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Interleukin-6 gene polymorphisms correlate with the progression of nephropathy in Chinese patients with type 2 diabetes: A prospective cohort study

Wen-Tsan Chang [a,b], Meng-Chuan Huang [c,d], Hsin-Fang Chung [e], Yen-Feng Chiu [f], Pao-Shan Chen [c], Fang-Pei Chen [c], Chun-Yi Lee [f], Shyi-Jang Shin [g], Shang-Jyh Hwang [h], Ya-Fang Huang [f], Chih-Cheng Hsu [f,i,j,*]

[a] Department of Surgery, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[b] Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[c] Department of Public Health and Environmental Science and Graduate Institute of Medicine, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Department of Nutrition and Dietetics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[e] School of Public Health, The University of Queensland, Brisbane, Australia
[f] Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan
[g] Division of Endocrinology and Metabolism, Department of Internal Medicine, Kaohsiung Medical University and University Hospital, Kaohsiung, Taiwan
[h] Division of Nephropathy, Department of Internal Medicine, Kaohsiung Medical University and University Hospital, Kaohsiung, Taiwan
[i] Department of Health Services Administration, China Medical University, Taichung, Taiwan
[j] Institute of Clinical Medicine, National Yang Ming University, Taipei, Taiwan

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ABSTRACT

Aims: Interleukin-6 (IL-6), an inflammatory cytokine, is considered a candidate gene possibly involved in susceptibility to nephropathy in diabetes. This study aimed to examine whether *IL-6* polymorphisms predict the progression of nephropathy in a prospective Chinese cohort of patients with type 2 diabetes.

Methods: A total of 568 type 2 diabetic patients with normoalbuminuria at baseline were followed up for a mean of 5.3 ± 1.5 years. Urinary albumin-to-creatinine ratio (ACR) ⩾30 mg/g in two consecutive urine tests were defined as progression to diabetic nephropathy ($n = 143$). Five polymorphisms of *IL-6* gene, rs1800795, rs1800796, rs1524107, rs2069837, and rs2069840, were genotyped. Cox proportional hazard models were used to estimate hazard ratio (HR) and 95% CI of progression to diabetic nephropathy under different genetic models.

Results: Almost all patients (99.6%) carried the rs1800795 GG homozygous genotypes. In the Cox proportional models adjusted for multiple covariates, the HR under recessive model was 2.02 for rs1800796 GG (vs. CC + CG, 95% CI: 1.08–3.75, $p = 0.027$), 2.37 for rs2069837 GG (vs. AA + AG, 95% CI: 1.15–4.87, $p = 0.019$), and 2.08 for rs1524107 CC (vs. TT + TC, 95% CI: 1.12–3.89, $p = 0.021$). These associations remained significant for rs1800796 and rs1524107 after correction for multiple testing ($\alpha = 0.017$). Overall, our results suggest that rs1800796

* *Corresponding author at:* Institute of Population Health Sciences, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 35053, Taiwan. Fax: +886 37 586 261.
E-mail address: cch@nhri.org.tw (C.-C. Hsu).
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GG and rs1524107 CC homozygous genotypes may confer a greater risk for development of nephropathy in type 2 diabetes.
Conclusions: *IL-6* gene polymorphisms rs1800796 and rs1524107 may serve as predictors of progression of nephropathy in Chinese patients with type 2 diabetes.

1. Introduction

Diabetic nephropathy is a major complication of diabetes mellitus and is the largest single and leading cause of end stage renal disease (ESRD) in the developed world [1,2]. In 2009, the incidence of ESRD in Taiwan was the third highest in the world and its prevalence was the highest [3]. Individuals with chronic kidney disease (CKD) have a prevalence of diabetes four times higher than those without CKD [4]. In addition, in Asia, a high prevalence of proteinuria (up to 60%) has been reported in type 2 diabetes, which suggests an impending pandemic of renal and cardiovascular diseases in this region [5].

Chronic inflammation is a common contributor to the pathogenesis of diabetic nephropathy leading to ESRD [6]. Levels of the inflammatory cytokine IL-6 have been found to be elevated in urine [7] and serum [8] samples obtained from diabetic patients with nephropathy. Diabetic nephropathy and its related clinical characteristics, including variations in glomerular filtration rate (GFR) and progression to ESRD, also can be, in part, influenced by genes [9]. Two recent genome wide scan studies have reported a gene locus on the human chromosome 7q21 and they identified *IL-6* as the prime candidate gene possibly affecting renal function using sib-pair linkage analysis [10,11]. A single nucleotide polymorphism of the *IL-6* gene, rs1800769, has been positively associated with renal dysfunction in Caucasians with chronic glomerulonephritis [12] and in Korean patients undergoing dialysis [13] and diabetic nephropathy (macroalbuminuria) [14]. Performing haplotype analyses, Ng et al. [15] found the haplotype (G-G-G-A-G-C) of 6 SNPs (rs2069827, rs1800796, rs1800795, rs2069837, rs2069840 and rs2069861) to be significantly more prevalent among Caucasian type 2 diabetes patients with CKD/ESRD than those without CKD/ESRD (25.5% vs. 15.4%).

To date, little research has been devoted to studying the association between polymorphisms of *IL-6* gene and risk of nephropathy in type 2 diabetic patients in Asian populations. This prospective study investigated whether *IL-6* polymorphisms (rs1800796, rs1800795, rs1524107, rs2069837, and rs2069840) might predict progression of nephropathy in a cohort of type 2 diabetes patients in Taiwan.

2. Methods

2.1. Study subjects

The study subjects were type 2 diabetic patients enrolled in the diabetes management through an integrated delivery system (DMIDS) Study (Identifier: NCT00288678, ClinicalTrials.gov). Description of the study design and selection criteria of original cohort have been published elsewhere [16]. Briefly, the baseline recruitment period was between August 2003 and December 2005 and the subjects were followed through the end of 2009 (Supplementary Fig. 1). We recruited patients between 30 and 70 years old who had been diagnosed or newly diagnosed as having type 2 diabetes by their primary physicians of primary healthcare clinics following criteria established by the American Diabetes Association (ADA) [17]. Pregnant women and participants with a history of myocardial infarction, cerebrovascular accident (e.g. stroke), foot amputation, and uremia under dialysis were not eligible at baseline recruitment. A total of 1209 type 2 diabetic patients aged 30–70 years participated in the original cohort. Of these subjects, 365 patients did not have available DNA specimens for genotyping and 276 had urinary albumin-to-creatinine ratio (ACR) $\geqslant$30 mg/g in the first two consecutive urine tests at baseline, leaving us with 568 patients who had normoalbuminuria at baseline for genetic association analyses. This study was approved by the Ethics Committee of the Kaohsiung Medical University and Taiwan National Health Research Institutes. Written informed consent was obtained from each subject.

2.2. Primary end point

The primary outcome of this prospective study was progression to diabetic nephropathy. The earliest clinical sign of diabetic nephropathy was the presence of microalbuminuria. Spot urine samples were collected every six months during the study period. Urinary albumin was measured using the immunoturbidimetric method (Hitachi 7060; Hitachi High Technologies, Tokyo, Japan), and urinary creatinine was measured using the Jaffe reaction method. Having a urinary albumin-to-creatinine ratio (ACR) $\geqslant$30 mg/g in two consecutive urine tests was defined as having microalbuminuria [16].

2.3. Anthropometrical and biochemical measurement

Trained research assistants measured height, body weight, and blood pressure, and collected venous blood specimen every 6 months under fasting conditions (overnight at least 8 h). All specimens were kept in 2–8 °C, delivered to a central laboratory, and measured within 8 h. Glycated hemoglobin (HbA1c) was performed by high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA, USA). Glucose, triglycerides, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), and serum creatinine were measured by an automatic analyzer (Hitachi 7060; Hitachi High Technologies, Tokyo, Japan). Estimated GFR (eGFR) was calculated using a 4-variable Modification of