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Allelic variations in superoxide dismutase-1 (*SOD1*) gene and renal and cardiovascular morbidity and mortality in type 2 diabetic subjects

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ABSTRACT

Background: Oxidative stress is involved in the pathophysiology of renal and cardiovascular complications of diabetes. Superoxide dismutase (SOD) enzymes play a major role in detoxification of reactive oxygen species and protection against oxidative stress. Associations of *SOD1* gene variants with diabetic nephropathy were reported in patients with type 1 diabetes. We investigated associations of allelic variations in *SOD1* gene with nephropathy and cardiovascular complications in patients with type 2 diabetes.
Methods: Seven SNPs in *SOD1* region were analyzed in 3744 type 2 European Caucasian diabetic patients from the DIABHYCAR (a 6-year prospective study) and DIABHYCAR_GENE cohorts. Odds ratios or hazard ratios for prevalence and incidence of diabetic nephropathy and cardiovascular events were estimated.
Results: We observed an association of rs1041740 with the prevalence of microalbuminuria at baseline (OR 1.51, 95% CI 1.10–2.10, $p = 0.01$). No association with the incidence of renal events (doubling of the serum creatinine levels or the requirement of hemodialysis or renal transplantation) or cardiovascular events (myocardial infarction or stroke) was observed during follow-up. However, three variants were associated with increased risk of death from cardiovascular causes (sudden death, fatal myocardial infarction or stroke) during the follow-up: rs9974610 (HR 0.64, 95% CI 0.46–0.88, $p = 0.005$), rs10432782 (HR 1.71, 95% CI 1.16–2.48, $p = 0.007$) and rs1041740 (HR 1.78, 95% CI 1.10–2.78, $p = 0.02$).
Conclusions: Our results are consistent with a major role for SOD1 in the mechanisms of cardiovascular protection against oxidative stress in type 2 diabetic subjects.

1. Introduction

Diabetic patients have a 3-fold higher risk than nondiabetic individuals of developing atherosclerosis and its clinical complications such as stroke, myocardial infarction, and peripheral vascular disease [1]. Cardiovascular disease accounts for up to 80% of the deaths of type 2 diabetic patients [2] and sudden death occurs frequently among diabetic patients [3]. Diabetic nephropathy is a leading cause of renal failure [4,5] and is associated with increased risk of cardiovascular morbidity and mortality in type 1 and in type 2 diabetic patients [6,7].

Reactive oxygen species (ROS), including free radicals such as superoxide and nonradical species such as hydrogen peroxide, are produced continuously in all cells as part of the normal cellular metabolism [8]. The major source of intracellular ROS is the mitochondrial respiratory chain, which produces large amounts of superoxide radicals [9]. Oxidative stress occurs when production of ROS exceeds local antioxidant capacity. In this situation, there is increased oxidation of proteins, lipids, carbohydrates and DNA, that can result in tissue and organ damage. Hyperglycemia increases the production of ROS and causes oxidative stress [10,11]. Oxidative stress influences multiple pathways implicated in diabetic nephropathy [12,13]. There is also compelling evidence that oxidative stress is associated with the metabolic syndrome and its components [14] and that it plays a key role in the pathophysiology of several cardiovascular diseases, including hypertension, myocardial infarction, stroke, and heart failure [15,16].

Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide [17]. They play an important role in antioxidant mechanism in nearly all cells exposed to oxygen. Three isoforms of SOD are expressed in

Abbreviations: ACE, angiotensin converting enzyme; ANOVA, analysis of variance; eGFR, estimated glomerular filtration rate; HR, hazard ratio; OR, odds ratio; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; SOD, superoxide dismutase; UAE, urinary albumin excretion at baseline; UTR, untranslated region.

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humans, encoded by different genes. SOD1 is mainly located in the cytoplasm, SOD2 in the mitochondria and SOD3 is extracellular. SOD1 accounts for ~85% of the total cellular SOD activity of most mammalian cells and is highly active in the human kidney and in the vascular wall [18,19].

Associations of *SOD1* gene variants with diabetic nephropathy have been observed in patients with type 1 diabetes [20,21], but no data regarding associations with nephropathy and cardiovascular complications in type 2 diabetes is available. In this study, we assessed the impact of *SOD1* allelic variation in the development and progression of diabetic nephropathy and in the morbidity and mortality of cardiovascular disease in individuals with type 2 diabetes followed prospectively for renal and cardiovascular events.

2. Methods

2.1. Participants

We studied unrelated French type 2 diabetic patients from the DIABHYCAR (n = 3137) and the DIABHYCAR_GENE (n = 607) cohorts. DIABHYCAR was a 6-year clinical trial conducted in men and women with type 2 diabetes selected on the basis of persistent microalbuminuria (urinary albumin excretion, UAE = 20–200 mg/l) or macroalbuminuria (UAE > 200 mg/l) without renal failure (plasma creatinine < 150 μmol/l) at baseline. The trial tested whether a low dose of ramipril, an angiotensin converting enzyme (ACE) inhibitor, able to reduce UAE would also reduce cardiovascular and/or renal events such as myocardial infarction, stroke, acute heart failure, end-stage renal failure, and cardiovascular death. For the purpose of the trial, a renal event was defined as the doubling of the serum creatinine levels or the requirement of hemodialysis or renal transplantation during follow-up. Myocardial infarction was diagnosed as the occurrence of at least 2 out of 3 of the following criteria: constrictive chest pain lasting 20 min or longer, increased serum creatinine phosphokinase and/or troponine levels, or typical electrocardiographic changes. Sudden death was defined as death occurring instantaneously or within 1 h after the onset of new cardiac symptoms (arrhythmia, myocardial infarction) or non-witnessed death, when the body was found and no cause of death could be discovered. Fatal stroke was not included in this group. Results were negative regarding the drug effect and were published previously [22,23]. The DIABHYCAR_GENE cohort was recruited concomitantly to DIABHYCAR and included men and women with T2DM presenting with normal UAE (UAE < 20 mg/l) at baseline, and who remained normoalbuminuric at the end of the follow-up. An independent committee reviewed all case records from both cohorts to validate selection criteria, to grade the renal involvement of each patient, and to adjudicate the clinical events during follow-up [22]. Urinary albumin was measured by nephelometry [24]. Estimation of the glomerular filtration rate (eGFR) was computed with the Modification of Diet in Renal Disease (MDRD) formula [25]. Participants gave written informed consent and study protocols were approved by the ethics committee of Angers University Hospital.

2.2. DNA studies

The *SOD1* gene is located on chromosome 21q22.11 and has a genomic size of 9307 bp. It consists of five exons, with untranslated regions (UTR) in exons 1 and 5, separated by four introns. The 5′ flanking region containing transcription factor binding sites and known regulatory elements extends up to −500 bp [26]. Seven SNPs in the *SOD1* region were analyzed [26]: rs9974610 (~13.6 kb 5′ from transcription start site), rs2173962 (~9.9 kb 5′ from transcription start site), rs10432782 (intron 2), rs2070424 (intron 3), rs1041740/rs17880196 (intron 4), rs17880135 (~0.7 kb 3′ from the end of exon 5/UTR) and rs202449 (~5.0 kb 3′ from the end of exon 5/UTR). The SNPs were chosen in HapMap (public release #23) on the basis of giving information on ~90% of the allelic variation of SNPs with minor allele frequency ≥5% at $r^2 > 0.8$ in haplotype blocks containing *SOD1*. Genotypes were determined by an Assay by Design (ABD) kit from Applied Biosystems (rs17880135) or by competitive allele-specific PCR genotyping system assays (KASPar, Kbioscience, Hoddeston, UK). Genotyping success rate was >95%. Genotyping was repeated in 5% of subjects with 100% coherence. All genotypes were in Hardy–Weinberg equilibrium.

2.3. Statistical analysis

Results are expressed as mean ± SD except when stated otherwise. Differences between groups were assessed by Pearson's chi-squared test and by ANOVA. When ANOVA was significant, comparisons between pairs were made using the Tukey–Kramer HSD test. Allelic associations with renal or cardiovascular traits were assessed by regression models. Adjustments for clinical and biological parameters were carried out by including these parameters as covariables in the regression model. Cox proportional hazards survival regression analyses were used to examine the effect of explanatory variables on time-related survival (or disease-free) rates in prospective analyses. Logistic regression analyses were used for cross-sectional analyses. Hazard ratios or odds ratios, respectively, with their 95% confidence intervals were computed in these analyses for the minor alleles. Data were log-transformed for the analyses when the normality of the distribution was rejected by the Shapiro–Wilk W test. Correction for multiple comparisons due to multiple SNP testing took into account the effective number of independent tests (M_{eff}) based on the degree of linkage disequilibrium between SNPs [27,28]. The adjusted significance threshold was determined by dividing M_{eff} into the nominal significance threshold ($p = 0.05$). Thus, $p \leq 0.02$ was considered significant, unless stated otherwise. The power to detect associations of the SNPs with diabetic nephropathy at baseline and with incidence of renal events, cardiovascular events and cardiovascular death during follow-up was 0.95, 0.67, 0.70 and 0.74 respectively, for odds ratio or hazard ratio equal or higher than 1.5 and alpha = 0.02. Statistics were performed with the JMP software (SAS Institute Inc., Cary, NC).

3. Results

3.1. SOD1 variants and UAE status at baseline

In a first step, participants were divided into 3 groups according to UAE status at baseline: normal UAE (DIABHYCAR_GENE cohort), microalbuminuria and macroalbuminuria (DIABHYCAR cohort, both groups). Characteristics of participants are shown in Table 1. Subjects with microalbuminuria or with macroalbuminuria were younger, had a shorter duration of diabetes and were more often of male sex than subjects with normal UAE. Individuals with macroalbuminuria as compared to those with normal UAE had higher body mass index (BMI), higher blood pressure levels, increased levels of HbA1c, total cholesterol and triglycerides, and lower levels of HDL cholesterol, while individuals with microalbuminuria presented with intermediate values of these parameters. Genotype frequencies according to UAE status at baseline are shown in Table 2. We observed an association of the minor T-allele of rs1041740 with microalbuminuria (odds ratio 1.51, 95% CI 1.10–2.10, p = 0.01) in a dominant model, adjusted for sex, age, BMI, duration of diabetes, HbA1c and presence of arterial hypertension. No association of the variant with macroalbuminuria was observed. Allele and genotype frequencies of the other six SNPs were similar in the 3 groups of subjects.

3.2. SOD1 variants and incidence of renal events during follow-up

Next, we assessed the impact of allelic variations on the renal outcomes of the original DIABHYCAR study. A renal event defined as the doubling of the serum creatinine levels or the requirement of