 1.35±0.37 | 0.72 |
| Post-BD $FEV_1$ predicted, % | 59.1±16.5 | 58.8±16.7 | 0.89 |
| Post-BD $FEV_1$/FVC, % | 53.5±10.1 | 52.3±12.7 | 0.46 |
| BMI, kg/m$^2$ | 23.0±2.9 | 22.9±2.8 | 0.85 |
| 6MWD, min | 292.1±49.9 | 291.5±53.1 | 0.94 |
| Yearly exacerbation rate | 1.80±0.78 | 1.76±0.76 | 0.70 |
| SGRQ score | | | |
| Total | 36.9±12.1 | 38.0±13.1 | 0.54 |
| Symptom | 40.9±12.3 | 41.7±13.5 | 0.67 |
| Activity | 47.2±12.1 | 48.7±13.3 | 0.43 |
| Impact | 26.3±10.9 | 28.1±11.7 | 0.29 |

**Note:** Data are presented as n (%) or mean ± standard deviation, unless otherwise stated.
**Abbreviations:** COPD, chronic obstructive pulmonary disease; EPHX1, microsomal epoxide hydrolase; $FEV_1$, forced expiratory volume in one second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; SABA, short-acting beta$_2$ agonist; LABA, long-acting beta$_2$ agonist; ICS, inhaled corticosteroid; SAMA, short-acting muscarinic antagonist; LAMA, long-acting muscarinic antagonist; BD, bronchodilator; BMI, body mass index; 6MWD, 6-minute walking distance; SGRQ, St George's Respiratory Questionnaire; min, minutes.

**Table 5** Characteristics of subjects with COPD in different EPHX1 enzyme activity groups before and after treatment with NAC for 12 months

| | Baseline | After NAC |
|---|---|---|
| Post-BD $FEV_1$ predicted, % | | |
| Slow activity group (n=157) | 59.1±16.5 | 63.2±17.8# |
| Fast activity group (n=62) | 58.8±16.7 | 61.9±18.4 |
| Post-BD $FEV_1$, L | | |
| Slow activity group (n=157) | 1.37±0.35 | 1.46±0.39# |
| Fast activity group (n=62) | 1.35±0.37 | 1.38±0.41 |
| BMI, $kg/m^2$ | | |
| Slow activity group (n=157) | 23.0±2.9 | 23.1±2.7 |
| Fast activity group (n=62) | 22.9±2.8 | 22.8±2.7 |
| 6MWD, min | | |
| Slow activity group (n=157) | 292.1±49.9 | 298.8±57.1 |
| Fast activity group (n=62) | 291.5±53.1 | 297.4±54.6 |
| Yearly exacerbation rate | | |
| Slow activity group (n=157) | 1.80±0.78 | 0.88±0.59§ |
| Fast activity group (n=62) | 1.76±0.76 | 1.21±0.60§ |
| SGRQ score | | |
| Total | | |
| Slow activity group (n=157) | 36.9±12.1 | 35.1±12.7 |
| Fast activity group (n=62) | 38.0±13.1 | 35.8±14.8 |
| Symptom | | |
| Slow activity group (n=157) | 40.9±12.3 | 37.8±13.1# |
| Fast activity group (n=62) | 41.7±13.5 | 38.7±15.4 |
| Activity | | |
| Slow activity group (n=157) | 47.2±12.1 | 45.8±12.8 |
| Fast activity group (n=62) | 48.7±13.3 | 49.1±14.0 |
| Impact | | |
| Slow activity group (n=157) | 26.3±10.9 | 24.9±11.5 |
| Fast activity group (n=62) | 28.1±11.7 | 26.5±12.1 |

**Notes:** Data are presented as the mean ± standard, unless otherwise stated. #Comparison of slow activity group after NAC treatment versus baseline, $P<0.05$. §Comparison of yearly exacerbation rate after NAC versus baseline, $P<0.001$.
**Abbreviations:** COPD, chronic obstructive pulmonary disease; EPHX1, microsomal epoxide hydrolase; NAC, N-acetylcysteine; BD, bronchodilator; $FEV_1$, forced expiratory volume in one second; BMI, body mass index; 6MWD, 6-minute walking distance; SGRQ, St George's Respiratory Questionnaire; min, minutes.

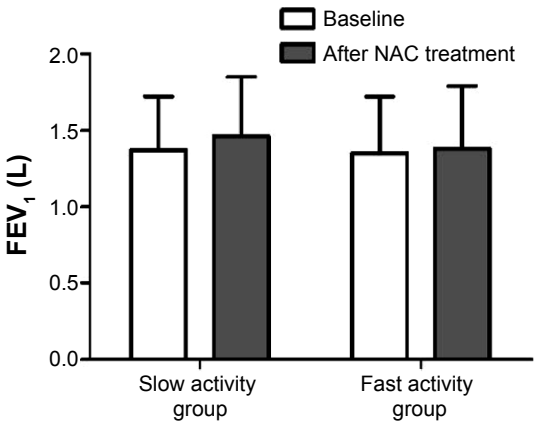


**Figure 1** $FEV_1$ versus NAC treatment in the two groups.
**Abbreviations:** $FEV_1$, forced expiratory volume in one second; NAC, N-acetylcysteine.

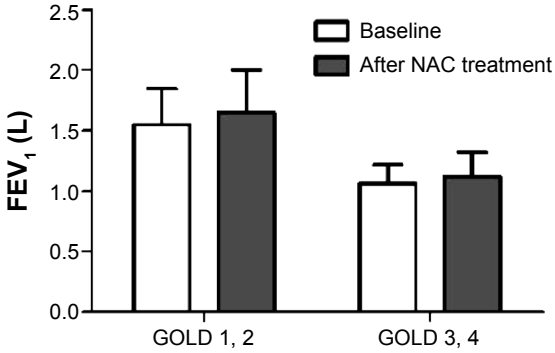


**Figure 2** $FEV_1$ versus NAC treatment in the two subgroups of the slow activity group.
**Abbreviations:** $FEV_1$, forced expiratory volume in one second; GOLD, Global Initiative for Chronic Obstructive Lung Disease stage; NAC, N-acetylcysteine.

after treatment with NAC for one year. Moreover, the mean exacerbation rate was lower in the slow activity group than in the fast activity group (0.88±0.59 versus 1.21±0.60, respectively, $P<0.001$).

There was a significant difference in SGRQ symptom score in the slow activity group after one year of treatment with NAC when compared with baseline (40.9±12.3 versus 37.8±13.1, $P=0.033$), but there was no significant difference in the fast activity group. Further, the change in SGRQ symptom score after treatment with NAC was statistically significant compared with baseline in the mild to moderate subgroup (34.3±8.9 versus 31.2±9.8, $P=0.018$), but there was no statistically significant change after treatment with NAC in the severe to extremely severe subgroup (52.7±7.8 versus 49.8±9.2, $P=0.071$). There were no statistically significant differences in SGRQ total score or any other domains in the two groups between baseline and after treatment with NAC. Changes from baseline in body mass index and 6MWD did not differ in the two groups after one year of treatment with NAC.

Except for mild gastrointestinal complaints, no major adverse events occurred in each of the groups. Three patients died during the trial, one in the slow activity group (pneumonia) and two in the fast activity group (acute exacerbation of COPD and acute heart failure). None of these deaths were thought to be related to NAC.

## Discussion

Our study demonstrated significant improvements in $FEV_1$, $FEV_1$% predicted, and SGRQ symptom score in COPD patients with extremely slow/slow activity of EPHX1 who were treated with NAC 600 mg twice daily for one year. Unlike in previous studies, this study of NAC treatment was carried out in COPD patients with different EPHX1 phenotypes for enzyme activity, and that may be one of the

reasons why findings such as ours have not been reported previously. In an earlier study of 256 COPD patients and in this study, we found that the extremely slow/slow activity EPHX1 phenotype exhibited decreased enzyme activity and thus had an increased risk of COPD.[6] Other studies have also revealed a significant association between the extremely slow/slow activity EPHX1 phenotype and an increased risk of COPD.[18,20–23] The EPHX1 enzyme detoxifies the harmful epoxides produced by smoking and hydrolyzes various exogenous pro-oxidants to form water and soluble dihydrodiol compounds, so has a protective effect on the lung.[24] The extremely slow/slow activity phenotype has a decrease in EPHX1 enzyme activity of more than 50%.[25] This decreased EPHX1 enzyme activity may result in an oxidant/antioxidant imbalance within the lung. The body's antioxidant capacity may be enhanced by antioxidant drugs such as NAC in COPD patients with decreased EPHX1 enzyme activity. This may be the reason why NAC was more effective in the group with the extremely slow/slow EPHX1 activity phenotype than in the group with fast/normal EPHX1 activity phenotype.

Numerous studies have been performed over the last two decades to assess whether NAC has potential therapeutic effects in patients with COPD. No previous studies have shown a significant increase in $FEV_1$ with either high-dose or low-dose NAC, although some studies reported improvements in some parameters of lung function after treatment with NAC. For example, Stav and Raz found that inspiratory capacity and FVC were higher, in particular, post exercise, after treatment with NAC 600 mg twice daily compared with placebo,[15] and Tse et al found that high-dose NAC resulted in significantly improved small airway function.[13] Some of the interindividual differences in effect associated with NAC treatment might be explained by genetic polymorphism of the antioxidase.

Another important finding of this study is that the changes in $FEV_1$, $FEV_1$% predicted, and SGRQ symptom score were statistically significant in the mild to moderate subgroup after treatment with NAC when stratified by disease severity. Although there was also a small improvement in the severe to very severe subgroup, the change was not statistically significant. Our analysis of the treatment effects in COPD stratified by severity showed a greater improvement in $FEV_1$ in less advanced COPD. This suggests that NAC might have a more crucial preventive effect in patients in the early stages of COPD. The effect of early intervention with NAC in COPD patients might be better with regard to controlling symptoms, slowing disease progression, and improving the quality of life and prognosis.

There was also a significant reduction in exacerbation rate in the slow activity group and in the fast activity group after treatment with NAC, but there the exacerbation rate was lower in the slow activity group than in the fast activity group (0.88±0.59 exacerbations per patient-year versus 1.21±0.60 exacerbations per patient-year, respectively, $P<0.001$). There is increasing evidence that use of NAC can decrease the risk of exacerbation in patients with COPD,[26–30] and our findings is also in agreement with recent studies.[13,14]

Except for SGRQ symptom score, there were no significant differences in BMI, 6MWD, or SGRQ total score or other domains in the two groups between baseline and after treatment with NAC. The reason for this might be two-fold. Firstly, COPD is a complex polygenic disease, and previous studies have shown that the hereditary susceptibility to COPD may depend on the coincidence of several gene polymorphisms acting together,[31,32] but our study only observed the effect of NAC treatment in COPD patients stratified by EPHX1. Will the effect of NAC treatment be better in COPD patients with deficiencies in several antioxidant enzyme genes? Secondly, some studies have shown that it takes more than 6 months for the antioxidant drug to go into effect,[14,33] and suggested that the therapeutic effect of the antioxidant drug was slow but progressive, so that longer treatment times might be required to improve BMI and exercise capacity in patients with COPD.

In conclusion, we have found preliminary evidence that $FEV_1$, SGRQ symptom score, and risk of exacerbation can be improved by NAC in COPD patients with extremely slow/slow EPHX1 enzyme activity, which means that polymorphism in the EPHX1 gene may have a significant role in the different responses to NAC seen in patients with COPD.

Our findings have crucial clinical implications with regard to our understanding of the hereditary factors determining responsiveness to antioxidant treatment with NAC in patients with COPD. More and larger prospective studies in groups of COPD patients with different ethnic origins and various antioxidant enzyme gene polymorphisms will help to define which patients may benefit more from treatment with NAC.

## Acknowledgments

We are grateful to Shen Ling from the School of Public Health, Kunming Medical University, for help with the statistical analysis and to Gan Ping, Kunming Institute of Zoology, for providing technical assistance. This study was supported by a grant from the National Natural Science Foundation of China (81160006).

## Disclosure

The authors report no conflicts of interest in this work.

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