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Letter to the Editor

Endothelial Nitric Oxide Synthase 4a/B Polymorphism and Its Interaction with Enos G894T Variants in Type 2 Diabetic Patients: Modifying the Risk of Diabetic Nephropathy

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Dear Editor-in-Chief

Diabetic Nephropathy (DN) is one of the most important microvascular complications of T2DM. A candidate gene implicated to play a major role in DN susceptibility, is endothelial nitric oxide synthase (*eNOS*) gene (1). *eNOS* polymorphisms is associated with endothelial dysfunction and diabetic complications (retinopathy and neuropathy). In recent years, several polymorphisms of the *eNOS* gene have been identified. G894T, the missense mutation in exon 7 accurs in position 894 of the *eNOS* gene corresponds to a guanine to thymine conversion and leads to a glutamine to aspartate substitution in position 298 of the protein (Glu298Asp). Another studied polymorphism is 5' end of *eNOS* gene (27bp repeats). This polymorphism regulates *eNOS* expression through small interference RNA (sirRNA) (2).

Some studies have confirmed the relationship between *eNOS* genetic polymorphisms and DN (3, 4). So we investigated the association of 4a/b polymorphism and the risk of T2DM and DN in diabetic patients in southwest of Iran. Furthermore, we wanted to measure the kidney function. A total number of 132 diabetic patients (66 patients with DN, 66 patients without DN) of Arab ethnicity referred to the Golestan Hospital of Ahvaz, southern Iran were enrolled. Informed consent for participation was obtained from all the subjects.

The study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1395.200). Blood samples were taken in the morning after overnight fasting for measuring Fasting blood glucose, HbA1c, BUN, Serum creatinine, microalbumin, Serum cystatin C levels and eGFR. Total genomic DNA was extracted from leukocyte fraction using a standard technique. Genotyping for 4a/4b was performed by PCR and genotyping for G894T based on the PCR-RFLP methods using Ban II digestion.

Elevated serum creatinine, BUN, cystatin C, microalbumin levels and decreased estimated GFR (as markers of kidney damage) were observed in people with the T2D as compared to healthy control group. Significant associations with the susceptibility to T2DM were detected for G894T and 4a/4b polymorphisms, (OR=1.86 95%CI=1.16-2.99, *P*=0.01) and (OR=1.92 95%CI=1.18-3.2, *P*=0.0009), respectively. Further we did not find a significant association with DN as the microvascular complication of T2DM, (OR = 1.1 95% CI 0.7–1.8, *P*=0.6) and (OR = 0.92 95% CI 0.6–1.5, *P*=0.7), respectively.

In contradiction to our study in Indian and Malaysian people (3, 5), revealed *eNOS* genotypes have significant effects on the development of nephropathy in type 2 diabetes patients. Furthermore we demonstrated the risk of DN in T2DM in the presence of *eNOS* T alleles and *eNOS* a alleles up to 1.8-fold (*P*=0.049) and 4.6-fold(*P*=0.029), respectively. Concomitant presence of the two variants was not associated with increased risk of T2DM and DN (Table 1).

Table 1: Interaction of *eNOS* a with *eNOS* T alleles in association with diabetic nephropathy

eNOS a	***eNOS*** **T**	**T2DM patients without nephropathy** N(%)	**T2DM patient with nephropathy** N(%)	**OR(95% CI)**	***P*-value**	**Adjusted OR[a](95% CI)**	***P*-value[a]**
+	+	27(40.9)	12(18.2)	0.89(0.3-2.9)	0.8	1.04(0.7-1.6)	0.858
-	+	17(25.8)	29(43.9)	3.4(1.1-10.8)	0.036	1.8(1-3.4)	0.049
+	-	10(15.2)	19(28.8)	3.8(1.1-13.2)	0.035	4.6(1.2-18.5)	0.029
-	-	12(18.2)	6(9.1)	1.0(ref)		1.0(ref)	

Data in n (%),[a]Adjusted for age, sex, BMI, HbA1c

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Conflict of interest

The authors declare that there is no conflict of interest.

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