

INTRODUCTION

While heavy episodic drinking has been shown to be harmful to the heart, moderate alcohol consumption is thought to be protective against cardiovascular disease, through the regulation of rising HDL cholesterol levels.¹ The cardio-protective effect of alcohol is now not thought to vary by beverage type. In fact, evidence for an additional cardio-protective effect of antioxidant polyphenols in red wine is weak.²⁻³ The study of genetics variants of the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) genes involved in alcohol metabolism is important to understand the patterns of drinking habits and its effects in stroke susceptibility. The enzyme alcohol dehydrogenase (ADH), which oxidizes alcohol to acetaldehyde, has proven to play an important role in alcohol metabolism. Seven genes encoding ADH are found in a tight cluster on chromosome 4 and some are polymorphic in white European populations.⁴ More active variants of ADH cause higher concentrations of acetaldehyde in the body following alcohol consumption and are therefore protective against drinking. Functional variants in both ADH1B and ADH1C have been associated with alcohol consumption or alcohol dependence.⁵⁻⁹ The ADH1B variant (rs1229984) has emerged as the most strongly associated with alcohol phenotypes and is therefore the most suitable instrument for Mendelian randomization studies in Europeans.⁶ The A allele has an allele frequency of approximately 2-5% in Europeans and plays a protective role against heavy drinking because it confers an higher alcohol metabolic rate and consequent accumulation of toxic acetaldehyde.¹⁰⁻¹¹

Hence we analyzed if a possible association of the SNP rs1229984 with stroke susceptibility is mediated by the patterns of alcohol consumption.

METHODS

We genotyped SNP rs1229984 using a TaqMan Drug Metabolism Genotyping Assay in 569 stroke patients with extended clinical and lifestyle information and in 433 controls with matching clinical and lifestyle information (Table 1). Case-control analysis was performed in SPSS (v20.0) and p-values and odds ratio were calculated using Pearson's *chi*-squared test. The interpretation of the results allowed to determine the effect of the genetic variant on stroke susceptibility. All genotyping plates contained quality control samples: no-template controls, HapMap individuals and duplicated samples within and across genotyping plates.

Table 1 – Characterization of the studied population

Characteristic	Controls	Patients	P
Age and Gender			
Age, mean±SD (years)	64.0±5.5	51.6±8.9	<10 ⁻⁴ *
Gender (male), n/N (%)	201/433 (46.4)	362/569 (63.6)	<10 ⁻⁴ *
Stroke type			
Ischemic stroke, n/N (%)	-	451/569 (79.3)	-
Hemorrhagic stroke, n/N (%)	-	107/569 (18.8)	-
Unknown type of stroke, n/N (%)	-	11/569 (1.9)	-
Stroke Risk Factors			
Hypertension (>85-140mmHg), n/N (%)	157/426 (36.9)	313/500 (62.6)	<10 ⁻⁴ *
Diabetes, n/N (%)	52/412 (12.6)	85/526 (16.2)	0.128 [†]
Hypercholesterolemia, n/N (%) (cholesterol > 200mg/dl)	296/433 (68.3)	329/519 (63.4)	0.108 [†]
Smoking, n/N (%)	120/423 (28.4)	257/557 (46.1)	<10 ⁻⁴ *
Drinking, n/N (%)~			<10 ⁻⁴ *
None	254/407 (62.4)	242/558 (43.4)	
Moderate consumption of alcohol	82/407 (20.1)	49/558 (8.8)	
Excessive consumption of alcohol	71/407 (17.4)	267/558 (47.8)	

SD – standard deviation; ~Alcohol consumption was divided in three categories, according to the amount of alcohol consumed/day. None: <1 beverage/day; Moderate consumption: 1 beverage/day for females and 1-2 beverages/day for males; Excessive consumption: >1 beverage/day for females and >2 beverages/day for males (Rimm & Moats, 2007); * Mann-Whitney test; [†] Pearson's *Chi*-squared test.

RESULTS

The SNP met quality control criteria and was further evaluated.

Genotyping results are shown in Table 2. AA and AG genotypes were taken together for further analysis to enable a better statistical analysis. Association analysis between each two variables was performed and its results are shown in Table 3.

Table 2 – Genotyping results

Stroke	Alcohol Consumption	Genotype			Total
		GG	AG	AA	
Patients	None	208	32	2	242
	Moderate consumption	43	6	0	49
	Excessive consumption	242	23	2	267
	Total	493	61	4	558
Controls	None	214	37	3	254
	Moderate consumption	63	17	2	82
	Excessive consumption	58	12	1	71
	Total	335	66	6	407
Total	None	422	69	5	496
	Moderate consumption	106	23	2	131
	Excessive consumption	300	35	3	338
	Total	828	127	10	965

Table 3 – Association analysis results

Genotype	Stroke	Alcohol consumption
	Stroke	0.008
Stroke	-	<10 ⁻⁴

On the associations analysis with significant results (genotype-stroke and alcohol consumption-stroke) we tested whether the third variable (alcohol consumption and genotype, respectively) was modifying the observed effect. As Tables 4 and 5 illustrate alcohol consumption modifies the association genotype-stroke ($P=0.008$) with excessive alcohol consumption playing the largest effect on this modification ($P=0.034$). Genotype also modifies the association alcohol consumption-stroke ($P<10^{-4}$).

Table 4 – Modifying effect of alcohol consumption on the association genotype-stroke

Alcohol Consumption	Genotype	Stroke		Total	P	OR
		Patients	Controls			
None	AA and AG	N 34 % 14.0%	N 40 % 15.7%	74 14.9%	0.596	1
	GG	N 208 % 86.0%	N 214 % 84.3%	422 85.1%		
	Total	N 242 % 100.0%	N 254 % 100.0%	496 100.0%		
Moderate Consumption	AA and AG	N 6 % 12.2%	N 19 % 23.2%	25 19.1%	0.124	0.68
	GG	N 43 % 87.8%	N 63 % 76.8%	106 80.9%		
	Total	N 49 % 100.0%	N 82 % 100.0%	131 100.0%		
Excessive Consumption	AA and AG	N 25 % 9.4%	N 13 % 18.3%	38 11.2%	0.034	4.17
	GG	N 242 % 90.6%	N 58 % 81.7%	300 88.8%		
	Total	N 267 % 100.0%	N 71 % 100.0%	338 100.0%		
Total	AA and AG	N 65 % 11.6%	N 72 % 17.7%	137 14.2%	0.008	
	GG	N 493 % 88.4%	N 335 % 82.3%	828 85.8%		
	Total	N 558 % 100.0%	N 407 % 100.0%	965 100.0%		

Table 5 – Modifying effect of genotype on the association alcohol consumption-stroke

Genotype	Alcohol Consumption	Stroke		Total	P	OR
		Patients	Controls			
AA and AG	None	N 34 % 52.3%	N 40 % 55.6%	74 54.0%	0.005	0.85
	Moderate consumption	N 6 % 9.2%	N 19 % 26.4%	25 18.2%		
	Excessive consumption	N 25 % 38.5%	N 13 % 18.1%	38 27.7%		
	Total	N 65 % 100.0%	N 72 % 100.0%	137 100.0%		
GG	None	N 208 % 42.2%	N 214 % 63.9%	422 51.0%	<10 ⁻⁴	1
	Moderate consumption	N 43 % 8.7%	N 63 % 18.8%	106 12.8%		
	Excessive consumption	N 242 % 49.1%	N 58 % 17.3%	300 36.2%		
	Total	N 493 % 100.0%	N 335 % 100.0%	828 100.0%		
Total	None	N 242 % 43.4%	N 254 % 62.4%	496 51.4%	<10 ⁻⁴	
	Moderate consumption	N 49 % 8.8%	N 82 % 20.1%	131 13.6%		
	Excessive consumption	N 267 % 47.8%	N 71 % 17.4%	338 35.0%		
	Total	N 558 % 100.0%	N 407 % 100.0%	965 100.0%		

DISCUSSION AND CONCLUSIONS

- (1) In this study we did not find an association between the SNP rs1229984 and alcohol phenotype. However, further studies in larger populations are needed to confirm these results. Also it would be interesting to observe whether the activity of ADH1B varies within groups of individuals sharing the same genotype, which might influence individual alcohol tolerance.
- (2) We tested whether the amount of alcohol consumed was modifying the association genotype-stroke and we observed, as expected¹², that heavy drinking increases in about four times (OR=4.17) stroke risk in the group of A allele non-carriers. On the other hand, A carriers present a decrease to about half (OR=0.46) stroke risk despite the excessive alcohol consumption, which suggests that the A allele plays a protective role on stroke susceptibility. Further studies are needed to validate the same trend in groups of individuals with lighter alcohol consumption.
- (3) We confirmed the importance of alcohol as a stroke risk factor. While moderate consumption appears to be protective (OR<1), heavy drinking is potentially harmful (OR>1), as previously mentioned.¹ However, when testing whether the genotype was modifying the association alcohol consumption-stroke, we observed a protective role of A allele, which is consistent with previous results. In fact, considering individuals with none or moderate alcohol consumption, A carriers show a decreased stroke risk when compared with non-carriers (0.85>OR_{A carriers}>0.32 vs 1>OR_{A non carriers}>0.68). Individuals consuming excessive amounts of alcohol, despite an increased stroke risk, have larger probability of developing an event if they are A non carriers (OR_{A carriers}=1.92 vs OR_{A non carriers}=4.17).

In conclusion, A allele of the SNP rs1229984 appears to be protective against stroke. However, further studies are needed to replicate these results in other populations. Because the associations analyzed are complex, functional studies including other relevant genetic variants, such as ALDH, should be performed. Physiological studies including variables such as hypertension and hypercholesterolemia would also be interesting.