AJH 2005; 18:1549–1555

Genetics

Atherosclerosis and the Glu298Asp Polymorphism of the eNOS Gene in White Patients With End-Stage Renal Disease

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Background: We investigated whether the eNOS G/T polymorphism (Glu298Asp variant) is linked to the severity of carotid atherosclerosis and whether it is independent of asymmetric dimethylarginine (ADMA) in determining vascular damage in patients with end-stage renal disease (ESRD).

Methods: The eNOS polymorphism, ADMA, carotid intima–media thickness (IMT), and carotid artery (CCA) wall-to-lumen ratio (an indicator of arterial remodeling) were determined/measured in 131 patients with ESRD.

Results: Both in the co-dominant and dominant model approach, IMT as well as CCA wall-to-lumen ratio were directly related to the T allele ($P \leq .009$) and these relationships held true in multiple linear regression analyses including ADMA and traditional and emerging risk factors. The relationship between eNOS genotypes and CCA wall-to-lumen ratio was further analyzed by a categorical approach and in a multiple logistic regression analysis, the odds ratio (OR) of increased CCA wall-to-lumen ratio was strongly associated to the T allele (codominant model: GG, OR = 1; GT, OR = 2.1; TT, OR = 8.2; P for trend = .01; dominant model: GG, OR = 1; GT and TT, OR = 2.7; $P = .02$).

Conclusions: The T allele of eNOS gene is an independent predictor of intimal lesions and vascular remodeling and it is associated with the severity of atherosclerosis independently of ADMA. Am J Hypertens 2005;18:1549–1555 © 2005 American Journal of Hypertension, Ltd.

Key Words: Asymmetric dimethylarginine (ADMA), atherosclerosis, dialysis, Doppler ultrasound, eNOS gene.

The unique severity of atherosclerotic complications in patients with advanced renal diseases was recognized by Lindner et al[1] 30 years ago and evidence that accelerated atherosclerosis is the most concerning sequela of end-stage renal disease (ESRD) is now overwhelming.[2] Because hypertension, diabetes, hypercholesterolemia, and other traditional risk factors often cluster in ESRD, these factors are considered of primary importance in arterial damage in patients with chronic renal insufficiency.[3] Traditional risk factors apart, other disease-specific factors—hyperparathyroidism, anemia, and accumulation of endogenous inhibitors of the nitric oxide (NO) system—and emerging risk factors like hyperhomocystenemia and inflammation appear strongly associated with cardiovascular complications in these patients. Furthermore, disturbed regulation of the NO system and endothelial dysfunction resulting from increased plasma concentration of asymmetric dimethylarginine (ADMA) appear to be a prominent feature of uremic vasculotoxicity.[4–6] In view of the particular relevance of NO system alterations in the pathogenesis of atherosclerosis in ESRD we wondered whether genetic polymorphisms in the eNOS gene are linked to atherosclerosis in this condition and whether this hypothetical link is independent of ADMA accumulation. We focused our attention on the G/T polymorphism, coding for Glu298Asp variant, be-

Received December 22, 2004. First decision May 9, 2005. Accepted June 9, 2005.

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This study was supported by grants of the C.N.R., Regione Calabria and by the Fondazione Bonino-Puleio Messina, Italy.

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0895-7061/05/$30.00
doi:10.1016/j.amjhyper.2005.06.007

cause it is the only known polymorphism in this gene associated with an altered protein sequence in the resulting enzyme and with compromised NO synthesis.[7] In this regard it is important noting that this polymorphism is associated with atherosclerotic plaques[8] as well as with atherosclerotic complications in patients without renal insufficiency.[9] Furthermore it was shown that the T allele is predominant in patients with ESRD as compared to healthy controls.[10,11] Here we report a detailed multivariate analysis of the relationship between intima–media thickness (IMT), arterial remodeling, the Glu298Asp variant, and ADMA in a large group of patients with ESRD.

Methods

The protocol conformed with the local ethical guidelines of our institution and informed consent was obtained from each participant.

Patients

One hundred thirty-one (78 men and 53 women, all white) dialysis patients (86 on hemodialysis [HD] and 45 on chronic ambulatory peritoneal dialysis [CAPD]) who had been on regular dialysis treatment (RDT) for at least 6 months and who were free of overt infections (fever, infected vascular access or peritonitis, or exit site infection) were recruited for the study. Hemodialysis patients were being treated three times weekly with standard bicarbonate dialysis (in millimoles per liter: Na 138, HCO_3 5, K 1.5, Ca 1.25, Mg 0.75) either with Cuprophan or semisynthetic membranes. The average urea fractional urea clearance (Kt/V) in these patients was 1.28 $\pm$ 0.30. The remaining 45 patients were on CAPD (weekly Kt/V 1.67 $\pm$ 0.33). All HD patients were virtually anuric (24-h urine volume $<$200 mL/d), whereas a minority of CAPD patients ($n = 6$) had a 24-h diuresis of $>$200 mL/d. Nineteen patients were diabetics and 65 were habitual smokers (22 $\pm$ 18 cigarettes/d). Ninety-two patients were hypertensive (blood pressure [BP] $>$140/90 mm Hg or on antihypertensive treatment). Among them 76 patients were treated with various antihypertensive drugs (51 on monotherapy with angiotensin-converting enzyme [ACE] inhibitors, angiotensin-1 antagonists, calcium channel blockers, α- and β-blockers and the remaining 25 on double or triple therapy with various combinations of these drugs), and the remaining 16 were untreated. Sixty-four patients were on treatment with erythropoietin.

Carotid Ultrasonography

In all patients ultrasonographic studies on common carotid arteries (CCA) were performed bilaterally by a single observer (FAB) who was blinded as to the clinical and biochemical data. All studies were performed with a Hewlett Packard Sonos 1500 using a 7.5-MHz high resolution probe. Intima–media thickness was defined as a low level echo gray band that does not project into the arterial lumen and was measured during end-diastole as the distance from the leading edge of the second echogenic line of the far walls of the distal segment of the CCA, the carotid bifurcation, and the initial tract of internal carotid artery on both sides.[12] Measurements were performed 0.5, 1, and 2 cm below and above the bifurcation (six measurements on each side) and the average measurement was taken as the IMT. The internal diameter of the CCA was measured bilaterally 2 cm below the bifurcation during end-diastole and the average measurement was taken as the internal diameter. The number of atherosclerotic plaques[13] (either as faint gray echoes [soft plaques] or bright white echoes [calcified plaque] protruding into the lumen) detected in the bulbar area (from 2 cm below to 2 cm above the bifurcation) of the carotid arteries was recorded on both sides and summed up. The IMT and internal diameter of the CCA measurements were always performed in plaque-free arterial segments. Repeated studies in 105 dialysis patients by a blinded observer in our laboratory showed that the IMT and the internal diameter of the CCA represent reliable measurements because their coefficient of variation was 5.5% and 3.2%, respectively. The CCA wall-to-lumen ratio was calculated by the standard formula: $2 \times$ *IMT/Internal diameter of the CCA*. As upper limit of the normal range of CCA wall-to-lumen ratio we considered the threshold of 0.325, which corresponds to the mean + 2 SD of this parameter in a series of 30 healthy subjects (aged 55.4 $\pm$ 7.9 years; 11 men and 19 women) studied in our laboratory.

Laboratory Measurements

For HD patients fasting blood sampling was performed during the midweek nondialysis day and for CAPD patients at empty abdomen. Serum cholesterol, albumin, calcium and phosphate, and hemoglobin measurements were made using standard methods in the routine clinical laboratory. The C-reactive protein (CRP) was measured by using a commercially available kit (Behring, Scoppito, L'Aquila, Italy). Plasma homocysteine[14] and ADMA[6] were determined as previously reported.

Glu298Asp Polymorphism on Exon 7 of eNOS Gene

Genomic DNA was extracted from peripheral blood leukocytes by the salting-out technique.[15] The G/T polymorphism of the eNOS gene was identified with a polymerase chain reaction, according to Rankinen et al,[16] followed by restriction enzyme analysis with *Mbo*I and *Eco*24I that cut mutant and wild allele, respectively, as previously reported.[17]

Statistical Analysis

Data are expressed as mean $\pm$ SD or SE, median (interquartile range), or as percent frequency, as appropriate. Between groups comparisons were made by P for trend.

The relationship between eNOS genotypes (defined