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The *ALDH2* Glu504Lys polymorphism is associated with coronary artery disease in Han Chinese: Relation with endothelial ADMA levels

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ABSTRACT

Objectives: We studied the association between mitochondrial aldehyde dehydrogenase (ALDH2) Glu504Lys (rs671 or *ALDH2*2*) polymorphism and coronary artery disease (CAD), and sought to clarify the mechanisms underlying this association.
Methods: The *ALDH2* rs671 polymorphism was genotyped in 417 CAD patients and 448 age- and gender-matched controls. All participants were Han Chinese. Human umbilical vein endothelial cells (HUVECs) isolated from 11 human umbilical cords were genotyped, cultured, and exposed to angiotensin II (Ang II, 10^{-7}–10^{-5} mol/L). Dimethylarginine dimethylaminohydrolase 1 (*DDAH1*) mRNA expression levels were determined by real-time PCR. Levels of asymmetric dimethylarginine (ADMA) in culture media and cell lysates were determined by high performance liquid chromatography-mass spectrometry (HPLC–MS).
Results: The frequency of carriers of the *ALDH2* rs671 A allele (GA + AA) was significantly higher in patients with CAD (47.5%) than in controls (35.0%, $p = 0.0002$). After adjustment for potential confounders, the odds ratio (OR) for CAD for carriers of the rs671 A allele was 1.85 (95% confidence interval [CI]: 1.38–2.49, $p = 0.00005$) in the entire study cohort, and 1.95 (95% CI: 1.40–2.70, $p = 0.00007$) in non-drinkers. In non-drinking controls, the homozygous rs671 AA genotype was associated with significantly lower high-density lipoprotein cholesterol (HDL-C) concentrations compared with rs671 GG homozygotes ($p = 0.015$). HUVEC cells homozygous for the G allele of rs671 showed a significantly higher *DDAH1* mRNA expression and lower intracellular ADMA levels compared with heterozygous GA cells ($p < 0.05$, respectively). In homozygous GG cells, high concentrations of Ang II (10^{-5} mol/L) decreased *DDAH1* mRNA expression and increased intracellular ADMA concentrations.
Conclusions: The rs671 polymorphism of *ALDH2* is associated with CAD in Han Chinese, possibly by influencing HDL-C levels and endothelial ADMA levels.

1. Introduction

Coronary artery disease (CAD) is a major cause of morbidity and mortality in humans worldwide. The mortality from ischemic heart disease has increased steadily in most industrialized countries, and a better understanding of the pathophysiology of coronary atherosclerosis is paramount to develop strategies of early intervention.

Atherosclerosis is the consequence of complex interactions between genetic and environmental factors. Oxidative stress and reactive oxygen species (ROS) are deemed to play an important role in the pathogenesis of CAD [1]. Of note, oxidative stress may be a common link between CAD and traditional vascular risk factors, including cigarette smoking, heavy alcohol drinking, diabetes, hypercholesterolemia, and hypertension [2,3]. In addition, ROS may promote inflammation [4], low-density lipoprotein cholesterol oxidation, and endothelial dysfunction [5]. Interestingly, ROS can react with cellular lipids to generate lipid peroxides, including the major toxic aldehyde 4-hydroxy-2-nonenal (4-HNE). Evidence has also suggested that 4-HNE is involved in atherosclerotic vascular disease through the induction of endothelial cell barrier dysfunction [6,7].

Endothelial dysfunction, characterized by decreased bioavailability of nitric oxide (NO), promotes the initiation and development of atherosclerotic lesions. Increased levels of asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of NO synthase, have recently emerged as an independent

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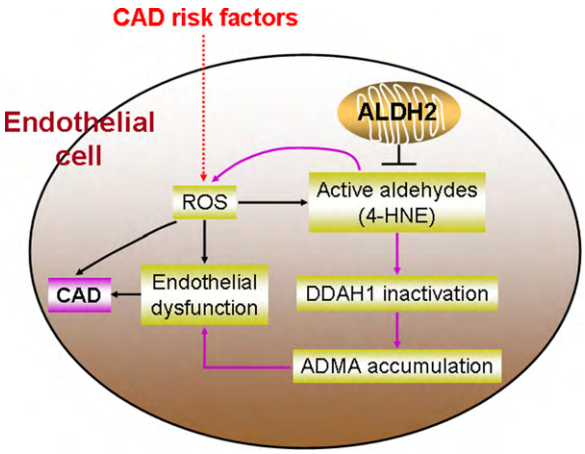

Fig. 1. Potential relationship between mitochondrial dehydrogenase 2 and active aldehydes. The figure shows the potential role of this pathway in atherosclerosis through its influence on endothelial DDAH1 and ADMA. ROS, reactive oxygen species; 4-HNE, 4-hydroxy-2-nonenal; DDAH1, dimethylarginine dimethylaminohydrolase 1; ADMA, asymmetric dimethylarginine; AS, atherosclerosis.

cardiovascular risk factor [8]. The major route of elimination of ADMA is metabolism by the enzymes dimethylarginine dimethylaminohydrolase-1 and -2 (DDAH1 and DDAH2). DDAH1 is strongly expressed in coronary endothelium [9], and – differently from DDAH2 – can modulate endothelial NO bioavailability through ADMA-dependent mechanisms [10]. It has been shown that 4-HNE can form Michael adducts with DDAH1 in cultured vascular endothelial cells, as well as decrease DDAH1 activity, increase ADMA formation, and decrease NO generation in a dose-dependent fashion. In addition, DDAH1 overexpression can completely restore endothelial NO production following 4-HNE exposure [11–13]. Of note, plasma concentrations of 4-HNE have been shown to be positively correlated with circulating levels of ADMA in patients with major depression [13].

Mitochondrial aldehyde dehydrogenase (ALDH2) is an enzyme responsible for the detoxification of aldehydes generated by alcohol drinking and lipid peroxidation, including 4-HNE [14]. Interestingly, ALDH2-deficient mice are more susceptible to oxidative stress damage compared with wild-type animals [15]. Moreover, PC12 cells overexpressing *ALDH2*-deficient transfectants are more vulnerable to 4-HNE and oxidative stress [16]. In contrast, induction of ALDH2 activity can decrease the formation of 4-HNE adducts and exerts cardioprotective effects [17]. Since ALDH2 is important for protection against oxidative stress, we speculated that a decrease in ALDH2 activity may contribute to the development of CAD through an effect on 4-HNE detoxification and ADMA accumulation in endothelial cells (Fig. 1).

A genetic variant which decreases the activity of ALDH2, the *ALDH2* rs671 polymorphism (*Glu504Lys* in exon 12, *ALDH2*2*), is transmitted in an autosomal dominant fashion and is common in the Asian population [14,18]. It has been reported that serum concentrations of lipid peroxides are significantly higher in carriers of the rs671 A allele (*ALDH2* Lys504 or *ALDH2*2*) in Japanese women, even after correction for alcohol drinking [19]. In addition, a study among alcohol abstainers from Japan has shown that carriers of the rare rs671 A allele have lower serum levels of high-density lipoprotein cholesterol (HDL-C) [20]. Interestingly, carriers of the AA homozygous genotype are at increased risk of myocardial infarction, possibly due to the effect of this genotype on serum HDL-C levels [21]. It has also been demonstrated that carriers of the rs671 A allele are at an increased risk of myocardial infarction and diabetes mellitus in Chinese patients with CAD [22]. To our knowledge, however, no previous study has assessed the association of this polymorphism with CAD.

The purpose of the present study is twofold. First, we sought to investigate whether the *ALDH2* rs671 polymorphism is associated with CAD in Han Chinese. Second, we tested the hypothesis that the effect of this polymorphism on susceptibility to CAD can be mediated by its effects on the DDAH/ADMA system.

2. Subjects and methods

2.1. Study subjects

Between November 2007 and September 2009, a total of 417 patients with angiographically proven CAD were recruited from the outpatient clinics of the Xiangya Hospital, Hunan province, China. CAD was defined as luminal stenosis ≥50% in at least one major coronary artery branch. The control group consisted of 448 subjects were also recruited from individuals attending a routine health screening in the same Hospital. To made the cases and controls matched better in age, gender, and CAD risk factors, the recruitment of the controls was started when general information about the demographic characters and distribution of CAD risk factors in the initially collected cases (approximately 200) were obtained. The controls were apparently healthy, and all had a negative history of hypertension, diabetes mellitus, ischemic heart disease, and chronic heart failure. Information regarding age, gender, duration of CAD, cigarette smoking, and alcohol drinking was collected by questionnaire. Venous blood samples were drawn from all participants. All subjects underwent routine blood and biochemistry tests, including serum total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), creatinine, and fasting plasma glucose. All participants were Han Chinese from Changsha or the surrounding counties. All subjects signed an informed consent to the study, which was approved by the Ethics Committee of the School of Pharmaceutical Sciences, Central South University, Changsha.

2.2. Isolation and culture of human umbilical vein endothelial cells (HUVECs)

Cords taken at vaginal deliveries were used for this study. Signed informed consent was obtained from each mother. All women were healthy non-smokers. The cords were cut from the placenta soon after delivery and transported to the lab in cold PBS within 2 h. Endothelial cells were isolated from umbilical veins using 0.25% trypsin at 37 °C. HUVECs were cultured in EGM-2 medium containing 20% fetal calf serum in a humidified atmosphere of 5% CO_2 in air. Fourth-passage cells were exposed to a concentration gradient of angiotensin II (Ang II, from 10^{-7} mmol/L to 10^{-5} mmol/L) for 24 h. The medium was then collected to determine the ADMA concentration. Cells were then harvested to extract RNA. For determination of ADMA levels in HUVECs, cells were harvested, washed with PBS, and counted with a hemocytometer. Lysis was then promoted by treating cells with 50 μL of cell lysis buffer. Cell lysates were stored at −20 °C until analysis.

2.3. ALDH2 genotyping

Genomic DNA was extracted from peripheral blood leukocytes and umbilical cord tissues by the standard phenol/chloroform protocol. Genotyping of the *ALDH2* rs671 polymorphism was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [14]. Primers used for PCR amplification were as follows: 5′-CCTGGGCAACAGAGAAAGAT-3′ (forward), and 5′-AAACACTGATGGCCTCAAGC-3′ (reverse). PCR products (5 μL) were digested with 5 U *Eco57* I overnight at 37 °C. The digested