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Nodular glomerulosclerosis and renin angiotensin system in Chinese patients with type 2 diabetes

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ABSTRACT

Background: Diabetic nephropathy (DN) is a multifactorial and polygenic disease with nodular glomerulosclerosis (NGS) pathognomonic for diabetes and hypertension. Patients with type 2 diabetes and hypertension have characteristic renin-angiotensin system (RAS) gene polymorphisms.
Methods and results: In this retrospective cohort study, we correlated the presence of NGS with renal function, angiotensin-converting enzyme (ACE) genotypes (DD, DI, and II), angiotensinogen (AGT) genotypes (MM, MT, and TT) and immunohistochemical staining characteristics of RAS components in 847 patients and 172 consecutive autopsy cases with type 2 diabetes. T allele of AGT was associated with macroalbuminuria ($P = 0.040$). Multitude regression analysis revealed ACE insertion (I)/deletion (D) polymorphism as an independent determinant for estimated glomerular filtration rate (eGFR) less than 60 mL min^{-1}·1.73 m^{-2} (DD carriers: odds ratio [OR] = 3.46, 95% confidence interval [CI] = 1.08–11.07; DI carriers: OR = 3.51, 95% CI = 1.63–7.56). A significant association between NGS and eGFR less than 60 mL min^{-1}·1.73 m^{-2} also persisted after adjusting for nonlinear relationship ($P < 0.001$). In NGS patients, immunoreactivity of angiotensin I converting enzyme 2 (ACE2) significantly decreased in glomeruli with mesangial nodules compared with glomeruli without the mesangial nodules.
Conclusions: These data suggest associations of ACE D allele with glomerular filtration impairment, and NGS with glomerular ACE2 down-regulation and reduced glomerular filtration in Chinese patients with type 2 diabetes.

1. Introduction

Diabetic nephropathy (DN) occurs in 30–40% of patients within 25 years from the diagnosis of diabetes and is the most common cause of end-stage renal disease (Shulman et al., 1996). Progression of nephropathy varies tremendously in patients with type 2 diabetes, due to the heterogeneity in renal histopathological changes occurred with proteinuria (Fioretto et al., 1996; Gambara et al., 1993). A subset of them have nodular glomerulosclerosis (NGS) characterized by mesangial nodule formation of the Kimmelstiel-Wilson (KW) lesion (Fioretto et al., 1996; Kimmelstiel and Wilson, 1936). Most likely, a prognostic impact of structural alterations in renal dysfunction is strongly related to the severity of glomerulopathy (Solini et al., 2002).

NGS is pathognomonic in patients with diabetes and hypertension (Kimmelstiel and Porter, 1948; Kimmelstiel and Wilson,

Abbreviations and acronyms: ACE, angiotensin-converting enzyme; ACE2, angiotensin I converting enzyme 2; ACR, albumin creatinine ratio; AER, albumin excretion rate; AGT, angiotensinogen; ANCOVA, analysis of covariance; AT1, angiotensin II type 1 receptor; AT2, angiotensin II type 2 receptor; BMI, body mass index; CI, confidence interval; D, deletion; DBP, diastolic blood pressure; DN, diabetic nephropathy; DNA, deoxyribonucleic acid; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; I, insertion; KW, Kimmelstiel-Wilson; LDL-C, low-density lipoprotein cholesterol; M, methionine; NGS, nodular glomerulosclerosis; ORs, odds radio; PAS, periodic acid-Schiff; RAS, renin-angiotensin system; SBP, systolic blood pressure; SD, standard deviation; T, threonine; TC, total cholesterol; TG, triglyceride; WHR, waist hip rate.

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1936; Zhao et al., 2004a). Similarly, we have found NGS is related to hypertension and advanced renal disease in patients with type 2 diabetes (Zhao et al., 2004a). And, tight glycemic control and aggressive antihypertensive treatment in combination with the use of renin-angiotensin system (RAS) inhibitors should substantially delay the progression of nephropathy (Critchley et al., 2002). Recently, we have reported the activation of RAS and lipogenic peroxisome proliferator-activated receptor-gamma plays an important role in the renal adipogenesis and lipid metabolism (Sui et al., 2010). Additionally, genetic factors such as polymorphisms of aldose reductase and apolipoprotein E genes had been explored in our previous studies (Guan et al., 2009; Zhao et al., 2004b). Obviously, the clinical and pathologic features of nephropathy in type 2 diabetes are associated with renal hemodynamics and blood pressure regulation (Adler, 2004). Therefore, the RAS polygenic defects may have impact on renal structural abnormities.

RAS, a key mediator of diabetes, hypertension and DN (Siragy and Carey, 2010), is partly under genetic control (Costacou et al., 2006). An insertion (I)/deletion (D) polymorphism in intron 16 of angiotensin-converting enzyme (ACE) gene has been identified as a potential marker for the differential response to the development and progression of diabetic kidney disease (Rudberg et al., 2000; Schmidt et al., 1995). Similarly, a causal association might exist between the angiotensinogen (AGT) gene polymorphism and plasma and tissue AGT levels, and a mass of unfavorable clinical features such as higher blood pressure in patients with DN and early onset of essential hypertension (Pontremoli et al., 2000). Thus, the ACE and AGT genes have been considered as the primary candidates in association analyses regarding early or overt glomerulopathy in patients with type 2 diabetes.

However, large-scale data from both clinical cohorts and autopsies studies on the association of NGS with genetic RAS polymorphisms and RAS component immunolocalization remain rare. Therefore, we conducted this clinical-genetic-immunopathological correlation study to examine the interrelationship among NGS, RAS genotypes, estimated glomerular filtration rate (eGFR), proteinuria, and RAS immunoreactivity in Chinese patients with type 2 diabetes.

2. Methods

2.1. Subjects and clinical measurements

All patients with type 2 diabetes who were classifiable according to their renal impairments and had been entered into the database of the university affiliated hospital by 2012 participated in this study. Consequently, 847 patients were involved in this clinical-genetic study with their ascertainable renal status and ACE or AGT genotypes. Among these patients, 796 had genotyping for ACE I/D and 757 for AGT methionine/threonine (M/T) polymorphism. Analogously, we conducted an autopsy cohort, a total of 172 consecutive autopsy cases with type 2 diabetes, adult Chinese subjects performed at the affiliated hospital. Patients with type 1 diabetes, acute infection, neoplastic and/or hepatic diseases, chronic heart failure, or severe obesity were excluded, as previously (Zhao et al., 2004b).

The Institutional Review Board of the Guilin Medical University approved the study (GLMC191211HL). Informed consent was obtained from all subjects or the next of kin.

All patients underwent complete physical examination. In addition to hospital records of demographic data and clinical assessment of diabetic complications, fasting blood samples were taken to measure plasma glucose, glycated hemoglobin, lipid profile (triglyceride, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol) and renal functions. At least two urinary collections from all patients were used to measured albumin creatinine ratio (ACR) and albumin excretion rate (AER).

2.2. ACE and AGT genotyping

Genomic DNA (deoxyribonucleic acid) was extracted by standard phenol/chloroform methods from a 5 mL peripheral blood samples collected on ethylene diaminetetra acetic acid. Genomic DNA of autopsy cases was extracted from spleen tissue as previously described (Zhao et al., 2004b). ACE gene I/D and AGT gene M/T polymorphisms was determined by polymerase chain reaction (PCR) using primers flanking the polymorphic region of intron 16 and 354 bp of exon 2, respectively. Primers for the ACE I/D polymorphism were 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and 5′-GATGTGGCCATCACATTCGTCAGAT-3′, and a second insertion-specific PCR was performed to avoid DD/ID mistyping using a pair of primers, 5′-TGGGACCACAGCGCCCGCCACTAC-3′ and 5′-TCGCCAGCCCTCCCATGCCCATAA-3'; for AGT M/T polymorphism, 5′-CAGGGTGCTGTCCACACTGGACCCC-3′ and 5′-CCGTTTGTGCAGGGCCTGGCTCTCT-3' (Pontremoli et al., 2000). PCR amplification revealed a 190 bp fragment (ACE I allele) and/or a 490 bp fragment (ACE D allele); a 266 bp fragment (AGT M235 allele) and/or a 303 bp fragment (AGT 235T allele).

2.3. Histochemical and immunohistochemical staining

Specimens of kidney were taken at postmortem examination, fixed in 4% buffered paraformaldehyde, and embedded in paraffin blocks. These blocks were cut into 3–4 μm sections, and stained by hematoxylin-eosin, periodic acid-Schiff (PAS), periodic acid-silver metheramine and Masson's trichrome. The degree of nodular glomerulosclerosis was assessed by the PAS-positive mesangial matrix.

In order to characterize the RAS immunophenotypes of diabetic NGS, kidney cortical tissue blocks from 172 consecutive autopsy cases (79 NGS cases and 93 NGS-free diabetic controls) were stained with antibodies to ACE, angiotensin I converting enzyme 2 (ACE2), angiotensin II type 1 receptor (AT1), and angiotensin II type 2 receptor (AT2) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The immunohistochemical staining method were described previously (Zhao et al., 2004b). Stained slides were examined with a Zeiss A2 imaging microscope (Carl Zeiss, Hamburg, Germany). For each slide, 10 images at magnification 200 × were randomly acquired by our technicians who were blinded to grouping codes. Quantitative analyses of immunoreactive intensity were carried out with Image-Pro Plus software 6.0 (Media Cybernetics, MD, USA). In order to exclude any potential effects from treatments with RAS blockers, the stained glomeruli of the same field were further stratified into two groups based on the presence or absence of KW lesion. Hereby the KW lesion in the NGS cases was the sole determinant of the RAS immunoreactivity. Comparisons were also done between the 79 NGS cases and 93 NGS-free diabetic controls.

2.4. Definitions and calculations

Hypertension was defined as an average blood pressure ≥140/90 mm Hg at least three different occasions at rest state or by the presence of antihypertensive treatment. Renal status was defined on the basis of AER and ACR: normoalbuminuria (AER < 20 μg/min or ACR < 30 mg/g), microalbuminuria (20 ≤ AER < 200 μg/min or 30 ≤ ACR < 300 mg/g), macroalbuminuria (AER ≥ 200 μg/min or ACR ≥ 300 mg/g). Creatinine clearance was estimated using the following formula, which expressed in $ml \cdot min^{-1} \cdot 1.73\ m^2$ and calculated as (140 − age in years) × body weight in kg ÷ 72 ÷