

Plasma concentration, kinetic constants, and gene polymorphism of angiotensin I-converting enzyme in centenarians

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We have determined serum activity and kinetic constants of angiotensin I-converting enzyme (ACE), parallel to an insertion/deletion (I/D) polymorphism in its gene, in French centenarians and controls 20–70 years of age because this enzyme could have an impact on cardiovascular risk, and thus on longevity. Both the ACE D allele and ACE D/D genotype were more frequent in centenarians in comparison with controls, without sex-related differences nor significant correlation with a cardiovascular pathology. In centenarians, I/D polymorphism was correlated with circulating ACE activity (D/D genotype, 89.0 ± 36.8 U/L; I/D genotype, 63.5 ± 26.0 U/L; and I/I genotype, 55.1 ± 39.4 U/L). The Michaelis constants for two substrates were identical whatever the genotype and were not different between centenarians and controls, i.e., 0.30 ± 0.03 mmol/L for furylacryloyl-phenylalanyl-glycyl-glycine and 1.35 ± 0.05 mmol/L for hippuryl-histidyl-leucine; for the latter, the optimal pH and activating concentration of chloride did not depend on I/D polymorphism. The maximal velocities with both substrates reflected the distribution of serum ACE activity as a function of the genotypes, in centenarians and in controls. In conclusion, plasma ACE activity is subject to a similar genotypic influence in centenarians as in adults 20–70 years of age; however, ACE itself appears to be functionally similar for each genotype. Furthermore, the D allele as well as the higher serum ACE activities associated with the D/D genotype

cannot discriminate individuals at high risk for cardiovascular diseases, major causes of mortality before the age of 100 years.

There is increased evidence supporting the hypothesis that genetic components contribute to the determination of human longevity. Because cardiovascular diseases are major causes of death, we investigated possible associations between longevity and the alleles of candidate genes coding for proteins that could be incriminated in the pathogenesis of cardiovascular diseases. In particular, we previously found that the D allele of an insertion/deletion (I/D)⁴ polymorphism in angiotensin I-converting enzyme (ACE, EC.3.4.15.1) gene is significantly associated with longevity because its distribution was shifted in centenarians with an increase of the D/D homozygote genotype (1). Curiously, a number of epidemiological studies have identified the D allele as a risk factor for myocardial infarction (2), cardiomyopathy (3), and coronary heart disease in diabetes mellitus (4), all being causes of shortened longevity. On the other hand, this ACE I/D polymorphism would account for almost one-half the variance in the plasma ACE concentration, probably by modulating ACE gene transcription (5). Because the D/D genotype is linked to higher ACE concentrations, we now complete our previous observation by the determination of ACE activity in sera from a population of centenarians for correlation with I/D polymorphism. Our results show that mean serum ACE activity is not different in centenarians than in controls 20–70 years of age and that, also in centenarians, this activity is correlated to I/D polymorphism and should limit the usefulness of serum ACE as a phenotypic marker for discriminating individuals at high

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Received January 16, 1998; revision accepted June 23, 1998.

⁴ Nonstandard abbreviations: I/D, insertion/deletion; ACE, angiotensin I-converting enzyme; FAPGG, furylacryloyl-phenylalanyl-glycyl-glycine; and HHL, hippuryl-histidyl-leucine.

risk of cardiovascular disease. We also show that enzymatic constants of serum ACE are identical whatever the I/D genotype, in controls as well as in centenarians.

Materials and Methods

POPULATIONS

A population of 394 French centenarians (individuals in their 100th year and beyond) was recruited and collected as previously detailed and without exclusion criteria (1, 6). This population was 13% men and 87% women, with a mean age of 100.6 years (range, 99–122 years). Controls (238) from 20 to 70 years of age were chosen from unrelated healthy individuals; 140 were men (mean age, 50.3 years) and 98 were women (mean age, 51.9 years).

The ACE I/D genotype was determined in each individual of both populations; however, serum ACE activity was determined in 150 centenarians (15 men and 135 women from the total population) and 74 controls (42 men and 32 women). For K_m and kinetic analyses, 8–10 genotyped sera from each population, centenarians and controls, were pooled.

ACE GENOTYPE

The ACE diallelic polymorphism was genotyped by polymerase chain reaction for amplification of the variable segment, located in a repetitive Alu sequence in intron 16; resolution of the 190- and 490-bp alleles on 1.5% agarose gel was as described (7).

ACE ACTIVITY

Serum ACE activity was determined in duplicate on the synthetic specific substrate furylacryloyl-phenylalanyl-glycyl-glycine (FAPGG), using a method that we previously developed and automatized. In particular, the final FAPGG concentration was 0.8 mmol/L, and controls were included in each series (8). FAPGG was also used for the Michaelis constant (K_m) determination; however, the absorbance was read manually at 340 nm on a DU-70 spectrophotometer from Beckman at 37 °C and with a time-drive program. The final concentrations were as

follows: FAPGG, 0.1–2 mmol/L in 25 mmol/L HEPES–0.3 mol/L NaCl, pH 8.2, buffer. The ACE K_m was also measured with hippuryl-histidyl-leucine (HHL) in a radiometric assay that we described previously (9). The final concentrations were as follows: HHL (isotopic dilution of ^{14}C -HHL in cold substrate), 1–6 mmol/L in 250 mmol/L potassium phosphate–0.375 mol/L NaCl, pH 8.3, buffer at 37 °C. All the concentrations of both substrates maintained steady-state conditions; one unit (1U) of ACE activity is the amount of enzyme that hydrolyzes 1 μmol of substrate per minute.

Results and Discussion

We first confirmed in a larger cohort than the one we previously reported (1), that the ACE D allele and D/D genotype are more frequent in French centenarians in comparison with controls 20–70 years of age (Table 1) and are without sex-related differences in both genotype and allele frequencies. Both populations were in Hardy-Weinberg equilibrium for ACE alleles; however, we can note that the significance of association ($P < 0.05$) is weaker than the first result on a small population (1). In centenarians, we found no significant correlation between the D allele or D/D genotype and a cardiovascular pathology such as angora pectoris, arteritis, or hypertension ($P = 0.07$).

We demonstrated that, in centenarians also, I/D polymorphism is correlated ($\chi^2 = 23$) with circulating ACE activity; i.e., the D/D genotype is associated with higher serum ACE concentrations (89.0 ± 36.8 U/L), the I/I genotype with lower concentrations (55.1 ± 39.4 U/L), and the heterozygosity associated with intermediate concentrations (63.5 ± 26.0 U/L; Fig. 1). The mean serum ACE activity of the global cohort of centenarians was 69.2 ± 34.1 U/L, which is not statistically different from that of controls (71.2 ± 35.0 U/L). Thus, ACE concentration does not significantly vary with aging, whereas we previously showed that it increases during childhood to reach a maximum near puberty and then slowly returns to a definitive reference value, but ever more slowly in boys than in girls (10). This reason led us to select controls

Table 1. Distribution of ACE genotypes and alleles in centenarians and controls.^a

	Centenarians ^b		Controls ^c	
	Number	Frequency	Number	Frequency
ACE genotypes				
D/D	47	31.3	20	27.0
I/D	82	54.7	38	51.4
I/I	21	14.0	16	21.6
ACE alleles				
D	176	58.7	78	52.7
I	124	41.3	70	47.3

^a Statistical analysis of association between longevity and ACE polymorphism: ACE genotypes (D/D genotype more frequent in centenarians than in controls), $P < 0.05$; ACE alleles (D allele more frequent in centenarians than in controls), $P = 0.025$.

^b $n = 150$.

^c $n = 74$.

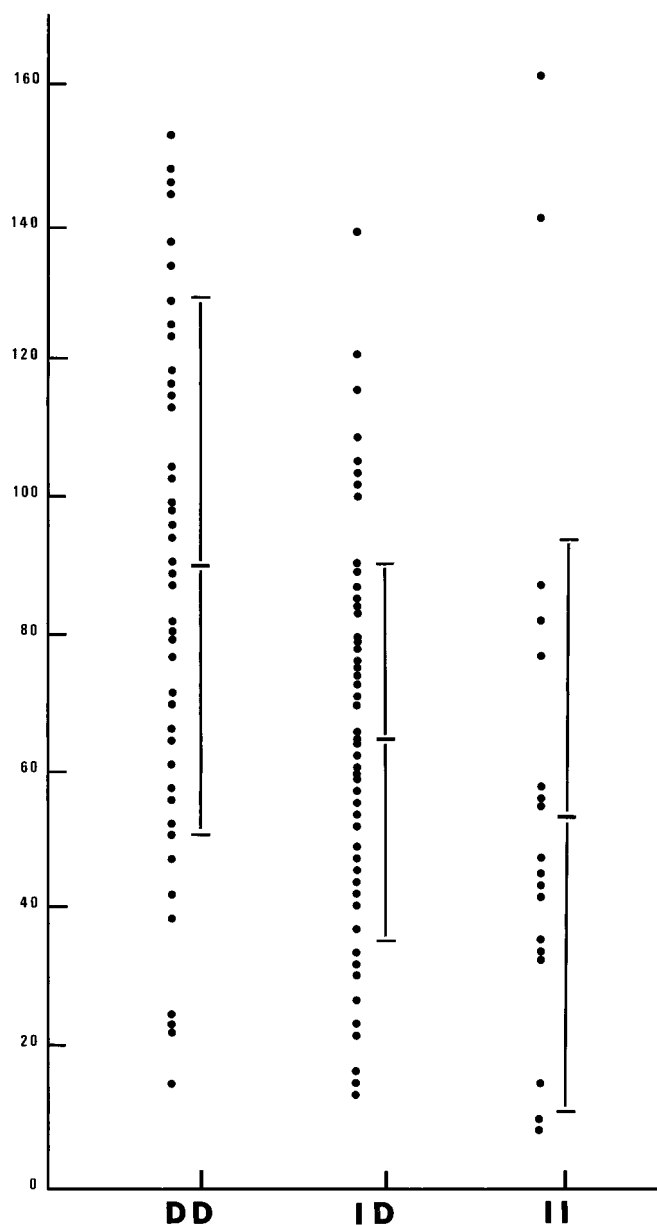


Fig. 1. Distribution of serum ACE activity (U/L) in centenarians as a function of the I/D polymorphism genotype.

Vertical bars represent the mean \pm SD.

among individuals ≥ 20 years of age. Nevertheless, the large excess of the D/D genotype of centenarians and the association of this genotype with higher ACE concentrations could increase the mean serum ACE activity in this population that we have not noted, perhaps because a slight decrease of ACE concentration as a function of age could counterbalance the genotypic effect.

We also characterized ACE kinetics in the serum of individuals who have been genotyped for the I/D polymorphism because Lee (11) reported that this polymorphism could modify the ACE molecule and thus be observable as a change in the kinetic behavior of the enzyme. On pools of sera from genotyped centenarians,

the K_m for FAPGG was 0.297 ± 0.035 for the D/D genotype, 0.288 ± 0.028 for the I/D genotype, and 0.325 ± 0.04 mmol/L for the I/I genotype, without statistical difference between them. The maximal velocities (V_{max}) were 127.3, 92.2, and 51.9 U/L, respectively (Fig. 2A), with, as could be expected, the same differences in function of I/D polymorphism as for ACE activity in individuals. For the same pooled samples, the K_m for HHL was strictly identical, at 1.35 ± 0.05 mmol/L, for the three genotypes; the V_{max} values were 25.6, 23.8, and 17.2 U/L for the D/D, I/D, and I/I genotypes, respectively (Table 2). With this substrate, the optimal pH was 8.8 for all the samples, and the optimal activating concentration of chloride was 0.375 mol/L (Fig. 3).

The K_m for FAPGG was also determined in controls: on pools of sera from genotyped controls, the K_m was 0.30 ± 0.02 mmol/L without statistically significant differences between the three genotypes or with the K_m of centenarians sera, genotypically matched. The V_{max} values for these control pools were 136.5, 91.2, and 70.7 U/L for the D/D, I/D, and I/I genotypes, respectively (Fig. 2B); in comparison with centenarians, the only statistical difference was for the I/I genotype, with a higher value in controls than in centenarians ($P < 0.05$).

These results show that the V_{max} of both HHL and FAPGG hydrolyses by human serum ACE are under genetic control in adults 20–70 years of age as well as in centenarians, and especially in relation with I/D polymorphism in an Alu sequence in intron 16 of the somatic ACE gene. This variation in V_{max} does not depend on optimal pH or chloride concentration; it depends only on the ACE concentration in plasma, as shown by Rigat et al. (12) with an immunological assay. Moreover, our data obviously demonstrate that ACE I/D polymorphism is not associated with altered substrate affinity, which refutes the data of Lee (11), who reported a higher K_m for the D/D genotype using HHL as substrate in a fluorometric assay. Alternatively, Morris et al. (13) did not find any variation in the ACE K_m of plasma from hypertensive patients, using another substrate, hippuryl-glycyl-glycine. Taken all together, these data presume that the part played by I/D polymorphism in controlling ACE transcription does not touch the enzyme active site; however, they cannot exclude that the plasma ACE concentration could be posttranscriptionally controlled, in particular at the step of precursor mRNA splicing, consequently altering the stability of mature mRNA and thus the translation step (5, 12). The superimposition of the ACE activities of the three genotyped groups, D/D, I/D, and I/I (see Fig. 1) confirms that the deletion is not functional. Other polymorphisms of the ACE gene (14) or other quantitative-trait loci (15) have been demonstrated and as has their relationship to plasma ACE concentrations; however, the functional variant, located within or close to the ACE locus, has not been definitely characterized.

On the other hand, it is well recognized that ACE

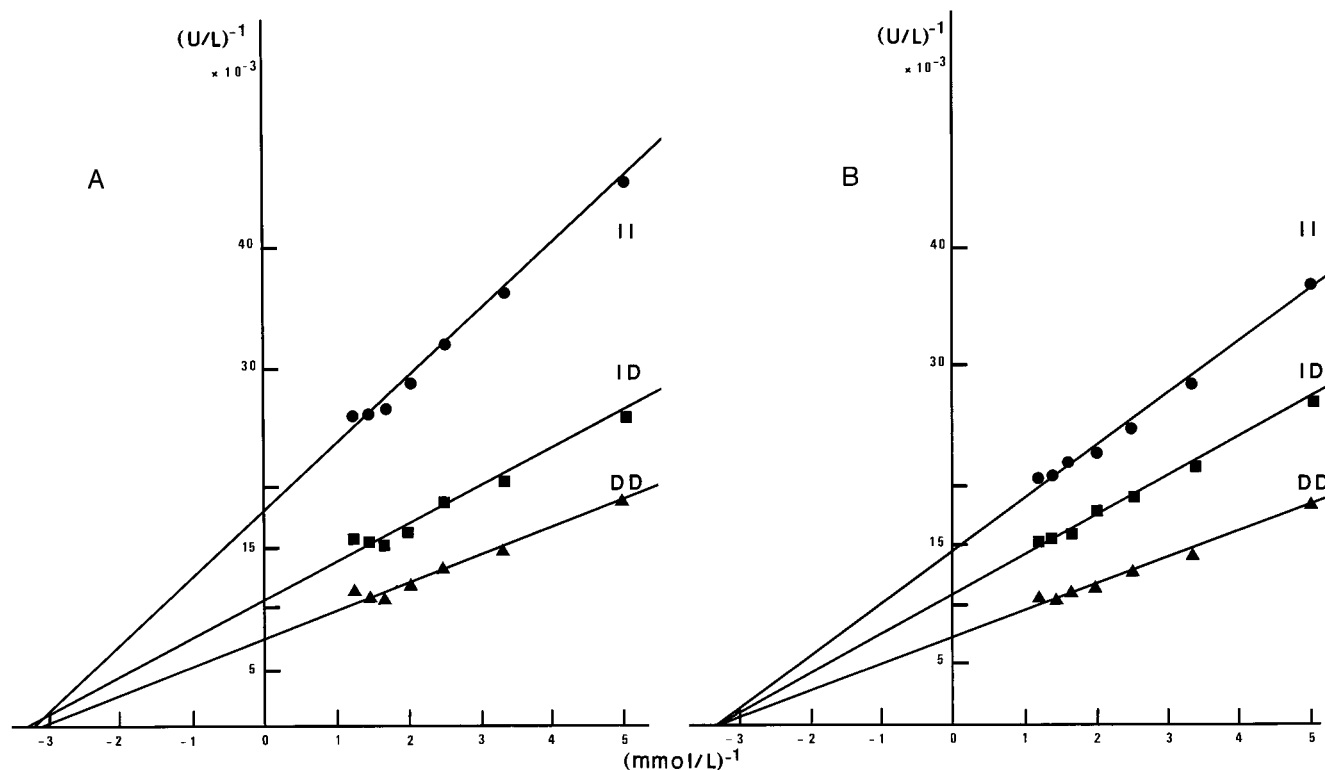


Fig. 2. Lineweaver-Burk plots of ACE activity, determined on FAPGG as the substrate, in genotyped sera pooled from centenarians (A) or controls (B).

Each point is the mean of three determinations.

inhibitors protect against cardiovascular diseases, certainly by enhancing bradykinin concentration in the vascular wall; however, it is difficult to relate this observation to the fact that the centenarians have no particular low ACE concentrations. The I/D polymorphism of the ACE gene does not seem to be a marker for either form of hypertension, as was shown in our study as in a large elderly cohort (16).

Our results particularly limit the interest in considering the D allele as a genetic marker or plasma ACE concentration as a phenotypic marker, related or not to the former, for identifying individuals at high risk for morbid cardiovascular diseases. In centenarians recruited on their longevity characteristics whatever their clinical presentation, we particularly show that the ACE D allele is not associated with one of the major causes of mortality before the age of 100 years. Our results were unexpected

because the D/D genotype was previously identified as conferring a risk for myocardial infarction in an otherwise low-risk population (2). It is the paradox of aging for which other genetic and biochemical results have been recently obtained, specially by our group, e.g., for coagulation or thrombotic factors such as fibrinogen, factor V, and homocysteine, and also lipoproteins, in particular lipoprotein a and the *APOE* $\epsilon 4/APOE$ $\epsilon 4$ genotype of apolipoprotein E (1, 6). All these studies indicate that the relative allele-specific effects on survival are age-dependent; therefore, age conditions the response to cardiovascular risk factors associated with genotypes with one or two D alleles. Perhaps it is true for other disease-associated genetic factors that, along with the ACE locus, may contribute to longevity. Thus ACE may have both beneficial and deleterious functions, and the balance between each may change during the human lifetime.

Table 2. Kinetic constants of ACE activity on two substrates in genotyped sera pooled from centenarians (means of three determinations).

ACE genotypes	FAPGG		HHL	
	K_m , mmol/L	V_{max} , U/L	K_m , mmol/L	V_{max} , U/L
D/D	0.297 ± 0.035	127.3	1.34 ± 0.04	25.6
I/D	0.288 ± 0.028	92.2	1.34 ± 0.05	23.8
I/I	0.325 ± 0.040	51.9	1.35 ± 0.05	17.2

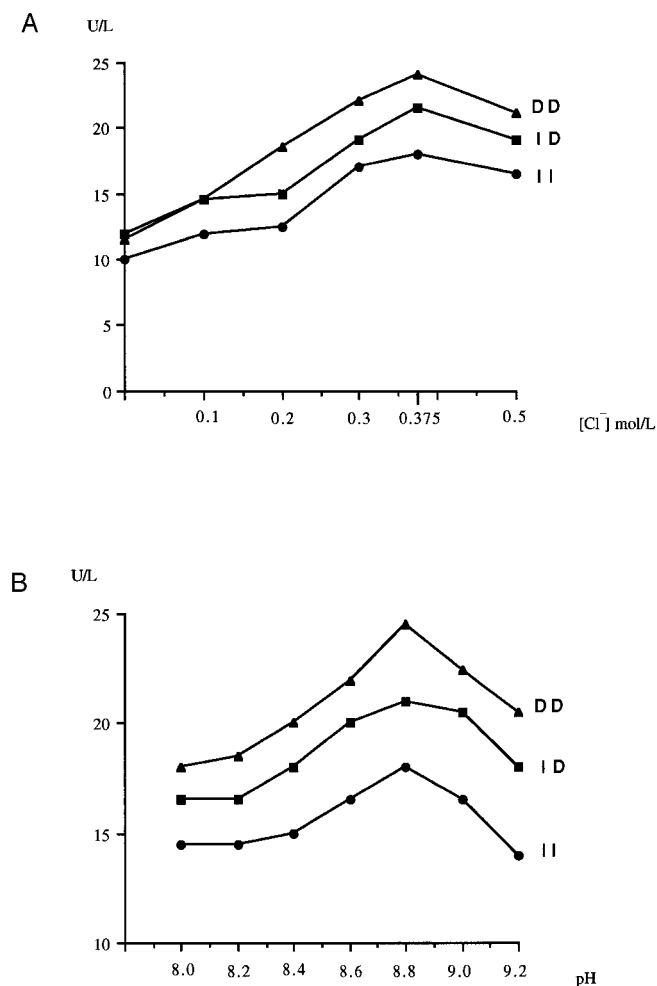


Fig. 3. Effects of chloride concentration (A) and pH (B) on ACE activity, determined on HHL as the substrate, in genotyped sera pooled from centenarians.

Each point is the mean of three determinations.

We thank R. Prothée and L. Bowen for their statistical analysis; ARCOO, AGIRC, Fondation IPSEN, B. Forette, R. Moulias, F. Piette, and the participating clinicians in France for help in collecting the centenarians; J. Zouali, C. Billon, L. Casez, C. Dross, F. Dufour, and M. Legrand for technical assistance; P. Jue for manuscript preparation; and J. Feingold for support and discussion.

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