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ANGIOTENSIN I - CONVERTING ENZYME GENE POLYMORPHISM AND ACTIVITY IN PATIENTS WITH ISCHEMIC STROKE

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Abstract

The possible association of ACE polymorphism with ischemic stroke (IS) was evaluated in 65 patients with IS and 330 age and BMI-matched controls. ACE genotypes were determined by polymerase chain reaction (PCR). There was no significant difference in ACE genotype/allele frequencies between case and control group ($p>0.05$). Patients with D allele had 4,7 times higher risk for large vessel IS than healthy persons D allele possessors. Persons with D allele had 9.2 times higher risk for large vessel disease than small vessel disease. These data suggest a possible association of ACE gene polymorphism with pathogenesis of large vessel IS.

INTRODUCTION

Stroke is a complex, multifactorial disease. It is one of the leading causes of disability and death. About 80% of all strokes are ischemic and have a polygenic basis. Advances in genetic epidemiology have revealed that some genetic variants increase the risk for IS. Evidence suggests that genetic variation in the rennin angiotensin-aldosterone system (RAAS) contributes to the risk of ischemic stroke. Among the various sequence variations in RAAS, the insertion/deletion (*I/D*) polymorphism in angiotensin I- converting enzyme (ACE) is the most extensively studied.

ACE is a dipeptidyl carboxylase that converts angiotensin I into the antinatriuretic vasoactive angiotensin II, an octapeptide involved in vasoconstriction, aldosterone production, and norepinephrine release from sympathetic nerve endings and inactivates bradykinin, a vasodilator and natriuretic substance. ACE is encoded by a 21-kb 26 exons gene located on chromosome 17 at q23. A deletion polymorphism in the ACE gene consists of the absence of a 287-bp Alu repetitive sequence in reverse orientation near the 3' end of intron 16. Mean ACE activity concentration in DD carriers are around twice those found in II carriers (1, 2).

The purpose of this study was: a) to report the distribution of ACE genotypes and alleles in patients with ischemic stroke (IS), b) to investigate the impact of ACE polymorphism on serum lipid and apolipoprotein concentrations, as well as on ACE activity and arterial blood pressure level.

Materials and Methods

Blood samples were obtained from 65 unrelated subjects with acute IS (mean age 41). These were proven by computed tomography or magnetic resonance of the brain. Acute stroke patients were separated into two groups: one with large vessel disease (45 patients) and the other with small vessel disease (lacunar stroke) (20 patients). The age and BMI-matched control group consisted of 330 unrelated Serbian people. They did not show any signs of cerebrovascular disease from their health questionnaires and clinical examinations. Informed consent was obtained from each participant in the study; and all procedures were in accordance with the Helsinki Declaration.

DNA was extracted by Triton X-100 lysis, proteinase K digestion and phenol/chloroform extraction. The reaction pertaining to ACE was performed with 20 pmol of the sense strand-specific primer SA66, 7 pmol of the anti-sense strand-specific primer 167, and 1.5 pmol of another I allele sense strand specific primer ACE3, as previously described (3). Amplification products pertaining to I/D polymorphism were separated by electrophoresis in 1.8 % (w/V) agarose gels. Alleles were identified according to their expected lengths. All gels were visualized and analyzed by GDS8000 gel documentation system (Ultra Violet Products Inc, Upland). ACE activity was determined in serum samples using commercial test (Trinity, Biotech). Lipid and apolipoprotein concentrations were determined by standard methods.

Statistical Analysis

Results are presented as mean ± S.D. for continuous normally distributed variables, and as percent for categorical data. Comparisons between normally distributed continuous variables were performed by the Student's t-test and ANOVA. If the distribution of quantitative variables was skewed, log-transformed values were used for the analysis. Association between categorical variables was tested by the chi-square test for contingency tables. Frequencies of genotypes/alleles were determined by the gene counting method. Analyses were conducted with the SPSS (version 11.1) software package.

Results

According demographic and clinical data of stroke patients and controls, we found that stroke patients were more often female, smokers, and hypertensive compared with controls. ACE genotypes and allele distribution among cases and controls were also determined and presented in Table 1. There was no significant difference in the distribution of ACE gene variant between cases and controls.

TABLE 1. GENOTYPE AND ALLELE FREQUENCIES OF ACE IN SERBIAN STROKE PATIENTS AND CONTROL SUBJECTS.

ACE		Stroke patients		Control subjects	
Genotypes	*II*	8	(0,123)	59	**(0,179)**
	ID	37	(0,569)	168	**(0,509)**
	DD	20	(0,308)	103	**(0,312)**
Allele frequency	*I*	52	(0.400)	286	**(0.433)**
	D	78	(0.600)	374	**(0.567)**

Table 2 shows the ACE genotype and allele distribution among patients with large vessel stroke and small vessel (lacunar) stroke. Patients with D allele had 9.21 (95% CI 1.67–50.96) times higher risk for large vessel disease than

lacunar stroke. Patients with D allel had 4,68 (95% CI 1.10–19.86) times higher risk for large vessel stroke than healthy persons D allele possessors.

Table 2. Genotype and Allele frequencies of ACE in subgroups of IS patients.

ACE	Genotip ACE	Large vessel IS		Lacunar stroke	
Genotypes	*II*	2	(0,044)	6	**(0,300)**
	ID	28	(0,600)	9	**(0,500)**
	DD	15	(0,356)	5	**(0,200)**
Allele frequency	*I*	32	(0,344)	21	**(0,550)**
	D	58	(0,656)	19	**(0,450)**

Patients-carriers of DD genotypes had significantly higher ($p<0,05$) tryglicerides, apolipoprotein B, higher ACE catalitic activity, and significantly lower ($p<0,05$) HDL-choleterol and apolipoprotein AI concentrations compared with control group. Patients with IS had higher levels of systolic blood pressure, diastolic blood pressure, mean arterial pressure and pulse pressure regardless of ACE genotype they possess. There was no significant difference in serum lipids, apolipoproteins and blood pressure levels according ACE genotype in stroke patients group. Patients with DD genotype had significantly higher catalitic ACE activity than patients with II or ID genotype. (Table 3)

TABLE 3. LIPID PROFILE, BP LEVELS AND ACE ACTIVITY IN RELATION TO ACE GENOTYPE IN IS PATIENTS AND CONTROLS.

	ACE genotype	Stroke patients		Control subjects		p, Student t-test
		Srednja vrednost	SD	Srednja vrednost	SD	
Total cholesterol (mmol/L)	*II*	5,10	0,96	5,77	1,32	>0,05
	ID	6,00	1,31	5,77	1,15	>0,05
	DD	6,60	1,86	5,76	1,36	>0,05
p (ANOVA)		>0,05		>0,05		
HDL-choleterol (mmol/L)	*II*	1,08	0,30	1,37	0,37	<0,05
	ID	1,18	0,55	1,38	0,36	<0,05
	DD	1,10	0,52	1,45	0,39	<0,05
p (ANOVA)		>0,05		>0,05		
LDL-cholesterol (mmol/L)	*II*	3,07	0,91	3,81	1,11	>0,05
	ID	3,61	1,41	3,75	1,00	>0,05
	DD	4,00	1,81	3,66	1,17	>0,05
p (ANOVA)		>0,05		>0,05		
Triglycerides (mmol/L)	*II*	1,76	0,83	1,31	0,86	>0,05
	ID	2,11	0,92	1,39	0,81	<0,05
	DD	2,08	0,89	1,43	0,80	<0,05
p (ANOVA)		>0,05		>0,05		
Apolipoprotein AI (g/L)	*II*	1,35	0,27	1,51	0,35	>0,05
	ID	1,33	0,35	1,47	0,33	>0,05
	DD	1,31	0,40	1,53	0,30	<0,05
p (ANOVA)		>0,05		>0,05		
Apolipoprotein B (g/L)	*II*	1,25	0,31	1,09	0,39	>0,05
	ID	1,40	0,55	1,19	0,42	<0,05
	DD	1,57	0,67	1,02	0,29	<0,05
p (ANOVA)		>0,05		<0,05		
Apolipoprotein E (g/L)	*II*	53,30	10,12	41,45	20,18	>0,05
	ID	40,13	11,83	39,77	19,52	>0,05
	DD	51,12	23,75	44,68	22,04	>0,05
p (ANOVA)		>0,05		>0,05		
Lipoprotein (a) (g/L)	*II*	0,25	0,22	0,18	0,16	>0,05
	ID	0,26	0,42	0,22	0,26	>0,05
	DD	0,17	0,16	0,19	0,13	>0,05
p (ANOVA)		>0,05		>0,05		
ACE (U/L)	*II*	18,83	10,91	18,78	6,59	>0,05
	ID	38,81	11,78	35,28	12.29	>0,05
	DD	67,95	20,21	57,40	13,25	<0,05
p (ANOVA)		<0,05		<0,05		
Systolic blood pressure (mmHg)	*II*	149,38	21,95	126,27	22,30	<0,05
	ID	164,32	29,93	128,54	20,48	<0,05
	DD	160,00	31,50	131,41	23,14	<0,05
p (ANOVA)		>0,05		>0,05		
Diastolic blood pressure (mmHg)	*II*	96,88	15,34	81,10	12,83	<0,05
	ID	97,30	13,57	81,49	11,06	<0,05
	DD	96,50	16,23	83,33	12,26	<0,05
p (ANOVA)		>0,05		>0,05		
Mean arterial pressure (mmHg)	*II*	114,37	16,08	96,16	15,29	<0,05
	ID	119,64	18,26	97,17	13,47	<0,05
	DD	117,67	20,97	99,36	14,85	<0,05
p (ANOVA)		>0,05		>0,05		
Pulse pressure (mmHg)	*II*	52,50	16,26	45,17	13,71	>0,05
	ID	67,03	19,91	47,05	13,40	<0,05
	DD	63,50	17,33	48,08	16,19	<0,05
p (ANOVA)		>0,05		>0,05		

DISCUSSION

This is the first study in Serbia that has examined the effect of ACE I/D gene polymorphism on IS risk. No significant evidence was provided to support our hypothesis that ACE gene polymorphism may represent a susceptibility mutation for IS. Previous reports on ACE gene polymorphism and IS produced conflicting results as to the importance of ACE alleles in predisposition to IS. We compiled results from 7 meta-analyses and 53 case-control studies (Table 4).

Table 4. Genetic association studies of ACE I/D polymorphism and ischemic stroke.

Study	Year	Methodology	Phenotype	Result
Sharma i sar. (4)	1994	Case–control: 100 patients, 73 controls	IS	Negative
Castellano i sar. (5)	1995	Cross-sectional: 199 patients	IMT	Positive
Dessi-Fulgheri i sar.(6)	1995	Case–control: 193 patients, 147 controls	IS	
Markus i sar. (7)	1995	Case–control: 100 patients, 137 controls	IS	Positive
Ueda i sar. (8)	1995	Case–control: 488 patients, 188 controls	IS	Negative
Catto i sar. (9)	1996	Case–control: 418 patients, 231 controls	IS	Negative
Hosoi i sar. (10)	1996	Cross-sectional: 288 patients	IMT NIDDM	Positive
Kario i sar. (11)	1996	Case–control: 138/90 patients, 90/104 controls	IS, lacunar stroke in hypertensive. Hypertensive/normotensive control	Positive
Margaglione i sar. (12)	1996	Case–control: 101 pacijent, 109 controls	IS	Positive
Pullicino i sar. (13)	1996	Case–control: 60 patients, controls	Lacunar stroke	Negative
Sertic i sar. (14)	1996	Case–control: 50 patients, 25 controls	CS	Negative
Agerholm-Larsen i sar. (15)	1997	Case–referent: 184 pacijent, 5028 referenata	IS	Negative
Agerholm-Larsen i sar. (15)	1997	Case–referent: 268 patients, 4015 referenata	IS	Negative
Doi i sar. (16)	1997	Case–control: 181 patients, 271 controls	IS	Positive
Nakata i sar. (17)	1997	Case–control: 55 patients, 61 controls	IS	Positive
Watanabe i sar. (18)	1997	Cross-sectional: 169 patients	CS/ lacunar stroke	Positive
Aalto Setala i sar. (19)	1998	Cross-sectional: 234 patients	IS	Negative
Molyaka i sar. (20)	1998	Case–control: 52 patients, 80 controls	IS	Negative
Pfohl i sar. (21)	1998	Case–control: 388 patients	IS	Negative
Seino i sar. (22)	1998	Case–control: 26 patients, 28 controls	IS	Positive
Shen i sar. (23)	1998	Case–control: 44 patients, 62 controls	IS	Positive
Xu i sar. (24)	1998	Case–control: 65 patients, 117 controls	IS	Positive
Heijmans i sar. (25)	1999	Cross-sectional: 79 patients	CVD	Negative
Kostulas i sar. (26)	1999	Case–control: 100 patients, 100 controls	IS/CS	Negative
Notsu i sar. (27)	1999	Case–control: 175 patients, 213 controls	IS	Negative
Zee i sar. (28)	1999	Nested case–control: 348 patients, 348 controls	IS/PICH	Negative
Lin i sar. (29)	2000	Case–control: 306 patients, 300 controls	IS	Negative
Wei i sar. (30)	2000	Case–control: 87 patients, 257 controls	IS	Positive
Szolnoki i sar. (31)	2001	Case–control: 664 patients, 199 controls	IS	Negative Positive (small vessel)
Um i sar. (32)	2001	Case–control: 106 patients, 498 controls	IS	Negative
Zhang i sar. (33)	2001	Case–control: 152 patients, 72 controls	IS	Positive
Zhang i sar. (34)	2001	Case–control: 165 patients, 106 controls	IS	Positive
Li i sar. (35)	2002	Case–control: 143 patients, 154 controls	IS	Negative

Ohkubo i sar. (36)	2002	Case–control: 69 patients, 294 controls	IS	Positive
Szolnoki i sar. (37)	2002	Case–control: 292 patients, 652 controls	IS, large vessel	Negative
		Case–control: 211 patients, 652 controls	IS, small vessel	Positive
		Case–control: 186 patients, 652 controls	IS, mzxed type	Negative
Thomas i sar. (38)	2003	Case–control: 218 patients, 490 controls	IS	Negative
Um i sar. (39)	2003	Case–control: 365 patients, 319 controls	IS	Negative
Yuan i sar. (40)	2003	Case–control: 122 patients, 1229 controls	IS	Negative
Karagiannis i sar. (41)	2004	Case–control: 100 patients, 100 controls	IS	Negative
Wang i sar. (42)	2004	Case–control: 46 patients, 43 controls	IS	Positive
Zee i sar. (43)	2004	Case–control: 319 patients, 2092 controls	IS	Negative
Szolnoki i sar. (44)	2005	Case–control: 407 patients, 295 controls	IS	Negative
Um i sar. (45)	2005	Case–control: 211 patients, 319 controls	IS	Positive
Dikmen i sar. (46)	2006	Case–control: 185 patients, 50 controls	IS	Negative
Gao i sar. (47)	2006	Case–control: 100 patients, 100 controls	IS	Negative
Pera i sar. (48)	2006	Case–control: 368 patients, 456 controls	IS	Negative
Szolnoki i sar. (49)	2006	Case–control: 272 patients, 308 controls	IS	Positive
Tuncer i sar. (50)	2006	Case–control: 108 patients, 79 controls	IS	Negative
Lalouschek i sar. (51)	2007	Case–control: 450 patients, 817 controls	IS/TIA	Negative
Li i sar. (52)	2007	Case–control: 454 patients, 334 controls	IS	Positive
Munchi i sar. (53)	2008	Case–control: 162 patients, 150 controls	IS	Positive
Hong i sar. (54)	2008	Case–control: 232 patients, 225 controls	IS	Positive
Celiker i sar. (55)	2008	Case–control: 162 patients, controls	IS	Positive
Saidi i sar. (56)	2009	Case–control: 228 patients, 323 controls	IS	Negative

The first meta-analysis has evaluated the risk of stroke in 1196 subjects versus 722 controls from seven studies. It was concluded that the ACE genotype conferred a small but modest effect, with an odds ratio of 1.31 (95% CI 1.06–1.62), according to a dominant model of inheritance. A weaker association was seen under a recessive model (57). In a meta-analysis of Casas et al. (58) including 2990 predominantly white patients and 11 305 controls, the DD genotype was shown to confer a small but significant risk of ischemic stroke (odds ratio 1.21; 95% CI 1.08–1.35). From 2007-2009 five meta-analysis were published. Ariyaratnam et al. (59) investigated the association of *ACE* I/D in three ethnic groups of non-European descent (a total of 3572 Chinese individuals, 1601 Japanese individuals, and 2750 Korean individuals). The overall OR for the nine studies in the Chinese population was 1.90 (95% CI 1.23–2.93) and for six Japanese studies the OR was 1.74 (95% CI 0.88–3.42). The overall OR in the Asian group (Chinese and Japanese) was 1.82 (95% CI 1.28–2.60). Meta-analysis of Banerjee et al. (60) included six studies and did not detect a significant association with ACE gene insertion/deletion polymorphism. Xu et al. (61) identified statistically significant associations with IS and ACE I/D (OR = 1.87, 95% CI =1.45–2.42 (15 studies, 1935 patients/1485 controls). Tao i sar. (62) analyzed 29 studies in Chinese population (3654 patients/3058 controls) and found that ACE DD genotype is a risk factor cerebral infarction in Chinese population (OR 1,91 (95% CI 1,56–2,34), P<0,00001). Rao et al. (63) investigated the association of IS subtypes of subjects with the ACE I/D polymorphism published in 11 papers and gave ACE/DD OR 1.31 for small vessel stroke (95% CI 0.96–1.79, p = 0.09) compared to large vessel stroke (OR 1.02, 95% CI 0.82–1.26, p = 0.88) showed a preferential association for the DD genotype with small compared to large vessel disease.

Twenty three out of 53 studies (Table 4) examined the association of ACE gene polymorphism and ischemic stroke yielded a significant association with IS. Only a few studies have been done on the association of the ACE I/D polymorphism with lacunar infarction. Markus et al. (7) studied this polymorphism in a small series of lacunar infarction cases (n=18) in which there was a positive association between D alele and lacunar infarction; OR associated with the DD genotype for lacunar stroke was 5.60 (95% CI, 2.00 to 15.71). Study on a Japanese population found that,

in hypertensive patients only, the ACE D allele was associated with silent brain infarction (11). However, this result was not replicated in another study using a larger population of lacunar infarction patients (n=130) (9). Watanabe et al. (18) showed no association of the ACE polymorphism with SBI in a smaller population (n=36). Takami et al. (64) failed to show an association with number of lacunae in 134 Japanese patients.

Discrepancy in the obtained results can be explained by heterogeneity of study designs employed, differences in inclusion and exclusion criteria, ethnically different patient populations, small sample sizes, unmatched controls, combinations of ischemic and hemorrhagic strokes, different stroke subtypes and age of stroke onset, type of statistical evaluation, covariates, correction for multiple testing, and other factors that affect many publications.

There is less literature data about the association of serum lipids and apolipoproteins with ACE *I/D* polymorphism in patients with IS. The results of our study are consistent with two previously published studies. Del Ser et al. (65) showed that serym triglycerides concentration is elevated in stroke patients with *DD* ACE genotype. Sertić et al. (14) found significant association of triglycerides concentration and *DD* genotype (DD>ID=II). It can be hypothesized that the increased serum triglycerides level in *DD* ACE genotype could be a feature of an insulin resistence syndrome and determine atherogenic and thrombogenic risk (65).

Agerholm-Larsen et al. (66) determined the effect of *ACE* genotype on serum ACE activity in 900 women and men from the Copenhagen City Heart Study and found that ACE gene polymorphism explains 30-40% of variability in serum ACE activity and this effect was codominant, with *DD* subjects having the highest, *ID* subjects intermediate, and *II* subjects the lowest serum ACE activity. The relatively small number of published studies with contradictory results examined the association between ACE activity and ACE I/D polymorphism in patients with IS compared to the number of studies that examined only the association of ACE I/D polymorphism polymorphism with IS.

Our study showed expected trend in ACE activity (DD>ID>II) in patient and control groups. Patients with DD genotype had significantly higher ACE activity ($p<0,05$) compared with controls. This result is in accordance with croatian study (patients with angiographicaly proved cerebral atherosclerosis) (14). Dikmen et al. (46) examined Turkish acute stroke patients (185) although ACE activity was high in DD genotype, no difference was determined to ACE enzyme activity according to ACE genotypes in patients and controls. Some studies suggested that D allele of the ACE gene is a marker of an elevated circulation ACE level**.** Catto et al. (9) reported plasma ACE activity significantly lower in stroke patients (cerebral infarction or cerebral hemorrhage) than in controls, and levels of ACE activity were significantly lower during the acute phase of stroke but were similar to level of control activity after 3 months. Markus et al. (7) found that patients with IS and DD genotype has higher ACE activity compared with carriers of II genotype.

We expect that subsequent genome-wide association studies in large and well-characterized groups of patients of different ethnic origin will contribute substantially in the near future to a better understanding of the pathophysiology underlying ischemic stroke and permit the identification of new therapeutic targets aimed at stroke prevention and neuroprotection from ischemic injury.

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References

1. Mattei MG, Hubert C, Alhenc-Gelas F, Roeckel N, Corvol P, Soubrier F. Angiotensin I converting enzyme gene is on chromosome 17. Cytogenet Cell Genet 1989; 51: 1041.
2. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990; 86: 1343–6.

3. Stanković A, Ilić N, Žunić Z, Glišić S, Alavantić D. Association of the insertion/deletion polymorphism at the angiotensin I-converting enzyme locus with arterial blood pressure: population-based study. Jugoslov Med Biohem 1999; 18(4): 141–7.
4. Sharma P, Carter ND, Barley J, Brown MM. Molecular approach to assessing the genetic risk of cerebral infarction: deletion polymorphism in the gene encoding angiotensin 1-converting enzyme. J Hum Hypertens 1994; 8: 645–8.
5. Castellano M, Muiesan ML, Rizzoni D, Beschi M, Pasini G, Cinelli A, Salvetti M, Porteri E, Bettoni G, Kreutz R, Lindpaintner K, Rosei EA. Angiotensin-converting enzyme I/D polymorphism and arterial wall thickness in a general population. The Vobarno Study. Circulation 1995; 91: 2721–4.
6. Dessi-Fulgheri P, Catalini R, Sarzani R, Sturbini S, Siragusa N, Guazzarotti F, Offidani M, Tamburrini P, Zingaretti O, Rappelli A. Angiotensin converting enzyme gene polymorphism and carotid atherosclerosis in a low-risk population. J Hypertens. 1995; 13: 1593–6.
7. Markus HS, Barley J, Lunt R, Bland M, Jeffery S, Carter ND, Brown MM. Angiotensin-converting enzyme gene deletion polymorphism: a new risk factor for lakunar stroke but not carotid atheroma. Stroke 1995; 26: 1329–33.
8. Ueda S, Weir CJ, Inglis GC, Murray GD, Muir KW, Lees KR. Lack of association between angiotensin converting enzyme gene insertion/deletion polymorphism and stroke. J Hypertens 1995; 13: 1597–601.
9. Catto A, Carter AM, Barrett JH, Stickland M, Bamford J, Davies JA, Grant PJ. Angiotensin-converting enzyme insertion/deletion polymorphism and cerebrovascular disease. Stroke 1996; 27: 435–40.
10. Hosoi M, Nishizawa Y, Kogawa K, Kawasaki T, Konishi T, Maekawa K, Emoto M, Fukumoto S, Shioi A, Shoji T, Inaba M, Okuno Y, Morii H. Angiotensin-converting enzyme gene polymorphism is associated with carotid arterial wall thickness in non-insulin-dependent diabetic patients. Circulation 1996; 94: 704–7.
11. Kario K, Kanai N, Saito K, Nago N, Matsuo T, Shimada K. Ischemic stroke and the gene for angiotensin-converting enzyme in Japanese hypertensives. Circulation 1996; 93: 1630–3.
12. Margaglione M, Celentano E, Grandone E, Vecchione G, Cappucci G, Giuliani N, Colaizzo D, Panico S, Mancini FP, Di Minno G. Deletion polymorphism in the angiotensin-converting enzyme gene in patients with a history of ischemic stroke. Arterioscler Thromb Vasc Biol 1996; 16: 304–9.
13. Pullicino P, Kwen PL, Greenberg S, Becker AL, Glenister N. Angiotensin-converting enzyme gene and lakunar stroke letter. Stroke 1996; 27: 569–70.
14. Sertic J, Hebrang D, Janus D, Salzer B, Nikšić M, Čvorišćec D, Stavljenić-Rukavina A. Association between deletion polymorphism of the angiotensin-converting enzyme gene and cerebral atherosclerosis. Eur J Clin Chem Clin Biochem 1996; 34: 301–4.
15. Agerholm-Larsen B, Tybjærg-Hansen A, Frikke-Schmidt R, Grønholdt M-LM, Jensen G, Nordestgaard BG. ACE gene polymorphism as a risk factor for ischemic cerebrovascular disease. Ann Intern Med 1997; 127: 346–55.
16. Doi Y, Yoshinari M, Yoshizumi H, Ibayashi S, Wakisaka M, Fujishima M. Polymorphism of the angiotensin-converting enzyme (ACE) gene in patients with trombotic brain infarction. Atherosclerosis 1997; 132: 145–50.
17. Nakata Y, Katsuya T, Rakugi H, Takami S, Sato N, Kamide K, Ohishi M, Miki T, Higaki J, Ogihara T. Polymorphism of angiotensin converting enzyme, angiotensinogen, and apolipoprotein E genes in a Japanese population with cerebrovascular disease. Am J Hypertens 1997; 10:1391–5.
18. Watanabe Y, Ishigami T, Kawano Y, Umahara T, Nakamori A, Mizushima S, Hibi K, Kobayashi I, Tamura K, Ochiai H, Umemura S, Ishii M. Angiotensin-converting enzyme gene *I/D* polymorphism and carotid plaques in Japanese. Hypertension 1997; 30:569–73.
19. Aalto-Setala K, Palomaki H, Miettinen H, Vuorio A, Kuusi T, Raininko R, Salonen O, Kaste M, Kontula K. Genetic risk factors and ischaemic cerebrovascular disease: role of common variation of the genes encoding apolipoproteins and angiotensin-converting enzyme. Ann Med 1998; 30: 224–33.
20. Molyaka IuK, Petruk SV, Kirianov SA, Dzhibladze DN, Chechetkin AO, Scherbatykh TV, Rogaev EI. Association study of polymorphism in the gene of angiotensin-converting enzyme in ischemic stroke. Zh Nevrol Psikhiatr Im S S Korsakova. 1998; 98(6): 35–7.
21. Pfohl M, Fetter M, Koch M, Barth CM, Weiss R, Haring HU. Association between angiotensin I-converting enzyme genotypes, extracranial artery stenosis, and stroke. Atherosclerosis 1998; 140: 161–6.
22. Seino Y, Ikeda U, Maeda Y, Haga Y, Yashima H, Shimada K. Angiotensin-converting enzyme gene polymorphism and plasminogen activator inhibitor 1 levels in subjects with cerebral infarction. J Thromb Thrombolysis 1998; 5(3): 263–7.
23. Shen D, Ha D. The relationship between angiotensin-converting enzyme gene polymorphism and brain infarction in Chinese hypertensives. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 1998; 15: 136–8.

24. Xu Y, Wang X, Zhu J. Angiotensin converting enzyme gene polymorphism and cerebrovascular disease. Zhonghua Shen Jing Ge Za Zhi 1998; 31: 152–5.
25. Heijmans BT, Westendorp RGJ, Knook DL, Kluft C, Slagboom PE. Angiotensin I-converting enzyme and plasminogen activator inhibitor-1 gene variants: risk of mortality and fatal cardiovascular disease in an elderly population-based cohort. J Am Coll Cardiol 1999; 34: 1176–83.
26. Kostulas K, Huang WX, Crisby M, Jin YP, He B, Lannfelt L, Eggertsen G, Kostulas V, Hillert J. An angiotensin-converting enzyme gene polymorphism suggests a genetic distinction between ischaemic stroke and carotid stenosis. Eur J Clin Invest 1999; 29(6): 478–83.
27. Notsu Y, Nabika T, Park HY, Masuda J, Kobayashi S. Evaluation of genetic risk factors for silent brain infarction. Stroke 1999; 30: 1881–6.
28. Zee RY, Ridker PM, Stampfer MJ, Hennekens CH, Lindpaintner K. Prospective evaluation of the angiotensin-converting enzyme insertion/deletion polymorphism and the risk of stroke. Circulation 1999; 99: 340–3.
29. Lin JJ, Yueh KC, Lin GY, Chang DC, Chang CY, Shieh HL, Harn HJ. Lack of association between angiotensin I-converting enzyme gene deletion polymorphism and cerebrovascular disease in Taiwanese. J Formos Med Assoc 2000; 99(12): 895–901.
30. Wei X, Wang G, Jiang C, Li D, Zhao G. Association between hypertensive cerebrovascular stroke and renin-angiotensin system gene polymorphism from Chinese cohort in Shanghai. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2000; 17: 256–8.
31. Szolnoki Z, Somogyvari F, Szabo M, Fodor L. A clustering of unfavourable common genetic mutations in stroke cases. Acta Neurol Scand 2000; 102(2): 124–8.
32. Um JY, Kim HJ, Choi TJ, Jin CS, Park ST, Lee KC, Rhee HS, Lee KM, Lee YM, Kim HM, An NH, Kim JJ. Polymorphism of the angiotensin-converting enzyme gene in patients with cerebral infarction in Koreans. J Mol Neurosci. 2001; 17(3): 279–83.
33. Zhang X, Wang D, Xu L, Ma Y, Zhang S. Association between reninangiotensin system gene polymorphism and type 2 diabetics with stroke in China. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2001; 18: 462–6.
34. Zhang X, Xia J, Jin D. The relationship between angiotensin converting enzyme gene polymorphism and risk factors for cerebral infarct. Zhonghua Liu Xing Bing Xue Za Zhi 2001; 22: 435–8.
35. Li CM, Zhang C, Lu XL, Feng HY, Su QX, Zeng Y, Zhang HL, Qiu SL. Relationship between angiotensin converting enzyme gene and ischemic stroke. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2007; 19: 321–4.
36. Ohkubo R, Nakagawa M, Ikeda K, Kodama T, Arimura K, Akiba S, Saito M, Ookatsu Y, Atsuchi Y, Yamano Y, Osame M. Cerebrovascular disorders and genetic polymorphisms: Mitochondrial DNA5178C is predominant in cerebrovascular disorders. J Neurol Sci 2002; 198: 31–5.
37. Szolnoki Z, Somogyvari F, Kondacs A, Szabo M, Fodor L. Evaluation of the interactions of common genetic mutations in stroke subtypes. J Neurol 2002; 249: 1391–7.
38. Thomas GN, Lin JW, Lam WW,.Tomlinson B, Yeung V, Chan JC, Wong KS. Middle cerebral artery stenosis in type II diabetic Chinese patients is associated with conventional risk factors but not with polymorphisms of the renin-angiotensin system genes. Cerebrovasc Dis 2003; 16: 217–23.
39. Um JY, Moon KS, Lee KM, Cho KH, Heo Y, Moon BS, Kim HM. Polymorphism of Angiotensin-converting enzyme, angiotensinogen, and apolipoprotein E genes in Korean patients with cerebral infarction. J Mol Neurosci. 2003; 21(1): 23–8.
40. Yuan XD, Hou QX, Wu SL, Pei HZ, Li HF. A cross-sectional study on angiotensin-converting enzyme and angiotensin II type I receptor gene polymorphism and cerebral infarction. Zhonghua Liu Xing Bing Xue Za Zhi 2003; 24: 822–6.
41. Karagiannis A, Balaska K, Tzimolos K, Tokalaki-Nikolaidou L, Papayeryiou A, Zamboulis C. Lack of an association between angiotensin-converting enzyme gene insertion/deletion polymorphism and ischemic stroke. Eur Neurol 2004; 51(3): 148–52.
42. Wang YM, Liu XD, Dong WW, Yang ZC. The relationship between angiotensin-converting enzyme gene polymorphism and heart rate variability in cerebral stroke. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2004; 21: 156–60.
43. Zee RYL, Cook NR, Cheng S, Reynolds R, Erlich HA, Lindpaintner K, Ridker PM. Polymorphism in the P-selectin and interleukin-4 genes as determinants of stroke: a population-based, prospective genetic analysis. Hum Mol Genet 2004; 13: 389–96.
44. Szolnoki Z, Havasi V, Bene J, Komlosi K, Szoke D, Somogyvari F, Kondacs A, Szabo M, Fodor L, Bodor A, Gati I, Wittman I, Melegh B. Endothelial nitric oxide synthase gene interactions and the risk of ischaemic stroke. Acta Neurol Scand 2005; 111: 29–33.

45. Um JY, Moon KS, Lee KM, Cho KH, Heo Y, Moon BS, Kim HM. Polymorphism of Angiotensin-converting enzyme, angiotensinogen, and apolipoprotein E genes in Korean patients with cerebral infarction. J Mol Neurosci 2003; 21: 23–8.
46. Dikmen M, Veysi Gunes H, Degirmenci I, Ozdemir G, Basaran A. Are the angiotensin-converting enzyme gene and activity risk factors for stroke? Arq Neuropsiquiatr 2006; 64: 211–6.
47. Gao X, Yang H, ZhiPing T. Association studies of genetic polymorphism, environmental factors and their interaction in ischemic stroke. Neurosci Lett 2006; 398: 172–7.
48. Pera J, Slowik A, Dziedzic T, Wloch D, Szczudlik A. ACE I/D polymorphism in different etiologies of ischemic stroke. Acta Neurol Scand 2006; 114: 320–2.
49. Szolnoki Z, Maasz A, Magyari L, Horvatovich K, Farago B, Somogyvari F, Kondacs A, Szabo M, Fodor L, Bodor A, Hadarits F, Melegh B. Coexistence of angiotensin II type-1 receptor A1166C and angiotensin-converting enzyme D/D polymorphism suggests susceptibility for small-vessel-associated ischemic stroke. Neuromolecular Med 2006; 8: 353–60.
50. Tuncer N, Tuglular S, Kihc G, Sazci A, Us O, Kara I. Evaluation of the angiotensin-converting enzyme insertion/deletion polymorphism and the risk of ischaemic stroke. J Clin Neurosci 2006; 13: 224–7.
51. Lalouschek W, Endler G, Schillinger M, Hsieh K, Lang W, Cheng S, Bauer P, Wagner O, Mannhalter C. Candidate genetic risk factors of stroke: results of a multilocus genotyping assay. Clin Chem 2007; 53: 600–5.
52. Li C, Zhang C, Qiu S, Lu X, Zeng Y, Wu H, Chen W, Luo W, Liu J. Polymorphisms of ACE-1 and MTHFR genes and genetic susceptibility of ischemic stroke. Zhonghua Yi Xue Za Zhi 2002; 82: 1046–9.
53. Munshi A, Sultana S, Kaul S, Reddy BP, Alladi S, Jyothy A. Angiotensin-converting enzyme insertion/deletion polymorphism and the risk of ischemic stroke in a South Indian population. J Neurol Sci 2008; 272(1-2): 132–5.
54. Hong SH, Park HM, Ahn JY, Kim OJ, Hwang TS, Oh D, Kim NK. ACE I/D polymorphism in Korean patients with ischemic stroke and silent brain infarction. Acta Neurol Scand 2008; 117(4): 244–9.
55. Celiker G, Can U, Verdi H, Yazici AC, Ozbek N, Atac FB. Prevalence of thrombophilic mutations and ACE ID polymorphism in Turkish ichemic stroke patients. Clin Appl Thromb Hemost 2008 Apr 15.
56. Saidi S, Zammiti W, Slamia LB, Ammou SB, Almawi WY, Mahjoub T. Interaction of angiotensin-converting enzyme and apolipoprotein E gene polymorphisms in ischemic stroke involving large-vessel disease. J Thromb Thrombolysis 2009; 27(1): 68–74.
57. Sharma P. Meta-analysis of the ACE gene in ischaemic stroke. J Neurol Neurosurg Psychiatry 1998; 64: 227–30.
58. Casas JP, Hingorani AD, Bautista LE, Sharma P. Meta-analysis of genetic studies in ischemic stroke. Thirty-two genes involving approximately 18000 cases and 58 000 controls. Arch Neurol 2004; 61: 1652–62.
59. Ariyaratnam R, Casas JP, Whittaker J, Smeeth L, Hingorani AD, Sharma P. Genetics of Ischaemic Stroke among Persons of Non-European Descent: A Meta-Analysis of Eight Genes Involving ~ 32,500 Individuals. PLoS Med 2007; 4: e131.
60. Banerjee I, Veena Gupta V, Ganesh S. Association of gene polymorphism with genetic susceptibility to stroke in Asian populations: a meta-analysis. J Hum Genet 2007; 52: 205–9.
61. Xu X, Li J, Sheng W, Liu L. Meta-analysis of genetic studies from journals published in China of ischemic stroke in the Han Chinese population. Cerebrovasc Dis 2008; 26(1): 48–62.
62. Tao HM, Shao B, Chen GZ. Meta-analysis of the ACE gene polymorphism in cerebral infarction. Can J Neurol Sci 2009; 36(1): 20–5.
63. Rao R, Tah V, Casas JP, Hingorani A, Whittaker J, Smeeth L, Sharma P. Ischaemic stroke subtypes and their genetic basis: a comprehensive meta-analysis of small and large vessel stroke. Eur Neurol 2009; 61(2):76–86.
64. Takami S, Imai Y, Katsuya T, Ohkubo T, Tsuji I, Nagai K, Satoh H, Hisamichi S, Higaki J, Ogihara T. Gene polymorphism of the renin-angiotensin system associated with risk for lacunar infarction: The Ohasama study.AJH 2000; 13: 121–7.
65. Del Ser T, Bornstein B, Barba R, Cemillan C. Relationship of angiotensin converting enzyme genotype with serum triglyceride concentration in stroke patients. Neurosci Lett 2001; 316: 21–4.
66. Agerholm-Larsen B, Tybjærg-Hansen A, Schnohrb P, Nordestgaard BG. *ACE* gene polymorphism explains 30–40% of variability in serum ACE activity in both women and men in the population at large: the Copenhagen City Heart Study. Atherosclerosis 1999; 147: 425–7.