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A functional variant of the dimethylarginine dimethylaminohydrolase-2 gene is associated with chronic kidney disease

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ABSTRACT

Objective: Studies reported a relationship between elevated asymmetric dimethylarginine (ADMA) concentrations and adverse renal outcomes. There is evidence that the rs9267551 variant in the *DDAH2* gene has a functional impact with the C allele having a higher transcriptional activity resulting in increased expression of DDAH2 in endothelial cells and lower plasma ADMA levels in C allele carriers.
Methods: To address whether this variant is associated with chronic kidney disease (CDK), 2852 *White* European were studied. CKD was defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2.
Results: The proportion of subjects with CKD was significantly lower in C allele carriers than in GG genotype carriers (OR 0.49, 95%CI 0.25–0.97; $P = 0.03$). In a logistic regression model adjusted for age, gender, BMI, blood pressure, total and HDL cholesterol, triglyceride, and fasting plasma glucose, C allele carriers have a lower risk of CKD compared with GG genotype carriers (OR 0.38, 95%CI 0.18–0.78; $P = 0.008$). This association was maintained after addition to the logistic regression model of other confounders including glucose tolerance status, presence of dyslipidemia, anti-hypertensive and anti-diabetic drugs (OR 0.35, 95%CI 0.15–0.80; $P = 0.01$).
Conclusion: The rs9267551 functional variant of the *DDAH2* gene is associated with CKD with carriers of the C allele having a lower risk of renal dysfunction independently from several confounders. Because ADMA predicted progression of renal disease, it is possible that, in GG carriers, ADMA may accumulate at the renal level causing endothelial dysfunction as a consequence of reduced nitric oxide availability and potentiating micro-vascular damage.

Chronic kidney disease (CKD) is a global health problem due to its adverse outcomes, including cardiovascular events and all-cause mortality [1,2]. Decreased estimated glomerular filtration rate (eGFR), the primary measure used to define CKD (eGFR <60 ml/min/1.73 m^2), and cardiovascular disease share common atherosclerotic risk factors including hyperglycemia, hypertension, dyslipidemia, smoking, overweight/obesity, and insulin resistance [3–10]. Endothelial dysfunction is considered an early alteration in the development and progression of atherosclerosis, and is already present at early stages of renal dysfunction [11–13]. In addition, results of longitudinal studies have demonstrated that endothelial dysfunction is associated with a decline in eGFR independently of traditional cardiovascular risk factors and antihypertensive treatment [14]. Endothelial dysfunction is a wide term that entails diminished production and/or availability of nitric oxide (NO). Inhibition of NO synthesis by endogenous inhibitors of the endothelial NO synthase (eNOS) may have a main role in inducing endothelial dysfunction [15–17].

Although initial studies found increased plasma ADMA concentrations in patients with end-stage renal disease (ESRD) [18,19], subsequent studies have reported a relationship between elevated ADMA concentrations and adverse renal outcomes over time, which could imply a causal role for elevated ADMA levels in the progressive decline of kidney function [20,21]. ADMA is generated by proteins methylation by the enzyme protein arginine N-methyltransferases (PRMTs), and is degraded by the dimethylarginine dimethylaminohydrolase (DDAH) [22,23]. There are two isoforms

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of DDAH, with DDAH1 found in tissues expressing neuronal NOS, and DDAH2 highly expressed in the endothelium [24]. We have recently shown that the rs9267551 variant in the *DDAH2* gene has a functional impact with the C allele having a higher transcriptional activity resulting in increased expression of DDAH2 in primary human endothelial cells naturally carrying the C allele. In addition, subjects carrying the C allele exhibit lower levels of circulating ADMA, and higher insulin sensitivity [25]. Interestingly, in genome-wide association studies (GWAS) carried out by the Diabetes Genetics Replication And Meta-analysis (DIAGRAM+), the rs9267551 variant in the *DDAH2* gene was nominally associated with type 2 diabetes ($P = 3 \times 10^{-5}$) with the G diabetogenic risk allele conferring an odds ratio (OR) of 1.12 (95%CI 1.06–1.19) (Andrew Morris and Mark McCarthy personal communication for the DIAGRAM+). In view of the important role of ADMA in regulating endothelium-dependent vasodilation and, thereby, renal function, we hypothesized that the rs9267551 polymorphism in the *DDAH2* gene may be associated with CKD. To this aim, we investigated the association of rs9267551 polymorphism with CKD in a cohort of subjects of European ancestry.

1. Materials and methods

1.1. Study subjects

The study group consisted of 2852 adult individuals of European ancestry consecutively recruited at the Department of Systems Medicine of the University of Rome-Tor Vergata and at the Department of Medical and Surgical Sciences of the University "Magna Graecia" of Catanzaro [26]. Recruited subjects participated to a campaign for assessment of cardio-metabolic risk factors. Recruitment mechanisms include word-of-mouth, fliers, and newspaper advertisements. The inclusion criteria were: age ≥23 years, and presence of one or more cardio-metabolic risk factors including elevated fasting glucose levels, hypertension, dyslipidemia, overweight/obesity, and family history for diabetes. Subjects were excluded if they had end-stage renal disease (ESRD), chronic gastrointestinal diseases, chronic pancreatitis, history of any malignant disease, history of alcohol or drug abuse, positivity for antibodies to hepatitis C virus (HCV) or hepatitis B surface antigen (HBsAg), and hepatic failure. After 12-h overnight fasting, subjects underwent anthropometrical evaluation and a venous blood sample was drawn for laboratory determinations. Body mass index (BMI) was calculated as body weight (kilograms) divided by the square of height (meters). Waist (at the midpoint between the lateral iliac crest and lowest rib) and hip circumference (at the level of the trochanter major) were measured to the nearest 0.5 cm. Readings of clinic blood pressure (BP) were obtained in the left arm of the supine patients, after 5 min of quiet rest, with a sphygmomanometer. Normal fasting glucose (NFG) was defined as fasting plasma glucose <100 mg/dl, impaired fasting glucose (IFG) as fasting plasma glucose ≥100 and <126 mg/dl, and type 2 diabetes mellitus (T2DM) as fasting plasma glucose ≥126 mg/dl or current treatment with anti-diabetic drugs. Atherogenic dyslipidemia and hypertension were defined according to the criteria utilized for definition of the metabolic syndrome released in 2009 by a joint statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity [27]. Thus, atherogenic dyslipidemia was defined as triglyceride ≥150 mg/dl, HDL <40 mg/dl in men and <50 mg/dl in women or drug treatment. Hypertension was defined as systolic blood pressure ≥130 and/or diastolic ≥85 mmHg or antihypertensive drug treatment in the subjects with a history of hypertension.

The protocol was approved by the Institutional Ethics Committees and informed written consent was obtained from participants. All the investigations were performed in accordance with the principles of the Declaration of Helsinki.

1.2. Analytical determinations

Serum creatinine was measured in the routine laboratory by an automated technique based on a Creatinine Jaffè compensated Method for serum and plasma (Roche Diagnostics) method implemented in an autoanalyzer. The laboratory references ranges for this assay are 0.7–1.2 mg/dl for males, 0.5–1.0 mg/dl for females. Glucose, triglyceride, total and HDL-C concentrations were determined by enzymatic methods (Roche, Basel, Switzerland).

1.3. Calculations

Estimated glomerular filtration rate (eGFR) was calculated by using the CKD-EPI equation [28]: $eGFR = 141 \times \min(Scr/k, 1)^{\alpha} \times \max(Scr/k, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female], where Scr is serum creatinine, k is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of Scr/k or 1, and max indicates the maximum of (Scr/k or 1).

1.4. DNA analysis

DNA was isolated from whole blood using commercial DNA isolation kit (Promega, Madison, WI). Screening of the rs9267551 polymorphism was performed using a TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). TaqMan genotyping reaction was amplified on a GeneAmp PCR system 2700 and fluorescence was detected using an ABI Prism 7000 sequence detector (Applied Biosystems, Foster City, CA). Genotyping quality was tested by including 3 HapMap samples in each 96-well plate. The agreement rate with the HapMap database genotypes was >99%.

1.5. Statistical analysis

The results for continuous variables are given as means ± SD. Unpaired Student's t test was used to compare differences of continuous variables between two groups, and the χ^2-test for non-continuous variables. The Hardy–Weinberg equilibrium between the genotypes was evaluated by χ^2 test. There was no deviation from the Hardy Weinberg equilibrium in genotype distributions ($P = 0.003$) using the same threshold of statistical significance utilized by the DIAGRAM + investigators in the replication studies of the GWAS (defined as a $P > 0.001$) [29,30]. A multivariate logistic regression analysis was used to determine the association between the rs9267551 polymorphism and CKD. The case-control study has 80% power to detect an association conferring a 0.60-fold reduced risk of CKD for protective C allele according to a dominant model. All tests were two-sided, and a P value <0.05 was considered statistically significant. All analyses were performed using the SPSS software program Version 16.0 for Windows.

2. Results

Clinical characteristics of study subjects according to the rs9267551 polymorphism are shown in Table 1. Because of the small number of CC individuals ($n = 13$) and the a priori hypothesis based on the dominant effect observed in our previous functional studies [25], GC and CC individuals were pooled and analyzed together as C carriers, according to a dominant genetic model. The rs9267551 polymorphism did not show any significant association