Enhanced Diabetic Nephropathy in Mice Lacking Bradykinin B2 Receptors

Nobuyo Maeda
University of North Carolina

October 17, 2005
Vascular effects of diabetes in apoE deficient mice

ApoE-/- on B6 and 129 backgrounds

Vitamin C deficiency
Dominant negative mutation of PPARG
Overexpression of ANGII type 1a receptor
Ahaphtoglobinemia
Connective tissue growth factor

Dietary Supplement of Lipoic Acid
Enhanced Diabetic Nephropathy in Mice Lacking Bradykinin B2 Receptors

Oliver Smithies

Masao Kakoki
Nobuyuki Takahashi

Charles Jennette
Prognostic Value of Angiotensin-I Converting Enzyme I/D Polymorphism for Nephropathy in Type 1 Diabetes Mellitus: A Prospective Study

SAMY HADJADJ,† RIADH BELLOUM,* BÉATRICE BOUHANICK,* YVES GALLOIS,† GERARD GUILLOTTEAU,* GILLES CHATELLIER,‡ FRANÇOIS ALHENC-GELAS,§ and MICHEL MARRE**

†Médecine B and ‡Laboratoire de Biochimie B, Centre Hospitalier Universitaire, Angers; †Service d’informatique Médicale, Hôpital Broussais, Paris; §Institut National de la Recherche Médicale (INSERM U367), Paris; and ‡Diabétologie, Endocrinologie, Hôpital Bichat, Paris, France.

Abstract. Angiotensin-I converting enzyme (ACE) regulates renal hemodynamics. Its insertion/deletion (I/D) polymorphism, which determines most of ACE interindividual variance, was proposed as a genetic marker for diabetic nephropathy. A substitution (M235T) polymorphism in angiotensinogen (AGT) may interact with ACE I/D polymorphism for the risk of diabetic nephropathy, but their prognostic values have to be established by follow-up studies. A total of 310 type 1 diabetes mellitus patients who attended the diabetic clinic in Angers (France) took part in a prospective, observational, follow-up study. Glycohemoglobin, BP, plasma creatinine, and urinary albumin excretion were determined periodically. Nephropathy was classified as absent, incipient (microalbuminuria), established (proteinuria), advanced (plasma creatinine ≥ 150 μmol/L), and terminal (renal replacement therapy). The main end point was the occurrence of a renal event defined as the progression to a higher stage of diabetic nephropathy. At baseline, 251 (81%) patients had no nephropathy, 35 (11%) had incipient nephropathy, 18 (6%) had established nephropathy, and 6 (2%) had advanced nephropathy. The ACE I/D and M235T AGT polymorphisms were in Hardy-Weinberg equilibrium in the patients. The median duration of follow-up was 6 yr (range, 2 to 9 yr). The occurrence of renal events was significantly influenced by ACE genotype (log-rank II versus I/D versus DD, P < 0.03) with a dominant deleterious effect of the D allele: ID or DD versus II (adjusted hazard ratio, 5.0; 95% confidence interval, 1.5 to 16.6). Other contributors were high glycohemoglobin and systolic BP. In the patients who initially were free of nephropathy, baseline plasma ACE concentration was higher in patients who progressed to microalbuminuria (571 ± 231 versus 466 ± 181 μg/L; P = 0.0032); the D allele independently favored the occurrence of incipient nephropathy (adjusted hazard ratio, 4.5; 95% confidence interval, 1.1 to 19.4); other contributors were male gender, baseline systolic BP, and urinary albumin excretion. The AGT M235T polymorphism was not associated with renal events. The D allele of the ACE I/D polymorphism is an independent risk factor for both the onset and the progression of diabetic nephropathy in type 1 diabetes mellitus patients.

The AGT M235T polymorphism was not associated with renal events. The [D allele of the ACE I/D polymorphism is an independent risk factor for both the onset and the progression of diabetic nephropathy in type 1 diabetes mellitus patients.
Diabetic Nephropathy and Ace Genotype

Albumin Excretion (ug/24hr)

Duration of Diabetes (weeks)

F Ahlenc-Gelas

3 copy diabetic p< 0.0001

1 or 2 copy diabetic

1, 2 or 3 copy non-diabetic

F Ahlenc-Gelas
A simple computer simulation suggests that small changes in ACE levels due to human D/I polymorphisms alter bradykinin but not angiotensin II levels.
Prediction

If reduced ACE preserves bradykinin which protects the kidney from damage in diabetics,

then absence of the bradykinin receptor in the kidney should increase nephropathy in a diabetic animal.
The two tightly linked genes code for bradykinin receptors, B2R and B1R.

The B2 receptor is constitutively expressed on many cell types and mediates most of the vascular functions of bradykinin.

Mice lacking the B2 receptor survive with relatively minor phenotypic changes. (Borkowski et al., 1995)

The B1 receptor is expressed at very low levels under normal conditions but is induced during inflammation and in pathologic conditions such as diabetes and hypertension.
Akita x B2R-/- mice

Part I. 6 month old males
The absence of B2R does not affect blood pressures of mice or alter blood glucose levels in diabetic mice.
Food intake and urine volume are increased by diabetes but are not greatly influenced by absence of B2R
Kidney weight is increased by diabetes and is significantly affected by the lack of B2R.
B2R absence increases albumin excretion significantly in diabetic mice
Mesangial expansion in kidney glomeruli

Akita

B2R-/- Akita

PAS stain for glycoproteins
Akita diabetic mice lacking the bradykinin B2 receptor develop overt albuminuria accompanied by marked kidney pathology including mesangial expansion and interstitial fibrosis.

Bradykinin system plays a critical role in protecting the kidney from damage during diabetes mellitus.

(Kakoki et al., PNAS 101,13302, 2004)
Akita x B2R-/- mice

Part II. 12 month old males
alopecia
Kyphosis
skin atrophy
Reduction of femur mineral bone density
Accumulation of pigmented material in the testicular Leydig cells (lipofuscin: indicator of cellular senescence)
lipofuscin in proximal tubule cells
Autophagolysosomes in the proximal tubule cells of B2R -/- Akita mice
Increased oxidative stress measured by plasma TBAR and erythrocyte GSH
Increased oxidative changes in mitochondrial DNA
Increased mutations in mitochondrial DNA

### Point Mutations (per 10 kb mtDNA)

- **Wildtype**: 9
- **Bdcrb2−/−**: 9
- **Ins2Akita+/−**: 9

### D-17 Deletion (% Wildtype)

- **Wildtype**: 5
- **Bdcrb2−/−**: 4
- **Ins2Akita+/−**: 5
- **Bdcrb2−/− Ins2Akita+/−**: 5

Statistical significance:
- $P < 10^{-4}$
- $P < 0.001$
- $P < 0.05$
- $P < 0.05$
- $P < 0.05$
- $P < 0.05$
Protective effect of BK is partly mediated by NO
Increased expression of TGF β1 and CTGF in kidney
Expression of B1 receptor is markedly enhanced in the double mutants (25X wild type)

Compensatory increase of B1R expression: Good or Bad?
Conclusions

Absence of the B2 receptor, or diabetes or absence of the receptor plus diabetes, progressively accelerate senescence-associated phenotypes in multiple tissues.

Indicators of premature senescence include alopecia, skin atrophy, kyphosis, osteoporosis, testicular atrophy and lipofuscin accumulation that are clearly detectable by 12 months of age.
Absence of the bradykinin B2 receptor increases the oxidative stress and mitochondrial damage that are already present to a lesser degree in untreated Akita diabetic mice.

Protective effects of bradykinin is at least partly mediated by NO
Hyperglycemia

Oxidative Stress

Mitochondrial Damage

Bradykinin

NO

Diabetic Complications + Premature Senescence