Angiotensin-Converting Enzyme *I/D* and α-Adducin *Gly460Trp* Polymorphisms

From Angiotensin-Converting Enzyme Activity to Cardiovascular Outcome

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Abstract—The angiotensin-converting enzyme (*ACE*) *I/D* and the α-adducin (*ADD1*) *Gly460Trp* polymorphisms are associated with cardiovascular risk factors. In a prospective population study and in cell models, we investigated the combined effects of these 2 polymorphisms. We randomly recruited 1287 white subjects (women: 50.0%; mean age: 55.9 years). We obtained outcomes from registries and repeat examinations (median 3). Over 9.0 years (median), 178 fatal or nonfatal cardiovascular events occurred. In *ADD1 Trp* allele carriers, the multivariate-adjusted hazard ratios associated with *ACE DD* versus *I* were 1.72 ($P=0.007$) for total mortality, 2.35 ($P=0.02$) for cardiovascular mortality, 2.02 ($P=0.005$) for all cardiovascular events, and 2.59 ($P=0.03$) for heart failure. In contrast, these hazard ratios did not reach significance in *ADD1 GlyGly* homozygotes ($0.08 \leq P \leq 0.90$). The positive predictive value and attributable risk associated with *ACE DD* homozygosity combined with mutated *ADD1* were 36.2% and 10.3%, respectively. To clarify our epidemiological observations, we investigated the effects of mutated human *ADD1* on the membrane-bound ACE activity in fibroblasts from 51 volunteers and in transfected human embryonic kidney cells (31 experiments). In fibroblasts (5.10 versus 3.63 nanomoles of generated hippuric acid per milligram of protein per minute; $P=0.0021$) and human embryonic kidney cells (1.086 versus 0.081 nmol/mg per minute; $P=0.017$), the membrane-bound ACE activity increased in the presence but not absence of the *ADD1 Trp* allele. In conclusion, the combination of *ACE DD* homozygosity and mutated *ADD1* worsened cardiovascular prognosis to a similar extent as classic risk factors, possibly because of increased membrane-bound ACE activity in subjects carrying the *ADD1 Trp* allele. **(*Hypertension*. 2007;49:1291-1297.)**

Key Words: adducin ■ angiotensin-converting enzyme ■ clinical genetics ■ epidemiology ■ risk factors

In monogenic diseases with typical Mendelian inheritance, such as phenylketonuria[1] or thalassanemia,[2] penetrance is under the influence of endogenous modulators, lifestyle, and environmental factors. The same mutation can, therefore, produce a wide spectrum of clinical manifestations, ranging from early onset debilitating disease to just mild symptoms at advanced age. This principle is even more applicable to polygenic cardiovascular disorders, in which many genes to a small and variable extent contribute to a common disease.

The insertion–deletion polymorphism of the angiotensin-converting enzyme (ACE) gene (*ACE I/D*) is among the most frequently examined genetic variants in cardiovascular medicine.[3] It affects the plasma level of ACE[3] and the generation of angiotensin II in the kidney,[4–6] arterial wall,[7] and heart.[8] The sodium content of the body modulates the effects of angiotensin II.[9] Substitution of glycine by tryptophan in the cytoskeleton protein α-adducin (*ADD1 Gly460Trp*) enhances tubular sodium reabsorption in the kidney.[9,10] Studies of never-treated hypertensive patients[11,12] and European[13–15] and Chinese populations[16] suggested that cardiovascular risk factors are associated with the *ACE D* allele, but only in the presence of mutated *ADD1*. Here, we report the results of a prospective population study and cell experiments, which further clarify the interaction between these 2 polymorphisms from a prognostic and mechanistic point of view.

Methods

Prospective Population Study

The University of Leuven Ethics Committee approved the Flemish Study on Environment, Genes and Health Outcomes.[13,17] From

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August 1985 until December 2005, we randomly recruited a family based population sample from a geographically defined area in Northern Belgium. The study population included 3108 subjects. The participation rate among the subjects contacted averaged 64.3%. Blood for DNA extraction could not be obtained from 422 participants, because they did not consent (n=150), had died (n=110), were terminally ill (n=30), or had moved out of the area (n=132). We also excluded 323 teenagers and 1076 adults with lower than median age at enrollment (41.4 years), because during follow-up only 12 (0.9%) of these 1399 subjects had experienced a fatal or nonfatal cardiovascular event. Thus, the number of participants statistically analyzed totaled 1287.

At the enrollment home visit, trained nurses measured anthropometric characteristics and blood pressure. They also administered a questionnaire to collect information about each subject's medical history, smoking and drinking habits, and intake of medications. Blood pressure was the average of 5 consecutive readings. Hypertension was defined as a blood pressure of ≥140 mm Hg systolic or ≥90 mm Hg diastolic or as the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters. Venous blood samples were drawn for DNA extraction and the measurement of serum cholesterol. Hypercholesterolemia was a serum level of ≥5.16 mmol/L (200 mg/dL)[18] or treatment with lipid-lowering drugs. Genomic DNA from white blood cells was amplified and genotyped for *ACE* and *ADD1*, as described previously.[13]

Via the National Population Registry (Rijksregister) in Brussels, Belgium, we ascertained vital status of all of the participants until December 31, 2005. We obtained the International Classification of Disease codes for the immediate and underlying causes of death from the Flemish Registry of Death Certificates. In 1089 subjects, we also collected information on the incidence of nonfatal events via follow-up visits with repeat administration of the same standardized questionnaire as that used at baseline. Follow-up was available at 1, 2, 3, or more occasions in 346, 286, 315, and 340 subjects, respectively. Physicians blinded with regard to the genetic results ascertained the diseases reported on the death certificates or via the questionnaires against the records held by general practitioners or hospitals. Coronary events included fatal and nonfatal myocardial infarction and procedures for coronary revascularization. Cardiac events consisted of fatal and nonfatal heart failure and coronary events. Fatal and nonfatal cardiovascular events were composed of cardiac end points, stroke not including transient ischemic attacks, aortic aneurysm, cor pulmonale, and pulmonary embolism. For all of the end points, we censored subjects from further analysis after the occurrence of a first event.

We used the SAS 9.1 software (SAS Institute) for database management and statistical analysis. We compared means and proportions by the standard normal z test and the χ^2 statistic, respectively, and survival curves by Kaplan–Meier survival function estimates and the log-rank test. We used Cox proportional hazard regression as implemented in the PROC SURVIVAL procedure of the SUDAAN 9.0 software (Research Triangle Institute) to calculate hazard ratios, while allowing for covariates, confounders, and family clusters. The baseline characteristics considered as covariates in Cox regression were sex, age, systolic blood pressure, body mass index, smoking, intake of alcohol, use of antihypertensive drugs, serum cholesterol, and previous cardiovascular complications. For all of the cardiovascular events, we computed the positive predictive value of a risk factor as $[R\times D]/[(G/100)\times(R-1)+1]$, where R is the multivariate-adjusted hazard ratio, D is the incidence of cardiovascular disease (19.7%), and G is the prevalence of the risk factor expressed in percent.[19] The attributable risk is given by $[(G/100)\times(R-1)\times 100]/[(G/100)\times(R-1)+1]$.[19]

Measurement of ACE Activity in Experimental Studies

The ethics committee of the University Vita Salute, Milan, approved the study. We genotyped fibroblast harvested from forearm skin biopsies for *ACE* and *ADD1*. Fifty-one volunteers gave informed written consent. The fibroblasts were grown to confluence in minimum Eagle's medium with 10% FBS and 100 IU/mL of penicillin/streptomycin. At least 72 hours before the ACE assay, the fibroblasts were made quiescent in minimum Eagle's medium with 0.3% FBS. We measured ACE activity in cell homogenates[20] and the membrane-bound activity[21] by hydrolysis of the synthetic substrate hippuryl-L-histidyl-L-leucine (Sigma-Aldrich),[22] followed by a high-performance liquid chromatography quantification of the hippuric acid formed during incubation.[23] We expressed ACE activity in nanomoles of hippuric acid produced per milligram of protein per minute. We used Student's t test for unpaired observations with 2-tailed P levels to compare ACE activity across genotypes.

Human embryonic kidney (HEK) cells, purchased from the European Collection of Animal Cell Cultures (85120602), were cultured in monolayer in DMEM (Gibco BRL) with 5% bovine serum (Cambrex), 1% non-essential amino acids (Sigma-Aldrich), and 100 IU/mL of penicillin/streptomycin (Gibco BRL). We obtained human wild-type (*Gly460* and *Ser*586) and mutated (*Trp460* and *Cys586*) *ADD1* by site-directed mutagenesis of an expression vector for hemagglutinin-tagged human adducin (aa1–737, pCMVneoHA-ADD1) using the QuickChange mutagenesis kit (Stratagene). For transfection, we used the Cal Phos mammalian transfection kit (Clontech). We selected stable transfectants by addition of 0.9 mg/mL of G418 sulfate (Stratagene). We quantified the overexpression of protein in Western blots using a monoclonal antibody against ADD1 and a polyclonal antibody against the hemagglutinin tag (Upstate) and the Odyssey Infrared Imaging System (Li-Cor Biosciences). For each of the 2 *ADD1* variants, we generated 3 lines of transfected HEK cells. In each cell line, we measured membrane-bound ACE activity[21] in 4 to 6 separate experiments. After being grown to confluence, the HEK cells were scraped, gently centrifuged, and resuspended in assay buffer containing 1 mmol/L of hippuryl-L-histidyl-L-leucine.

Results

Prospective Population Study

The 1287 participants included 643 women (50.0%); 522 hypertensive patients (40.6%) of whom 257 (49.2%) were on antihypertensive drug treatment; and 976 hypercholesterolemic patients (75.8%) of whom 44 (4.5%) were on treatment with lipid-lowering drugs. Women compared with men ($P<0.05$) had lower systolic (130.3 versus 132.5 mm Hg) and diastolic (77.8 versus 79.8 mm Hg) blood pressures and less frequently reported smoking (22.6% versus 35.4%), intake of alcohol (12.1% versus 38.4%), and previous cardiovascular complications (4.0% versus 7.9%). The frequencies of the *ACE* genotypes (*DD* 24.6%, *ID* 51.6%, and *II* 23.8%; $P=0.25$) and the *ADD1* genotypes (*GlyGly* 58.6%, *GlyTrp* 36.1%, and *TrpTrp* 5.3%; $P=0.74$) did not deviate from Hardy–Weinberg proportions. The baseline characteristics of the study participants by *ACE* and *ADD1* genotypes appear in Table 1.

Median follow-up was 9.0 years (5th to 95th percentile interval: 0.9 to 20.2 years) for fatal end points and 7.2 years (5th to 95th percentile interval: 1.3 to 16.7 years) for all of the fatal and nonfatal cardiovascular events combined. Participants with follow-up information available at 1 or 2 occasions (n=632) as compared with those with more frequent follow-up (n=655) had similar baseline characteristics with the exception of small differences in age (57.7 versus 54.2 years, respectively; $P<0.001$), systolic blood pressure (132.6 versus 130.2 mm Hg; $P=0.02$), and serum cholesterol (5.84 versus 6.06 mmol/L; $P=0.001$). Of 220 deaths, 90 (40.9%) were because of cardiovascular causes. The incidence of fatal

TABLE 1. Clinical Features by *ACE* and *ADD1* Genotypes

Characteristic	*ACE* Genotypes			*ADD1* Genotypes		
	II + ID (n=971)	*DD* (n=316)	*P*	*GlyGly* (n=754)	*GlyTrp + TrpTrp* (n=533)	*P*
Mean±SD of entry characteristic						
Age, y	55.7±10.6	56.4±10.6	0.36	55.3±10.5	56.7±10.7	0.03
Body mass index, kg/m^2	26.8±4.3	26.9±4.4	0.74	26.9±4.6	26.8±4.0	0.50
Systolic blood pressure, mm Hg	131.0±17.9	132.4±18.5	0.26	131.3±17.9	131.5±18.3	0.82
Diastolic blood pressure, mm Hg	78.8±9.7	79.0±9.1	0.69	79.0±9.4	78.6±9.8	0.43
Serum cholesterol, mmol/L	5.96±1.21	5.92±1.16	0.62	5.92±1.19	5.99±1.21	0.30
No. (%) with entry characteristic						
Women	494 (50.9)	149 (47.2)	0.27	369 (48.9)	274 (51.4)	0.40
Current smokers	296 (30.5)	77 (24.4)	0.04	221 (29.3)	152 (28.5)	0.80
Consuming alcohol	245 (25.2)	80 (25.3)	0.99	195 (25.9)	130 (24.4)	0.56
Hypertensive patients	388 (40.0)	134 (42.4)	0.47	294 (39.0)	228 (42.8)	0.19
On antihypertensive medication	193 (19.9)	64 (20.3)	0.87	144 (19.1)	113 (21.2)	0.36
Cardiovascular history*	60 (6.2)	17 (5.4)	0.68	44 (5.8)	33 (6.2)	0.81

P values are for the differences between genotypes.

*Previous cardiovascular complications consisted of myocardial infarction, coronary revascularization, heart failure, stroke, aortic aneurysm, cor pulmonale, and pulmonary embolism.

and nonfatal cardiovascular outcomes totaled 178, including 91 coronary events, 46 cases of heart failure, and 41 strokes.

In single-gene analyses (Table 2) with adjustments applied for family clusters and baseline characteristics including sex, age, body mass index, systolic blood pressure, smoking, alcohol intake, serum cholesterol, use of antihypertensive drugs, and previous cardiovascular disease, *ACE DD* homozygosity compared with the *I* allele predicted total mortality (hazard ratio: 1.43; $P=0.014$) and heart failure (hazard ratio: 2.33; $P=0.006$). Although not formally significant, trends were similar for cardiovascular mortality (hazard ratio: 1.61; $P=0.062$) and all of the cardiovascular events (hazard ratio: 1.34; $P=0.09$). In similarly adjusted single-gene analyses, the *ADD1 Trp* allele compared with *GlyGly* homozygosity predicted heart failure (hazard ratio: 2.21; $P=0.006$) but none of the other outcomes (Table 2).

The Figure shows Kaplan–Meier survival function estimates for all of the cardiovascular events. In subjects with mutated *ADD1* ($P=0.03$), but not in *GlyGly* homozygotes ($P=0.70$), *ACE DD* homozygosity conferred a higher overall cardiovascular risk than the *I* allele. Table 3 shows that with similar adjustments applied as in the single-gene analyses, none of the hazard ratios associated with *ACE DD* homozygosity reached significance ($0.08 \leq P \leq 0.90$) in *ADD1 GlyGly* homozygotes. In contrast, in *ADD1 Trp* allele carriers, the corresponding hazard ratios were significant for total mortality (hazard ratio: 1.72; $P=0.007$), cardiovascular mortality (hazard ratio: 2.35; $P=0.02$), all cardiovascular events (hazard ratio: 2.02; $P=0.005$), and heart failure (hazard ratio: 2.59; $P=0.03$), with a similar trend for cardiac events (hazard ratio: 1.79; $P=0.057$). The interaction between the *ACE* and *ADD1* genes reached statistical significance for all of the fatal

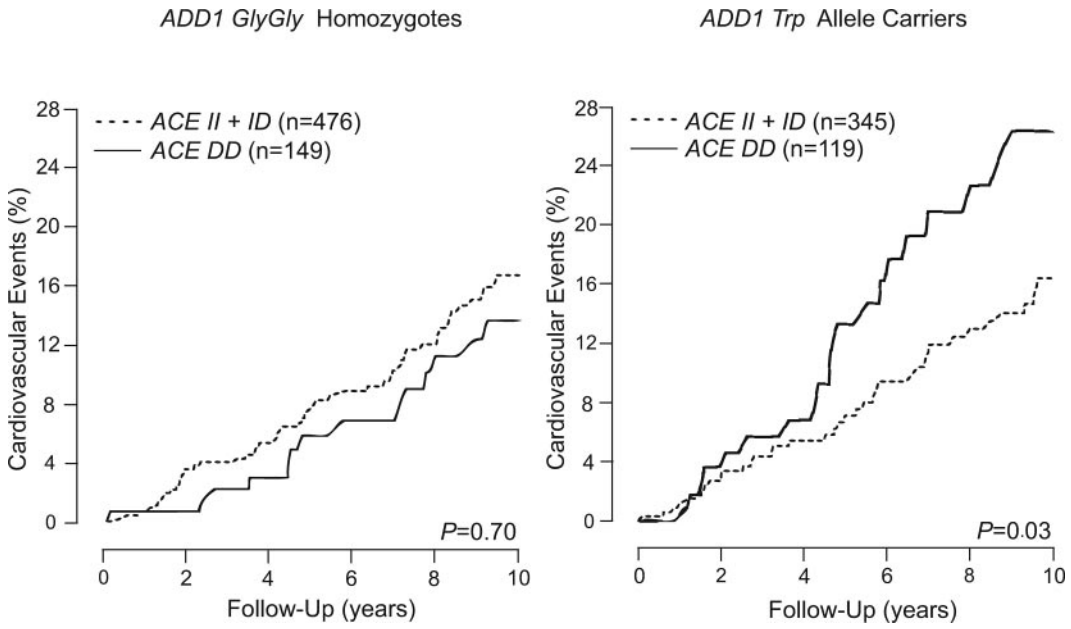
TABLE 2. Hazard Ratios for Cardiovascular Outcomes in Single-Gene Analyses

Event Type	*ACE DD* vs *I*			*ADD1 Trp* vs *GlyGly*		
	Cases *DD/I**	Hazard Ratio (95% CI)†	*P*	Cases *Trp/GlyGly**	Hazard Ratio (95% CI)†	*P*
Mortality						
Total	62/158	1.43 (1.08 to 1.90)	0.014	96/124	1.16 (0.88 to 1.54)	0.30
Cardiovascular	28/62	1.61 (0.98 to 2.64)	0.062	41/49	1.25 (0.79 to 1.97)	0.34
Fatal and nonfatal events‡						
Cardiovascular	48/130	1.34 (0.95 to 1.90)	0.09	80/89	1.19 (0.88 to 1.60)	0.26
Cardiac	34/86	1.36 (0.88 to 2.11)	0.17	57/63	1.26 (0.86 to 1.85)	0.24
Coronary	22/69	1.04 (0.63 to 1.72)	0.88	38/53	0.93 (0.59 to 1.48)	0.77
Heart failure	19/27	2.33 (1.28 to 4.25)	0.006	28/18	2.21 (1.25 to 3.91)	0.006
Stroke	12/29	1.29 (0.66 to 2.52)	0.45	14/27	0.74 (0.38 to 1.44)	0.38

*Number of *ACE DD/I* subjects at risk amounted to 316/971 for mortality and 268/821 for fatal and nonfatal events. For *ADD1 Trp/GlyGly*, these numbers were 533/754 and 464/625, respectively.

†Hazard ratios for *ACE DD* vs *I* were adjusted for family clusters and baseline characteristics: sex, age, body mass index, systolic blood pressure, serum cholesterol, smoking, alcohol intake, use of antihypertensive drugs, and previous cardiovascular diseases.

‡A total of 198 participants were alive on December 31, 2005, but were not followed up for nonfatal events.



Kaplan–Meier survival function estimates for all of the cardiovascular events in *ACE DD* homozygotes compared with *ACE I* allele carriers in *ADD1 GlyGly* homozygotes (left) and in *ADD1 Trp* allele carriers (right). *P* values were derived from the log-rank test.

and nonfatal cardiovascular events ($P=0.02$) but not for the other end points under study ($P\geq0.20$). Sensitivity analyses showed consistent results after exclusion of the participants who, at enrollment, had a history of cardiovascular complications (Table S1 available online at http://hyper.ahajournals.org) or who were on antihypertensive drug treatment at baseline (see Table S2).

In all of the participants, the positive predictive value and attributable risk associated with the combination of *ACE DD* homozygosity and mutated *ADD1* were 36.2% and 10.3%, respectively. Considering classic risk factors at enrollment, these estimates were 24.5% and 10.4% for smoking, 21.1% and 25.0% for hypercholesterolemia, and 25.7% and 21.8% for hypertension, respectively.

Experimental Studies

In human fibroblasts, the membrane-bound ACE activity was similar irrespective of the presence (n=37; 3.97 nmol/mg per minute; SE: 0.28 nmol/mg per minute) or absence of the *ACE D* allele (n=14; 4.39 nmol/mg per minute; SE: 0.45 nmol/mg per minute; $P=0.43$). In contrast, the membrane-bound ACE activity was higher in fibroblasts carrying the *ADD1 460Trp* allele (n=21; 5.10 nmol/mg per minute; SE: 0.35 nmol/mg per minute) compared with those not carrying the mutated *ADD1* allele (n=30; 3.63 nmol/mg per minute; SE: 0.29 nmol/mg per minute; $P=0.0021$). In homogenates of human fibroblasts, the ACE activity tended to be higher in *ACE D* allele carriers (n=32; 3.48 nmol/mg per minute; SE: 0.24 nmol/mg per minute) than in noncarriers (n=13; 2.76 nmol/mg per minute;

TABLE 3. Hazard Ratios for Cardiovascular Outcomes in *ACE DD* vs *I* Carriers by *ADD1* Genotype

	ADD1 GlyGly Homozygotes			*ADD1 Trp* Allele Carriers		
Event Type	Cases *DD/I**	Hazard Ratio (95% CI)†	*P*	Cases *DD/I**	Hazard Ratio (95% CI)†	*P*
Mortality						
Total	31/93	1.24 (0.82 to 1.88)	0.31	31/65	1.72 (1.16 to 2.54)	0.007
Cardiovascular	14/35	1.51 (0.75 to 3.04)	0.24	14/27	2.35 (1.14 to 4.77)	0.02
Fatal and nonfatal events‡						
Cardiovascular	22/76	0.97 (0.58 to 1.62)	0.90	26/54	2.02 (1.24 to 3.29)	0.005
Cardiac	15/48	1.20 (0.65 to 2.24)	0.56	19/38	1.79 (0.98 to 3.26)	0.057
Coronary	11/42	1.07 (0.52 to 2.21)	0.85	11/27	1.14 (0.56 to 2.29)	0.72
Heart failure	7/11	2.42 (0.89 to 6.56)	0.08	12/16	2.59 (1.08 to 6.21)	0.03
Stroke	8/19	1.38 (0.60 to 3.17)	0.44	4/10	1.28 (0.43 to 3.83)	0.66

*Among *ADD1 GlyGly* homozygotes, the number of *ACE DD/I* subjects at risk amounted to 180/574 for mortality and 149/476 for fatal and nonfatal events. Among *ADD1 Trp* allele carriers, these numbers were 136/397 and 119/345, respectively.

†Hazard ratios for *ACE DD* vs *I* were adjusted for family clusters and baseline characteristics: sex, age, body mass index, systolic blood pressure, serum cholesterol, smoking, alcohol intake, use of antihypertensive drugs, and previous cardiovascular diseases.

‡A total of 198 participants were alive on December 31, 2005, but were not followed up for nonfatal events.

SE: 0.26 nmol/mg per minute; $P=0.089$) and in *ADD1 Trp* carriers (n=19; 3.69 nmol/mg per minute; SE: 0.36 nmol/mg per minute) compared with the corresponding noncarriers (n=23; 3.03 nmol/mg per minute; SE: 0.20 nmol/mg per minute; $P=0.091$). HEK cells transfected with mutated human *ADD1* had higher membrane-bound ACE activity (n=16; 1.086 nmol/mg per minute; SE: 0.064 nmol/mg per minute) than those transfected with the wild-type *ADD1* (n=15; 0.081 nmol/mg per minute; SE: 0.055 nmol/mg per minute; $P=0.017$).

Discussion

The key finding of our study was that in a randomly recruited sample of middle-aged and older white subjects, *ACE DD* homozygosity predicted total and cardiovascular mortality and the incidence of cardiovascular events in carriers of the mutated *ADD1* but not in subjects carrying the wild-type *ADD1*. These findings translate previously observed[11–15] associations of cardiovascular risk factors with these 2 polymorphisms into hard cardiovascular outcomes. Furthermore, in ex vivo studies, human fibroblasts showed higher membrane-bound ACE activity in the presence of the *ADD1 Trp* allele. We corroborated the latter observation in cell membranes of HEK cells transfected with the human mutated and wild-type *ADD1*. Based on this replication, we believe that our ex vivo finding might be extrapolated to human kidney cells.

In line with expert opinion,[24] we opted for the candidate gene approach, which relies on solid knowledge of pathophysiologic pathways and accounts for the context dependency of genetic associations.[25] Previous clinical studies[11–15] informed our present analysis. In 2001, we noticed in untreated hypertensive patients that the *ACE D* allele and mutated *ADD1* synergistically enhanced the pressor effect of sodium loading.[12] In a subsequent article,[13] we reported that the incidence of new-onset hypertension more than doubled in *ACE DD* homozygotes compared with *I* allele carriers but only in the presence of the *ADD1 Trp* allele. In cross-sectional analyses of the same population, carriers of the 2 risk-conferring alleles had a thickened intima–media of the femoral artery,[15] as well as a higher serum creatinine concentration and an increased 24-hour proteinuria.[14] In never-treated hypertensive patients, *ACE DD* homozygosity was associated with microalbuminuria, but only in carriers of the *ADD1 Trp* allele.[11] Our current prospective findings with hard events as the outcome of interest span a median follow-up of 9 years and consolidate the concept that the combination of *ACE DD* homozygosity and the *ADD1 Trp* allele define a genetic constellation associated with a substantially increased cardiovascular risk.

Our current findings not only clarified the combined effects of the *ACE I/D* and *ADD1 Gly460Trp* polymorphisms from a prognostic point of view but also provided the first experimental evidence linking the known functionality of these 2 polymorphisms.[3,8–10,20,26,27] In line with clinical data[3] and other studies of human tissues,[8,20] we found a slightly increased ACE activity in homogenates of human fibroblasts carrying the *ACE D* allele. Rat experiments,[26] in vitro transfection studies,[27] and studies of never-treated hypertensive patients[12] revealed that mutation of *ADD1* leads to a cellular dysfunction characterized by higher Na^+,K^+-ATPase activity, hence, increased tubular sodium reabsorption in the kidney.[9,10] Expression of the hypertensive rat or human variant of *ADD1* into normal renal epithelial cells impaired Na^+,K^+-ATPase endocytosis, which explains the higher sodium pump activity at the basolateral membrane[10] and sodium retention and hypertension in the intact organism.

Cell transfection and molecular experiments in cell-free conditions demonstrated that the *ADD1 Trp* allele compared with the *Gly* allele modulates actin polymerization and thereby leads to stiffening of the cytoskeleton supporting the cell membrane.[9] This effect, along with other molecular mechanisms discussed in detail elsewhere,[9,10] slows the rate of endocytosis of membrane-bound proteins and lengthens their residential time at the cell surface. Our current finding that the membrane-bound ACE activity increased in the presence of the *ADD1 Trp* allele in human fibroblasts, as well as in transfected HEK cells, is therefore consistent with the already available experimental evidence on the sodium pump.[9,10]

In the circulating blood, renin is the rate-limiting enzyme controlling the generation of angiotensin II.[28] However, the production of angiotensin II within tissues is regulated in a different way. ACE at the cell surface locally generates angiotensin II in the kidney,[4–6] the arterial wall,[7] and the heart.[8] The concentration of angiotensin II is much higher in the renal interstitium and in the proximal tubular fluid than in the circulating blood.[4] Renal tubular cells express the biochemical machinery, including ACE, which is necessary to synthesize angiotensin II.[4–6] Locally generated angiotensin II probably plays a role in the tubuloglomerular feedback mechanism.[6] Expression of ACE in the arterial wall also increases the local generation of angiotensin II.[7] As suggested by others,[29–31] increased target organ damage in carriers of the *ACE DD* genotype might be attributable to the exaggerated generation of angiotensin II within tissues. We suggest that the *ADD1 Trp* allele probably facilitates this pathogenetic pathway.

The present study must be interpreted within the context of its possible limitations. First, in comparison with other prospective studies, our sample size and the number of events was relatively small. As exemplified by the wide CIs (Table 3), the number of some end points might have been too small to pick up a synergistic effect of *ACE DD* homozygosity and mutated *ADD1*. Second, we could not study the effects of *ADD1 Trp* homozygosity, because our population study included only 68 homozygotes and because none of the donors of fibroblasts was homozygous. Third we did not prove causality and cannot exclude that genetic polymorphisms in linkage disequilibrium with the *ACE* or *ADD1* genes contributed to our findings. Fourth, in long-term surveys, the definition of events is likely to be less precise than in short-term studies or clinical trials, in which end points are collected via a single channel of information. On the other hand, our outcome results showed internal consistence, including those for total mortality, which is a binary outcome not requiring any medical diagnosis. Use of a prospective design in a cohort protects against confounding by population stratification. Furthermore, our prospective

epidemiological results corroborate a hypothesis independently raised by studies in never-treated hypertensive patients,[11,12] previous population surveys,[13–16] and pharmacogenomic studies,[32,33] and they are supported by animal experiments[26] and an established pathogenetic mechanism.[9,10] Moreover, we replicated our initial findings in human fibroblasts in kidney cells of human origin transfected by the wild-type and mutated human *ADD1*.

Perspectives

Our present findings might have repercussions for clinical practice. The positive predictive value and attributable risk associated with the combination of *ACE DD* homozygosity and mutated *ADD1* were 36.2% and 10.3%, respectively. These estimates suggest that this particular genetic constellation carries a risk comparable to that of established cardiovascular risk factors, such as smoking, hypercholesterolemia, and hypertension. The factors explaining the high positive predictive value associated with the combination of *ACE DD* homozygosity and mutated adducin were the relative risk of 2.05 and the prevalence of 10.9%, which were substantially higher and lower, respectively, than the corresponding values for the classic risk factors. In a single-center study, we observed that never-treated hypertensive patients investigated under highly standardized conditions showed the largest decrease in blood pressure in response to diuretic therapy if they carried both the *ACE I* and *ADD1 Trp* alleles.[32] Hypertensive carriers of mutated *ADD1* experience more cardiovascular complications than noncarriers,[17,34] and they are better protected if they are treated with diuretics instead of drugs that do not antagonize the enhanced tubular sodium reabsorption.[33] We believe that our current findings might help to pave the way to a pharmacogenomic and cost-saving approach for the management of hypertension.

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Disclosures

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