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Original Article

Apolipoprotein A1 rs5070 A/G polymorphism with stroke subtypes in Taiwan

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Abstract

Background: Apolipoprotein A1 (ApoA1) is a structural protein of high-density lipoprotein cholesterol; ApoA1 is involved in lipid and cholesterol metabolism. This study evaluated the association of ApoA1 polymorphism (rs5070) with different stroke subtypes in Taiwanese individuals.

Methods: A total of 2139 cases, including 614 controls and 708 large artery atherosclerosis (LAA), 377 small-vessel occlusion, and 440 hypertensive intracranial hemorrhage cases, were enrolled in this study. ApoA1 polymorphism was genotyped through polymerase chain reaction amplification and then subjected to mass-assisted laser desorption ionization time-of-flight mass spectrometry using a Bruker SNP genotyping system in the National Center for Genome Medicine (Academia Sinica, Taipei, Taiwan; http://ncgm.sinica.edu.tw/).

Results: The frequency of ApoA1 rs5070 dominant genotype (AA vs. AG + GG) was not significantly different among LAA, small-vessel occlusion, and hypertensive intracranial hemorrhage groups compared with that of the control group. Compared with diabetic patients with the AA allele, those with the AG + GG allele of ApoA1 rs5070 polymorphism showed a 1.58-fold likelihood of developing LAA (odds ratio = 1.58; 95% confidence interval = 1.00–2.42; $p = 0.046$), but not small-vessel occlusion or hypertensive intracranial hemorrhage. In male diabetic patients, the odds ratio increased to 1.90-fold.

Conclusion: Our findings suggested that genetic polymorphisms of ApoA1 rs5070 A/G may play a role in the susceptibility to LAA among male diabetic patients.

Keywords: apolipoprotein A1; diabetes mellitus; genetics; polymorphism; stroke

1. Introduction

Stroke is a leading cause of death and the main cause of serious disability worldwide.[1] In Taiwan, stroke is the third leading cause of death. Stroke can be divided into three subtypes, namely, ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage.[2,3] Although each subtype presents distinct pathogenic factors,[2] several conventional common risk factors, including obesity, hypertension, diabetes, dyslipidemia, and smoking, are responsible for stroke development.

Dyslipidemia, characterized by high triglyceride, high total cholesterol, high low-density lipoprotein cholesterol (LDL-C) and low high-density lipoprotein cholesterol (HDL-C) levels, is associated with an increased risk of major cardiovascular disease (CAD) and stroke.[3] HDL-C mediates the transport of cholesterol from peripheral tissues to the liver; as a result,

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plasma cholesterol levels decrease. HDL-C also elicits protective effects against CAD and stroke because HDL-C exhibits antiatherogenic, antioxidant, and anti-inflammatory properties.[3] Hence, high HDL-C levels may prevent the occurrence of CAD and stroke.[4] Apolipoprotein A1 (ApoA1) is a major component of HDL-C and may be correlated with dyslipidemia, insulin resistance, and metabolic syndromes.[5] Several ApoA1 polymorphisms correlated with serum HDL-C level are also associated with human diseases.[6,7] A high A allele frequency of ApoA1-75G/A polymorphism is related to lower HDL-C and ApoA1 levels in northern Indians.[8] CAD prevalence is significantly higher in carriers with the A allele than in carriers with the G allele.[8] Morcillo et al[9] demonstrated that the AA genotype of ApoA1-75G/A polymorphism is a risk factor for type 2 diabetes. HDL-C level is high in healthy Chinese people with the AA genotype.[10] The HDL-C level is also high in healthy male carriers with the CT heterogenotype of ApoA1 + 83 C/T.[11] HDL-C and glycosylated hemoglobin levels are similarly high in carriers with the C allele of APoA1 rs12721026 in Brazil.[7] Chen et al[6] demonstrated that the G allele of ApoA1-75G/A polymorphism is associated with hypertension; furthermore, the C allele of +83 C/T polymorphism is correlated with hypertension in CAD carriers. *ApoA1* gene polymorphism also predicts the cardiometabolic risk of South Asian immigrants in the USA.[12] However, studies have yet to determine the relationship between *ApoA1* gene polymorphism and stroke in Taiwanese patients. Thus, this study aimed to investigate the association of ApoA1 rs5070 polymorphism and different stroke subtypes in Taiwanese people.

2. Methods

2.1. Study population

All the patients hospitalized in the Chang Gung Memorial Hospital due to stroke were enrolled in this study. This study included 1082 patients with ischemic stroke and 440 with hypertensive intracranial hemorrhage (HICH). In addition, 614 individuals with normal neurological examination and physical checkup in the same hospital were recruited as normal controls. The study protocol was performed in compliance with the Helsinki Declaration and approved by the institutional review board of Chang Gung Medical University Hospital. Informed consent was signed by eligible patients and controls.

Based on the results of investigative examinations (brain computed tomography or magnetic resonance imaging, electrocardiogram, and Holter monitor), those patients with ischemic stroke were subclassified on the basis of the definitions presented by the Trial of Org 10172 in Acute Stroke Treatment definition[13]: large artery atherosclerosis (LAA; hypodense lesions with a diameter of >15 mm and >50% stenosis in the appropriate extra- or intracranial arteries) and small-vessel occlusion (SVO; small, subcortical, hypodense lesions with a diameter of <15 mm and corresponding to clinical lacunar syndrome). Those with cardioembolic strokes were excluded from this study because these cases could result from different etiologies. Individuals with strokes occurring in the course of systemic conditions, such as coagulopathies, iatrogenic causes, immunological disorders, or undetermined or unclassified etiologies, were also excluded to ensure the solely atherosclerotic etiology of our patients. HICH was defined if hematoma was located in the basal ganglion (putamen and thalamus), cerebellum, and pons; HICH was also considered if the hematoma was mainly caused by hypertension.

A detailed medical history was obtained, and the participants were subjected to a thorough neurological examination. Demographic data, laboratory findings, and established vascular risk factors were recorded. Arterial hypertension was documented when patients yielded a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg; arterial hypertension was also recorded when patients currently received antihypertensive medication. Diabetes mellitus was considered present if fasting plasma glucose level was >126 mg/dL and HbA1C was ≥6.5%, or if patients currently received antidiabetic drug treatment. Smoking activity within the last 6 months was considered as current. Alcohol consumption was defined as consumption of ≥30 g of alcohol per day within the last 6 months. Whole-blood samples from the participants were used to isolate peripheral blood leukocytes for genetic analysis.

2.2. Genetic analysis

In genotyping, ApoA1 rs5070 polymorphism was determined using the Bruker SNP genotyping system. Genomic DNAs were prepared from patients' peripheral blood samples using GentraPuregene DNA purification kits (Qiagen, Frankfurt, Germany). The genomic DNAs (50 ng) were amplified through polymerase chain reaction and allele-specific primer extension reaction. Primer extension products were purified using a GenePureolig purification system (Bruker Corporation, Billerica, Massachusetts, USA) and subjected to matrix-assisted laser desorption ionization time-of-flight mass spectrometry analysis. The procedures were performed in the National Center for Genome Medicine (Academia Sinica, Taipei, Taiwan; http://ncgm.sinica.edu.tw/).

2.3. Statistical analysis

Genotyping and allele frequency between controls and stroke patients were analyzed using chi-square test and Hardy–Weinberg equilibrium. Logistic regression analysis was performed to estimate the odds ratio (OR) and 95% confidence interval (CI), which were adjusted with several risk factors as confounders. Data were analyzed with Statistical Analytic System for Windows (SAS Institute Inc., Cary, NC, USA). Significant difference was set at $p < 0.05$.

3. Results

A total of 2136 cases, including 614 controls, and 707 LAA, 375 SVO, and 440 HICH cases, were enrolled in this study. Clinical parameters between the control individuals and

patients with different stroke subtypes are listed in Table 1. The average age and serum HDL-C level were significantly lower in all the stroke subtypes than those of the control group ($p < 0.001$). Female gender, hypertension, smoking, alcohol consumption, lower cholesterol level, and no family history of CAD were high risk factors of stroke development. LAA ($p < 0.001$) and SVO ($p < 0.001$) frequencies, but not HICH frequency ($p = 0.956$), were significantly higher in patients with diabetes than in the control individuals. Low serum LDL-C and no family history of stroke were associated with HICH, but not with LAA and SVO. Serum triglyceride level was associated with SVO, but not with LAA or HICH.

Table 2 presents the rs5070 genotypes in the control individuals and patients with different stroke subtypes. The frequency of ApoA1 rs5070 polymorphism in all the stroke subtypes was not significantly different from that in the control group. After age, gender, hypertension, diabetes, triglyceride level, cholesterol level, smoking, alcohol consumption, family history of stroke, family history of CAD, and LDL-C and HDL-C levels were adjusted, the difference of frequency in the polymorphisms between the two groups was not significant.

The association of rs5070 dominant genotype (AA/AG + GG) with the clinical parameters is summarized in Table 3. Patients with AG + GG genotypes showed a more significant family history of CAD than those with the AA genotype. Age, gender, hypertension, diabetes, serum cholesterol, smoking, alcohol, triglyceride, family history of stroke, LDL-C, and HDL-C were not associated with ApoA1 rs5070 polymorphism. The HDL-C levels were 49.9 ± 13.2, 41.6 ± 12.7, 44.8 ± 12.6, and 44.2 ± 16.3 in the control individuals with the GG genotype, LAA, SVO, and HICH, respectively.

Compared with that of the patients with the AA allele, the OR of the diabetic patients with the AG + GG allele was 1.58 (95% CI = 1.00–2.42; $p = 0.046$; Table 4), associated with the development of LAA but not with the development of SVO (OR = 1.21; 95% CI = 0.71–2.07; $p = 0.428$) or HICH (OR = 0.62; 95% CI = 0.91–2.87; $p = 0.100$). Furthermore, the OR of male diabetic patients with the AG + GG allele associated with the development of LAA was increased to 1.90 (95% CI = 1.04–3.46; $p = 0.035$). The OR of female diabetic patients with the AG + GG allele associated with the development of LAA was not significant (OR = 1.23; 95% CI = 0.59–2.59; $p = 0.577$; Table 5).

4. Discussion

Stroke is the final event in a complex interplay between the environment and genes.[14] In this study, several conventional risk factors, such as age, gender, hypertension, diabetes, and cholesterol levels, were associated with stroke. Epidemiological studies have shown that the prevalence of stroke is lower

Table 1
Clinical parameters in controls and different stroke subtypes.

	Control ($n = 614$)	LAA ($n = 707$)	SVO ($n = 375$)	HICH ($n = 440$)
Age (y)	62.19 ± 11.90	66.64 ± 11.45**	59.26 ± 12.14**	56.3 ± 12.74**
Gender				
Female	279 (45.4%)	141 (19.9%)**	127 (33.9%)**	122(27.7%)**
Male	335 (54.6%)	566 (80.1%)	248 (66.1%)	318 (72.3%)
Hypertension				
No	295 (48.0%)	138 (19.5%)**	81 (21.6%)**	40 (9.1%)**
Yes	319 (52.0%)	569 (80.5%)	294 (78.4%)	400 (90.9%)
Diabetes				
No	499 (81.3%)	438 (62.0%)**	263 (70.1%)**	357 (81.1%)
Yes	115 (18.7%)	269 (38.0)	112 (29.9%)	83 (18.9%)
Triglyceride	149.93 ± 112.23	160.17 ± 113.47	172.59 ± 223.75*	152.73 ± 150.52
Cholesterol	197.15 ± 39.103	186.10 ± 45.04**	193.77 ± 45.68	181.40 ± 39.97**
Smoking				
No	464 (75.6%)	306 (43.3%)**	204 (54.4%)**	254 (57.7%)**
Yes	150 (24.4%)	401 (56.7%)	171 (45.6%)	186 (42.3%)
Alcohol				
No	520 (84.7%)	529(74.8%)**	287 (76.5%)**	313 (71.1%)**
Yes	94 (15.3%)	178(25.2%)	88 (23.5%)	127 (28.9%)
Stroke history				
No	367 (59.8%)	432 (63.1%)	217 (57.9%)	292 (66.4%)**
Yes	247 (40.2%)	275 (36.9%)	158 (42.1%)	148 (33.6%)
CAD history				
No	500 (81.4%)	621 (87.8%)**	331 (88.3%)**	411 (93.4%)**
Yes	114 (18.6%)	86 (12.2%)	44 (11.7%)	29 (6.6%)
LDL	117.03 ± 34.96	115.66 ± 35.43	117.01 ± 36.63	108.05 ± 36.65**
HDL	50.25 ± 13.48	41.10 ± 13.15**	45.06 ± 13.94**	44.88 ± 15.20**

* $p < 0.05$ compared with the control group.
** $p < 0.001$ compared with the control group.
CAD = cardiovascular disease; HDL = high-density lipoprotein; HICH = hypertensive intracranial hemorrhage; LAA = large artery atherosclerosis; LDL = low-density lipoprotein; SVO = small-vessel occlusion.

Table 2
Frequencies of RS5070 genotypes in 614 controls and 707 LAA, 375 SVO, and 440 HICH cases.

	Control	LAA	SVO	HICH
AA	342 (55.7%)	375 (53.0%)	211 (56.3%)	250 (56.8%)
AG	237 (38.6%)	290 (41.1%)	134 (35.7%)	165 (37.5%)
GG	35 (5.7%)	42 (5.9%)	30 (8.0%)	25 (5.7%)
AA	342 (55.7%)	375 (53.0%)	211 (56.3%)	250 (56.8%)
AG + GG	242 (44.3%)	332 (47.0%)	164 (43.7%)	190 (43.2%)
OR (95% CI)	1	1.11 (0.90−1.38)	0.98 (0.75−1.27)	0.96 (0.75−1.22)
AOR[a] (95% CI)	1	1.281 (1.00−1.64)	1.048 (0.79−1.39)	1.052 (0.79−1.40)
AG + AA	579 (94.3%)	665 (94.1%)	345 (92.0%)	415 (94.3%)
GG	35 (5.7%)	42 (5.9%)	30 (8.0%)	25 (5.7%)
OR (95% CI)	1	1.05 (0.57−1.66)	1.44 (0.87−2.39)	1.00 (0.58−1.70)
AOR[a] (95% CI)	1	1.19 (0.71−2.00)	1.44 (0.84−2.47)	1.15 (0.62−2.10)

AOR = adjusted odds ratio; CAD = cardiovascular disease; CI = confidence interval; HICH = hypertensive intracranial hemorrhage; LAA = large artery atherosclerosis; OR = odds ratio; SVO = small-vessel occlusion.
[a] Adjusted for gender, hypertension, diabetes, smoking, alcohol, stroke history, and CAD history.

in premenopausal females than in age-matched males.[15] In this study, the incidence of different stroke subtypes was significantly lower in women than in men. Hypertension is a well-known critical risk factor of stroke development.[16] In Japan, the hazard ratios of stroke in normal (<120/80 mmHg), normal-to-high [(120−139) mmHg/(80−89) mmHg], hypertension stage I [(140−159) mmHg/(90−99) mmHg], and hypertension stage II (>160/100 mmHg) are 2.12, 2.43, 2.62, and 4.38 in men and 1.05, 1.29, 1.21, and 2.02 in women, respectively.[17] The Asia Pacific Cohort Studies Collaboration also indicated that an increase in systolic and/or diastolic blood pressure can increase the hazard ratio of stroke development.[18] Diabetes is also an independent risk factor of stroke development.[19] The risk of stroke is higher in diabetic patients than in normal individuals.[20] However, intensive glucose control in diabetic patients is not associated with a decreased incidence of CAD.[20] Hypercholesterolemia is associated with an increased risk of stroke.[18] Ebrahim et al[21] demonstrated that a high serum cholesterol level is associated with ischemic stroke, and a low cholesterol level is correlated with hemorrhagic stroke. By contrast, our results indicated that low cholesterol levels were associated with both ischemic and hemorrhagic strokes. We could not exclude the possibility that low lipid levels in stroke patients were attributed to lipid-lowering medications or other treatments because drug information was not available in our study. Further research should be conducted to elucidate the relationship between low cholesterol levels and ischemic stroke. In Korea and Japan, smoking of a large number of cigarettes per day increases the risk of ischemic stroke and subarachnoid hemorrhage, but not that of intracerebral hemorrhage.[22,23] An increased HDL-C level reduces not only the risk of CAD and stroke, but also the recurrence of stroke.[24] In our study, the HDL-C level of the stroke patients was significantly lower than that of the control group. Therefore, conventional risk factors, such as gender, age, hypertension, diabetes, serum cholesterol level, smoking, and low HDL-C, were associated with different stroke subtypes.

Dyslipidemia is characterized by increased total cholesterol, LDL-C, and triglyceride levels, and decreased HDL-C levels. However, the association of ApoA1 polymorphism with serum HDL-C level remains controversial. The polymorphisms of ApoA1 + 84 TC, but not MspI, are associated with serum HDL-C.[25] The -75AA genotype of ApoA1 is independently associated with a high serum HDL-C in Turkish adults.[26] By contrast, the -75AA genotype of ApoA1 is associated with low serum HDL-C in Tunisians.[27] In our study, HDL-C level was not associated with ApoA1 rs5070 polymorphism in Taiwanese stroke patients. Likewise, Minnich et al[28] demonstrated that ApoA1-75A/G polymorphism was

Table 3
Association of RS5070 AA/AG + GG with clinical parameters.

	AA	AG + GG
Age (y)	61.74 ± 12.57	62.22 ± 12.59
Gender		
Male	360 (53.8%)	309 (46.2%)
Female	818 (55.8%)	649 (44.2%)
Hypertension		
No	303 (54.7%)	251 (45.3%)
Yes	875 (55.3%)	707 (44.7%)
Diabetes		
No	855 (54.9%)	702 (45.1%)
Yes	323 (55.8%)	256 (44.2%)
Triglyceride	163.076 ± 135.02	151.37 ± 158.77
Cholesterol	403.43 ± 577.44	401.78 ± 573.50
Smoking		
No	664 (54.1%)	564 (45.9%)
Yes	514 (56.6%)	394 (43.6%)
Alcohol		
No	903 (54.8%)	746 (45.2%)
Yes	275 (56.5%)	212 (43.5%)
Familial stroke history		
No	713 (54.5%)	595 (45.5%)
Yes	465 (56.2%)	363 (43.8%)
Familial CAD history		
No	1051 (56.4%)	812 (43.6%)*
Yes	127 (46.5%)	146 (53.5%)
LDL	113.83 ± 35.44	116.00 ± 36.57
HDL	45.15 ± 14.04	45.28 ± 14.57

* $p < 0.001$ compared with the control group.
CAD = cardiovascular disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table 4
Association of ApoA1 rs5070 AA/ AG + GG in controls and different types of strokes in nondiabetes and diabetic patients.

	Controls	LAA	OR (95% CI)	SVO	OR (95% CI)	HICH	OR (95% CI)
Nondiabetes							
AA	269 (53.9%)	234 (53.4%)	1	145 (55.1%)	1	207 (58.0%)	1
AG + GG	230 (46.1%)	204 (46.6%)	1.02 (0.79–1.32)	118 (44.9%)	0.95 (0.0–1.23)	150 (42.0%)	0.85 (0.64–1.11)
Diabetes							
AA	73 (63.5%)	114 (52.4%)	1	66 (58.9%)	1	43 (51.8%)	1
AG + GG	42 (36.5%)	128 (47.6%)	**1.58 (1.00–2.47)***	46 (41.1%)	1.21 (0.71–2.07)	40 (48.1%)	1.62 (0.91–2.87)

* $p < 0.05$.
ApoA1 = apolipoprotein A1; CI = confidence interval; HICH = hypertensive intracranial hemorrhage; LAA = large artery atherosclerosis; OR = odds ratio; SVO = small-vessel occlusion.

Table 5
Association of ApoA1 rs5070 AA/AG + GG of different genders in diabetes without or with LAA.

	Controls	LAA	OR (95% CI)	*p*
Female				
AA	32 (59.3%)	33 (54.1%)	1	0.577
AG + GG	22 (40.7%)	28 (45.9%)	1.23 (0.59–2.59)	
Male				
AA	41 (67.2%)	108 (51.9%)	**1**	**0.035**
AG + GG	20 (32.8%)	100 (48.1%)	**1.90 (1.04–3.46)**	

Bold indicates $p < 0.05$.
ApoA1 = apolipoprotein A1; CI = confidence interval; LAA = large artery atherosclerosis; OR = odds ratio.

not correlated with serum HDL-C level. ApoA1-75A/G is also not associated with 83 C/T polymorphism with HDL-C.[29] These observations suggested that the association of ApoA1 polymorphism with HDL-C level differs among distinct ethnic regions. Our findings also demonstrated that ApoA1 rs5070 polymorphism was correlated with a family history of stroke. Chien et al[30] revealed that a low risk of triglyceride was found in ApoA1-75A/A haplotype compared with the G/G haplotype. Furthermore, ApoA1-3013C and ApoA1-75G haplotypes were significantly associated with high triglyceride levels in Taiwanese people.[31]

Diabetes is a major risk factor of CAD and stroke; diabetes is also associated with high mortality among patients with such diseases.[1] Megherbi et al[32] revealed that ischemic stroke occurs more frequently in diabetic patients than in hemorrhage stroke or nonstroke patients. Ischemic stroke (45.4%) also yields a higher percentage than hemorrhage stroke (37.0%) among diabetic patients in Taiwan.[33] In our study, a significantly higher number of patients with LAA ($p < 0.001$) and SVO ($p < 0.001$), but not those with HICH, were diabetic. Compared with the AA allele, the ApoA1 AG + GG genotype in male diabetic patients, but not in female diabetic patients, was 1.90-fold more likely to develop LAA, but not SVO or HICH, although ApoA1 rs5070 A/G polymorphism was not significantly associated with stroke subtypes. To our knowledge, this study is the first to demonstrate the association of apolipoprotein gene polymorphism with stroke in male diabetic patients. The gender-specific association of APOA1 polymorphism with clinical presentations has also been reported; in particular, ApoA1-75GA heterogenotype is associated with high blood pressure and a low fasting serum glucose level in males but not in females.[9] APOA1-75 polymorphism also contributes to plasma cholesterol efflux capacity in females.[34] Therefore, the significant association of rs5070 AG + GG genotype with LAA is gender dependent.

One strength of our study is that all samples came from a population of pure Han origin, avoiding confounding factors of different ethnic backgrounds. Additionally, all ischemic stroke patients were carefully classified based on the results of investigations and the Trial of Org 10172 in Acute Stroke Treatment definition, leading to a safer conclusion regarding the relationship of the *ApoA1* gene and stroke. Nonetheless, our study had some limitations. First, although the current study is a retrospective analysis, all participants were collected prospectively, which might avoid some flaws of a retrospective study. Second, given the relatively small sample size, our study might have had insufficient detection of minor effects of risk factors other than diabetes.

In conclusion, stroke is a result of complex interactions among various environmental and genetic factors. Therefore, our study revealed that several conventional cardiovascular risk factors, such as age, gender, hypertension, diabetes, cholesterol level, smoking, alcohol, and HDL-C level, were associated with the risk of different stroke subtypes. In addition to serum triglyceride level and a family history of CAD, LAA development was significantly associated with ApoA1 rs5070 polymorphism in male diabetic patients. Further studies should be conducted to elucidate the interplay between the environmental and genetic factors of stroke.

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