Meta-analysis of the ACE gene in ischaemic stroke

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Abstract

***Objectives*—The angiotensin-1 converting enzyme (ACE) gene is known to have two polymorphic alleles I/D. People with the DD genotype have been shown to be at greater risk of myocardial infarction, but only in some studies. Similar studies in stroke patients also show inconsistent results, but most of these studies have been underpowered to detect a small contribution to stroke risk from the ACE gene. A meta-analysis was undertaken using all known publications of the ACE polymorphism in ischaemic stroke.**

***Methods*—Two computerised databases were searched for all publications relating to case-control studies using the ACE I/D variant in human ischaemic stroke. Seven association studies were identified and a meta-analysis was conducted using the Mantel-Haenszel estimate for odds ratio (OR) to determine whether the DD genotype predicted outcome in either a genetically dominant or recessive model.**

***Results*—1918 white subjects (1196 cases and 722 controls) were used in the meta-analysis. There was no difference in ACE genotype (χ^2=2.92, p>0.05) or I/D allele frequency (χ^2=3.28, p>0.05) in cases or controls. The overall OR for the D allele as an independent risk factor in ischaemic stroke was 1.31 (95% confidence interval (95% CI): 1.06–1.62, p=0.01) under a recessive model, and 1.14 (95% CI: 0.91–1.44, p=0.26) under a dominant model.**

***Conclusions*—This meta-analysis shows that the the D allele, acting recessively, is a modest but independent risk factor for ischaemic stroke onset. Meta-analysis may usefully be employed in allelic association studies for detecting small attributable risks of candidate genes in polygenic disorders.**

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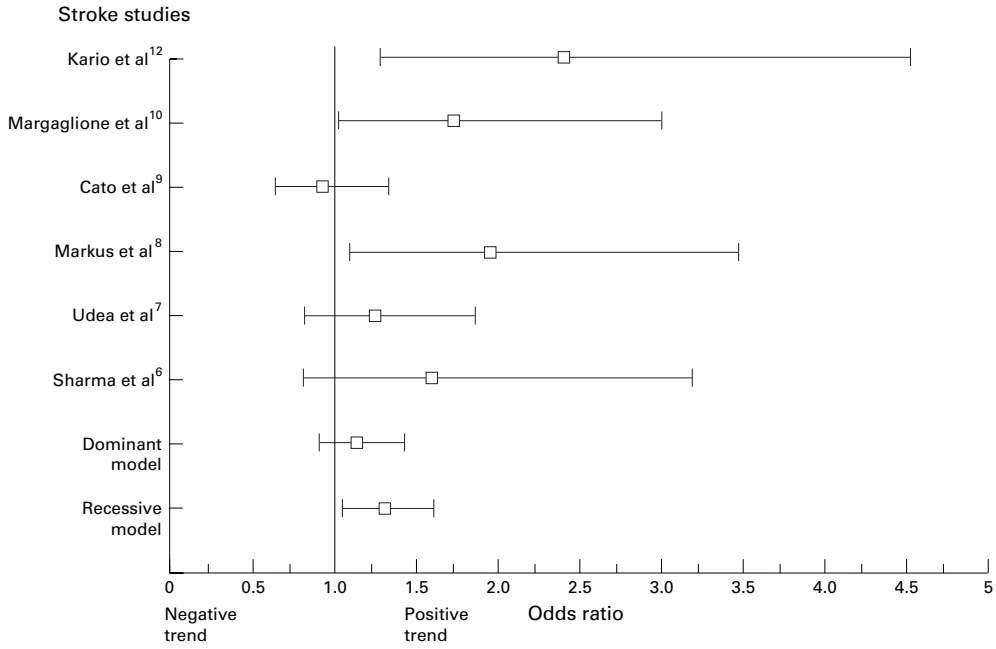
Angiotensin-1 converting enzyme (ACE) is the rate limiting enzyme of the renin angiotensin system and is known to be involved in vascular remodelling[1] and atherosclerosis.[2] A single copy gene encoding ACE lies on human chromosome 17q and has two polymorphic alleles depending on the insertion I, or the deletion D, of a 287 bp *alu* sequence within intron 16. 47% of the variance of plasma ACE is known to be determined by this allelic variation.[3] It was this background of knowledge that led to the work by Cambien *et al*[4] which suggested that the DD genotype may be associated with increased risk of myocardial infarction, particularly in those deemed to be least susceptible by conventional risk assessment. However, subsequent work on ischaemic heart disease has failed to confirm that original finding.[5]

The similarity in the pathophysiology of myocardial infarction with cerebral infarction prompted investigators to study the role of the ACE gene polymorphism in stroke, with varying results.[6–13] This is not surprising as any genetic component to stroke is likely to result from several genes interacting with each other (alongside environmental factors), with no single gene making any large contribution to overall stroke risk. Thus these studies, generally conducted with a few subjects, were often underpowered in their ability to detect a small contribution by the DD genotype to the aetiology of stroke. Combining the results of such studies can increase the power of detecting a smaller susceptibility risk by increasing the sample size.[14] Such meta-analyses have been criticised on various grounds[15] but the methodological principles remain sound, as long as its limitations are appreciated.[16] Indeed, the biggest criticism of meta-analysis is in avoiding biases such as selection of patients, publication bias, and reviewers' biases.[15] However, these biases are less likely to occur in studies of genetic variants with disease associations, because the dichotomy of such variants (presence or absence) is easier to assess than the heterogeneous clinical manifestations of patients evaluated in therapeutic trials,[15] which have been the source of many previous meta-analyses. Further, genetic studies showing no evidence of association tend to be reported, decreasing the chances of publication bias.[17]

To clarify the varying results of ACE/ID, I have undertaken a meta-analysis of all known publications involving the ACE polymorphism in ischaemic stroke.

Methods

Two computer databases (MEDLINE and BIDS) were searched for all publications relating to association studies using the ACE I/D polymorphism in ischaemic stroke. Eight studies were identified.[6–13] One related to ACE/ID in hypertensive subjects with parental history of stroke,[13] and was not included in any subsequent analysis. References from retrieved publications were scrutinised for any additional studies, and one published in abstract form was identified[18] but related to the same subjects as a



Odds ratios for all stroke studies.

later study already identified via the computerised database search.[11] Of the publications which included patients with ischaemic and haemorrhagic events, only results relating to ischaemic stroke were included. Details such as age, sex, ACE genotype, and allele frequencies were documented.

ACE genotype and allele frequencies were analysed using χ^2 analysis with MINITAB version 8.2. A combined odds ratio (OR) for the D allele using the Mantel-Haenszel test was determined under both a genetically dominant and recessive model. In all cases $p<0.05$ was considered to be significant.

Results

Seven case-control studies[6–12] (all in English) were identified totalling 2220 subjects (stroke/mean age 1394/67.9; controls/mean age 826/66.4) (table). Of these, three reported a positive association of the DD genotype with onset of ischaemic stroke whereas four reported no evidence of association. One related to a Japanese population[12] and was excluded in the overall OR estimate to maintain genetic homogeneity which otherwise can often give misleading results.[19] The study by Pullicino *et al*[11] had no control subjects but merely cited genotype frequencies in the stroke subtypes and so was also excluded from the overall OR estimate. Thus five studies were used in this meta-analysis incorporating 1918 subjects (1196 cases and 722 controls).

There was no significant difference between the combined ACE allelic (D/I cases: controls 0.55/0.45: 0.52/0.48; χ^2= 3.28; *df*1; $p>0.05$) and genotype (DD: ID: II cases and controls 364: 580: 252 and 194: 363: 165, respectively; χ^2=2.92; *df*2; $p>0.05$) frequencies from these five studies. The effect of the ACE gene variant was assessed using both genetic recessive and dominant models. In a recessive model 364 DD and 832 ID/II cases, and 194 DD and 528 ID/II controls were identified, respectively. For a dominant model 944 DD/ID and 252 II cases, and 557 DD/ID and 165 II controls were

Table 1 Summary of all stroke studies involving the ACE gene

Reference		*Subjects (n)*	*Males/ females*	*Mean age (y)*	*D/I*	*DD*	*ID*	*II*	*Study result*	*OR (95% CI)*
Sharma *et al* (1994)[6]	Cases	100	55/45	66.8	0.57/0.43	33	47	20	Negative	1.62 (0.82–3.22)
	Controls	73	33/40	65.4	0.48/0.52	17	36	20		
Ueda *et al* (1995)[7]	Cases	488	249/239	67.9	0.54/0.46	127	271	90	Negative	1.26 (0.84–1.88)
	Controls	188	83/105	65.2	0.50/0.50	41	105	42		
Markus *et al* (1995)[8]	Cases	101	69/32	64.8	0.59/0.41	36	47	18	Positive	1.98 (1.11–3.51)
	Controls	137	59/78	63.9	0.48/0.52	30	71	36		
Cato *et al* (1996)[9]	Cases	406	195/211	74.0	0.50/0.50	114	178	114	Negative	0.94 (0.65–1.35)
	Controls	215	91/124	72.5	0.53/0.47	63	102	50		
Margaglione *et al* (1996)[10]	Cases	101	51/50	63.6	0.72/0.28	54	37	10	Positive	1.76 (1.02–3.05)
	Controls	109	57/52	63.6	0.62/0.38	43	49	17		
Pullicino *et al* (1996)[11]	Cases	60	—	63	0.61/0.39	23	27	10	Negative	—
	Controls	0	—	—	—	—	—	—		
Kario *et al* (1996)[12]	Cases	138	66/72	70.0	0.47/0.53	34	63	41	Positive	2.44 (1.31–4.55)
	Controls	104	53/51	68.0	0.34/0.66	8	55	41		

identified, respectively. The overall OR estimate for the five studies is 1.31 (95% CI: 1.06–1.62; χ^2=6.27, 1 df, p=0.01) under a recessive model, and 1.14 (95% CI: 0.91–1.44; χ^2=1.26, 1 df, p=0.26) under a dominant model. With a combined number of 1918 subjects the power of this study to detect a doubling of relative risk is 99.99% under either a recessive or dominant model.

Discussion

Association studies are case-control studies which compare the frequency of a variant allele within a candidate gene between unrelated affected and unaffected persons, from a given population. The ACE (candidate) gene molecular variant has been widely investigated in a variety of cardiovascular diseases, with varying results. The aim of this study was to undertake a meta-analysis of all publications relating to the ACE polymorphism in ischaemic stroke.

Seven studies were identified of which five were deemed suitable to be used in the final analysis, totalling 1918 subjects. There was no significant difference in ACE genotype or allele frequency between cases and controls. Overall OR estimates showed that possession of the DD genotype conferred a modest additional risk for ischaemic stroke onset. The power of this meta-analysis to detect a relative risk of 2 is 99.99%. Even if a more conservative relative risk to stroke of 1.5 is assumed, the power of this study is 96.4% under a recessive model and 93% under a dominant model, at the 5% significance level. Similar studies in patients with ischaemic heart disease have had conflicting results[4 5] but a recent meta-analysis by Samani *et al*[14] also found that the DD genotype conferred a small additional risk to myocardial infarction, the risk being greater in a Japanese population.

ACE allelic frequencies are known to be racially dependent.[20] To maintain ethnic homogeneity in this meta-analysis the study of Kario *et al*[12] on a Japanese population was excluded in the overall OR estimate. It is of course entirely possible that the results of this meta-analysis would be more significant in a Japanese population, as illustrated by Kario *et al*.[12] However, association studies are notoriously sensitive to the homogeneity of the unaffected and affected cohorts,[19] and Kario *et al* were criticised methodologically for combining various heterogeneous groups.[21] Notwithstanding, Markus *et al*[8] were criticised by both Pullicino *et al*[11] and Cato *et al*[9] for his subgroup analysis. However, the stroke group used by Cato *et al*[9] was, inexplicably, not in Hardy-Weinberg proportion (suggesting that this group was an unrepresentative cohort), whereas Margaglione *et al* recruited only subjects who survived a cerebral ischaemic insult. No prospective stroke study (which would go a long way towards reducing selective advantages or disadvantages) on ACE/ID to date has been published. Further, all ischaemic strokes—for example, lacunar, middle cerebral, etc—were considered together in the meta-analysis but stroke is phenotypically a heterogenous disease and it is possible that different genes may play different roles in the differing subtypes of strokes. However, the validity of this meta-analysis rests on the assumption that any genes contributing to stroke risk contribute roughly equally to all types of stroke and that our pathological classification of stroke, although clinically useful, is not sound in terms of molecular genetics. The genetic component disclosed by this meta-analysis might act through a change in the blood, or the vessel wall, which promotes thrombosis irrespective of the type of stroke. In this type of model, environmental factors would determine the type of stroke that occurred in those with predisposing prothrombic genes. It is important to note these findings because the result and interpretation of any meta-analysis is dependent on the quality of the studies being reviewed. However, the increased power of the combined studies in a meta-analysis can minimise (although admittedly not eliminate) these individual shortcomings.

Plasma ACE concentrations have been shown to be codominantly related to the number of D alleles in normal subjects[22] as well as patients with stroke[23] and sarcoidosis.[24] ACE converts angiotensin 1 to angiotensin 2 which is known to be involved in vascular hypertrophy, vasoconstriction, and atherosclerotic processes.[2] Thus the ACE gene is a good candidate gene for ischaemic stroke and with this a priori hypothesis the results of this meta-analysis are the more interesting. It was not possible to determine any genetic influence of blood pressure on stroke onset because not all investigators measured blood pressure in these stroke studies. However, this is unlikely to effect the result of this meta-analysis because despite intensive investigation only a few studies have shown any relation between the ACE variant and blood pressure.[25 26] Thus it is unlikely that the ACE gene influences stroke onset through blood pressure, except possibly in the Japanese population.[12 26] However, an epistatic (gene-gene) effect (or more complex interaction) of the ACE gene with blood pressure controlling genes cannot be excluded.

Of course the results of any meta-analysis must always be viewed with caution, especially where publication bias cannot be excluded.[15] It is thought that all studies to date investigating the ACE gene in stroke were included in this work. However, unpublished negative work may result in selection bias, but this in unlikely as even so called negative genetic associations still attract interest. It is more likely that ongoing studies have not been included, but the combined power of this meta-analysis is probably greater than any single continuing study.

The OR of the D allele in ischaemic stroke using a recessive model was greater (and more significant) than with a dominant model, but this is not surprising. The genetic contribution of any single gene towards a complex disease (such as stroke) is unlikely to act in a simple Mendelian dominant fashion. The question of which strategy to adopt in maximising investigators' chances of detecting such several recessively inherited "stroke risk genes" has recently been reviewed.[19] Linkage studies using affected

sibling pairs can detect dominantly inherited genes if large numbers of sib pairs can be recruited, but have little power and low resolution although they can cover the entire human genome. A candidate-gene based approach can detect recessively inherited disease alleles with greater power and resolution, with the disadvantage that the pathophysiology of the disease must be understood to identify which candidate genes to investigate, along with the need to identify a suitable control population. However, the resources collected for the studies cited in this paper will be very useful in investigating other potential candidate genes as they arise. There is a merging consensus of opinion that the two strategies however, are complementary—for example, the use of association studies to positionally clone any anonymous loci identified using linkage.[19] Notwithstanding, the fact that such a likely candidate gene such as ACE confers only a minor attributable risk to stroke, and the fact that a meta-analysis was necessary to show this, provides a sobering thought for those investigators currently involved in trying to unravel the genetics of complex diseases.

This meta-analysis shows that the D allele, acting in a recessive fashion, is a modest, independent risk factor for ischaemic stroke. Meta-analysis is a useful tool that can be employed to increase the power of small genetic studies to detect minor attributable risks, as long as its limitations are recognised.

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