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Genetic disposition and modifiable factors independently associated with anemia in patients with type 2 diabetes mellitus

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ABSTRACT

Aims: Anemia is prevalent but under-recognized in patients with diabetes mellitus (DM). Genetic variants in angiotensin-converting enzyme (*ACE*), tumor necrosis factor-alpha (*TNF-α*) and erythropoietin (*EPO*) have been associated with diabetic nephropathy. In the present study, we investigated the associations between anemia and polymorphisms in *EPO* promoter (rs1617640), *TNF-α G-308A* and *ACE* Insertion/Deletion in Chinese patients with type 2 diabetes.

Methods: Polymorphisms in *ACE*, *TNF-α* and *EPO* were genotyped in 1142 patients. Anemia was defined as hemoglobin (Hb) levels below 12 g/dL for women and 13 g/dL for men.

Results: 286 (25%) patients had anemia. Patients with anemia were older, had longer duration of diabetes, worse renal function and more albuminuria. *ACE* Insertion/Deletion and *TNF-α G-308A* were not associated with anemia. The frequencies of *EPO* polymorphism (rs1617640) were significantly different between anemic and nonanemic patients. Patients with *TT* genotype had higher prevalence of anemia than those with *TG* and *GG*. Regression analysis identified *EPO* SNP, duration of DM, serum albumin, albuminuria and renal function independently associated with anemia. After adjusting for multiple variables, *TT* and *TG* genotypes were associated with 3–5-fold increased risk for anemia compared to *GG*.

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Conclusions: The *EPO* genotype in Chinese patients with type 2 diabetes is associated with anemia and may help to identify those at risk. Further evaluation of its effect on clinical outcomes in prospective studies may be useful to predict the outcomes of erythropoiesis stimulating therapy, and to individualize anemia management.

1. Introduction

Type 2 diabetes mellitus is one of the most common metabolic diseases in the world, and its prevalence, especially in Asians is increasing rapidly [1,2]. Biological factors contributing to this trend include higher insulin resistance, visceral adiposity, impaired pancreatic β-cell function and genetic predisposition. In addition, physical inactivity and dietary changes have led to increased risk for the development of diabetes and associated complications.

Anemia is a prevalent but under-recognized condition in patients with type 2 diabetes [3,4]. In patients with chronic kidney disease (CKD), anemia occurs earlier and is more severe in those with type 2 diabetes compared to those with non-diabetic CKD [5]. Anemia aggravates the development and progression of both microvascular (nephropathy, retinopathy, neuropathy) and macrovascular (ischemic heart disease, cerebrovascular disease, peripheral vascular disease) complications of diabetes, leading to increased hospitalization and mortality [6,7]. Moreover, anemia leads to fatigue, exercise intolerance and poor quality of life.

The etiology and pathogenesis of anemia in diabetes are multifactorial and include CKD, nutritional deficiency, chronic inflammation, drugs and inadequate erythropoietin responses [8]. However, whether genetic predisposition affects the risks of developing anemia has not been well explored in patients with type 2 diabetes. Genetic variants in angiotensin-converting enzyme (ACE) and tumor necrosis factor-alpha (TNF-α) have been significantly associated with diabetic nephropathy. In genetic studies involving Chinese patients with type 2 diabetes, those with ACE Deletion alleles or GG genotype in the promoter region of the TNF-α gene (G-308A polymorphism) tended to have higher albuminuria [9,10]. Single nucleotide polymorphisms (SNP) in the promoter region of the erythropoietin (EPO) gene have also been associated with proliferative diabetic retinopathy and end stage renal disease [11]. Furthermore, genetic polymorphisms in key factors of redox regulation may alter the risks of myocardial infarction in patients with type 2 diabetes mellitus [2].

We hypothesized that genetic variants in key factors of erythropoiesis, inflammation and diabetic nephropathy may also be important determinants for risk of anemia. The aim of this study was to investigate the associations of anemia with polymorphisms in *EPO* promoter (rs1617640), *TNF-α G-308A* and *ACE* Insertion/Deletion in Chinese patients with type 2 diabetes.

2. Subjects, materials and methods

Patients with type 2 diabetes attending the Division of Metabolism Outpatient Clinic at the Kaohsiung Chang Gung Memorial Hospital were recruited for this study. All study subjects did not have a history of diabetic ketoacidosis at the onset of diabetes nor dependent on insulin therapy within first year of diagnosis. All patients were over the age of 18 years old, and have been regularly followed for more than one year. Medical records were reviewed meticulously and patients were excluded if they had any of the following conditions: gastrointestinal bleeding within one year, history of malignancy, liver cirrhosis, dialysis dependence, use of erythropoietin stimulating agents, abnormal mean corpuscular volume (MCV $<$ 80 or $>$100 fL), white blood cell count (WBC $<$ 3500 or $>$11000/μL) or platelet count ($<$150,000 or $>$400,000/μL). The Human Research Ethics Committee of our hospital approved this study and all patients gave informed consent (IRB N0.98-2971B).

Anemia was defined according to the World Health Organization criteria: hemoglobin (Hb) levels below 12 g/dL for women and 13 g/dL for men. For each patient, we reviewed and recorded demographic, clinical and laboratory data, including blood pressures (BP), complete blood count, serum creatinine (Cr), high sensitivity C-reactive protein (hsCRP), glycated hemoglobin (HbA1c), urinary albumin and creatinine ratio (UACR) and body mass index. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula [12]. Renal function and stages of CKD were stratified according to eGFR, using the Kidney Disease Outcomes Quality Initiative classifications [13]. UACR was determined from spot urine specimens and classified as normoalbuminuria if UACR was $<$0.03 mg/mg once, microalbuminuria if UACR was 0.03–0.299 mg/mg (in two urine samples on two different occasions), and macroalbuminuria if UACR was equal to or $>$0.3 mg/mg (in two urine samples on two different occasions).

The genomic DNA was extracted from peripheral blood samples using the Gentra Puregene Blood Kit (Qiagen, Maryland). The *ACE* Insertion/Deletion polymorphisms in all patients were genotyped by PCR. *EPO* promoter (rs1617640) SNP were genotyped by real-time PCR. Genotyping for the *TNFa G-308A* polymorphism was performed using a PCR–restriction fragment length polymorphism method as described by Skoog et al. [14]. Quality control for genotyping was ensured by testing for Hardy Weinberg equilibrium and by sequencing six individuals for *EPO*.

Statistical analysis was performed using SPSS software version 12.0. Results are expressed as mean ± standard deviation for normally distributed data and as median (interquartile range) for nonparametric data. Student's *t*-test was used for comparison of means between two groups, and ANOVA used if more than two groups. Categorical variables were examined using Chi-square test. Logistic regression analysis was performed to examine the independent relationships between anemia and covariates. Significant factors from

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