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► **To cite this version:**

Giulia Dell'Omo, Giuseppe Penno, Laura Pucci, Daniela Lucchesi, Carmen Fotino, et al.. ACE gene insertion/deletion polymorphism and capillary permeability in hypertension. *Clinical Science*, 2006, 111 (6), pp.357-364. 10.1042/CS20060165 . hal-00479331

HAL Id: hal-00479331

<https://hal.science/hal-00479331>

Submitted on 30 Apr 2010

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Dell'Omo et al: ACE polymorphism and capillary permeability in hypertension, 8/2/2006 CS2006/0165

**ACE GENE INSERTION/DELETION POLYMORPHISM MODULATES
CAPILLARY PERMEABILITY IN HYPERTENSION**

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SHORT TITLE : ACE polymorphism and capillary permeability in hypertension

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ABSTRACT

RATIONALE: A D/D polymorphism within the ACE gene increases the risk of microalbuminuria, a predictor of atherosclerotic vascular disease, in essential hypertension. It is unknown, however, whether that genetic profile is accompanied by disturbed macromolecular permeability of systemic capillary endothelium, possibly in the context of a generalized endothelial dysfunction. **METHODS:** The ACE gene polymorphism was determined by polymerase chain reaction in 79 never treated, uncomplicated hypertensive and 16 normotensive men as controls. Evaluation variables were the transcapillary escape rate of albumin (TER_{alb}, the 1-hour decline rate of intravenous ¹²⁵I-albumin, a measure of integrity of systemic capillary endothelium), albuminuria, forearm vasodilation to intra-arterial acetylcholine, an index of nitric oxide-mediated vasomotion, in addition to a series of sensitive parameters for albumin permeation (BP, metabolic status, smoking habits). **RESULTS:** Analyses were done by comparing D/D homozygotes vs grouped I/D and I/I subjects. TER_{alb} was higher in D/D hypertensives characterized also by higher albuminuria, more frequent microalbuminuria and comparable forearm responsiveness to intra-arterial acetylcholine. 24-hr BP, fasting glucose and insulin, insulin sensitivity, smoking habits, metabolic parameters did not differ. TER_{alb} and urine albumin values were positively associated. **CONCLUSIONS:** ACE D/D homozygosis associates with higher TER_{alb} independent of several confounding factors in essential hypertensive men. That behavior may reflect noxious genetic influences on systemic vascular permeability, a critical control mechanism for atherogenesis in absence of grossly impaired nitric-oxide mediated arteriolar responsiveness. The parallel behavior of TER_{alb} and albuminuria suggests some shared genetically mediated determinant of renal and systemic microvascular abnormalities in hypertension.

Key Words: ACE POLYMORPHISM, TRANSCAPILLARY ESCAPE RATE OF ALBUMIN, ALBUMINURIA, ENDOTHELIAL FUNCTION, HYPERTENSION

ABBREVIATIONS: ACE:ANGIOTENSIN CONVERTING ENZIME; ACH:ACETYLCHOLINE; AUC: AREA UNDER CURVE; BMI:BODY MASS INDEX; BP:BLOOD PRESSURE; I/D:INSERTION/DELETION; FBF:FOREARM BLOOD FLOW; HOMA-IR:HOMEOSTASIS MODEL ASSESSMENT OF INSULIN RESISTANCE; MA:MICROALBUMINURIA; METS: METABOLIC SYNDROME; NO:NITRIC OXIDE; OGTT:ORAL GLUCOSE TOLERANCE TEST; SNP:SODIUM NITROPRUSSIDE; TERALB:TRANSCAPILLARY ESCAPE RATE OF ALBUMIN; UAE:URINARY ALBUMIN EXCRETION

INTRODUCTION

Consistent evidence supports the role of the Angiotensin I Converting Enzyme (ACE) Insertion/Deletion (I/D) polymorphism in the modulation of urinary albumin excretion (UAE) in non diabetic patients with essential hypertension. Several previous reports [e.g. 1-5], in fact, associated the presence of the D allele within the ACE gene with higher UAE and more frequent microalbuminuria (MA), a sign of subclinical renal damage and a predictor of atherosclerotic cardiovascular events [6] in hypertension. It is unknown, however, whether that noxious genetic influence on UAE, which may reflect more permeant renal glomeruli, is also accompanied by systemic changes in systemic permeability of capillary endothelium, a key determinant of endogenous defense against vascular injury and atherosclerosis [7, 8].

To assess that possibility, we measured the trans-capillary albumin escape rate (TERalb), a measure of the integrity of systemic capillary permeability [9], and its relationship with the ACE I/D genotype and UAE, in a group of non diabetic, never treated uncomplicated hypertensive men. To evaluate a possible coexistence of an abnormal function of arteriolar endothelium, we also investigated the association of that genotype with the endothelial-mediated vasodilator response to intra-brachial acetylcholine (ACH), a nitric oxide (NO) releasing agonist [10].

MATERIAL AND METHODS

Subjects

Seventy-nine never treated sedentary, Caucasian, apparently genetically unrelated hypertensive men and 16 normotensive men age-matched as a group participated in the study. Exclusion criteria were diabetes (fasting plasma glucose levels >125 mg/dl and/or 2-hr post-glucose load \geq 200 mg/dl), renal insufficiency (serum creatinine >1.4 mg/dl), proteinuria at the dipstick test and impaired cardiac function (ejection fraction <50%). In all, coexisting coronary and vascular disease were excluded through history, physical examination, routine blood chemistry, baseline EKG or, when indicated, treadmill test, echocardiography, carotid and lower limb echo-Doppler sonography.

In accordance with the Declaration of Helsinki and institutional guidelines, the local Ethical Committee approved the protocol and subjects were aware of the investigational nature of the study and agreed to participate after signing a written consent.

MAIN EVALUATION VARIABLES

ACE genotype determination

Whole blood was collected in EDTA tubes and stored at -20°C until extraction. Genomic DNA was extracted by manual saline extraction from lysed white blood cells as described elsewhere [11]. The polymorphic region in intron 16 of the ACE gene (17q23) was amplified by polymerase chain reaction (PCR) performed with 10 ng DNA using 5 pmol of both primers: forward 5'-CTGGAGACCACTCCCATCCTTTCT-3' and reverse 5'-GATGTGGCC ATCACAT TCGTCAGAT-3'. Since the amplification of I allele is less efficient than D allele, the specificity of D/D genotyping was increased by amplifying again all D/D samples using a pair of primers specific for the insertion sequence (forward 5'-TGGGACCACAGCGCCCGCCATTAC-3' and reverse 5'-TCGCCAGCCCTCCCATGCC CATAA-3') in presence of DMSO 5%. Two different operators (L.P. and D.L.) unaware of the patient clinical status read gels independently.

TERalb

TERalb studies were performed between 2.00 and 4.00 pm after a 4 hour fast with patients withdrawing from tea, coffee, alcohol, tobacco from the early morning as previously described [12]. In short, ¹²⁵I-labeled human serum albumin (6-8 μCi, 222-296 kBq, SARI-125 A-2, SORIN Biomedica) was obtained by electrolytic technique, a procedure that does not alter the biological behavior of albumin *in vivo*. Free ¹²⁵I was eluted by passage in a Sephadex G-25-M column (Column PD-10, Pharmacia), a purification step that reduced free ¹²⁵I content in the injected dose to less than 1%. Labeled albumin was injected after a 30-min rest in the sitting position as a bolus, and blood samples taken from the contralateral arm every 5 min during the hour following the injection. Radioactivity was measured (Cobra 5000 γ-counter, Packard) in duplicate in whole blood samples centrifuged for 10 min at 3000 g for 40 min. Hematocrit (Coulter Counter 55, Coulter Electronics) was determined in each sample. Serum albumin was measured by immunonephelometry (Behring Laser Nephelometer System, Behring, interassay variation coefficient: 5.2%).

UAE

Urinary albumin was measured by nephelometry (Behring Laser Nephelometer System, Behring) using a commercially available kit (Roche, Italy, 0.1 mg/dl detection limit). Intra- and inter-assay variation coefficients at different concentrations of urine albumin were: 2.7% and 3.2% (0.3 mg/dl), 2.4% and 2.9% (1 mg/dl) 1.9% and 2.6% (10 mg/dl), 1.8% and 3.2% (30 mg/dl) respectively. To minimize the confounding influence of daily physical activity and facilitate the collection procedure, urine was collected from 8.⁰⁰ p.m. to 8.⁰⁰ a.m. during three consecutive days. Urinary and serum creatinine was measured by standard colorimetric methods.

Forearm infusion

Forearm studies (n=66 hypertensive patients and n=12 normotensive controls) were performed in the morning in a quiet, air-conditioned room 24-48 hours apart from TERalb determination. Subjects were fasted and instructed to restrain from heavy exercise and to avoid smoking and emotional excitement from the day before the experiment. A 22-gauge polyethylene catheter (Angiocath, Becton Dickinson) was inserted into the right brachial artery, the arterial line connected to an infusion pump (Perfusor, Secura FT, Braun) and subjects were then allowed to rest for approximately 30 minutes. Total forearm blood flow (FBF) was measured by venous plethysmography with a strain-gauge apparatus (Hokanson, EC 5R Plethysmograph). Strands made of silastic tubing of 0.4 mm I.D. and 0.8 mm O.D filled with mercury were used. The gauge was applied on the arm, 5-6 cm distal to the elbow at a tension sufficient to keep the gauge in the same position throughout the experiment. Patient forearm was kept on a table, slightly flexed and inclined at about 45° to the horizontal plane with the wrist and hand supported by sand bags. One minute before FBF determination, a pneumatic paediatric cuff was placed around the wrist and inflated to suprasystolic arterial blood pressure in order to exclude the hand vascular region. A second cuff was placed proximal to the plethysmograph and automatically inflated to a pressure of 40 mmHg to allow FBF measurement according to the venous occlusion method. BP was measured every 5 minutes throughout the study at the contralateral arm by an automated device (NIBP KO 7267.004, Kontron Instruments). Further details of the method have been published elsewhere [13].

Fresh solutions of ACH HCl (Miovisin™, Farmigea, 7.5, 15 and 30 µg/min, five minutes each), a nitric oxide releasing compound [10], and sodium nitroprusside (SNP, Nipride™, Malesci, used as an internal control for NO-independent mechanisms [14], 0.8, 1.6 and 3.2 µg/min, five minutes each in syringes protected from light through aluminium foil were infused intra-arterially. Preliminary studies had shown that those infusion periods were sufficient to reach a plateau of FBF response without changes in systemic arterial pressure and contralateral FBF. Local administration of ACH and SNP was preceded and followed by saline infusion (0.6 ml/min). The infusion sequence was randomized and a 30-minute interval was left between the first and second drug administration.

Ancillary parameters

Office systolic and diastolic blood pressure (BP) was the mean of several indirect recordings. 24 hour BP (08³⁰ - 08⁰⁰ A.M) was measured through an oscillometric monitor (Diasys Integra, Novacor).

OGTT (75 g glucose) was performed in the morning after an overnight fast. Upon baseline samples, specimens for plasma glucose and insulin were drawn 0.5, 1, 1.5 and 2 hours after administration of the glucose load. Plasma glucose was measured by the glucooxidase method

Dell'Omo et al: ACE polymorphism and capillary permeability in hypertension, 8/2/2006 CS2006/0165

using a Beckman Glucose Analyzer I/I (Beckman Instruments), and plasma insulin by immunoradiometric assay (Biosource, no crossreactivity with human proinsulin) with an interassay variation coefficient of 5%.

Total, high density (HDL) and low density lipoprotein (LDL) cholesterol, triglycerides were assessed in fasting conditions by enzymatic colorimetric techniques (Roche, Modular system).

Anthropometric measurements (height and weight) were made after each participant had removed his shoes and upper garments. Body weight was measured to the nearest 0.1 Kg on a scale with attached height measure (SECA 207).

Smoking status was defined as active smokers vs non smokers, without distinction between former- and never smokers.

Data processing

Plasma ^{125}I -albumin concentration (cpm/ml) was plotted on a semi-logarithmic scale, and the transcapillary escape rate (%/h) was calculated from the mono-exponential disappearance rate constant of the ^{125}I curve from 10 to 60 min. Plasma volume ($\text{ml}/1.73 \text{ m}^2$) was determined by retropolation to zero time of the disappearance curve corrected for the injected dose of tracer obtained by weighing the syringes before and after the injection [12].

UAE ($\mu\text{g}/\text{min}$) was the average of three consecutive overnight collections (mean variation coefficient of the triplets: 22%). MA was defined as $\text{UAE} \geq 15 \mu\text{g}/\text{min}$, an accepted threshold for overnight collections [15]. Because of its skewed distribution, albuminuria was log-transformed to normalize its distribution. Creatinine clearance ($\text{ml}/\text{min} \times 1.73\text{m}^2$) was calculated according to standard formulae¹

FBF ($\text{ml } 100 \text{ ml}^{-1} \text{ forearm volume min}^{-1}$) represents the mean of four-five determinations obtained over the last two minutes of each experimental period. Percent FBF changes from baseline were the evaluation variable.

OGTT data were summarised as the area under curve (AUC, trapezoidal rule) values. Insulin sensitivity was assessed through the homeostasis model assessment of insulin resistance (HOMA-IR)² [16]. Increasing HOMA-IR values denote progression from normal to impaired insulin

¹ $[(12\text{-HR URINE VOLUME} \times \text{URINE CREATININE})/\text{SERUM CREATININE} \times 1.73]/\text{BODY SURFACE AREA}$

² $\text{FASTING SERUM INSULIN } [\mu\text{U}/\text{ML}] \times \text{FASTING PLASMA GLUCOSE } [\text{MMOL}/\text{L}]/22.5$

sensitivity. BMI was calculated as weight/height² (Kg/m²). Metabolic syndrome (MetS) was diagnosed according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III [17] (elevated BP and at least 2 of the following: triglycerides \geq 150 mg/dL, HDL cholesterol $<$ 40 mg/dL, fasting plasma glucose \geq 110 mg/dL, BMI $>$ 30 Kg/m²).

Statistics

Proper ANOVA models and χ^2 statistics tested the statistical significance of continuous and categorical parameters respectively. Intraindividual association between variables was tested by correlation coefficients analysis. The association of TERalb (coded as 1 for upper-quartile values and 0 for the three remaining ones, cut-off: 11.1 %/hr) with the ACE D/I genotype and other continuous and categorical covariates was analyzed by multivariate logistic regression and odds ratios (ORs) and 95% confidence interval (CI)s were used to estimate relative risks. Statistical significance was set at $p < 0.05$. Descriptive statistics were means \pm SD or geometric means (interquartile range) for skewed parameters.

RESULTS

As a whole, 42 (45%) subjects showed a D/D genotype vs 42 (45%) and 7 (10%) with a I/D and I/I genotype respectively. Allelic distribution was in Hardy-Weinberg equilibrium (chi-square $<$ 0.271, df=1, $p > 0.6$) and did not differ by blood pressure status (**table 1**). Because of their low number making separate statistical comparisons not meaningful, I/I subjects were grouped with I/D in statistical analyses.

TERalb by ACE genotype

Fasting and stimulated glucose and insulin, HOMA index, triglycerides, BMI, UAE was higher and MetS more frequent in hypertensive patients than normotensive controls (table 1). As compared with normotensive controls, TERalb was higher in hypertensive patients, either as a whole ($7.4 \pm 1.5\%/hr$, $n=16$, vs $9.8 \pm 2.4\%/hr$, $n=79$, $p=0.0002$) or stratified by ACE genotype (D/D: $7.0 \pm 2.0\%/hr$, $n=6$ vs $10.4 \pm 2.7\%/hr$, $n=36$, $p=0.005$; I/D+I/I: $7.8 \pm 1.1\%/hr$, $n=10$ vs 9.3 ± 2.1 , $n=43$, $p=0.03$).

In the hypertensive sample, TERalb was higher ($p=0.03$) in D/D homozygotes than I/D+I/I subjects (**figure 1**) independent of age, BP levels, metabolic parameters, renal function and MetS frequency that matched closely in the two hypertensive subgroups (table 2). Hematocrit (I/D+I/I: $38.3 \pm 4.7\%$ vs D/D: $39.5 \pm 8.3\%$), serum albumin (I/D+I/I: 4.3 ± 0.3 vs D/D: 4.6 ± 0.3 g/dl) and plasma volume (I/D+I/I: 2837.3 ± 359.4 vs D/D: 2900.5 ± 400.5 ml/1.73 m²) did not differ.

When TERalb (as a dichotomized variable, cutoff: $11.1\%/hr$) was used as the dependent variable in a multivariate logistic regression model, the allelic odds ratio for ACE D/D vs pooled D/I+II was 3.1 (95% CI: 1.1-7.5, $p=0.04$) after accounting for BP, LDL cholesterol, smoking status, presence of MetS, HOMA index.

UAE and FBF responses by ACE genotype

UAE (12.1 [16] vs 8.0 (7.3), $p=0.03$) was higher and MA (42% vs 14%, $p=0.01$) more frequent in D/D hypertensive homozygotes in whom TERalb correlated positively with UAE ($r=0.23$, $p=0.03$, $n=79$) but not office and 24hr BP, BMI, lipids, fasting and post-load plasma glucose and insulin, HOMA index. No correlation was found among normotensive controls (figure 2).

Baseline FBF (I/D+I/I: 3.7 ± 1.3 , $n=35$ vs D/D: 3.6 ± 1.1 ml 100 ml⁻¹ forearm volume min⁻¹, $n=31$) and FBF responses to intra-brachial ACH and SNP did not differ by ACE genotype nor by hyper- vs normotensive status (**figure 3**)

DISCUSSION

The major and original finding of this cross-sectional genetic association study in never treated, uncomplicated hypertensive men was the independent association between ACE D/D homozygosis and faster TERalb. The data is consistent with a more deranged endothelial barrier function at the level of the systemic capillary circuit where most of the albumin permeation process takes place [7-9 for reviews]. Faster TERalb also coexisted with higher UAE suggesting some common genetically-mediated determinant for the abnormal behavior of renal and systemic

microvessels without evidence of NO-mediated endothelial dysfunction of systemic arterioles as assessed by forearm ACH stimulation.

Pathophysiological implications

Although the mechanistic links between the ACE polymorphism and a remote and complex phenotype such as capillary permeability cannot be elucidated, some plausible pathophysiological inferences may still be drawn from our data. First, whatever the underlying biological mechanism, the unfavorable influence of the ACE D/D genotype should not be seen as the cause but rather as a modulating factor for the defective systemic capillary permeability featuring human hypertension [9, 18, 19] since irrespective of the ACE genotype, TERalb was higher in hypertensive patients than normotensive controls. One might also wonder whether the influence of the ACE D/D polymorphic extends to normotensive subjects but the limited number of control subjects included in our sample makes this point irrelevant. As a second point, the effect of a series of sensitive parameters for capillary albumin permeation, such as elevated BP [19] and LDL cholesterol [13], hyperinsulinemia [20], smoking habits [21], MetS phenotype [22], was likely excluded because of the homogeneous distribution of those parameters across ACE genotypes. Atherosclerotic vascular disease also affects TERalb [23] but our patients were carefully selected for absence of clinical vascular disease. Given the above considerations, other possibilities should be considered and renin angiotensin system activation appears as a plausible candidate mechanism. In fact, D/D homozygosis associates with higher circulating and tissue ACE levels [24], the rate limiting step in biologically active angiotensin II production [25], and also stimulates its local production [26]. In turn, angiotensin II increases oxidative stress through NADH/NADPH oxidase stimulation and, in concert with other mediators such as cytokines, NO, endothelin-1, prostaglandins and Rho-protein pathway, promotes vascular inflammation, increases capillary permeability and impairs endothelial function [27]. In that latter perspective, it was of interest the homogeneous forearm vasodilatory responsiveness to ACH, a negative result in agreement with previous reports [28], suggestive of a different impact of the ACE genotypic variants on the capillary endothelial cell monolayer localized at the blood-vessel interface and endothelial cells covering resistance-sized forearm arterioles. Other possibilities cannot, however, be excluded including that, while TERalb measures systemic capillary permeability in basal conditions under the influence of several and interacting stimuli [7-9], the infused forearm model test only endogenous NO bio-availability in response to ACH, a NO-releasing stimulus [10]. Furthermore, several and heterogeneous organs and tissues contribute to the transvascular albumin leakage determination while forearm responses represent a single, mainly muscular vascular bed. Finally, NO-mediated relaxation needs only endothelial synthesis and diffusion of the substance to underlying smooth muscle cells, while capillary permeability requires a more complicated functional and structural interaction of cell junctions and extra-cellular matrix with endothelial cells [7-9]. On the

other hand, the preserved forearm responsiveness to locally infused ACH, concordant with previous data in forearm [e.g. 29, 30], coronary [e.g. 31] and subcutaneous [e.g. 32] arterioles, shows that impaired endothelial-mediated vasodilatation is not universal among essential hypertensive patients but may rather develop at more complicated stages [33] or with longer duration of hypertensive disease [34]. However, we have no arguments in favor or against those possibilities, and, on the other hand, our study did not address those specific issues.

Comment also deserves the confirmed [1-5] association of higher UAE with the ACE D/D genotype, and more importantly in this context, its association with TERalb suggestive of some shared genetically-promoted determinant for the abnormal behavior of renal and systemic microvessels in ACE D/D hypertensive patients. Previous data showed that more albumin leaks through exaggeratedly permeant glomeruli characterized by decreased size- and charge-selectivity [35] and that albuminuria may reflect a generalized transvascular albumin leakiness in clinically healthy and diabetic subjects [36, 37]. It should be noted, however, that the correlation between TERalb and UAE, albeit statistically significant, was rather weak in our present group. We also could not find a similar behavior in previous series [19], perhaps because the modulation of both parameters by multiple hemodynamic, metabolic and behavioral factors [6, 8] may obscure that link. It may not be irrelevant to note that higher UAE predicts cardiovascular events even in non hypertensive individuals [38] and MA is frequent among patients with mildly elevated BP, lean body size and no evidence of metabolic abnormalities and subclinical inflammation [39]. In those subsets, perhaps, genetic influences may be more influential on the renal and systemic microvascular system, an attractive possibility to be tested in the future.

Limitations of the study

A first obvious limitation of our study regards the strength of conclusions based upon a sample size of 79 patients. However, calculations accounting for effect size and allele frequency for predisposing alleles [40] indicate that, albeit limited, those numbers have a high statistical power to detect significant association when, as in our conditions, homozygous susceptibility genotype has a large biological impact (allelic OR=3.1) and the prevalence of the susceptibility allele in the control group is high (41%). On the other hand, we are aware that small initial studies frequently overestimate the true effect size thus increasing sample size requirements to thousands of cases and controls [e.g. 41], a goal difficult to be achieved dealing with a laborious parameter such as TERalb. For this reason, our present work should be seen as a biologically plausible, hypothesis-generating contribution waiting for support from larger trials. Second, we studied an all-male hypertensive group and the influence of the ACE I/D polymorphism may differ by gender [42]. Third, our sample was recruited cross-sectionally making it difficult to assess cause-effect

relationships. Fourth, allelic association studies based on a single polymorphism in few candidate genes are subject to bias and confounding and we cannot exclude the influence of some unmeasured factors in our patients.

In conclusion, an ACE D/D homozygosis associates with a higher elevated trans-capillary escape rate of albumin and urine albumin levels suggesting a common origin for the two phenomena. That abnormal systemic capillary permeability may accelerate atherosclerotic vascular disease, for example by retaining more atherogenic lipoproteins in the subendothelial space and/or preventing their egress, a morbid evolution to which D/D subjects may be more exposed [43]. That conceivable but by now speculative hypothesis needs, however, to be tested prospectively.

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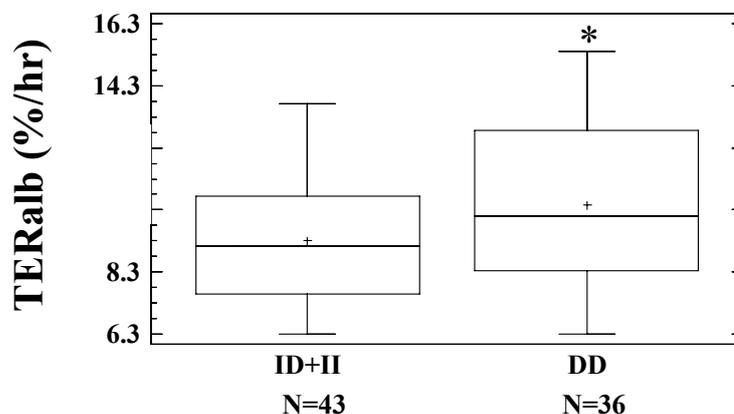
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FIGURES

Figure 1 : TERalb by ACE genotype in hypertensive men. Data reported as Box-and-whisker plots³. * p=0.03. For further details see text.



³ **Box-and-Whisker plot:** The central box encloses the middle 50 percent of the data ; the horizontal line inside the box represents the median and the mean is plotted as a cross. Vertical lines [whiskers] extend from each end of the box and cover four interquartile ranges.

Figure 2: The relationship between TERalb and urinary albumin excretion [UAE, log-scale] in hypertensive patients [n=79, □]. For a comparison, the scatterplot also show data obtained in control subjects [n=16, ●]. For statistics see text.

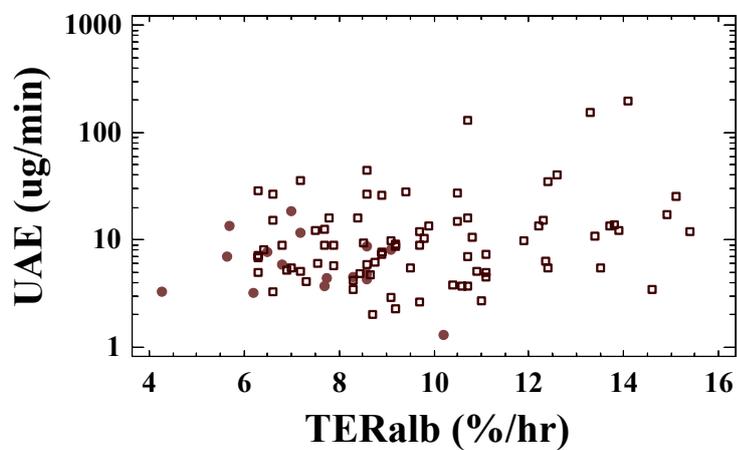
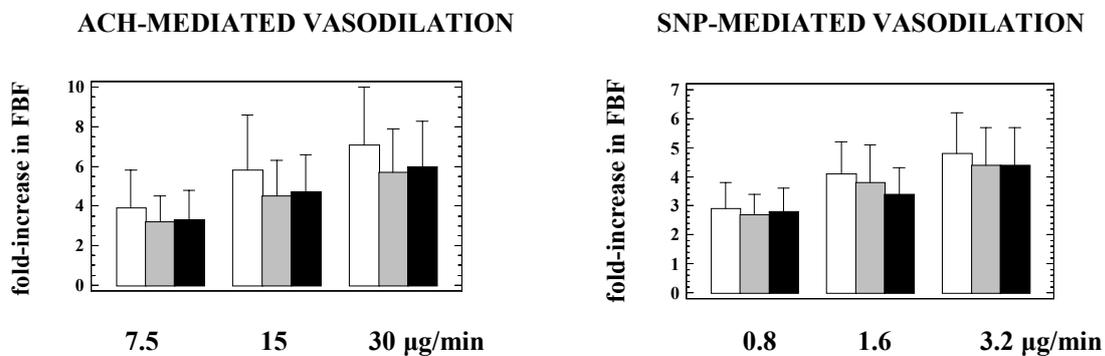


Figure 3: FBF responses to ACH and SNP by ACE genotype in hypertensive patients. As a standard of comparison, the figure also reports data for control subjects [□ Controls, n=12; ■ I/D+I/I, n=35; ■ D/D, n=31]. Means±SD. For further details see text.



TABLES

Table 1: Comparison of hypertensive patients and normotensive controls [Means±SD or geometric means [interquartile range]]. For statistics, definitions and abbreviations see text.

<i>VARIABLES</i>	<i>CONTROLS</i> <i>N=16</i>	<i>HYPERTENSIVES</i> <i>N=79</i>	<i>P VALUE</i>
Age [yrs]	47±12	48±10	NS
DD/ID/II	6/8/2	36/36/7	NS
Clear _{creat} [ml/min*1.73 m ²]	104±29	110±36	NS
Active smokers	7/16 [43%]	26/79 [33%]	NS
Office systolic BP [mm Hg]	126±10	149±14	N/A
Office diastolic BP [mm Hg]	76±6	96±11	N/A
24 hr systolic BP [mmHg]	121±7	137±16	N/A
24-hr diastolic BP [mmHg]	75±6	88±9	N/A
Fasting plasma glucose [mg/dL]	88±12	98±11	p=0.002
Glucose AUC ^{0-120min} [[mg/dl x 2h ⁻¹] $\times 10^{-3}$]	14.3±2.1	16.1±3.1	p=0.02
Fasting insulin [μ U/ml]	7.7 [2.3]	12.3 [10.4]	p=0.004
Insulin AUC ^{0-120min} [[μ U/ml x 2h ⁻¹] $\times 10^{-3}$]	5.3 [6.1]	12.3 [10.4]	p=0.014
HOMA-IR [Units]	1.7 [0.9]	2.9 [2.8]	p=0.0013
HDL cholesterol [mg/dL]	46±9	45±14	NS
Serum triglycerides [mg/dL]	94 [42]	160 [124]	p=0.004
BMI [Kg/m ²]	24.1±1.7	27.3±3	p=0.0001
MetS	0/16 [0%]	32/79 [41%]	p=0.005
Total cholesterol [mg/dL]	193±47	208±42	NS
LDL cholesterol [mg/dL]	127±43	126±38	NS

Table 2 : Comparisons by ACE genotype in hypertensive patients [Means±SD or geometric means [interquartile range]]. For definitions and abbreviations see text. None of the parameters showed significant differences.

VARIABLES	ID +I/I N=43	D/D N=36
Age [yrs]	47±9	49±11
D Allele frequency [%]	42%	100%
Clear _{creat} [ml/min*1.73 m ²]	106 [35]	110 [28]
Active smokers	16/43 [37%]	10/36 [28%]
Office systolic BP [mm Hg]	149±14	151±16
Office diastolic BP [mm Hg]	96±11	96±9
24 hr systolic BP [mmHg]	138±17	136±14
24-hr diastolic BP [mmHg]	88±10	88±8
Fasting plasma glucose [mg/dL]	99±11	96±11
Glucose AUC ^{0-120min} [[mg/dl x 2h ⁻¹] $\times 10^{-3}$]	16.7±2.7	15.1±3.2
Fasting insulin [μ U/ml]	13.9 [2.5]	10.4 [7]
Insulin AUC ^{0-120min} [[μ U/ml x 2h ⁻¹] $\times 10^{-3}$]	13.9 [7.4]	7.4 [8.2]
HOMA-IR [Units]	3.3 [3.3]	2.6 [1.7]
HDL cholesterol [mg/dL]	45±15	44±12
Serum triglycerides [mg/dL]	159 [147]	151 [113]
BMI [Kg/m ²]	27.3±3	27.4±3.1
MetS	20/43 [47%]	12/36 [33%]
Total cholesterol [mg/dL]	208±42	213±37
LDL cholesterol [mg/dL]	126±38	134±32