JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Prospective study: Aldehyde dehydrogenase 2 gene is associated with cardio-cerebrovascular complications in type 2 diabetes patients

Qingfang He, Jin Pan, Lixin Wang, Yujia Fang, Ruying Hu
Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China

Keywords
Aldehyde dehydrogenase 2, Cardio-cerebrovascular complications, Type 2 diabetes mellitus

***Correspondence**
Ruying Hu
Tel.: +86-571-8711-5160
Fax: +86-571-8711-5160
E-mail address:
ruyinghu01@163.com

J Diabetes Investig 2021; 12: 1845–1854

doi: 10.1111/jdi.13538

ABSTRACT

Aims/Introduction: Most studies have shown that aldehyde dehydrogenase 2 (*ALDH2*) mutations were associated with cardio-cerebrovascular complications of diabetes based on cross-sectional investigations, but few studies based on cohorts were carried out. The aim of this study was to explore the correlation between the *ALDH2* gene and the occurrence of and death from cardio-cerebrovascular complications in type 2 diabetes patients through a prospective cohort study.

Materials and Methods: According to a community-based and disease-based prospective cohort study design, a baseline cohort of 10,339 persons with type 2 diabetes was established in 2016, and the occurrence of and death from cardio-cerebrovascular complications were followed up until December 2018. A total of 2,500 diabetes patients without cardio-cerebrovascular complications were randomly selected from the baseline cohort to detect the rs671 polymorphism of the *ALDH2* gene. Cox regression analysis was carried out on the effect of different *ALDH2* rs671 single-nucleotide polymorphisms on the risk and survival time of cardio-cerebrovascular complications among diabetes patients.

Results: There were 215 cardio-cerebrovascular complications, including 10 deaths, that occurred in the 2,500 diabetes patients during the follow-up period. Cox regression analysis showed that rs671 GA/AA genotype, sex (male), poor control of high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, blood pressure and glycated were risk factors, whereas drinking alcohol was a protective factor for cardio-cerebrovascular complications ($P < 0.05$). After adjusting for age and sex, the risk of the rs671 GA/AA genotype was 1.314-fold (95% confidence interval 1.000–1.727) that of the GG genotype.

Conclusion: The G-A mutation of *ALDH2* rs671 is a risk factor for the occurrence of and death from cardio-cerebrovascular complications in type 2 diabetes patients. Further studies with larger cohorts and longer follow up will be necessary to reveal a consensus.

INTRODUCTION

The harm of diabetes mainly lies in its complications, such as cardio-cerebrovascular disease, kidney disease, eye disease and so on, which seriously threaten people's health, and are the main cause of death and disability in persons with diabetes[1,2]. In 1998, 249 Swedish diabetes patients were followed by Adlerberth *et al.*[2] for 16 years, and they found that cardiovascular causes accounted for 62.6% of diabetes deaths.

The occurrence of cardio-cerebrovascular complications in persons with diabetes is closely linked to glycemic control, diabetes duration and age; however, it has been clinically observed that complications occurred in some persons with short diabetes duration and good glycemic control, but not in persons with poor glycemic control and long diabetes duration. Therefore, in addition to environmental factors, genetic factors might play an important role in the occurrence and development of type 2 diabetes.

The significant aldehyde dehydrogenase 2 (*ALDH2*) rs671 single-nucleotide polymorphism (SNP; Glu504Lys) in exon 12, which causes a replacement of glutamate to lysine at position 504, results in the decreased catalytic ability of the enzyme encoded by this gene[3]. As a key enzyme in the ethanol

Received 18 October 2020; revised 2 February 2021; accepted 28 February 2021

metabolism pathway and an important anti-oxidant stress molecule, Glu504Lys polymorphism of *ALDH2* is significantly associated with the occurrence and development of cardio-cerebrovascular diseases[3-5]. It has been reported that *ALDH2* is an independent risk factor for ethanol-independent cardiovascular disease, and can inhibit the apoptosis of cardiomyocytes caused by acetaldehyde or hypoxia by reducing reactive oxygen species or detoxifying the 4-hydroxynonenal pathway, thus playing a protective role in cardiac dysfunction and cardiomyopathy[6].

ALDH2 is an enzyme that detoxifies reactive aldehydes, such as methylglyoxal and 4-hydroxynonenal. These aldehydes usually come from lipids and glucose. It was reported that methylglyoxal and 4-hydroxynonenal caused protein carbonylation and mitochondrial dysfunction, forming advanced glycation end-products. With long-term hyperglycemia, the blood lipid and carbohydrate in persons with diabetes would easily be peroxidized, which leads to the production of large numbers of toxic aldehyde substances, thus causing multiple organ damage[7,8]. The ALDH2 detoxification of aldehyde might be effective against cardiovascular disease in diabetes and promote cardiovascular function, which would be of benefit to prevent cardio-cerebrovascular complications and treatment among diabetes patients, and thus improve the survival rate of persons with diabetes. In addition, the protective role of ALDH2 in cell signal transduction can reverse the abnormalities caused by hyperglycemia, thus protecting the heart function of patients with diabetes[9]. However, the A allele mutation of *ALDH2* leads to the decrease of the inhibitory effect of acetaldehyde by its detoxification 4-hydroxynonenal pathway, and affect the myocardial protective function. Studies reported that the GA/AA genotype of *ALDH2* is relevant to the thickening of carotid artery intima-media thickness (various media thickness) among elderly diabetes patients, which might increase the risk of complicated cardio-cerebrovascular diseases[10]. People with type 2 diabetes carrying the *ALDH2* A mutation showed a higher macrovascular complication prevalence irrespective of alcohol consumption[11].

At present, studies on the relationship between the *ALDH2* gene and cardio-cerebrovascular complications in diabetes patients are mostly based on cross-sectional investigations, whereas few are based on cohorts. The present study was a community-based and disease-based prospective cohort study, carried out to evaluate the effect of *ALDH2* gene polymorphism (rs671) on the risk of cardio-cerebrovascular complications and survival time among diabetes patients, by using Cox proportional hazards model, and providing more reliable data and assessing the risk of cardio-cerebrovascular complications of diabetes.

MATERIALS AND METHODS

Participants

In 2016, a cohort and baseline database of 10,339 type 2 diabetes patients in Jiashan County, Suichang County and Yongkang City in Zhejiang Province, China, were established by Zhejiang Provincial Center for Disease Prevention and Control (Zhejiang CDC). All of the 10,339 type 2 diabetes patients met the 1999 World Health Organization diabetes diagnostic criteria, and were resident adult type 2 diabetes patients registered and receiving standardized management. Exclusion criteria included: (i) those who are bedridden or seriously ill and cannot complete laboratory testing; and (ii) pregnant or lactating women. The study was approved by the ethics committee of Zhejiang CDC, and all the participants signed informed consent. At the same time, the incidence and outcome of cardio-cerebrovascular complications in this cohort were followed up by multiple sources every year from 2016.

A total of 2,500 type 2 diabetes patients in the 2016 cohort who had never experienced cardio-cerebrovascular complications in the baseline database were randomly selected, and the *ALDH2* gene polymorphism (rs671) was detected.

Sample size

According to the diabetes monitoring data in Zhejiang province, the incidence of cardio-cerebrovascular complications among diabetes patients in 3 years was estimated to be approximately 8%, and the risk of a susceptible positive mutation was 1.5-fold of the non-mutant population. According to the sample size calculation of the cohort, a 10% loss to follow-up rate was considered, so 1,211 samples of the susceptibility genes mutation-positive group and negative group, respectively, were required.

Methods

Sample collection

When recruited in the cohort in 2016, the tubes of fasting venous blood samples were extracted: 1 mL sodium fluoride anticoagulant tube, 4 mL gel serum tube and 3 mL ethylenediaminetetraacetic acid anticoagulant tube.

Biochemical detection

Biochemical indexes were measured by automatic biochemical analyzer.

Fasting blood glucose (hexokinase method) was detected by using plasma samples in the sodium fluoride anticoagulant tube.

Total cholesterol (TC, oxidase method), triglyceride (TG; enzymatic method), low-density lipoprotein cholesterol (LDL-C; direct clearance method) and high-density lipoprotein cholesterol (HDL-C; direct clearance method) were detected by using serum samples.

Detection of glycated hemoglobin

Blood samples in EDTA tubes were used to test glycated hemoglobin (HbA1c; high-performance liquid chromatography, automatic HbA1c analyzer) first and then for *ALDH2* gene polymorphism (rs671) detection.

ALDH2 gene polymorphism (rs671) detection

DNA extraction and quality control

The QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) was used to extract the blood genomic deoxyribonucleic

Table 1 | Primer sequences of the aldehyde dehydrogenase 2 gene (rs671)

Primer	Primer sequences (5′ to 3′)	The length of the amplified fragment (bp)
rs671-F	AGGGGGTCCTGGGAGTGTAA	524
rs671-R	GGGCACGTCCCTCAGTATTT	

acid (DNA). NanoDrop ND-1000 (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure DNA concentration and detect DNA quality. The A260/A280 ratio was between 1.8–2.0, showing high DNA purity. It was diluted to a working solution concentration (25 ng/μL) for further study.

Amplification

The primers for the detection of G-A single-nucleotide mutation of the *ALDH2* gene (rs671) were designed by National Center for Biotechnology Information online. The length of the amplified fragment was approximately 524 bp. The primer working concentration was 10 μmol/L. Primer sequences are shown in Table 1.

Polymerase chain reaction (PCR) reaction system was 50 μL: upstream primer 2 μL, downstream primer 2 μL, DNA 1 μL and mix 45 μL. The reaction procedure was: 98°C for 2 min; 35 cycles of 98°C for 10 s, 60°C for 10 s and 72°C for 10 s; then, 72°C for 2 min. ABI 7500 real-time fluorescence quantitative PCR (Thermo Fisher Scientific) was used.

Sanger sequencing

PCR products were detected by agarose gel electrophoresis, and the qualified products were Sanger sequenced using an ABI 3730XL sequencer (Thermo Fisher Scientific). The rs671 SNPs were analyzed by Seqman software (DNASTAR Inc. Madison, WI, USA).

Data collection and medical examination

General information, such as sex, age, smoking, drinking, disease history and hypoglycemic drug use, of type 2 diabetes patients were collected through the residents' health records, diabetes community management system and questionnaires.

Physical examination, including height, fasting weight, fasting waist circumference (WC), systolic blood pressure and diastolic blood pressure was carried out. Height and weight were measured by a height scale, which were accurate to 0.1 cm and 0.1 kg, respectively. A waist ruler, which was accurate to 0.1 cm, was used to measure WC. Blood pressure was measured with an electronic sphygmomanometer (OMRON, Shanghai, China), which was accurate to 1 mmHg, and measured two consecutive times (interval 1 min), and the mean value was taken.

body mass index (BMI) = weight (kg) / (height $[m]^2$).

Criteria defined

All the cardio-cerebrovascular complications of diabetes were clinically confirmed cases. Cardio-cerebrovascular complications include ischemic heart disease and stroke. Ischemic heart disease includes angina pectoris, myocardial infarction and coronary heart disease (CAD). Stroke includes cerebral ischemia and cerebral hemorrhage.

Table 7 "Control Targets of Type 2 diabetes in China" in "Guidelines for the prevention and treatment of type 2 diabetes mellitus in China (2017) "[12] was referenced:

1. HbA1c control criteria: HbA1c <7.0% shows good blood glucose control, and HbA1c ≥7.0% shows poor blood glucose control.
2. The control criteria of hypertension is: systolic blood pressure <130 mmHg and diastolic blood pressure <80 mmHg are well controlled; systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 80 mmHg are not well controlled.
3. The control criteria for obesity and central obesity are: BMI <24.0 kg/m^2 shows well controlled, and BMI ≥24.0 kg/m^2 shows poorly controlled; Male WC ≥85 cm, female WC ≥80 cm are defined as central obesity.
4. Blood lipid control criteria: TC <4.5 mmol/L and TG <1.7 mmol/L, HDL-C >1.0 mmol/L for men and >1.3 mmol/L for women, and LDL-C <2.6 mmol/L for patients without atherosclerotic cardiovascular disease and <1.8mmol/L for patients with atherosclerotic cardiovascular disease, are well controlled. The other cases show poor lipid control.

Smoking: more than one cigarette a day for a continuous or cumulative period of 6 months were defined as smoking. The rest were defined as non-smoking.

Alcohol drinking: more than once a week (excluding once), but not daily, was defined as drinking; less than once a week (including once) was defined as less drinking; ever drinking, but quit drinking >6 months was defined as abstinence. There were just three abstinence cases in our cohort, so we defined both abstinence and never drinking as non-drinking.

Follow up

Three forms of follow up were carried out: (i) community follow up; (ii) monitoring system for chronic diseases and causes of death; and (iii) health insurance system data comparison. Data related to clinical complications and tumors, including the onset time, outcome, types and diagnosis, and treatment institutions of cardio-cerebrovascular complications, such as ischemic heart disease and stroke, were followed up and collected through electronic medical records, death cause monitoring, chronic disease incidence monitoring and other databases. Individual case information collected from multiple sources was matched and checked, and sorted out if inconsistent, and then feedback to investigate and verify.

Quality control

Before the investigation of this cohort study, the investigators were uniformly centralized trained, and the investigation and measurement could not be carried out until they were qualified. A quality control team was established by both the provincial and county (city) CDCs, with strict quality control on-site investigation, physical measurement, follow up, information entry and so on.

Statistical analysis

EpiData 3.0 software (The EpiData Association, Odense, Denmark) was used to establish the database, and SPSS 19.0 statistical software (SPSS, Chicago, IL, USA) for data analysis. The enumeration data were compared by the χ^2-test, and Fisher's exact probability was used when the theoretical frequency was <5. For 16 factors, including sex, age, HbA1c, blood pressure, TC, TG, HDL-C, LDL-C, BMI, WC, *ALDH2* polymorphism, region, smoking, drinking, diabetes duration and use of hypoglycemic drugs, first univariate Cox regression analysis was used to select the risk factors influencing the morbidity and outcome of cardio-cerebrovascular complications in persons with diabetes, and then variables with $P < 0.05$ in univariate analysis were entered into multivariate Cox regression analysis. After age and sex adjustment, risk factors influencing the onset and outcome of cardio-cerebrovascular complications in persons with diabetes were screened. Variables with $P < 0.05$ in multivariate analysis were entered into Cox regression to plot survival curve. The significance test level was $\alpha = 0.05$, and $P < 0.05$ was considered statistically significant.

RESULTS

Participants' characteristics and follow-up outcomes

Among the 2,500 participants, 1,110 (44.4%) were men, 1,390 (55.6%) were women. The age ranged from 32 to 91 years, with a mean of 67.26 ± 8.50 years. The disease course ranged from 0 to 37 years, with a mean of 6.43 ± 4.78 years, among which 1,029 patients (41.2%) had a course <5 years. There were 1,262 patients (50.5%) with good control of HbA1c <7.0%, whereas 1,238 patients (49.5%) had HbA1c >7.0%. There were 541 patients (21.6%) with good blood pressure control, but 1,959 (78.4%) patients with poor blood pressure control (78.4%). There were 1,981 patients treated with hypoglycemic drugs (79.2%). There were 1,431 patients of central obesity (57.2%), and 1,356(54.2%) were overweight or obese. A total of 2,208 patients (88.3%) had dyslipidemia. A total of 407 patients (16.3%) were smokers and 235 (9.4%) were drinkers. Details are shown in Table 2.

As of December 2018, 215 of 2,500 diabetes patients(153 strokes, 52 ischemic heart disease, and 10 both ischemic heart disease and strokes) had cardio-cerebrovascular complications that occurred during the observation period, including 10 deaths due to cardio-cerebrovascular complications. A total of 6,407.53 person/years were observed, the median observation time was 2.68 years and the incidence density was 335.54 people/10,000 people per year. During the observation period, 13 people died from non-cardio-cerebrovascular diseases.

Genotype distribution of *ALDH2* (rs671)

The observed *ALDH2* (rs671) genotype frequency distribution among the 2,500 participants was consistent with the Hardy–Weinberg equilibrium ($\chi^2 = 4.017$, $P = 0.134$), with 1,571 GG genotypes (62.84%) and 929 GA/AA genotypes (37.16%). GA/AA genotypes accounted for 34.47% in the good HbA1c control group, which was lower than that in the poor HbA1c control group (39.90%), and the difference was significant ($P < 0.05$). In the drinking group, the proportion of GA/AA genotype was 12.77%, which was lower than that of the less drinking group (33.53%) and non-drinking group (40.19%), and the difference was statistically significant ($P < 0.05$). There were also significant differences in genotype distribution among regions ($P < 0.05$). There was no significant difference ($P > 0.05$) in genotype distribution in sex, age, diabetes duration, blood pressure, blood lipid, BMI, WC, smoking and hypoglycemic drugs, as shown in Table 2.

Cox regression analysis of risk factors of cardio-cerebrovascular complications in diabetes patients

Univariate Cox regression analysis

Univariate Cox regression analysis on factors, including sex, age, area, diabetes duration, hypoglycemic drugs treated, HbA1c, blood pressure, TC, TG, HDL-C, LDL-C, BMI, WC, *ALDH2* genotypes, smoking and drinking, showed that poor HbA1c control, poor HDL-C control, poor LDL-C control, drinking, *ALDH2* GA/AA genotype and diabetes duration (≥10 years) were the risk factors of diabetic cardiovascular complications ($P < 0.05$; Table 3).

Multivariate Cox regression analysis and survival curve

We defined the occurrence of cardio-cerebrovascular complications in type 2 diabetes patients as the outcome of the observation event (0 = no occurrence, 1 = occurrence or death due to it), and the whole follow-up time before the occurrence of cardio-cerebrovascular complications or death due to it as the overall survival time. Taking the related professional variables (sex, age and blood pressure) and the significant factors from univariate analysis as covariates, the Cox proportional hazards model was thus used to examine the association between these factors and overall survival time. Univariate analysis showed that HbA1c, HDL-C, LDL-C, drinking, *ALDH2* rs671 polymorphism and diabetes duration were significantly associated with overall survival. After adjusting for other confounding factors, male ($P = 0.001$), *ALDH2* "GA/AA" genotype ($P = 0.050$), poor HbA1c control ($P = 0.009$), poor HDL-C control ($P = 1.89E\text{-}5$), poor blood pressure control ($P = 0.031$) and poor LDL-C control ($P = 3.76E\text{-}19$) remained as risk factors for shorter overall survival, whereas drinking ($P = 0.044$) performed as a protective factor (Table 4). The risk in the *ALDH2* GA/AA genotype was 1.314-fold that of the GG genotype (95%

Table 2 | Genotype distribution of aldehyde dehydrogenase 2 (rs671)

Characteristics	Patients *n* (%)	GG genotype (*n* = 1571) *n*	GA/AA genotype (*n* = 929) *n*	χ^2	*P*-value
Sex				0.393	0.531
Female	1,390 (55.6%)	881	509		
Male	1,110 (44.4%)	690	420		
Age (years)	67.26 ± 8.50			1.364	0.850
<45	20 (0.8%)	12	8		
45–55	192 (7.7%)	116	76		
55–65	691 (27.6%)	441	250		
65–75	1,099 (44.0%)	696	403		
≥75	498 (19.9%)	306	192		
Region				6.740	0.034
Jiashan	1,874 (75.0%)	1,189	685		
Yongkang	254 (10.2%)	141	113		
Suichang	372 (14.9%)	241	131		
Diabetes duration (years)	6.43 ± 4.78			1.907	0.385
<5	1,029 (41.2%)	637	392		
5–10	948 (37.9%)	592	356		
≥10	523 (20.9%)	342	181		
Hypoglycemic drugs				11.589	0.072
No	519 (20.8)	332	187		
Insulin Secretagogues agent	1,030 (41.2%)	654	376		
Metformin	626 (25%)	406	220		
α-Glycosidase inhibitor	82 (3.3%)	45	37		
Euglycemic agent	13 (0.5%)	5	8		
Insulin and its analogs	16 (0.6%)	9	7		
Chinese herbal medicine and others	214 (8.6%)	120	94		
Hba1c (%)				7.902	0.005
Good control	1,262 (50.5%)	827	435		
Poor control	1,238 (49.5%)	744	494		
Blood pressure				3.260	0.071
Good control	541 (21.6%)	332	219		
Poor control	1,959 (78.4%)	1,249	710		
TC (mmol/L)				0.052	0.820
Good control	685 (27.4%)	428	257		
Poor control	1,815 (72.6%)	1,143	672		
TG (mmol/L)				0.010	0.920
Good control	1,529 (61.2%)	962	567		
Poor control	971 (38.8)	609	362		
HDL-C (mmol/L)				0.019	0.890
Good control	1,627 (65.1%)	1,024	603		
Poor control	873(34.9%)	547	326		
LDL-C (mmol/L)				0.052	0.820
Good control	1,096 (43.8%)	686	410		
Poor control	1,404 (56.2%)	885	519		
BMI (kg/m^2)				0.000	0.993
Normal	1,144 (45.8%)	719	425		
Overweight and obesity	1,356 (54.2%)	852	504		
WC (cm)				0.537	0.464
Normal	1,069 (42.8%)	663	406		
Central obesity	1,431 (57.2%)	908	523		
Smoking				0.854	0.356
No	2,093 (83.7%)	1,307	786		
Yes	407 (16.3%)	264	143		
Drinking				69.087	9.95E-16

Table 2 (*Continued*)

Characteristics	Patients *n* (%)	GG genotype (*n* = 1571) *n*	GA/AA genotype (*n* = 929) *n*	χ^2	*P*-value
Non-drinking	2,095 (83.8%)	1,253	842		
Less drinking	170 (6.8%)	113	57		
Drinking	235 (9.4%)	205	30		

Total *n* = 2,500. BMI, body mass index; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

confidence interval [CI] 1.000–1.727). Figure 1 shows the survival curves corresponding to the seven risk/protective factors, "good LDL-C control" versus "poor LDL-C control" (Figure 1a), "good HDL-C control" versus "poor HDL-C control" (Figure 1b), "female" versus "male" (Figure 1c), "good HbA1c control" versus "poor HbA1c control" (Figure 1d), *ALDH2* rs671 gene polymorphism "GG" versus "GA/AA" genotype (Figure 1e), "good blood pressure control" versus "poor blood pressure control" (Figure 1f), and "non-drinking" versus "less drinking" and "drinking" (Figure 1g), and was carried out by Cox regression analysis.

DISCUSSION

Previous studies suggested that hyperglycemia, hypertension, dyslipidemia and unhealthy behavior were important risk factors for cardio-cerebrovascular diseases. However, hyperglycemia, hypertension and dyslipidemia alone cannot fully explain the mechanism of cardio-cerebrovascular complications in type 2 diabetes patients. Recent studies have shown that gene polymorphism plays an important role in the occurrence and development of diseases, such as diabetes and coronary heart disease[1-11,13].

The polymorphism of *ALDH2* rs671 can be divided into three genotypes: GG genotype with normal catalytic active enzyme, GA genotype with just 10~45% of GG genotype and AA genotype with just 1~5% of normal enzyme activity[14]. In the present study, the influence of different *ALDH2* genotypes on the occurrence of cardio-cerebrovascular complications was carried out based on a prospective community and disease cohort of 10,339 persons. The *ALDH2* (rs671) SNPs were detected in 2,500 randomly selected persons without cardio-cerebrovascular complications, with the GA/AA genotype accounting for 37.16%, which was higher than that of Wuhan (36.99%), Shanghai (34.6%) and Shandong (27.9%)[15,16], showing regional differences. This difference also exists among Jiashan, Yongkang and Suichang in the present study ($P = 0.034$, Table 2).

It has been reported that ALDH2 is not only one of the key enzymes in alcohol metabolism, but also has cardiac protective effects, such as anti-oxidant damage and inhibition of cell apoptosis. The *ALDH2* GG genotype shows relatively consistent protective effects on diabetic cardio-cerebrovascular diseases, whereas GA/AA genotypes are closely related to the occurrence and development of cardio-cerebrovascular diseases[3,17]. The Genome-wide Association Analysis in 2012 confirmed that the Glu504Lys allele is a genetic susceptibility locus for CAD[18], whereas *ALDH2*-deficient type was a risk factor for CAD[19]. Epidemiological studies have found that *ALDH2* mutation can increase the risk of diabetic cardiovascular diseases; a clinical study of 167 persons with CAD complicated with diabetes found that the prevalence of hypertension in *ALDH2* mutation carriers was significantly higher than that in wild homozygous persons[20]. The results of the present study supported that the GA/AA genotype of *ALDH2* mutation was a risk factor for cardio-cerebrovascular complications of diabetes, and the GA/AA genotype significantly accelerated the occurrence or death due to cardio-cerebrovascular complications of diabetes ($P = 0.050$, hazard ratio 1.314, 95% CI 1.000–1.727). However, Narita[21] in Japan reported a contradictory conclusion that after controlling traditional cardiovascular risk factors and drinking, *ALDH2* gene mutation showed a significantly negative correlation with carotid artery plaque score; namely, the *ALDH2* gene mutation might be a protective factor of carotid artery atherosclerosis, prompting that *ALDH2* gene mutation might be a protective factor of coronary atherosclerosis. Kato *et al.*[22] also reported a finding, which is counterintuitive based on what is known about the relationship between blood pressure and CAD, that in their general population of a large sample size, the rs671 allele associated with elevated blood pressure was associated with a reduced risk of CAD. This might suggest some balance of rs671, hypertension, HDL-C, LDL-C and CAD.

As the "gold standard" for diabetes monitoring, HbA1c level can reflect the patient's blood glucose control in the past 8–12 weeks [12]. Studies have shown that HbA1c is significantly correlated with end-point events of vascular complications in persons with diabetes, and an increase of 1% in HbA1c increases the risk of heart disease and stroke by 18% in persons with diabetes[23]. It was also confirmed by the present study, suggesting that good blood glucose control is the key to preventing or delaying the occurrence and development of chronic complications. We found that GA/AA genotypes in the good HbA1c control group accounted for 34.47%, which was significantly lower than that in the poor HbA1c control group (39.9%), indicating that there was a significant correlation

Table 3 | Univariate Cox regression analysis of cardio-cerebrovascular complications in type 2 diabetes patients

Characteristics	Cardio-cerebrovascular complications (n = 215)	No cardio-cerebrovascular complications (n = 2,285)	HR_c (95% CI)
Sex			
Female	117	1,273	1.000
Male	98	1,012	1.050 (0.803–1.374)
Age (years)			
<45	3	17	1.000
45–55	17	175	0.565 (0.166–1.929)
55–65	62	629	0.579 (0.182–1.846)
65–75	94	1,005	0.550 (0.174–1.736)
≥75	39	459	0.502 (0.155–1.624)
Region			
Jiashan	152	1,722	1.000
Yongkang	24	230	1.284 (0.832–1.981)
Suichang	39	333	1.366 (0.951–1.963)
Diabetes duration (years)			
<5	71	958	1.000
5–10	86	862	1.328 (0.970–1.819)
≥10	58	465	1.643 (1.161–2.324)
Hypoglycemic drugs treated			
No	45	474	1.000
Insulin secretagogues agent	85	945	0.957 (0.667–1.373)
Metformin	56	570	1.034 (0.698–1.531)
α-Glycosidase inhibitor	5	77	0.701 (0.278–1.765)
Euglycemic agent	2	11	1.848 (0.448–7.619)
Insulin and its analogs	2	14	1.446 (0.351–5.961)
Chinese herbal medicine and others	20	194	1.102 (0.651–1.866)
HbA1c (%)			
Good control	87	1,175	1.000
Poor control	128	1,110	1.525 (1.161–2.002)
Blood pressure			
Good control	35	506	1.000
Poor control	180	1,779	1.431 (0.996–2.055)
TC (mmol/L)			
Good control	52	633	1.000
Poor control	163	1,652	1.176 (0.861–1.607)
TG (mmol/L)			
Good control	123	1,406	1.000
Poor control	92	879	1.189 (0.908–1.558)
HDL-C (mmol/L)			
Good control	123	1,504	1.000
Poor control	92	781	1.426 (1.088–1.868)
LDL-C (mmol/L)			
Good control	27	1,069	1.000
Poor control	188	1,216	5.709 (3.814–8.545)
BMI (kg/m^2)			
Normal	89	1,055	1.000
Overweight and obesity	126	1,230	1.206 (0.920–1.583)
WC (cm)			
Normal	95	974	1.000
Central obesity	120	1,311	0.938 (0.717–1.228)

Table 3 (*Continued*)

Characteristics	Cardio-cerebrovascular complications (n = 215)	No cardio-cerebrovascular complications (n = 2,285)	HR_c (95% CI)
Smoking			
No	179	1,914	1.000
Yes	36	371	1.039 (0.726–1.486)
Drinking			
Non-drinking	194	1,901	1.000
Less drinking	10	160	0.619 (0.328–1.168)
Drinking	11	224	0.490 (0.267–0.899)
ALDH2 genotype			
GG	119	1,452	1.000
GA/AA	96	833	1.391 (1.063–1.820)

Total n = 2,500. *ALDH2*, aldehyde dehydrogenase 2; BMI, body mass index; CI, confidence interval; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HR_c, crude hazard ratio; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

Table 4 | Multivariate Cox regression analysis of cardio-cerebrovascular complications in type 2 diabetes patients

Variables	*B*	SE	χ^2	*P*-value	HR_a (95% CI)
Sex (male)	0.476	0.150	10.107	0.001	1.160 (1.200–2.160)
Poor HbA1c control	0.368	0.140	6.916	0.009	1.445 (1.098–1.900)
ALDH2 GA/AA genotype	0.273	0.139	3.846	0.050	1.314 (1.000–1.727)
Poor HDL-C control	0.619	0.145	18.298	1.89E-5	1.857 (1.398–2.466)
Poor LDL-C control	1.857	0.208	79.993	3.76E-19	6.406 (4.264–9.624)
Less drinking	–0.623	0.332	3.509	0.061	0.537 (0.280–1.029)
Drinking	–0.650	0.323	4.067	0.044	0.522 (0.277–0.982)
Poor blood pressure control	0.401	0.186	4.649	0.031	1.493 (1.037–2.148)

Total n = 2,500. HR_a: hazard ratio adjusted for age, sex (female = 0, male = 1) and blood pressure (good control = 0, poor control = 1), which were related professional variables; and glycated hemoglobin (HbA1c; good control = 0, poor control = 1), high-density lipoprotein cholesterol (HDL-C; good control = 0, poor control = 1), low-density lipoprotein cholesterol (LDL-C; good control = 0, poor control = 1), drinking (non-drinking = 0, less drinking = 1, drinking = 2), aldehyde dehydrogenase 2 (*ALDH2*) rs671 (GG = 0, GA/AA = 1) and diabetes duration (<5 years = 0, 5~10 years = 1, ≥10 years = 2), which were the significant factors from the univariate regression analysis. CI, confidence interval; SE, standard error.

between the *ALDH2* genotype and HbA1c control. The mechanism might be related to *ALDH2* gene mutation, and thus decreasing the enzyme activity and the metabolism of aldehydes, while increasing the inflammatory response of the pancreas and the deficiency of the insulin signal, leading to the occurrence of insulin resistance and the increase of blood glucose in hyperinsulinemia[24,25].

The Cox regression analysis results of the present study showed that drinking was a protective factor for the occurrence of cardio-cerebrovascular complications in diabetes patients, which was inconsistent with most of the results[11]. We also found that GG and GA/AA genotypes of rs671 were significantly different between the drinking, less drinking and non-drinking groups, and the percentage of GG genotypes in the drinking group was significantly higher than that in the non-drinking group (P = 9.95E-16), showing that ALDH2 had a strong detoxification effect on aldehyde and significantly reduced the occurrence of cardio-cerebrovascular events. Researchers believe that elevated levels of acetaldehyde in the blood of *ALDH2* mutation carriers after drinking alcohol are the main cause of hyperglycemia and hyperinsulinemia. These results confirmed the protective effect of ALDH2 on diabetic cardiovascular disease.

We used the Cox proportional hazards model to analyze and identify that poor HDL-C control, poor LDL-C control, poor HbA1c control, poor blood pressure control, drinking, *ALDH2* GA/AA genotype and being male were risk factors for cardio-cerebrovascular complications

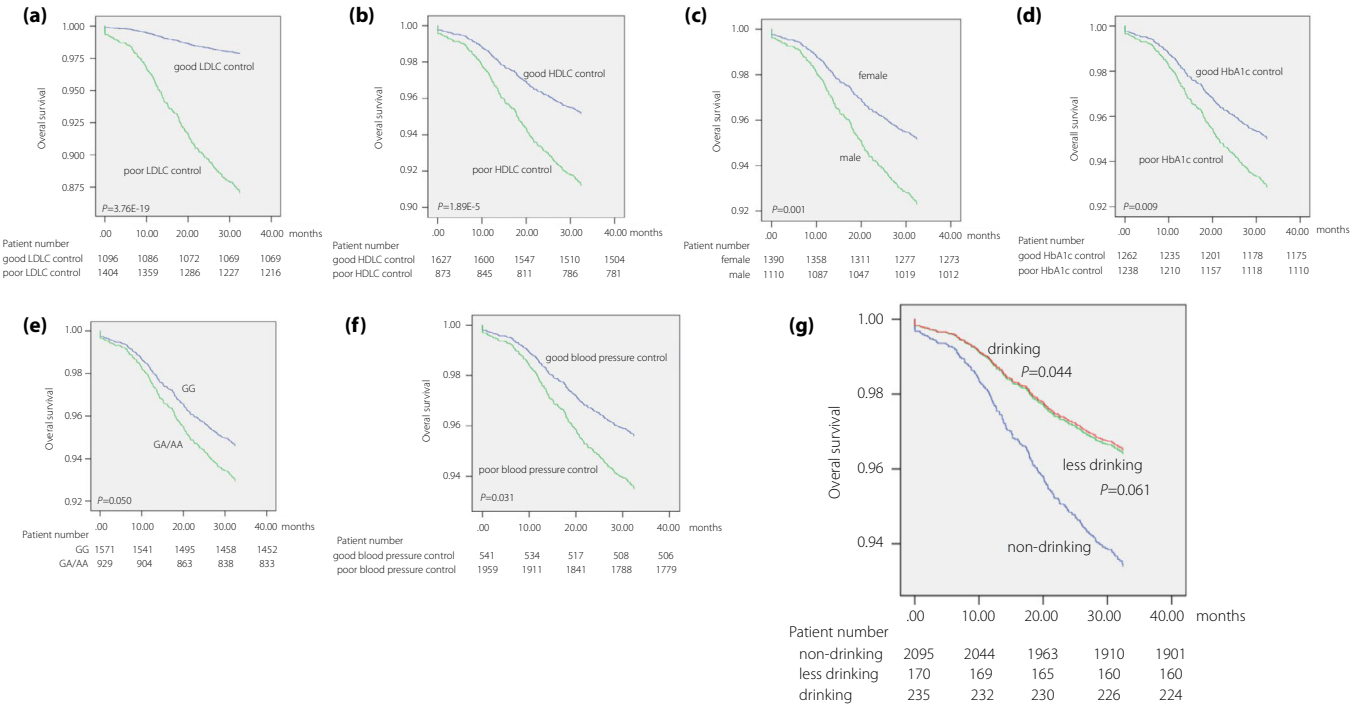

Figure 1 | Cox regression analysis of the effect of risk factors on the overall survival of cardio-cerebrovascular complications in type 2 diabetes. (a) "Good low-density lipoprotein cholesterol (LDL-C) control" versus "poor LDL-C control". (b) "Good high-density lipoprotein cholesterol (HDL-C) control" versus "poor HDL-C control". (c) "Female" versus "male". (d) "Good glycated hemoglobin (HbA1c) control" versus "poor glycated hemoglobin (HbA1c) control". (e) Aldehyde dehydrogenase 2 rs671 "GG" genotype versus "GA/AA" genotype. (f) "Good blood pressure control" versus "poor blood pressure control". (g) "Non-drinking" versus "less drinking" and "drinking".

($P < 0.05$). After adjusting for age, sex, HDL-C, LDL-C, blood pressure, drinking and HbA1c, the risk of cardio-cerebrovascular complications in the *ALDH2* GA/AA genotype was 1.314-fold (95% confidence interval 1.000–1.727) that of the GG genotype, which confirmed that the *ALDH2* gene GA/AA mutation increased the risk of cardio-cerebrovascular complications, and was consistent with most reports[26,27]. Poor control of HDL-C and LDL-C would affect the good control of HbA1c, thus, significantly affecting the occurrence or mortality rate of cardio-cerebrovascular complications in patients with diabetes. The present study showed that to prevent the occurrence of diabetic complications, prevention and treatment strategies should be comprehensive, including lowering blood glucose, blood pressure and regulating blood lipids. It is worth mentioning that in univariate Cox regression analysis, sex was not a statistically significant factor for cardio-cerebrovascular complications or death due to it ($P = 0.721$, 1.050, 95% CI 0.803–1.374), but after adjusting for HDL-C and LDL-C ($P = 0.011$, 1.440, 95% CI 1.087–1.906), it was shown that HDL-C, LDL-C and sex had strong collaborations. Liu *et al.*[28] found similar results. They recruited 38,989 participants without baseline cardiovascular disease from a multicenter longitudinal health management cohort in China, followed up from 2007 to 2015, and found that after multifactor adjustment, lower HDL-C and higher non-HDL-C, or TG alone, were associated with the increased risk of cardiovascular disease, in which the male adjustment hazard ratios were 1.77 (95% CI 1.36–2.30) and 2.08 (95% CI 1.30–3.34), respectively.

In summary, according to our community-based and disease-based prospective cohort study design, a baseline cohort of 10,339 type 2 diabetes patients and a baseline disease database were established. After continuous follow up of participants for approximately 3 years, we found that *ALDH2* SNPs (rs671) gene polymorphism was significantly associated with cardio-cerebrovascular complications in type 2 diabetes patients, and the *ALDH2* GG/GA genotype increased the risk of the occurrence of and death from cardio-cerebrovascular complications of diabetes.

ACKNOWLEDGMENTS

This work was supported by grants from the National Key Research and Development Program of China "precision medical research" (2016YFC0901200, 2016YFC0901205) from the Ministry of Science and Technology, and "Zhejiang Provincial Public Welfare Technology Applied Research Projects (no. 2017C33090)".

DISCLOSURE

All authors declare no conflict of interest.

REFERENCES

1. Afghahi H, Miftaraj M, Svensson AM, *et al*. Ongoing treatment with renin-angiotensin-aldosterone-blocking agents does not predict normoalbuminuric renal impairment in a general type 2 diabetes population. *J Diabetes Complications*. 2013; 27: 229–234.
2. Adlerberth AM, Rosengren A, Wilhelmsen L. Diabetes and long-term risk of mortality from coronary and other causes in middle-aged Swedish men. A general population study. *Diabetes Care* 1998; 21: 539–545.
3. Xu H, Zhang Y, Ren J. ALDH2 and Stroke: A Systematic Review of the Evidence. *Adv Exp Med Biol* 2019; 1193: 195–210.
4. Guo YJ, Chen L, Bai YP, *et al*. The ALDH2 Glu504Lys polymorphism is associated with coronary artery disease in Han Chinese: Relation with endothelial ADMA levels. *Atherosclerosis* 2010; 211: 545–550.
5. Guo JM, Liu AJ, Zang P, *et al*. ALDH2 protects against stroke by clearing 4-HNE. *Cell Res* 2013; 23: 915–930.
6. Yang H, Song Z, Yang GP, *et al*. The ALDH2 rs671 polymorphism affects post-stroke epilepsy susceptibility and plasma 4-HNE levels. *PLoS One* 2014; 9: e109634.
7. Zhai X, Zhang Z, Liu W, *et al*. Protective effect of ALDH2 against cyclophosphamide-induced acute hepatotoxicity via attenuating oxidative stress and reactive aldehydes. *Biochem Biophys Res Commun* 2018; 499: 93–98.
8. Gu XY, Fang TT, Gao Q, *et al*. Effect of high concentration glucose on the expression of ALDH2 in cardiac fibroblasts of rats. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 2017; 33: 267–270 (Chinese).
9. Munukutla S, Pan G, Palaniyandi SS. Aldehyde Dehydrogenase (ALDH) 2 in Diabetic Heart Diseases. *Adv Exp Med Biol* 2019; 1193: 155–174.
10. Li GCWY, Gong LN, Xu XB. Study on the correlation between ALDH2 genotype and carotid intima-media thickness in elderly diabetes patients. *Acta Universitatis Medicinals Nanjing* 2017; 37: 1307–1309 (Chinese).
11. Idewaki Y, Iwase M, Fujii H, *et al*. Association of Genetically Determined Aldehyde Dehydrogenase 2 Activity with Diabetic Complications in Relation to Alcohol Consumption in Japanese Patients with Type 2 Diabetes Mellitus: The Fukuoka Diabetes Registry. *PLoS One* 2015; 10: e0143288.
12. Chinese Diabetes Society. Chinese guidelines for the prevention and treatment of type 2 diabetes mellitus (2017 Edition). *Chin J Pract Int Med*. 2018; 38: 292–344.
13. Pan C, Xu F, Yuan QH. Research update on the relationship of aldehyde dehydrogenase 2 and cardio–cerebral–vascular diseases. *Chin J Cardiol* 2016; 44: 175–178 (Chinese).
14. Changfu Y, Min Ai, Fuchun X. Study on the Aldehyde Dehydrogenase2 Activity in Acute Myocardial Infarction Patients with Wild-Type ALDH2 Gene. *Sichuan Med J*. 2017; 38: 13–17 (Chinese).
15. Dong JL, Lin YK, Huang W, *et al*. Genetic polymorphism of ALDH2 among the Han population in Wuhan. *Central South Pharmacy* 2019; 17: 112–116 (Chinese).
16. Mi SL, Fan F, Li JM, *et al*. Distributed difference of aldehyde dehydrogenase 2(ALDH2) polymorphism in Shanghai and Shandong Han population. *Chin J Clin Med* 2011; 18: 735–737 (Chinese).
17. Chen CH, Ferreira JC, Gross ER, *et al*. Targeting aldehyde dehydrogenase 2: new therapeutic opportunities. *Physiol Rev* 2014; 94: 1–34.
18. Takeuchi F, Yokota M, Yamamoto K, *et al*. Genome-wide association study of coronary artery disease in the Japanese. *Eur J Hum Genet* 2012; 20: 333–340.
19. Chen L, Smith GD, Harbord RM, *et al*. Alcohol intake and blood pressure: a systematic review implementing a Mendelian randomization approach. *PLoS Medicine* 2008; 5: e52.
20. Wang H, Pan Q, Gao Q, *et al*. Correlation of aldehyde dehydrogenase-2 gene polymorphism with hypertension in patients with coronary heart disease complicated by diabetes mellitus. *Nan Fang Yi Ke Da Xue Xue Bao* 2013; 33: 542–545, 62.
21. Narita M, Kitagawa K, Nagai Y, *et al*. Effects of aldehyde dehydrogenase genotypes on carotid atherosclerosis. *Ultrasound Med Biol* 2003; 29: 1415–1419.
22. Kato N, Takeuchi F, Tabara Y, *et al*. Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. *Nat Genet* 2011; 43: 531–538.
23. Selvin E, Marinopoulos S, Berkenblit G, *et al*. Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. *Ann Intern Med* 2004; 141: 421–431.
24. Zhao LN, Hao LP, Yang XF, *et al*. The diabetogenic effects of excessive ethanol: reducing beta-cell mass, decreasing phosphatidylinositol 3-kinase activity and GLUT-4 expression in rats. *Br J Nutr* 2009; 101: 1467–1473.
25. Dembele K, Nguyen KH, Hernandez TA, *et al*. Effects of ethanol on pancreatic beta-cell death: interaction with glucose and fatty acids. *Cell Biol Toxicol* 2009; 25: 141–152.
26. Adeniji EA, Olotu FA, Soliman MES. Alcohol Metabolic Inefficiency: Structural Characterization of Polymorphism-Induced ALDH2 Dysfunctionality and Allosteric Site Identification for Design of Potential Wildtype Reactivators. *Protein J* 2018; 37: 216–222.
27. Koppes LL, Dekker JM, Hendriks HF, *et al*. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. *Diabetes Care* 2005; 28: 719–725.
28. Liu X, Yang Y, Kang F, *et al*. Cardiovascular Disease Risk Across a Spectrum of Adverse Plasma Lipid Combinations by Gender and Glycemic Status. *Am J Cardiol* 2019; 124: 702–708.