Journal of Gerontology: MEDICAL SCIENCES
2003, Vol. 58A, No. 9, 851–856

Angiotensin I-Converting Enzyme Gene Polymorphism in Two Ethnic Groups Living in Brazil's Southern Region: Association With Age

Ivana Beatrice Mânica Da Cruz,[1] Graziela Oliveira,[1] Maristela Taufer,[2] Ney Furhmann Leal,[1] Carla Helena Schwanke,[2] Luiz Glock,[2] Yukio Moriguchi,[1] and Emilio Hideyuki Moriguchi[1]

[1]Instituto de Geriatria e Gerontologia, Pontifícia Universidade Católica do Rio Grande do Sul, Brazil.
[2]Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Brazil.

Background. Several studies have been published on the association between ACE (angiotensin I-converting enzyme) polymorphism and longevity. However, the results are controversial.

Methods. We analyzed the association between ACE polymorphism and age in two different ethnic populations: a population originating from different European countries (Gaucha Population, GP) and a population originating from Japan (JP). Both populations live in Brazil's southern region.

Results. We determined the ACE genotype in 834 persons aged 10–104 years. The ACE genotype and allele frequencies differed between the two populations, with the D allele being more frequent in GP than JP. We found an association between the DD genotype and D allele and age in the GP group only. The ACE polymorphism–age association occurred at age >60 years in the GP population with decreasing II frequency.

Conclusion. We cannot dismiss the possibility of the association between ACE polymorphism and age involving linkage disequilibrium, since the nature of this phenomenon is still controversial. From our studies, it appears that there is a correlation between age, ethnicity, and ACE polymorphism. More of such studies are warranted, as further investigation in this area will have a high clinical relevance to cardiovascular disease and aging research.

ARE there gene polymorphisms that are truly longevity associated? In past decades, this question has become the main focus of several research groups studying longevity and aging processes. In an effort to dissect the genetic components of longevity, several studies have described a connection between gene polymorphism and advanced age (1,2).

The angiotensin-converting enzyme (ACE) gene was investigated and, despite its connection with the deletion (D) allele in cardiovascular diseases, the frequency of this allele seemed to be greater in the oldest individuals (3). The excess of D allele of the ACE gene was, at first sight, apparently paradoxical because previous reports had suggested an association of the D allele with a predisposition to myocardial infarction (MI) (4). A meta-analysis made by Samani and colleagues (5) of the association of the D allele with myocardial infarction suggested that limitations of the available data still exist.

Another study conducted in 10,150 individuals in the Danish population examined the association of ACE polymorphism with ischemic heart disease and longevity. The authors did not find any association between ACE polymorphism and longevity, with change of DD frequency as a function of age in participants aged 20 to $\geq$ 80 years (6). However, a limitation of the study cited could be the noninclusion of nonagenarians and centenarians in the analysis. Another study that also examined the association of ACE and apolipoprotein E (ApoE) polymorphism with longevity in French centenarians observed only an ApoE polymorphism association with longevity, but did not confirm an association with ACE polymorphism (7).

From the previous studies, we were able to see that the data obtained were compared according to different methodological designs, generally comparing the allele and genotype frequency between a young group (20 or 30 years old) and a much older group (nonagenarians or centenarians). If there is an association between ACE polymorphism and age, it is necessary to perform studies examining all ages to help us determine at which moment during aging ACE polymorphism could occur. Another point that needs to be clarified is whether age-associated ACE polymorphism is a universal phenomenon or dependent on ethnic group.

In this article, we studied the change in ACE polymorphism frequency as a function of age in two different ethnic populations that live in the Southern Brazil region.

METHODS

Populations

A total of 834 participants aged 10 to 104 years were studied. The population examined was composed of individuals mixed from different ethnic groups [Native South American, European Caucasian (Portuguese, Italians, Spaniards, and Germans)] originating a new genetic pool termed here the Gaucha population (GP). Participants were recruited by random selection from the Health and Social Assistance Program of Gravatai, Veranópolis, and Porto Alegre, Rio Grande do Sul, Brazil.

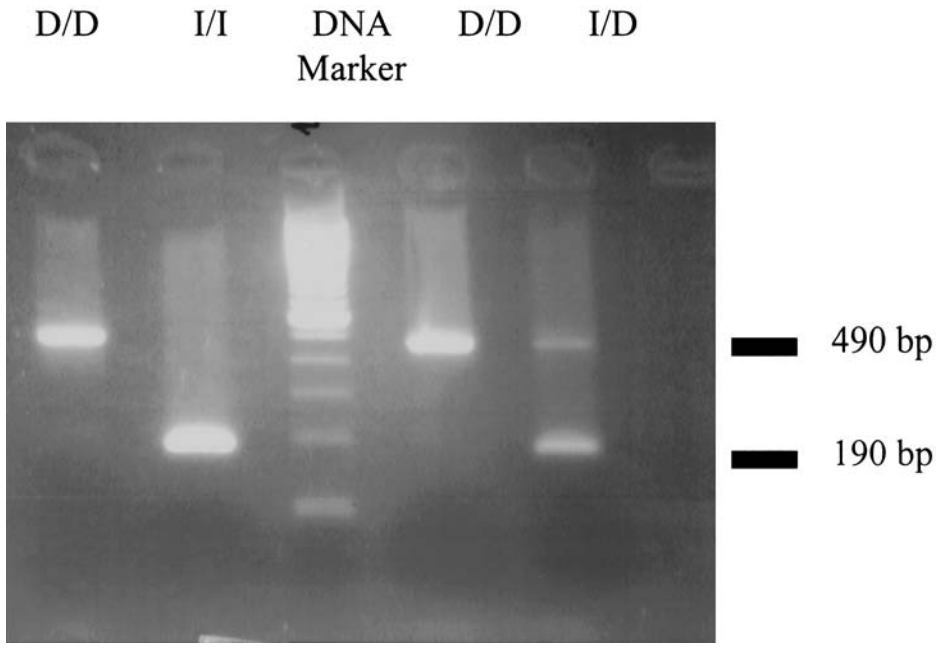

Figure 1. Angiotensin-converting enzyme (ACE) genotypes. Polymerase chain reaction amplification of ACE alleles insertion (I) and deletion (D) showed the presence of 490 bp and 190 bp products on 1.5% agarose gel electrophoresis.

The Japanese population came to Brazil's southern region after World War II (1950–1960) from several Japanese cities, mainly Nagasaki and Kumamoto. All Japanese participants included in this study were born in Japan or had both parents who were born in Japan (without any other ethnic miscegenation). The participants were recruited from the Japanese Assistant Association by random selection. All participants underwent a general physical examination. Blood samples were collected to confirm general health and to prepare DNA. The participants studied were not genetically related. The GP participants ranged in age from 10 to 104 years, and JP participants ranged in age from 20 to 100 years.

ACE Genotyping

Genomic DNA was isolated from peripheral blood leukocytes by standard methods (8). ACE genotyping was performed using the method described by Schachter and colleagues (3). ACE amplification of DNA was performed by polymerase chain reaction (PCR) with 0.1 mg of the DNA extract and thermostable *Taq* polymerase (Gibco, Inc., San Diego, CA) according to the manufacturer's instructions. PCR was performed in a thermal reactor using oligonucleotide primers 5′ CTG CAG ACC ACTCC C ATC CTT TCT 3′ and 5′GATGTG GCC ATC ACA TTC GTC AGA T 3′ (5). In addition, 10% (vol/vol) dimethyl sulfoxide was included in the reaction mixture to enhance the amplification of I allele. The PCR products were separated by electrophoresis on a 1.5% agarose gel (Figure 1).

Statistical Analysis

Allele frequencies were estimated by the gene-counting method. Chi-square (χ^2) analysis was used to estimate the Hardy-Weinberg equilibrium and to compare genotypic and allelic frequencies among the ethnic groups. Statistical analysis was performed by using the SPSS program (9). No parametric Spearman's correlation was made to test the association between ACE genotype, age, and sex. Additionally, to determine the starting point of a possible genotype association between age and ACE genotype, a percentile analysis of age (10,25,50,75,90) for each genotype was performed. A one-way analysis of variance followed by Duncan's test was used to compare the mean ages in the three different genotypes in each ethnic group studied. The chi-square likelihood test was used to compare genotype frequencies between ACE genotype and the following age points: 60, 70, and 80 years old. Also, the odds ratio (OR) and confidence interval (CI) (95%) were calculated. To test the possible association among ACE genotype, age, sex, and ethnic groups, we used the Foward Wald logistic regression. Similar statistics tests were used to analyze allele effect (D [deletion] and I [insertion]) associated with age.

Results

The frequencies of the D and I alleles are presented in Table 1. The genetic frequencies were in Hardy-Weinberg equilibrium for both ethnic groups investigated. Figure 1 shows a PCR product of the ACE polymorphism. The data analysis showed that genotype and allele frequencies were ethnic group related. The JP had a higher II genotype frequency than GP ($\chi^2 = 59.6$; df = 2, $p = .00$). We can see that there was a change in allele frequency between the two populations studied. While GP had a higher D allele frequency, JP showed a higher I allele frequency.

The mean age of all participants analyzed was 46.29 ± 24.34 years old. The percentile distributions of age considering ACE genotypes were different in the two populations studied (Figure 2).

In GP, all ACE genotypes had a similar age until the 10th percentile. However, from the 25th percentile, the values changed and the age values for II were less than the DD and ID genotypes. In the JP percentile distribution, the DD genotypes had age values less than II and ID genotypes until the 50th percentile.

When the mean ages were compared among ACE genotypes, the results found were related to ethnic group. The GP showed an association between age and ACE genotype, whereas the JP did not (Table 2). In the GP, the DD and ID genotypes correlated with higher ages, more than the II genotype ($F = 5.501$, df = 2, $p = .004$), suggesting a D allele-related dose effect.

Additionally, the following analysis considering different cut-off points was performed ($\geq$60, $\geq$70, and $\geq$80 years old) to determine at which moment the frequency of D allele changed significantly. The D allele frequency increased from $\geq$60 years old (Table 3) in the GP group. However, no significant differences were found among ACE genotype frequencies in JP at any age cut-points examined.

Also, we found in the GP group a D allele dose effect from $\geq$60 years old ($\geq$60 II = 13.9% and ID + DD = 32.7%, $\chi^2 = 10.38$, df = 2, $p = .001$, OR = 2.25, CI 95% = 1.05–4.83).

A multivariate analysis using logistic regression that included sex showed that, in the GP sample, the association between ACE polymorphism and age was independent.

Discussion

We found different results in the two ethnic groups studied. While GP showed genotype and allele frequencies of the ACE gene as a function of age, JP did not show this association. Some questions could be raised to try to understand the results obtained here. During the last years, several articles have been published reporting conflicting results about the association between ACE polymorphism and longevity.

Researchers have suggested that the association could be spurious due to biases as a result of small samples, which increase the risk of false positive reports. One case that supports this theory came from French centenarians. During the 1990s, an association between ACE and ApoE polymorphism and extremes of longevity in this population was published. However, a later study with a larger number of centenarians did not confirm the ACE association (7). Based on these data, the authors discussed recommendations to set up a rigorous experimental design involving analyses of association between gene polymorphism and longevity.

However, in Blanche's study (7), a comparison was made with ACE frequencies from a younger group to determine ACE polymorphism association with centenarians. There is probably no association between ACE polymorphism and centenarians as reported in Blanche's study. However, an association between ACE polymorphism and advanced age could exist.

A large retrospective cohort study in an ethnically homogeneous white population has been conducted (6). The authors did not observe any changes in ACE genetic frequencies as a function of age after examining participants aged 20 to 80 years. However, the authors did not include participants older than 80 years, and perhaps, this condition could have influenced the results obtained. For this reason, in the present study we included individuals with a broader range of ages (from 10 to 105 years) to analyze the possible association between D or DD frequencies and longevity.

Table 1. Angiotensin-Converting Enzyme Genotypes and Allele Frequencies in a Sample of the Japanese Ethnic Population and Gaucha Population (of European Origin) Living in the Southern Region of Brazil

	JP		GP	
	N	%	*N*	%
Genotype				
II	171	46.9	73	13.9
DD	43	11.8	223	42.5
ID	150	41.2	229	43.6
Allele				
I	492	0.66	375	0.36
D	236	0.34	675	0.64

Note: JP = Japanese population; GP = Gaucha population; I = insertion; D = deletion.

However, we actually observed a decrease in I and II frequencies in the older population ($\geq$60 years old) but not necessarily an increase in D and DD frequencies. If we examine the results from this point of view, we can explain the variation among associations obtained in different studies. In this case, we need to determine why the II participants die earlier than ID and DD participants. This question is paradoxical because several studies reported an association between D allele and age-related diseases, such as myocardial infarction, including studies in Japanese populations (10).

An association between II genotype frequencies and age, although apparent in the GP group, was not observed in the JP group. The different results obtained suggest that association could be influenced by linkage disequilibrium or some other hidden conditions. It is also possible that there were gene–environmental interactions that could be influencing the results obtained. A meta-analysis of the association of the D allele with myocardial infarction combining over 8500 participants from all currently available studies was carried out (5). The authors reported that an important confounding factor recognized in association studies is ethnicity, and they suggested two possibilities to explain this concern: (a) the existence of bias because several studies have been performed with small samples, and (b) differences in the relationship between DD genotype and myocardial infarction risks due to ethnic factors.

Considering the results of previously published studies and those of the present study, we cannot dismiss the possibility of this being due to a gene–environment or gene–gene interaction phenomenon. The gene–environment interaction was defined by Ottman (11) as "a different effect of an environmental exposure on disease risk in persons with different genotypes" or, alternatively, "a different effect of a genotype on disease risk in persons with different environmental exposures." She suggested five general models for the relation between a high-risk genotype and an environmental exposure, in terms of their effect on disease risk. The same concept could be applied to gene polymorphism associated with age (or longevity?). In this

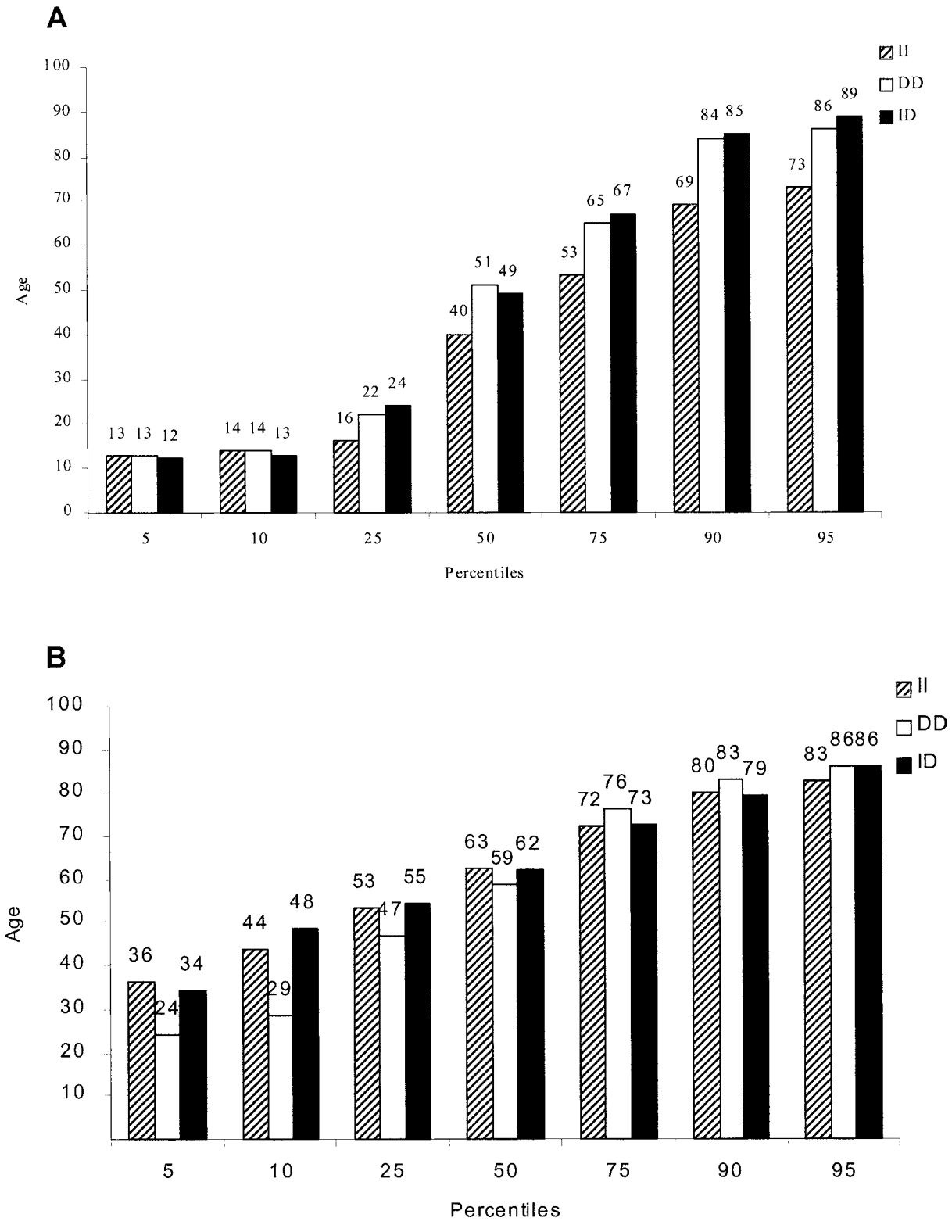

Figure 2. Percentile distribution of age as a function of angiotensin-converting enzyme polymorphism in two ethnically different populations living in the southern region of Brazil (RS). **A**, Gaucha population (GP); **B**, Japanese population (JP). Numbers represent the age estimated on different percentiles. I = Insertion; D = deletion.

Table 2. Comparison Among Mean Ages in Samples of the Japanese Ethnic Population and Gaucha Population Living in the Southern Region of Brazil With Different Angiotensin-Converting Enzyme Genotypes

	JP			GP		
Genotype	*n*	Mean ± *SD*	95% CI	*n*	Mean ± *SD*	95% CI
II	148	61.91 ± 14.25[a]	59.59–64.22	73	37.63 ± 20.23[a]	32.90–42.35
ID	125	62.22 ± 14.04[a]	59.73–64.71	222	47.617 ± 24.23[b]	44.46–50.88
DD	38	59.05 ± 18.46[a]	52.98–65.12	228	47.72 ± 24.97[b]	44.46–50.98
Total	311	61.69 ± 14.73	60.04–63.33	523	46.29 ± 24.26	44.20–48.37

Notes: Values with the same letter are not statistically significant ($p < .05$) in one-way analysis of variance followed by Student's *t* test in each population (JP and GP) analyzed separately.

n = Number of the sample; *SD* = standard deviation; JP = Japanese population; GP = Gaucha population; CI = confidence interval; I = insertion; D = deletion.

case, some polymorphism in the presence of different environmental conditions or in the presence of other genetic variables could possibly produce differential responses.

Luft's article (12) is a good example. This author studied the relationship among cardiovascular risk factors, such as hypertension, lipid disturbances, diabetes mellitus, left ventricular hypertrophy, and smoking, all of which are influenced by genetic variance. Luft postulated that genes that influence these factors could have a considerable bearing on longevity. Although mortality rates increase exponentially with increasing age, an interesting tendency toward a plateau occurs, suggesting that older individuals are somewhat protected from their inclination to die. This phenomenon is difficult to explain. One possibility is a model of repair, in which certain alleles exert a beneficial influence at an advanced age. An alternative explanation could be a mutation that exerts both negative and positive effects. The authors used the insertion/deletion (I/D) polymorphism in the ACE gene as an example leading to the notion that the ACE gene I/D polymorphism may be a genetic variant with both negative and positive effects. The study reported that, in a cohort of the German population aged older than 80 years, the D allele occurred at a frequency higher than expected, as seen in the results obtained here with the GP group.

In this case, Luft (12) reported an ACE polymorphism effect on heart size. The D allele was linked to a greater heart size, compared with the I allele in a modified sib-pair model. This potentially deleterious effect was counterbalanced by linkage of the D allele to increased heart rate variability, which is a potentially beneficial attribute.

Galinsky and colleagues (13) also found an association between ACE and age dependent on sex that indicated a gene interaction phenomenon. In this case, the I/I ACE was depleted in elderly men but not in elderly women, suggesting that the penetrance of loci that influence survival may vary according sex.

As we found an association between II frequencies and early age indicating that individuals with this genotype could die earlier, a complementary study testing Luft's hypothesis could help us understand the association between ACE polymorphism and longevity, or conversely, why II genotype individuals die earlier.

Other studies have also indicated the possibility of associations between ACE polymorphism and age involving gene interactions. Hibberd and colleagues (14) investigated the frequency of ACE polymorphism in 249 patients with type I diabetes and 162 healthy controls. An analysis of the ACE genotypes with respect to age and duration of diabetes showed that homozygosity for the insertion (I/I) genotype was significantly decreased with longer duration of diabetes in ACE and age association. The authors suggested that the ACE locus may be associated with longevity and survival in patients with type I diabetes. Thomas and colleagues (15) described an association between I allele and diabetes in a Chinese population. The results showed that the ACE-I allele was significantly more frequent in each group composed of participants with type 2 diabetes/glucose intolerance (GIT), and that the I allele was associated with higher fasting plasma glucose levels.

Alvarez and colleagues (16) studied the association between ACE polymorphism and Alzheimer's disease based on several lines of evidence suggesting that the endothelial constitutive nitric oxide synthase (ecNOS) and ACE may have a biological role in the disease. A total of 400 young healthy controls and 350 patients with Alzheimer's disease were genotyped for ACE and ecNOS polymorphisms. To define a possible role for these polymorphisms in longevity, 117 healthy controls older than 85 years were also analyzed. The results showed that the ACE-I allele was associated with a slightly increased risk of developing late-onset

Table 3. Comparison of Angiotensin-Converting Enzyme Genotype and Gene Frequencies by Different Age Cut-Off Points for the Japanese Population and Gaucha Population Living in the Southern Region of Brazil

Genotype	≥60 *n* (%)	≥70 *n* (%)	≥80 *n* (%)
GP Samples			
II	10 (13.9)	5 (6.9)	0
ID	75 (33.2)	46 (20.4)	32 (14.2)
DD	70 (32.1)	37 (16.9)	32 (14.6)
	$\chi^2 = 10.44$, df = 2, $p = .005$	$\chi^2 = 7.03$, df = 2, $p = .03$	$\chi^2 = 11.83$, df = 2, $p = .003$
JP Samples			
II	85 (57.4)	42 (28.4)	13 (8.8)
ID	72 (57.6)	34 (27.2)	10 (8.0)
DD	18 (47.4)	12 (31.6)	5 (13.2)
	$\chi^2 = 1.39$, df = 2, $p = .498$	$\chi^2 = 0.27$, df = 2, $p = .871$	$\chi^2 = 0.93$, df = 2, $p = .618$

Notes: χ^2 value; df = degrees of freedom; *p* = significance level.

GP = Gaucha population; JP = Japanese population; I = insertion; D = deletion.

Alzheimer's disease (OR = 1.28, 95% CI = 1.04, 1.58). These latter studies present some level of evidence for an association of I with diseases that could explain earlier mortality as found here for the GP group.

Recently, a study of ACE polymorphism and longevity was performed in three different ethnic Chinese groups totaling 424 participants (16). The authors found an association between DD and longevity that was similar to that described here for the GP group. To explain the D association with longevity, Rahmutula and colleagues (17) suggested that a genetic influence on differential survival may point to pleiotropic age-dependent effects on longevity.

At this time, the main reason for this association between ACE polymorphism and age is an open question (linkage disequilibrium? pleiotropic age-dependent effect? gene–gene or gene–environmental interactions?). Nonetheless, an association does appear to exist and is ethnic group dependent.

Acknowledgments

We thank research assistants Carin Gewer, Josiane Siviero, Gislaine Astir Flores, Alexandre Mânica da Cruz, Leni Araújo Leite, Ricardo Ehlers, and Margo Etiene do Canto, and Ivo Emilio da Cruz Jung for help with English translation. The study was supported by grants and fellowships from the Conselho de Desenvolvimento Científico (CNPq), Fundação de Amparo a Pesquisa do Rio Grande do Sul (FAPERGS), and Coordenação de Aperfeiçoamento do Ensino Superior (CAPES).

Address correspondence to Ivana B. M. Da Cruz, Instituto de Geriatria e Gerontologia, PUCRS, Av. Ipiranga, 6690, 90.610-000, Porto Alegre, RS-Brazil. E-mail: dacruz@pucrs.br

References

1. Schachter F. Causes, effects, and constraints in the genetics of human longevity. *Am J Hum Genet*. 1998;5:1008–1014.
2. De Benedictis G, Tan Q, Jeune B, et al. Recent advances in human gene-longevity association studies. *Mechan Aging Develop*. 2001;22: 909–920.
3. Schachter, F, Fuare-Delanef L, Guénot F, et al. Genetic Associations with human longevity at the APOE and ACE loci. *Nat Genet*. 1994; 6:29–31.
4. Beohar N, Damaraju S, Prather P. Angiotensin I-converting enzyme genotype DD is a risk factor for CAD. *J Invest Med*. 1985;43:275–280.
5. Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL. A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. *Circulation*. 1996; 94:708–712.
6. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Sorensen TIA, Jense GJ, Tybjaerg-Hansen A. ACE gene polymorphism: ischemic heart disease and longevity in 10,150 individuals. *Circulation*. 1997;5: 2359–2367.
7. Blanche H, Cabanne L, Sabattou M, Thomas G. A Study of French centenarians: are ACE and APOE associated with longevity. *C R Acad Sci*. 2001;29–135.
8. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988: 16:1215.
9. *SPSS for Windows: Base System User's Guide*. Release 9.0. Chicago: Norusis MJ/SPSS, Inc.; 1993.
10. Nakai K, Fusazaki T, Zhang T, et al. Polymorphism of the apolipoprotein E and angiotensin I converting enzyme genes in Japanese patients with myocardial infarction. *Pathophysiol Nat Hist*. 1998;9: 329–334.
11. Ottman, R. An epidemiologic approach to gene-environmental interaction. *Genet Epidemiol*. 1990;7:177–185.
12. Luft FC. Bad genes, good people, association, linkage, longevity and the prevention of cardiovascular disease. *Clin Exp Pharmacol Physiol*. 1999;26:576–579.
13. Galinsky D, Tysoe C, Brayne CE, et al. Analysis of the apoE/apo C-I, angiotensin converting enzyme and methylenetetrahydrofolate reductase genes as candidates affecting human longevity. *Atheros*. 1997; 129:177–183.
14. Hibberd ML, Millward BA, Demaine AG. The angiotensin I-converting enzyme (ACE) locus is strongly associated with age and duration of diabetes in patients with type I diabetes. *J Diabetes Compl*. 1997;11: 2–8.
15. Thomas GN, Tomlinson B, Chan JC, Sanderson JE, Cockram CS, Critchley JA. Renin-angiotensin system gene polymorphisms, blood pressure, dyslipidemia, and diabetes in Hong Kong Chinese: a significant association of the ACE insertion/deletion polymorphism with type 2 diabetes. *Diabetes Care*. 2001;24:356–361.
16. Alvarez R, Alvarez V, Lahoz CH, et al. Angiotensin converting enzyme and endothelial nitric oxide synthase DNA polymorphisms and late onset Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 1999;67: 733–736.
17. Rahmutula D, Nakayama T, Izume Y, et al. *J Gerontol Med Sci*. 2002; 54A:M57–M60.

Received October 7, 2002
Accepted December 5, 2002