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Association between the *aldehyde dehydrogenase 2*2* allele and smoking-related chronic airway obstruction in a Japanese general population: A pilot study

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HIGHLIGHTS

- Aldehyde dehydrogenase 2 (ALDH2) may protect airways against cigarette smoke injury.
- *ALDH2*2* allele and ever smoking affected airway obstruction in a dose-dependent manner.
- Pack-years smoking was a predictor of airway obstruction only in **2* allele carriers.
- **2* allele is associated with the incidence of smoking-related airway obstruction.
- Combined effect of smoking and **2* allele was prominent in the asthmatic subjects.

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ABSTRACT

Aldehyde dehydrogenase 2 (ALDH2) detoxifies exogenous and endogenous toxic aldehydes; however, its protective effect against cigarette smoke in airways is unknown. We therefore examined whether the inactive *ALDH2*2* allele is associated with smoking-related chronic airway obstruction. We conducted a cross-sectional study including 684 Japanese participants in a health screening program, and a retrospective longitudinal study in the elderly subgroup. The risks of airway obstruction in the ever-smokers with the *ALDH2*1/*2* and **2/*2* genotypes were two and three times higher, respectively, than in the never-smokers with the *ALDH2*1/*1* genotype. Moreover, the combined effect of smoking and the *ALDH2*2* allele was prominent in the asthmatic subjects. In a longitudinal association analysis, the combination of the *ALDH2* genotype and pack-years of smoking synergistically increased the risk of airway obstruction. The number of pack-years of smoking at baseline was identified to be a significant predictor of airway obstruction only in the *ALDH2*2* allele carriers. In addition, the *ALDH2*2* allele was also associated with the incidence of smoking-related airway obstruction, in the Cox proportional hazards model. This pilot study demonstrated for the first time a significant gene–environment interaction between the *ALDH2*2* allele and cumulative exposure to cigarette smoke on the risk of airway obstruction.

1. Introduction

A recent proteomic analysis demonstrated that aldehyde dehydrogenase 2 (ALDH2) is prominently elevated in the bronchoalveolar lavage fluid (BALF) obtained from chronic obstructive pulmonary disease (COPD) patients (Tu et al., 2014). ALDH2 is the primary enzyme that detoxifies exogenous and endogenous toxic aldehydes in the mitochondrial matrix of a range of cell types and prevents free radical-mediated oxidative stress

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(Chen et al., 2014; O'Brien et al., 2005; Tu et al., 2014). The *ALDH2*1* and *ALDH2*2* alleles of rs671 encode the active and inactive subunits of ALDH2, respectively, the latter of which determines an individual's tolerance for alcohol consumption (Chen et al., 2014; O'Brien et al., 2005). Furthermore, ALDH2 dysfunction may contribute to a variety of human diseases, *e.g.*, cardiovascular disease, diabetes, stroke and cancer, as well as aging (Chen et al., 2014; Guo et al., 2013; Morita et al., 2013, 2014a,b; Nakagawa et al., 2013; O'Brien et al., 2005).

A mainstream smoke analysis of cigarette smoke (CS) for aldehydes showed that acetaldehyde constitutes the major component, followed by acrolein, formaldehyde and crotonaldehyde (O'Brien et al., 2005). These aldehydes play an important role in the production of proinflammatory cytokines and recruitment of inflammatory cells into the airways (van der Toorn et al., 2013). Among these components, acrolein is found at level approximately 12-fold higher in sidestream smoke than in mainstream smoke and is a respiratory irritant (O'Brien et al., 2005), a powerful mediator of CS-induced macrophage activation and a key factor in the induction of pulmonary inflammation associated with CS (Facchinetti et al., 2007; O'Brien et al., 2005; Sun et al., 2014). On the other hand, aldehydes are generated endogenously, *e.g.*, acetaldehyde is the major metabolite of ethanol and 4-hydroxy-2-nonenal (4-HNE) is a byproduct of oxidative stress (Chen et al., 2014; O'Brien et al., 2005). 4-HNE diffuses within or without the cell and forms direct protein adducts resulting from its high affinity toward cysteine, histidine and lysine groups and thus mediates oxidant-induced cell signaling and apoptosis (Chen et al., 2014; O'Brien et al., 2005). The 4-HNE-modified protein levels in airways are elevated in subjects with COPD compared with those observed in subjects without COPD and are negatively associated with the forced expiratory volume in 1 s (FEV1) and positively correlated with the transforming growth factor-β1 and γ-glutamylcysteine synthetase levels in the airways in subjects with COPD (Rahman et al., 2002). Although acetaldehyde, acrolein and 4-HNE are all good substrates of ALDH2 (Chen et al., 2014; O'Brien et al., 2005; Tu et al., 2014; Yoval-Sanchez and Rodriguez-Zavala, 2012), the association between the inactive *ALDH2*2* allele and the risk of lung diseases has been found only in alcohol-induced asthma (Matsuse et al., 2001, 2007) and smoking-related lung cancer (Park et al., 2010), and this relationship has not yet been confirmed in COPD.

We herein report, for the first time, the combined effects of the smoking status and the *ALDH2* genotype on the risk for developing chronic airway obstruction with or without asthma in a Japanese general population.

2. Methods

We conducted a cross-sectional study including 684 (438 males and 246 females) participants in a health screening program and a retrospective longitudinal affiliated to the guideline study (observation period 5.4 ± 1.0 years) in the elderly subgroup from this study (60 years old or over, 197 males and 133 females). Participants with chronic lung diseases other than COPD and asthma were excluded. This study followed the principles of the Declaration of Helsinki. The protocol was approved by the ethics committee of the Faculty of Life Sciences, Kumamoto University (approval no. 169) according to the Ethical Guidelines for Human Genome/Gene Analysis Research of Ministries of Japan. All of the subjects provided their written informed consent to participate in the study.

Airway obstruction was defined as a FEV1/forced vital capacity (FVC) ratio (FEV1%) <70% without the use of a bronchodilator. Asthma was diagnosed by history. Information regarding smoking habits and alcohol intake was obtained *via* face-to-face interviews conducted by health care providers.

Genomic DNA was prepared from whole blood using a DNA purification kit (Flexi Gene DNA kit, QIAGEN, Hilden, Germany). The *ALDH2*1/*2* alleles were determined using a real-time TaqMan allelic discrimination assay (Step One Plus Real-Time PCR system version 2.1; Applied Biosystems, Tokyo, Japan) according to the protocols provided by the manufacturer (assay no. C_11703892_10).

The data are presented as the mean ± standard deviation or proportion for categorical variables. Student's *t*-test or a one-way analysis of variance and Fisher's exact test were used for comparisons of continuous and categorical variables, respectively. The associations between the *ALDH2* genotypes and the prevalence of airway obstruction were examined using a logistic regression analysis with calculations of the odds ratios (ORs) and 95% confidence intervals (95% CIs). The longitudinal associations of the *ALDH2* genotype with the risk of an airway obstruction were analyzed using the generalized estimating equations approach. The interactive effects of the *ALDH2* genotype and smoking status, drinking habits and/or asthma on the risk of airway obstruction were also analyzed. Receiver operating characteristic (ROC) curves were determined to evaluate the predictive performance of pack-years smoking for detecting an airway obstruction with calculations of the area under the curve (AUC). We determined the cut-off value for pack-years of smoking as the point with the shortest distance from the left upper corner of the graph. The airway obstruction-free survival was estimated using the Kaplan–Meier survival curves. Multivariate-adjusted hazard ratios (HRs) for the cumulative incidence of airway obstruction were examined using a Cox proportional hazards model. In all multiple regression models, age, smoking status and asthma were used as covariates. A p value of <0.05 was considered to be statistically significant. Multiple comparisons were corrected using Bonferroni's method, and p values $<0.05/n$ were considered to be statistically significant after correcting for the number of comparisons made. All statistical analyses were performed using the SPSS software package (version 17.0, IBM Japan Inc., Tokyo, Japan).

3. Results

The frequency of the *ALDH2*2* allele was 24.4%. The observed genotype frequency was consistent with the Hardy–Weinberg equilibrium. The clinical characteristics of the subjects at the end point of follow-up are shown in Table 1. The clinical features did not differ among the *ALDH2* genotype, except for the frequency of drinkers (Table 1). Using Bonferroni's multiple comparison, the prevalence of self-reported respiratory symptoms was higher in the **2/*2* genotype than the **1/*1* genotype (25.5% *vs*. 13.0%, $p = 0.031$).

Ninety-seven (14.2%) subjects were diagnosed with airway obstruction at the end point of follow-up (Table 1). In a logistic regression model, the *ALDH2* genotype alone did not affect the risk of airway obstruction, while age, ever-smoking and asthma were independent risk factor for airway obstruction (Table 2). The risk of airway obstruction was not influenced by drinking habit (Table 2). No interactive effect between the *ALDH2* genotype and smoking status on the risk of airway obstruction was observed. However, the *ALDH2* genotype and smoking status additively affected the risk of airway obstruction (Table 3). The risk of airway obstruction was significantly higher in the ever-smokers with the **1/*2* and **2/*2* genotypes than in the never-smokers with the **1/*1* genotype (Table 3). The FEV1% value was also significantly lower in the ever-smokers with the **2/*2* genotype than in the never-smokers with the **1/*1* genotype (Table 3). The risk of airway obstruction was significantly higher in the asthmatic subjects, irrespective of the *ALDH2* genotype (Table 4). However, the risk in the asthmatic subjects with the **2* allele was further increased among ever-