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Familial Resemblance of Plasma Angiotensin-converting Enzyme Level: The Nancy Study

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Summary

Plasma angiotensin I–converting enzyme (ACE) activity has been measured in a sample of 87 healthy families participating in a study of cardiovascular risk factors. The mean ± SD levels of plasma ACE were 34.1 ± 10.7, 30.7 ± 10.4 and 43.1 ± 17.2 units/liter in fathers (n = 87), mothers (n = 87) and offspring (n = 169), respectively. Plasma ACE was uncorrelated with age, height, weight, or blood pressure in the parents, but a negative correlation with age was observed in offspring (r = −.32). The age-adjusted familial correlations of plasma ACE were .038, .166, .323 and .303 for spouses, father-offspring, mother-offspring, and siblings, respectively. The results of the genetic analysis suggest that a major gene may affect the interindividual variability of plasma ACE, with different codominant effects in parents and offspring. According to this model, the major gene effect accounts for 4.8, 4.0, and 10.8 units/liter of the overall mean and for 29%, 29% and 75% of the variance of age-adjusted ACE in fathers, mothers, and offspring, respectively. The estimate of the probability of the less frequent allele is .26, and the major gene effect is approximately twice as great in high homozygotes than in heterozygotes and in offspring than in parents. The results of this study demonstrate the occurrence of a familial resemblance of plasma ACE activity in healthy families and suggest that this observation can be explained by the segregation of a major gene.

Introduction

The angiotensin I–converting enzyme (ACE) or kininase II (E.C.3.4.15.1) is a dipeptidylcarboxypeptidase that hydrolyzes angiotensin I in the circulation and converts it into the pressor peptide angiotensin II (Skeggs et al. 1956). It also inactivates bradykinin (Yang et al. 1970). The importance of ACE in circulatory homeostasis is well documented (Erdös 1980). Besides being present as a membrane-bound enzyme, on the surface of the vascular endothelial cells and in several types of epithelial cells, ACE also circulates in plasma. The cellular origin of plasma ACE remains unknown, but results of several studies suggest that the endothelial cells can secrete ACE and that the plasma enzyme may originate from the vascular endothelium (Das et al. 1977; Hayes et al. 1978).

In large series of normal individuals, plasma ACE levels can differ greatly from subject to subject, sometimes by more than fivefold (Lieberman 1975; Neels et al. 1982; F. Alhenc-Gelas and J. L. Richard, unpublished data). However, when measured repeatedly in a given subject, the ACE level remains remarkably constant (Dux et al. 1984; F. Alhenc-Gelas and J. L. Richard, unpublished data). The factors involved in the regulation of plasma ACE concentration in normal subjects are unknown. To test the hypothesis that differences among individuals in plasma ACE levels might have a genetic origin, we have performed a study of plasma ACE in a sample of "healthy" families.

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Material and Methods

Study Group

The families studied were recruited in the Center for Preventive Medicine of Vandoeuvre les Nancy, France, where members of families living in the Nancy area can volunteer to have a free health checkup examination. From January 1985 to June 1986, 87 families composed of two parents age ≤60 years and at least one offspring age >10 years agreed to participate in a study of familial cardiovascular risk factors where plasma ACE activity was measured. On the whole, 343 subjects, including 169 offspring, were examined. Many other variables, including height, weight, and blood pressure, were recorded on these subjects. Blood pressure was obtained at rest in the sitting position with a mercury sphygmomanometer. None of the individuals included in the study presented evidence of evolving disease, as attested by results of physical examination, chest X-ray, and routine laboratory tests.

Measurement of Plasma ACE Activity

Blood was drawn from the antecubital vein into heparinized tubes between 8 and 9 AM and centrifuged immediately. Plasma was stored at −196 C in aliquots of 0.2 ml. Plasma ACE activity was determined spectrophotometrically by a modification of the classical method of Cushman and Cheung (1971; Lieberman 1975). Measurements were performed in duplicate with zero blanks on 10–20 μl of plasma. The incubation was carried out for 2 h. Results are expressed in units per liter, one unit being the quantity of enzyme able to hydrolyze 1 μM substrate/1 min incubation (Cushman and Cheung 1971). Substrate consumption was kept <10% by using diluted plasma, and it remained apparently linear with time during the 2-h incubation. The interassay variances determined for six different plasmas of 21–49 units/liter were 6%–10%. In previous studies, we established that the results of the spectrophotometric assay correlated very well in plasma with those of the direct radioimmunoassay using specific antibodies to human ACE (Alhenc-Gelas et al. 1983; Yasui et al. 1983). The spectrophotometric assay, however, was used in the present study because of its high sensitivity and the limited quantity of plasma available. After collection of all samples, measurements were performed in the INSERM U36 laboratory in Paris, where the samples were processed in random order.

Statistical Methods

The purpose of the present study was, first, to establish whether there was a familial resemblance in the level of ACE and, second, if such was the case, to see whether this resemblance could be attributable to the presence of a polymorphic gene having a major effect on the phenotype.

Computation of familial correlations.—The familial correlations were computed jointly using the maximum likelihood method described by Donner and Koval (1981), which assumes that the distribution of the trait studied is multivariate normal within every family with identical parameters (means, variances, and correlations) among families. The parameters of this model were estimated as a special case of the more general model described in the next section.

Modelization of a major gene effect.—An extension of the method presented above, similar to the one proposed by Bonney (1984), was used to investigate the compatibility of the data with a major gene effect hypothesis. Let $x = (x_1, x_2, x_3, \ldots x_n)$ be the vector of phenotypes of a particular family. The number 1 indexes the father, 2 the mother, and 3 . . . n the offspring. The residual vector of phenotypes of a family after adjustment on major genotype is $y = x - \delta_g$; $\delta_g = (\delta_{g1}, \delta_{g2}, \delta_{g3}, \ldots \delta_{gn})$ is the familial vector of mean differences associated with the genotypes $g = (g1, g2, g3, \ldots gn)$. Since we assume a two-allele system, there are three possible genotypes, gk = (aa, aA, and AA, say), A being the less frequent allele. Conventionally $\delta_{aa} = 0$, whereas δ_{Aa} and δ_{AA} may differ from zero and may be different in parents and offspring.

The likelihood of a particular family may be written as $L(x) = \Sigma_{g1}\Sigma_{g2}\Sigma_{g3}, \ldots \Sigma_{gn}\, P(g)f(x|g)$. Under the simple case in which the parents are genetically unrelated and the genotypes of the offspring depend only on the genotypes of their parents,

$$P(g) = P(g_1, g_2, g_3, \ldots g_n)$$
$$= P(g_1)P(g_2)P(g_3|g_1g_2) \ldots P(g_n|g_1g_2).$$

If π is the population frequency of allele a and the population is panmictic, then according to the Hardy-Weinberg law $P(aa) = \pi^2$, $P(Aa) = 2\pi(1 - \pi)$, and $P(AA) = (1 - \pi)^2$. The conditional probabilities of the genotypes of the offspring are computed by defining a vector τ of three transmission probabilities from parent to offspring—$\tau(A|AA)$, $\tau(A|Aa)$, and $\tau(A|aa)$—which, under the Mendelian model of inheritance, are equal to 1, .5, and 0, respectively.

The $f(y)$ is assumed to be multivariate normal with mean vector $\mu = (\mu_1, \mu_2, \mu_3, \ldots \mu_n)$ and covariance matrix Σ. The likelihood of x is then a known function of the parameters μ, Σ, π, δ, and τ. The diagonal ele-

Table 1

Means (SD) of Some Variables

Variable	Fathers	Mothers	Offspring
Age	41.7 (5.0)	39.4 (5.2)	14.2 (2.6)
Height (cm)	173.5 (7.1)	160.6 (5.6)	160.0 (12.1)
Weight (kg)	75.4 (9.8)	60.2 (9.5)	49.4 (11.7)
Systolic blood pressure (mmHg)	125.9 (13.1)	117.8 (13.7)	112.8 (11.0)
Plasma ACE (units/liter)	34.1 (10.7)	30.7 (10.4)	43.1 (17.2)

ments of Σ are the residual variances of the trait, and the off-diagonal elements are the covariances among relatives after adjustment for major gene effect. Since the size of the families may differ, the size of the vectors μ and of the matrices Σ varies accordingly; however, in this model the parameter values of the offspring are assumed to be the same, whatever the size of the family.

If Ln L_i denotes the log likelihood for the ith of N families, the log likelihood of a sample of families Ln $L = \Sigma \ln L_i$ may then be maximized to estimate all or a subset of the above defined parameters and their asymptotic standard error. In any case, the likelihood function was maximized with the help of the program GEMINI (Lalouel 1981). Two nested models M_1 and M_2 (M_1 being the more general model, i.e., the one including the larger number of free parameters) with maximum log likelihood l_1 and l_2 and number of free parameters k_1 and k_2, respectively, can be compared, since $2(l_1 - l_2)$ is approximately distributed as a χ^2 with $k_1 - k_2$ df.

Results

In table 1 are presented the means and SDs of some variables used in this analysis. The mean value of ACE was much higher in offspring than in fathers ($P < 10^{-6}$) and mothers ($P < 10^{-6}$), with a more dispersed distribution. Furthermore, the ACE values were slightly lower in females than in males ($P < .05$ in parents and offspring). The kurtosis of the distribution of ACE was nonsignificant in any group of relatives; on the other hand, the distribution of ACE in offspring was significantly skewed toward high values ($P < .01$).

Table 2 reports the correlations between ACE and the other variables. In offspring, significant negative correlations between ACE activity and age, height, weight, and blood pressure were observed. Adjustment was performed by regressing the value of ACE on age and sex and by taking the residual as the working variable; no correlations were observed between this adjusted variable and the other covariates. No adjustment was performed on parents, since in this group of relatives ACE was not correlated with the covariates. Figure 1 shows the distribution of ACE in fathers and mothers—and in offspring after adjustments on age and sex.

Familial Correlations of ACE

The comparison of the model assuming no familial resemblance with the model including familial correlations (models 0 and 1, respectively, of table 3) indicates that the latter model is much better supported by the data ($\chi^2 = 26.9$ with 4 df; $P < .001$). The maximum likelihood estimates of the familial correlations adjusted for age were .038, .166, .323, and .303 for spouses, father-offspring, mother-offspring, and siblings, respectively.

Looking for a Major Gene Effect

Table 3 shows the results of the comparisons between the different models. Models 2 and 3 assume a major gene effect and no residual correlations; in model 2 the major gene effects are set identical in parents and offspring, whereas in model 3, they are not. Model 4 is identical to model 3 but allows for residual correlations. The last model is identical to model 4 except that the transmission probabilities are left free to test the hypothesis of Mendelian inheritance. Models 2 and

Table 2

Correlations between Plasma ACE and Other Variables

Variables	Fathers	Mothers	Offspring
Age	0.02	−0.01	−0.32**
Height	−0.15	0.04	−0.30**
Weight	−0.04	−0.05	−0.25**
Systolic blood pressure	−0.10	0.06	−0.16*

* $P < .05$.
** $P < .001$.

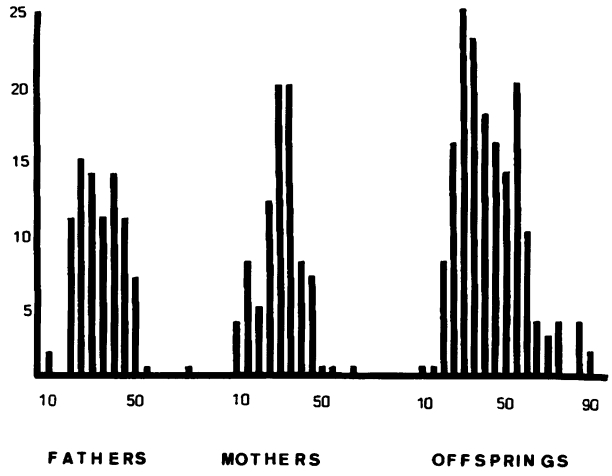

Figure 1 Frequency distribution of converting enzyme level

3 differ very significantly from the model including no familial resemblance, and model 3 is better supported by the data than is model 2 ($\chi^2 = 21.9$ with 2 df; $P < .001$), suggesting that the genetic effects are different in parents and offspring. Model 4 does not differ significantly from model 3, but the residual correlation between mother and offspring is significant when tested alone ($R = .244$; $P < .05$).

In the previous major gene models, the transmission parameters were fixed to the Mendelian values 1, .5, and 0. To test the hypothesis of Mendelian inheritance, these parameters were left free (model 5). The estimated values of the transmission parameters were 1.0, .453 and .311, respectively, with no significant improvement when compared with model 4 ($\chi^2 = 3.36$ with 3 df), the last value ($\tau(A|aa)$) not being statistically different from zero ($t = 1.2$). Another hypothesis of transmission was also tested by setting the transmission parameters to the estimated major allele probability. According to this hypothesis, the probability to transmit a is identical for subjects AA, Aa, and aa and is equal to the population probability of a. This model was less well fitted to the data than was the model assuming Mendelian segregation. Finally, two other models were compared with model 3, one to test the hypothesis of recessivity and the other to test the hypothesis of dominance, but both were rejected when compared with the codominant model.

Table 4 gives the parameter estimates, with their corresponding standard errors, for the more general model (model 5). An interesting feature of the model is that, after taking into account the major gene effects, the means and SDs of the ACE activity are almost identical in offspring and adults. According to this model, the major gene effect accounts for 4.8, 4.0, and 10.8 units of the overall mean and for 29.1%, 28.8%, and 74.7% of the variance of ACE in fathers, mothers, and offspring, respectively. The estimate of the probability of the less frequent allele is .26, and the major gene effect is approximately twice as great in high homozygotes than in heterozygotes and in offspring than in parents.

Discussion

In the present study, plasma ACE activity was measured in a large sample of adults and in their offspring. The measurement of plasma ACE activity by the method used here truly reflects the level of the circulating enzyme, since in previous studies the results of the enzymatic assay were always in very good agreement with the quantification, by the direct radioimmunoassay, of

Table 3

Tests of Genetic Hypotheses

Model[a]	Alternative Model	χ^2(df)
0: No familial resemblance		
1: Familial correlations, no major effect	0	26.9 (4)***
2: Major gene effect,[b] codominant, identical in parents and offspring, no residual familial correlations	0	31.6 (3)***
3: Same as model 2, with different major gene effects in parents and offspring	2	21.9 (2)***
4: Same as model 3, with residual familial correlations	3	6.0 (4)
5: Same as model 4, with free transmission parameters	4	3.4 (3)

[a] Adjusted on age and sex in offsprings.
[b] Except for model 5, all genetic models assume that the transmission parameters are set to the Mendelian values (0, .5, 1).
*** $P < .001$.

Table 4

Parameter Estimates of the Model including a Codominant Major Gene Effect, Different in Parent and Offspring, Residual Familial Correlations, and Free Transmission Parameters

	Parameter Estimate[a] (Standard Error)
Residual means:	
Fathers	29.6 (1.6)
Mothers	26.6 (1.5)
Offsprings	32.3 (.9)
Residual SD:	
Fathers	9.1 (.9)
Mothers	8.6 (.9)
Offsprings	7.8 (.6)
Familial correlations adjusted on major gene effect:	
Father-mother	.037 (.138)
Father-offspring	−.129 (.102)
Mother-offspring	.244* (.104)
Offspring-offspring	−.197 (.187)
Frequency of the more frequent allele	.76 (.05)
Major gene effects in heterozygotes:	
Parents	8.15** (2.56)
Offspring	21.42*** (1.46)
Major gene effects in high homozygotes:	
Parents	21.37*** (4.36)
Offspring	48.72*** (3.15)
Transmission probabilities:	
$\tau(A/AA)$	1.0[b] (. . .)
$\tau(A/Aa)$	.453 (.105)
$\tau(A/aa)$	.311 (.261)

[a] Adjusted on age and sex in offsprings.
[b] This parameter converged to the boundary value and was then fixed.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

immunoreactive ACE molecules in plasma (Alhenc-Gelas et al. 1983; Yasui et al. 1983; F. Alhenc-Gelas and J. L. Richard, unpublished data). The dispersion of plasma ACE measured in the 174 studied parents was in agreement with that observed in other series of healthy adult subjects (Lieberman 1975; Neels et al. 1982; F. Alhenc-Gelas and J. L. Richard, unpublished data). A slight difference was observed between men and women, as in some other studies (Lieberman 1975; Nakamura et al. 1982), but no association with age, weight, or blood pressure was detected. Offspring had higher levels of plasma ACE than did their parents. This is in agreement with most previous studies comparing children and adults (Lieberman 1975; Rodriguez et al. 1981; Neels et al. 1982). Furthermore, the interindividual variability of plasma ACE was much greater in offspring, especially those age <16 years, than in adults, suggesting that plasma ACE levels tend to decrease toward adult values at puberty or after. The reasons that would explain why levels of ACE are higher in children than in adults are unknown; they may be related to hyperactivity of the vascular endothelial cells during growth and angiogenesis or to the activity of any other cell type, such as macrophages, that may be responsible for ACE synthesis and secretion in plasma. A possible effect of thyroid hormones on ACE synthesis during growth can also be considered, since children have higher levels of thyroid hormones than do adults and since ACE level is known to be elevated in hyperthyroidism (Westgren et al. 1976; Nakamura et al. 1982; Yotsumoto et al. 1982). These hypotheses remain speculative, however.

The results of the present study demonstrate a familial resemblance of plasma ACE levels, a resemblance that is more likely to be explained by genetic factors than by environmental ones, for the following reasons: First, in another study, in a large group of adults plasma ACE level was found to be unrelated to several environmental factors, such as characteristics of the diet and alcohol or cigarette consumption (F. Alhenc-Gelas and J. L. Richard, unpublished data); second, the familial resemblance was only present between genetically related sub-

jects and not between spouses: third, the results of the genetic analysis indicated that the data were compatible with the presence of a major gene affecting the interindividual variability of plasma ACE. This compatibility, however, is in no way a definite proof of the presence of a major gene effect, and the limitations and many assumptions of the modelization should be kept in mind when interpreting the results.

The results indicate that the higher mean level and larger variance of plasma ACE in offspring than in parents could be entirely accounted for by a larger genetic effect in offspring. The different magnitudes of the genetic effects in parents and offspring can be mediated by a genetic mechanism and by environmental variables as well. Any correlate of age in offspring could be implicated; for example, as discussed above, any metabolic or hormonal factor negatively correlated with growth or maturation can possibly modulate the expression of the gene responsible for the genetic variability of ACE. The gene responsible for the genetic variability of ACE can be different from the gene(s) that is responsible for its synthesis. The plasma level of the enzyme is obviously determined by the equilibrium between synthesis in cells, secretion in plasma, and removal of the secreted protein from the circulation. The mechanisms involved in the regulation of these different events are largely unknown at the present time, and it can be hypothesized that any of them can be under genetic control. On the other hand, it is also logical to speculate that part of the interindividual variability of plasma ACE activity truly reflects a polymorphism in the gene(s) coding for ACE. It is interesting to note that two families have been observed in which an abnormal elevation in plasma ACE levels was genetically transmitted, apparently as an autosomal dominant trait. Plasma ACE levels in affected individuals in these kindreds were very high, much higher than the values observed in the present sample of healthy families (Le Pogamp et al. 1985; Okabe et al. 1985). This suggests the occurrence in some individuals of a rare but major abnormality in the genetic system(s) governing plasma ACE level.

In conclusion, the present study demonstrates a familial aggregation of plasma ACE level. The results are compatible with the presence of a genetic polymorphism explaining in part the interindividual variability of this physiologically important enzyme.

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