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Sex-specific differences in the association of a common aldehyde dehydrogenase 2 gene polymorphism and alcohol consumption with stroke risk in a Korean population: a prospective cohort study

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BACKGROUND/OBJECTIVES: It is well-known that alcohol consumption is associated with stroke risk as well as with aldehyde dehydrogenase 2 gene (*ALDH2*) polymorphisms. However, it is unclear whether *ALDH2* polymorphisms are associated with stroke risk independent of alcohol consumption and whether such association is modified by sex. We evaluated sex-specific associations of a common *ALDH2* polymorphism and alcohol consumption with stroke risk in a Korean population.
SUBJECTS/METHODS: We conducted a prospective cohort study involving 8,465 men and women, aged 40-69 years and free of stroke between June, 2001 and January, 2003, and followed for the development of stroke. We identified new cases of stroke, which were self-reported or ascertained from vital registration data. Based on genome-wide association data, we selected a single-nucleotide polymorphism (rs2074356), which shows high linkage disequilibrium with the functional polymorphism of *ALDH2*. We conducted Cox proportional hazards regression analysis considering potential risk factors collected from a baseline questionnaire.
RESULTS: Over the median follow-up of 8 years, 121 cases of stroke were identified. Carrying the wild-type allele of the *ALDH2* polymorphism increased stroke risk among men. The multivariate hazard ratio [95% confidence interval] of stroke was 2.02 [1.03-3.99] for the wild-type allele compared with the mutant alleles, but the association was attenuated after controlling for alcohol consumption. Combinations of the wild-type allele and other risk factors of stroke, such as old age, diabetes mellitus, and habitual snoring, synergistically increased the risk among men. Among women, however, the *ALDH2* polymorphism was not associated with stroke risk.
CONCLUSIONS: The prospective cohort study showed a significant association between a common *ALDH2* polymorphism and stroke risk in Korean men, but not in Korean women, and also demonstrated that men with genetic disadvantages gain more risk when having risk factors of stroke. Thus, these men may need to make more concerted efforts to control modifiable risk factors of stroke.

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INTRODUCTION

The aldehyde dehydrogenase 2 gene (*ALDH2*) encodes mitochondrial aldehyde dehydrogenase (ALDH2), which detoxifies acetaldehyde generated from the oxidation of ethanol by alcohol dehydrogenase. A common functional polymorphism, *ALDH2* Glu504Lys, has been identified; its allelic variants are known to be responsible for different enzyme activities, which affect the clearance rate of ethanol [1]. The *ALDH2* polymorphism is reportedly associated with alcohol consumption [2,3], cancer [4,5], hypertension [6,7], diabetes mellitus [8], and coronary artery disease (CAD) [9-11] primarily among Asian populations. Alcohol consumption is thought to underlie the association between the *ALDH2* polymorphism and these diseases, but the direct role of the *ALDH2* polymorphism in pathological mechanisms of disease cannot be ruled out [12]. Particularly, individuals with the *ALDH2* mutant allele with null or low enzyme activity are more likely to have CAD [9-11].

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Findings regarding the association between *ALDH2* variants and hypertension are inconsistent. A case-control study reported that individuals with the wild-type allele are more likely to have hypertension [6] whereas a prospective study showed that those possessing the mutant allele in *ALDH2* are at an increased risk of hypertension [7]. No data have been reported regarding the association between the *ALDH2* polymorphism and stroke. A previous study identified a significant association between a common *ALDH2* polymorphism and high alcohol consumption among patients with stroke, but was unable to investigate on association with stroke risk itself [13]. In a meta-analysis of accumulating data, a J-shaped relationship between alcohol consumption and stroke risk was observed; alcohol consumption less than 12 g/day was optimal for preventing stroke in prospective cohort studies whereas 15 to 30 g/day was shown as optimal consumption in case-control studies [14]. This discrepancy may be due to response errors in self-reported alcohol consumption, a lack of consideration of binge drinking behavior, or genetic variation, particularly allelic variation at alcohol metabolism genes.

Recently, we discovered common *ALDH2* polymorphisms highly associated with alcohol consumption as well as with alcoholism, such as rs2074356 and rs11066280, which are considered to reflect rs671, non-synonymous SNPs of *ALDH2* [3]. In particular, rs2074356 was discovered as a SNP associated with coronary artery disease risk [15]. In present study, we considered this SNP a surrogate of functional polymorphism of *ALDH2* and used to estimate stroke risk in a prospective cohort study. In analysis of 8-year follow-up data, we considered a number of potential risk factors including the presence of metabolic diseases, lifestyle factors, and daily amounts of alcohol consumed to identify the role of the *ALDH2* polymorphism in developing stroke. Furthermore, we evaluated the joint effects of genetic factors and other risk factors on stroke risk.

SUBJECTS AND METHODS

Study population

Study participants were from population-based cohorts embedded in the Korean Genome Epidemiology Study (KoGES), which is an ongoing longitudinal investigation. Cohort members included Korean men (n = 4,752) and women (n = 5,261), aged 40-69 years and residing in 2 cities, Ansan and Ansung, when they were enrolled between June, 2001 and January, 2003. Detailed information regarding enrollment of cohort members and study procedures is available in a previous report [3]. Briefly, cohort members were recruited based on a two-stage cluster sampling procedure and were enrolled after undergoing a comprehensive health examination and on-site interviews, which were administered by trained personnel. At the health examination, cohort members provided blood specimens for evaluation of biochemical parameters to measure lipid and glucose metabolism as well as for genetic assays. They completed questionnaire-based interviews to collect data on socio-demographics, medical history and health conditions, family history of disease, and lifestyle factors. They have also been followed biennially and have undergone similar health examination and interviews. At each visit, the participants sign an informed consent form, which was approved by the Human Subjects Review Committee either at the Ajou University Medical Center or at the Korea University Ansan Hospital. All procedures and protocols of the study were approved by both institutions.

Among a total of 8,716 individuals whose clinical and genetic data were available at baseline, we excluded 10 persons who reported pregnancy at baseline (n = 1), who did not complete the baseline anthropometric measurement (n = 3), or who did not provide information on alcohol consumption (n = 6). Because the outcomes of this study are new cases of stroke, we excluded 89 persons who reported at baseline a physician-diagnosis of stroke. Additionally, we further excluded 152 persons who reported at baseline a physician-diagnosis of coronary artery disease, congestive heart failure, or peripheral artery disease to minimize the effects of these diseases. After exclusions, a total of 8,465 participants (47% men and 53% women) were entered for analysis.

Outcome

The outcomes of this study were incident events or deaths due to stroke during a follow-up period from April, 2003 to December, 2010. Incident events were self-reported by interview-based questionnaires and deaths were ascertained with its cause and date based on vital registration data, which are periodically compiled by the Korea National Statistical Office. We excluded 10 persons who reported a diagnosis of both CAD and stroke during follow-up to distinguish risk factors of stroke from those of CAD. Concordance between self-reported diagnoses of stroke and those ascertained by brain magnetic resonance imaging was 91% in a subgroup of stroke cases.

Common ALDH2 gene polymorphism

To examine the *ALDH2* polymorphism, we focused on the most significant single-nucleotide polymorphism (SNP), rs2074356, which was reported to be highly associated with alcohol consumption (adjusted *P*-value $< 9.5 \times 10^{-59}$) and to be a surrogate marker of *ALDH2* Glu504Lys (rs671) in previous genome-wide association (GWA) studies ($r^2 = 0.8$) [3,15]. Genotypes of this SNP were observed to be CC, CT, and TT; its minor allele (T) frequency was 0.15 among male drinkers [3]. An earlier report provided information regarding the preparation of genomic samples, genotyping method, and quality control for the GWA data [16].

Potential risk factors

We collected information on age, sex, family history of stroke, smoking status, snoring during sleep, physical activity, alcohol drinking status, and amount of alcohol consumed from questionnaires used at the initial interview. Participants were asked whether their biological parents and sibling had been diagnosed with stroke. Regarding snoring status, they were asked if they snore; if so, they were further asked how frequently they snore (infrequently, 1 to 3 times per week, 4 to 5 times per week, 6 to 7 times per week). To collect information regarding daily physical activity, participants were asked to report hours spent in a typical day in sleep and 5 categories

of activity intensity (sedentary, very light, light, moderate, vigorous), given details on activities corresponding to each category. Specific metabolic equivalent (MET) values assigned to each category were multiplied by the reported hours spent to yield a total metabolic equivalent (MET-hours) score as a quantitative estimate of physical activity. Participants were asked whether they had ever consumed alcoholic beverages in their lifetime, whether there was a time in their life when they regularly consumed at least one drink of any alcoholic beverage in every month, and whether they drank in the past 30 days. They were also requested to complete a table to collect information on the average frequency of drinking and amount of alcoholic beverages consumed during a typical occasion in the past 30 days. Such information was used to calculate daily alcohol consumption (g/day).

At the health examination, height (cm) and body weight (kg) were measured by health professionals trained with a standardized protocol to yield body mass index (BMI, kg/m^2). Based on biochemical and clinical evaluations and self-reports on medications, the presence of hypertension (systolic/diastolic pressure $\geq$ 140/90 mmHg), diabetes mellitus (fasting glucose $\geq$ 126 mg/dL or 2-hour glucose $\geq$ 200 mg/dL), or hypercholesterolemia (serum total cholesterol $\geq$ 240 mg/dL) was determined.

Statistical analysis

Descriptive statistics on baseline characteristics of the study population were calculated. To analyze the association between stroke risk and *ALDH2* polymorphic variants and potential risk factors, we conducted Cox proportional hazards regression analysis. The person-years for each subject were calculated from the date when he or she participated in the baseline examination to the date when he or she reported the first stroke events in follow-up examinations or to death date or to December 31, 2010, whichever came first. Persons who died, refused further participation, or were lost to follow-up were censored. The length of follow-up period used for analysis was 9.5 years with a median follow-up period of 7.8 years. Stroke risk was expressed as a hazard ratio (HR) with its 95% confidence interval (CI). In multivariate models, age, BMI, and physical activity were adjusted as a continuous variable and other variables were treated as categorical variables, including current smoking status (nonsmoking, moderate smoking of 20 cigarettes per day or fewer, heavy smoking of 21 cigarettes per day or more), snoring status (no snoring, snoring for 1-3 nights per week, snoring for 4-6 nights per week or every night), and current alcohol drinking status (nondrinking in the past 30 days, light alcohol drinking of 1 drink per day or fewer, moderate alcohol drinking of 2 drinks per day, heavy alcohol drinking of 3 drinks per day or more). However, because the proportion of heavy smokers or heavy drinkers was trivial in women, categories for smokers and drinkers were united. *ALDH2* polymorphic variants (mutant type and wild-type), the presence of family history of stroke, and the presence of hypertension, diabetes mellitus, and hypercholesterolemia were fitted as binary variables in the model. Two multivariate Cox models stratified by sex were constructed in this study. The first multivariate model included *ALDH2* variants and potential risk factors except current alcohol drinking status. The second model included all variables described above and current alcohol status. Analyses stratified according to alcohol drinking status were also performed. Joint effects of *ALDH2* variants and significant risk factors of stroke were analyzed after taking other covariates into account. Proportional hazards assumptions were tested for the second model and no violation was confirmed. All testing was based on a two-sided level of significance. The SAS program (SAS 9.1.3, 2008, SAS Institute, Cary, NC, USA) was used to conduct statistical analyses.

RESULTS

Baseline characteristics

Over median follow-up of 7.8 years, 121 cases (67 men and 54 women) of stroke (99 incident events and 22 deaths due to stroke) were newly identified, with 53035.5 person-years accrued for analysis. A comparison of cohort members who were followed up until 2010 or ascertained as stroke cases with those lost during the follow-up period revealed no significant difference in the distribution of the wild-type (CC) and the mutant type (CT or TT) of the *ALDH2* polymorphism (rs2074356) ($P = 0.66$).

Characteristics of the study population according to *ALDH2* polymorphic variants are presented in Table 1. Of these, 27% had the mutant alleles. Persons with the wild-type allele were more likely to suffer from hypertension or diabetes mellitus

Table 1. Baseline characteristics of the study population (n = 8,465) according to the aldehyde dehydrogenase gene (*ALDH2*) polymorphism

Characteristics	*ALDH2* polymorphism		*P*-value
	Wild-type	Mutant type	
Number of subjects	6172	2293	
Stroke cases, %	1.5	1.2	0.23
Age, yrs	52.1 ± 8.8	52.3 ± 9.0	0.32
Male, %	47.7	46.7	0.42
Family history of stroke, %	9.8	10.4	0.42
Presence of diseases, %			
Hypertension	31.3	26.7	< 0.001
Diabetes mellitus	13.7	11.3	< 0.01
Hypercholesterolemia	13.4	13.9	0.57
Body mass index, kg/m^2	24.6 ± 3.1	24.5 ± 3.1	< 0.05
Smoking status, %			0.88
Nonsmoking	73	73.4	
Smoking $\leq$ 20 cigarettes/day	23.2	22.7	
Smoking > 20 cigarettes/day	3.8	3.9	
Snoring during sleep, %			0.29
Non-snoring	36.3	37.7	
Snoring 1-3 nights/week	49.6	47.7	
Snoring $\geq$ 4 nights/week	14.1	14.7	
Physical activity[1)]	30.9 ± 15.4	30.8 ± 15.4	0.95
Alcohol consumption, %			< 0.001
Nondrinking	43.7	73.6	
1 drink/day	32.8	21.1	
2 drinks/day	9.7	2.7	
$\geq$ 3 drinks/day	13.9	2.7	

Data are mean ± SD for continuous variables.
[1)] Average daily metabolic equivalents-hours (MET-hours)

Table 2. Hazard ratios (HR) and 95% confidence interval (CI) of stroke incidence among men (n = 4,011)

Potential risk factors	Categories	No of cases /non-cases	Unadjusted HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
ALDH2 variants	Mutant type	10/1,060	reference	reference	reference
	Wild-type	57/2,884	2.07 (1.06, 4.05)	2.02 (1.03, 3.99)	1.99 (0.96, 4.13)
Age, yrs		67/3,944	1.09 (1.06, 1.12)	1.10 (1.07, 1.14)	1.10 (1.07, 1.14)
Family history of stroke	Absence	58/3,531	reference	reference	reference
	Presence	9/413	1.26 (0.62, 2.54)	1.29 (0.63, 2.64)	1.30 (0.63, 2.65)
Hypertension	Absence	36/2,738	reference	Reference	reference
	Presence	31/1,206	2.03 (1.26, 3.28)	1.37 (0.83, 2.26)	1.36 (0.82, 2.25)
Diabetes mellitus	Absence	46/3,402	reference	reference	reference
	Presence	21/542	2.85 (1.70, 4.78)	1.93 (1.13, 3.29)	1.85 (1.08, 3.17)
Hypercholesterolemia	Absence	57/3,420	reference	reference	reference
	Presence	10/524	1.17 (0.60, 2.28)	1.20 (0.60, 2.37)	1.18 (0.60, 2.35)
Body mass index, kg/m^2		67/3,944	1.02 (0.94, 1.11)	1.02 (0.93, 1.11)	1.02 (0.93, 1.11)
Smoking status	Nonsmoking	34/1,968	reference	reference	reference
	≤ 20 cigarettes/day	26/1,661	0.96 (0.58, 1.60)	1.14 (0.67, 1.91)	1.11 (0.65, 1.89)
	> 20 cigarettes/day	7/315	1.35 (0.60, 3.04)	1.91 (0.84, 4.38)	1.83 (0.79, 4.24)
Snoring during sleep, %	No	14/1,253	reference	reference	reference
	1-3 nights/week	33/2,006	1.47 (0.79, 2.74)	1.75 (0.92, 3.32)	1.77 (0.93, 3.35)
	≥ 4 nights/week	20/685	2.50 (1.27, 4.96)	3.00 (1.47, 6.15)	2.89 (1.41, 5.92)
Physical activity, MET-hours		67/3,944	1.00 (0.99, 1.02)	1.00 (0.98, 1.01)	1.00 (0.98, 1.01)
Alcohol drinking status	Nondrinking	18/1,101	1.51 (0.76, 3.00)	-	1.64 (0.80, 3.34)
	1 drink/day	15/1,384	reference	-	reference
	2 drinks/day	15/592	2.09 (1.02, 4.27)	-	1.96 (0.95, 4.04)
	≥ 3 drinks/day	19/867	2.05 (1.04, 4.03)	-	1.84 (0.92, 3.66)

Model 1: Data are adjusted for all potential risk factors, except alcohol drinking status, listed in the table.
Model 2: Data are further adjusted for alcohol drinking status with covariates in Model 1.

Table 3. Hazard ratios (HR) and 95% confidence interval (CI) of stroke incidence among women (n = 4,454)

Potential risk factors	Categories	No of cases /non-cases	Unadjusted HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
ALDH2 variants	Mutant type	17/1,206	reference	reference	reference
	Wild-type	37/3,194	0.81 (0.45, 1.43)	0.83 (0.47, 1.48)	0.77 (0.42, 1.40)
Age, yrs		54/4,400	1.08 (1.05, 1.12)	1.08 (1.04, 1.12)	1.08 (1.05, 1.12)
Family history of stroke	Absence	47/3,983	reference	reference	reference
	Presence	7/417	1.36 (0.62, 3.01)	1.33 (0.60, 2.96)	1.32 (0.59, 2.94)
Hypertension	Absence	33/3,114	reference	reference	reference
	Presence	21/1,286	1.60 (0.93, 2.76)	1.02 (0.57, 1.83)	1.01 (0.56, 1.82)
Diabetes mellitus	Absence	46/3,870	reference	reference	reference
	Presence	8/530	1.36 (0.64, 2.88)	0.94 (0.44, 2.04)	0.95 (0.44, 2.06)
Hypercholesterolemia	Absence	44/3,796	reference	reference	reference
	Presence	10/604	1.49 (0.75, 2.97)	1.16 (0.57, 2.34)	1.15 (0.57, 2.33)
Body mass index, kg/m^2		54/4,400	0.99 (0.91, 1.07)	0.95 (0.87, 1.04)	0.95 (0.87, 1.04)
Smoking status	Nonsmokers	51/4,134	reference	reference	reference
	Smokers	3/266	1.05 (0.33, 3.38)	0.93 (0.29, 2.98)	0.84 (0.26, 2.75)
Snoring during sleep, %	No	14/1,825	reference	reference	reference
	1-3 nights/week	28/2,087	1.71 (0.90, 3.26)	1.65 (0.86, 3.16)	1.65 (0.86, 3.17)
	≥ 4 nights/week	12/488	3.19 (1.48, 6.91)	2.58 (1.16, 5.77)	2.56 (1.15, 5.73)
Physical activity, MET-hours		54/4,400	1.00 (0.98, 1.01)	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)
Alcohol drinking status	Nondrinkers	40/3,223	1.11 (0.59, 2.07)	-	0.75 (0.38, 1.47)
	1 drink/day	13/1,093	reference	-	reference
	≥ 2 drinks/day	1/84	1.23 (0.16, 9.44)	-	1.47 (0.19, 11.35)

Model 1: Data are adjusted for all potential risk factors, except alcohol drinking status, listed in the table.
Model 2: Data are further adjusted for alcohol drinking status with covariates in Model 1.

($P < 0.01$), have a greater BMI ($P < 0.05$), and consume more alcoholic beverages ($P < 0.001$) compared with those with the mutant alleles.

Associations between stroke risk and the ALDH2 Polymorphism and other risk factors by sex

Table 2 shows the association between stroke risk and *ALDH2* variants and potential risk factors among 4,011 men. Men with the wild-type of the *ALDH2* polymorphism showed an increased risk of stroke. Compared with the mutant alleles of the *ALDH2* polymorphism, the multivariate HR [95% CI] of stroke was 2.02 [1.03-3.99] for those with the wild-type before adjusting for alcohol consumption; values were not significant after adjustment. However, alcohol consumption itself was not significantly associated with stroke risk according to the multivariate model. The lowest risk of stroke was observed in light alcohol drinkers who consumed an average of 1 drink (15 g of alcohol) per day. In the second model that was further adjusted for alcohol consumption, aging, the presence of diabetes mellitus, and habitual snoring were observed to be significant risk factors of stroke. Men with diabetes mellitus had a 1.9-fold higher risk than those without. In addition, men who snored 4-7 nights per week had a 3-fold higher risk than non-snorers (Table 2). After adjusting for alcohol consumption, the previous results, except those for the *ALDH2* polymorphism, were not changed (Table 2).

Table 3 shows the association between stroke risk and *ALDH2* variants and potential risk factors for 4,454 women. The *ALDH2* polymorphism was not associated with the stroke risk, regardless of adjusting for alcohol consumption. In the second multivariate model that was further adjusted for alcohol consumption, aging and habitual snoring were observed to be significant risk factors of stroke. Women who snored 4-7 nights per week had a 2.6-fold higher risk of stroke than non-snorers (Table 3).

Associations between stroke risk and the ALDH2 polymorphism and other risk factors by Acohol drinking status

Multivariate results stratified by current status of alcohol drinking among men or women are presented in Table 4. Aging was found to be a significant risk factor, regardless of sex and alcohol drinking status. Although the association was not significant, carrying the wild-type of the *ALDH2* polymorphism generally increased the risk of stroke in male non-drinkers or drinkers, but generally decreased the risk in women. The presence of diabetes mellitus or snoring significantly increased the risk of stroke in male drinkers. Among women, the presence of snoring tended to increase the risk, but the association was not significant for either non-drinkers or drinkers.

Joint effects of the ALDH2 polymorphism and risk factors on stroke risk

Table 5 shows the joint effects of the *ALDH2* polymorphism and other significant risk factors such as aging, presence of diabetes mellitus, and snoring on stroke risk after taking into account other potential risk factors. Among men, the multivariate HR (95% CI) of stroke risk was 6.88 [2.78-17.05] for carriers of the wild-type aged 60 years or older compared with those with the mutant alleles aged 59 years or younger. Carriers of the wild-type allele with diabetes mellitus had a 3.6-fold [1.62-8.03] increased the risk of stroke compared with those with the mutant alleles without diabetes mellitus, while snorers with the wild-type had an 8.5-fold [1.16-61.74] increased risk compared with non-snorers with the mutant alleles among men. However, joining of the *ALDH2* polymorphism and snoring status was not significantly associated with stroke risk among

Table 4. Multivariate hazard ratios (HR) and 95% confidence interval (CI) of stroke incidence according to alcohol drinking status

Potential risk factors	Categories	Male nondrinkers (n = 1,119) HR (95% CI)	Male drinkers (n = 2,892) HR (95% CI)	Female nondrinkers (n = 3,263) HR (95% CI)	Female drinkers (n = 1,191) HR (95% CI)
ALDH2 variants	Mutant type	reference	reference	reference	reference
	Wild-type	2.26 (0.81, 6.25)	2.08 (0.74, 5.81)	0.83 (0.44, 1.58)	0.49 (0.11, 2.29)
Age, yrs		1.09 (1.02, 1.15)	1.10 (1.06, 1.14)	1.09 (1.04, 1.13)	1.07 (1.00, 1.15)
Family history of stroke	Absence	reference	reference	reference	reference
	Presence	2.27 (0.73, 7.06)	0.96 (0.37, 2.48)	1.57 (0.67, 3.76)	0.69 (0.09, 5.38)
Hypertension	Absence	reference	reference	reference	reference
	Presence	2.36 (0.89, 6.23)	1.17 (0.65, 2.11)	0.98 (0.50, 1.93)	1.23 (0.38, 3.99)
Diabetes mellitus	Absence	reference	reference	reference	reference
	Presence	1.55 (0.54, 4.50)	2.11 (1.14, 3.92)	0.85 (0.35, 2.07)	1.33 (0.28, 6.19)
Hypercholesterolemia	Absence	reference	reference	reference	reference
	Presence	0.85 (0.19, 3.81)	1.38 (0.64, 3.02)	1.04 (0.45, 2.40)	1.69 (0.45, 6.34)
Body mass index, kg/m^2		0.98 (0.83, 1.15)	1.03 (0.93, 1.14)	0.97 (0.87, 1.07)	0.93 (0.78, 1.11)
Smoking status	Nonsmokers	reference	reference	reference	reference
	Smokers	1.46 (0.51, 4.12)	1.29 (0.72, 2.30)	1.23 (0.29, 5.21)	0.51 (0.07, 4.06)
Snoring during sleep, %	No	reference	reference	reference	reference
	1-3 nights/week	2.20 (0.59, 8.16)	1.61 (0.77, 3.37)	1.70 (0.79, 3.67)	1.45 (0.41, 5.10)
	$\geq$ 4 nights/week	3.31 (0.75, 14.55)	2.86 (1.25, 6.52)	2.55 (0.99, 6.51)	2.46 (0.51, 11.97)
Physical activity, MET-hours		0.96 (0.93, 1.00)	1.00 (0.99, 1.02)	0.98 (0.96, 1.00)	1.02 (0.99, 1.05)

Data are adjusted for all potential risk factors listed in the table.

Table 5. Multivariate hazard ratios (HR) and 95% confidence interval (CI) for joint effects of the *ALDH2* polymorphism and other risk factors on stroke incidence

Risk factors		*ALDH2* variants	Unadjusted HR (95% CI)	Multivariate HR (95% CI)
Among men				
Age	< 60 yrs	Mutant type	reference	reference
	≥ 60 yrs	Mutant type	1.97 (0.56, 6.99)	2.27 (0.63, 8.19)
	< 60 yrs	Wild-type	1.50 (0.62, 3.65)	1.43 (0.59, 3.48)
	≥ 60 yrs	Wild-type	6.41 (2.67, 15.36)	6.88 (2.78, 17.05)
Diabetes mellitus				
	Absence	Mutant type	reference	reference
	Presence	Mutant type	1.03 (0.13, 8.13)	0.87 (0.11, 6.97)
	Absence	Wild-type	1.59 (0.77, 3.29)	1.75 (0.84, 3.65)
	Presence	Wild-type	4.76 (2.17, 10.46)	3.61 (1.62, 8.03)
Snoring status				
	Non-snoring	Mutant type	reference	reference
	Snoring	Mutant type	4.24 (0.54, 33.47)	5.07 (0.64, 40.16)
	Non-snoring	Wild-type	4.82 (0.63, 36.87)	4.73 (0.62, 36.27)
	Snoring	Wild-type	7.43 (1.02, 58.90)	8.45 (1.16, 61.74)
Among women				
Age	< 60 yrs	Mutant type	reference	reference
	≥ 60 yrs	Mutant type	2.38 (0.92, 6.16)	2.11 (0.79, 5.63)
	< 60 yrs	Wild-type	0.73 (0.33, 1.63)	0.73 (0.33, 1.63)
	≥ 60 yrs	Wild-type	2.14 (0.97, 4.73)	1.98 (0.87, 4.51)
Snoring status				
	Non-snoring	Mutant type	reference	reference
	Snoring	Mutant type	1.75 (0.62, 4.97)	1.51 (0.53, 4.33)
	Non-snoring	Wild-type	0.69 (0.23, 2.06)	0.67 (0.22, 2.01)
	Snoring	Wild-type	1.47 (0.57, 3.82)	1.30 (0.49, 3.41)

Data are adjusted for all potential risk factors listed in the Table 4.

women.

DISCUSSION

In this prospective cohort study, we found that carrying the wild-type *ALDH2* polymorphism is a risk factor of stroke in men. After taking into account age, family history of stroke, presence of hypertension, diabetes mellitus, and hypercholesterolemia, BMI, smoking status, snoring status, and physical activity as potential risk factors of stroke, men carrying the wild-type of the *ALDH2* polymorphism had a 2-fold higher risk of developing stroke compared with carriers of the mutant alleles. However, this association was attenuated after further adjusting for alcohol consumption. Stroke risk associated with the wild-type allele generally increased in both non-drinkers and drinkers with recent alcohol consumption. Thus, alcohol consumption may mediate this association and the *ALDH2* polymorphism may reflect not only recent alcohol consumption but also alcohol drinking behavior in the remote past. Among women, no association between the *ALDH2* polymorphism and stroke risk was observed.

It is well-known that alcohol drinking behavior is associated with the *ALDH2* polymorphism among Asian populations [1,3] and with the alcohol dehydrogenase gene (*ADH*) polymorphism among non-Asian populations [17]. The *ALDH2* * 504Lys variant is present in up to 41% of Asians, 0 to 2.5% of Africans, and 0 to 1.5% of other ethnicities [18]. Carriers of the heterozygous variant allele have low ALDH2 enzyme activity and those with the homozygous allele have null activity [1]. Thus, these carriers have no or a low capacity to eliminate toxic acetaldehyde and are likely to show aversive physiological responses such as facial flushing, hypotension, palpitations, tachycardia, and vomiting even with small amounts of alcohol consumed [1,19]. Such unpleasant reactions are advantageous in terms of alcohol drinking behavior because carriers of the *ALDH2* * 504Lys variant generally drink less alcohol or are less likely to become alcoholic [19]. However, some studies reported disadvantageous influences of the *ALDH2* * 504Lys variant on CAD [9-11].

To the best of our knowledge, the present investigation is a sole epidemiological study involving a large sample to evaluate the association between the *ALDH2* * 504Lys variant and stroke risk. A cross-sectional study first examined brain magnetic resonance imaging in 376 men and women and found that men without the *ALDH2* * 504Lys variant are more likely to have multiple lacunar infarcts [20]. No studies have reported the association between *ALDH2* polymorphisms and stroke, whereas some studies have investigated the association between *ADH* polymorphisms and stroke [21,22]. In our study, analysis stratified by sex showed that the risk of stroke was significantly associated with the wild-type *ALDH2* polymorphism in men, but not in women. This finding may be due in part to the social climate to tolerate heavy alcohol drinking for men, but not for women (particularly for house-wives), in Korea. Additionally, we speculate for men that the *ALDH2* polymorphism reflects alcohol consumption, not only in the past 30 days but also in the remote past, in its association with stroke. Because men carrying the wild-type polymorphism are expected to have biological tolerance towards heavy alcohol drinking, even those who did not drink alcohol recently or regularly may have drunk alcohol occasionally under the social climate to promote alcohol drinking. Thus, the increased stroke risk associated with the *ALDH2* polymorphism among male non-drinkers may be related to occasional binge drinkers in non-drinkers. A joint analysis of the presence of diabetes mellitus or snoring with carrying the wild-type allele showed additive effects on stroke risk. Some data support that alcohol drinking aggravates snoring [23], which was unclear in our data. Interestingly, the magnitude of the association between snoring and stroke risk appeared to be greater in this study than in other studies [24-26]. Other risk factors of stroke such as family history of stroke and hypercholesterolemia were not associated with stroke even in the univariate analysis and these results may be partly due to small numbers of cases.

Our findings support an association between alcohol drinking and stroke [14], although the independent effects of the *ALDH2* polymorphism on stroke cannot be ruled out. Previous studies showed a role for ALDH2 enzyme activity in protecting heart against ischemic damages [27] and in bioactivation of nitroglycerin, which is widely used to treat patients with heart disease [28]. However, these data imply disadvantageous effects of null or low activity of ALDH2 enzyme, which is a phenotype for carriers of the *ALDH2* * 504Lys variant. Thus, it is unclear whether carrying the *ALDH2* * 504Lys variant is protective against stroke independent of alcohol drinking. The biological mechanisms

underlying the association between heavy alcohol consumption and stroke includes alcohol-induced hypertension, cardiac arrhythmias, blood clotting, and reduction of cerebral blood flow [29].

Strengths of our study include its population-based design, the large sample analyzed with genetic data, prospective follow-up of stroke cases, and inclusion of a broad range of confounding factors. In the interpretation of our findings, however, some limitations should be noted. In our study, most events of stroke were documented based on questionnaire data since pathological information was unavailable, although we confirmed some cases through medical records. Vital registration data from which death cases of stroke were collected are quite accurate since the registration system requires a medical certificate of death. Therefore, misclassification of incident events may have occurred. Particularly, the incidence of stroke may be underestimated because of unreported cases, healthy cohorts, or persons lost during follow-up. The prevalence of self-reported stroke among Korean adults between 40 and 69 years from national survey data is approximately 1.5%, while it was 1% in our cohorts. However, potential under-reporting is likely to randomly occur, resulting in attenuation of the association. We were also unable to genotype the *ALDH2* Glu504Lys polymorphism. Instead, we utilized rs2074356, which appears to be a reasonable surrogate marker of *ALDH2* Glu504Lys, according to previous reports [3,15]. Although rs2074356 is an intronic SNP, its contribution to the development of stoke independent of *ALDH2* Glu504Lys cannot be ruled out. It is an emerging research field to identify the role of intronic SNPs in gene expression. Finally, it is difficult to generalize our results, and thus further investigations are necessary to analyze other ethnic groups.

In summary, this prospective cohort study showed that men carrying the wild-type *ALDH2* polymorphism had a 2-fold higher risk of developing stroke compared with carriers of the mutant allele after taking into account a broad range of confounding factors. Among women, no association between the *ALDH2* polymorphism and stroke risk was observed. Given the hypothesis that alcohol consumption mediates the association between the *ALDH2* polymorphism and stroke risk, heavy alcohol consumption should be avoided for high-risk populations with genetic predisposition to stroke.

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