

Small ubiquitin-like modifier 4 M55V polymorphism is not associated with diabetic nephropathy in Iranian type 2 diabetes patients

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INTRODUCTION: We studied the impact of small ubiquitin-like modifier 4 (SUMO4) M55V polymorphism on susceptibility to diabetic nephropathy in Iranian type 2 diabetes patients.

MATERIALS AND METHODS: The patient group consisted of 50 Iranian type 2 diabetes patients with nephropathy, and the control group consisted of 50 Iranian type 2 diabetes patients without nephropathy. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism method for the M55V.

RESULTS: The frequency of SUMO4 AA, AG, and GG genotypes were 23%, 18%, and 9% in the patient group and 10%, 22%, and 18% in the control group. There was no significant difference in frequency of SUMO4 genotypes in patients compared to controls.

CONCLUSION: These findings indicate that SUMO4 M55V polymorphism is not associated with diabetic nephropathy in Iranian type 2 diabetes patients.

Key words: Iranian, nephropathy, polymorphism, small ubiquitin-like modifier 4, type 2 diabetes

renal disease, in individuals with type 2 diabetes.^[1,2] It is suggested that genetic susceptibility plays an important role in the development and progression of diabetic nephropathy.^[3,4] The transcription factor of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) in most of the cell types can be activated by various molecules.^[5,6] The main determinant of the development of diabetic nephropathy is high glucose, and some studies have demonstrated that high glucose levels can rapidly activate NF- κ B in renal cells.^[7,8]

Small ubiquitin-like modifier 4 (SUMO4) gene is located in a type 1 diabetes susceptibility locus insulin-dependent diabetes mellitus 5 (IDDM5) and has been found to be involved in immune responses including autoimmunity and inflammation through NF- κ B regulation and heat shock transcriptional factor activation. It is found that SUMO4 be mainly expressed in the kidney and immune system.^[9,10] SUMO4 can modify immune response through the substrate inhibitor inhibitor of kappa light polypeptide gene enhancer in B-cells (IKK β), a negative regulator of NF- κ B.^[11] I κ B is bound to NF- κ B in the cytoplasm in unstimulated cells, and a variety of stimuli induce degradation of I κ B by the proteasome. After release from I κ B, NF- κ B translocates into the nucleus, where it induces the transcription of genes associated with the immune response, inflammation, and apoptosis.^[12] Human SUMO4 protein has been shown that can conjugate to the same site of I κ B.^[13] SUMO4-modified I κ B cannot be ubiquitinated and is resistant to degradation.^[14]

Introduction

Many factors may be associated with the pathogenesis of diabetic nephropathy, a leading cause of end-stage

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SUMO4 protein is encoded by the SUMO4 gene located at chromosome 6q25.^[15] A common single nucleotide polymorphism encoding a methionine-to-valine substitution at codon 55 (M55V) has been recently identified in SUMO4 gene.^[11] Recent reports showed that the SUMO4 M55V polymorphism is associated with increased susceptibility to type 2 diabetes and diabetic nephropathy in several populations,^[16,17] whereas our previous study^[18] indicated not association of the SUMO4 M55V polymorphism with the susceptibility of type 2 diabetes in Iranian population. These findings prompted us to investigate the impact of SUMO4 M55V polymorphism on susceptibility to diabetic nephropathy in Iranian type 2 diabetes patients.

Materials and Methods

Patients and controls

The patient group consisted of 50 type 2 diabetes patients with nephropathy, and the control group consisted of 50 type 2 diabetes patients without nephropathy. The individuals were recruited from different geographic area of the Tehran in Iran. The mean age individuals were range 25-45 years. The diagnosis of type 2 diabetes was established according to the Report of the Expert Committee on Diagnosis and Classification of Diabetes Mellitus.^[19] All samples were collected with the written consent of the patients or of their legal guardians. The DNA samples were prepared from peripheral blood leukocytes by the salting-out method.

Genotyping

The DNA samples were genotyped in a genotyping assay as previously described.^[18] Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR was used to amplify the fragments of SUMO4 that contained the site of 163A/G polymorphism. PCR reaction were carried out in a final volume of 25 μ l containing 50 ng/ μ l DNA, 2.5 μ l of each primer (Metabion Company, Forward: 5'TGTGAACCACGGGGATTGTCG3', and Reverse: 5'TCAGTAGACACCTCCCGTAC3') and 12.5 of \times 10 buffer, dNTP, and Taq polymerase master kit. All

genotyping was reproduced by PCR-RFLP methods with a second non-polymorphic cutting site in the same PCR products using restriction enzyme TSpR1 (boilable company). At 65°C for 16 h then separated on a 3% Agarose gel. Three possible genotypes were defined by three distinct patterns of bands seen on the gel: AG (134 bp, 66 bp), GG (134 bp), and AA (66 bp).^[18]

Statistical analysis

The frequencies for SUMO4 genotypes were calculated by direct counting. The significance of associations was determined using the Chi-square (χ^2) test and $P(p) < 0.05$ were considered statistically significant. The Chi-square test was used for Hardy-Weinberg equilibrium (HWE) by comparing the observed number of subjects for each genotype with the expected number of subjects, assuming the existence of HWE.

Results

The frequency of SUMO4 AA, AG, and GG genotypes were 23%, 18%, and 9% in type 2 diabetes patients with nephropathy and 10%, 22%, and 18% in type 2 diabetes patients without nephropathy. There was no significant difference in frequency of SUMO4 genotypes in patient group compared to control group. As a results of frequency matching, the observed genotype frequency among the controls were in agreement with the HWE SUMO4 ($\chi^2 = 1.5$, $df = 2$, $CI = 95\%$, $P = 0.6$).

Discussion

The human population is heterogeneous in terms of risk of disease. This is due to differences in the genetic and environmental characteristics. Another illustration, which may support genetic heterogeneity across population, is the ethnic difference.^[20] In this way, it has been demonstrated that a number of genetic factors are involved in the development of diabetic nephropathy.^[3,4]

Lin et al.^[17] have recently reported that the SUMO4 gene M55V variant is associated with severity of diabetic nephropathy in an Taiwanese cohort of 430 patients with type 2 diabetes. In present study,

we also investigated the association of SUMO4 M55V polymorphism with diabetic nephropathy in Iranian type 2 diabetes patients. In contrast to Lin *et al.*, we report no significant association between the SUMO4 M55V polymorphism and diabetic nephropathy in Iranian patients with type 2 diabetes.

We suggest that the reason for such opposing results may be related to different ethnic groups of the studied cohorts, for example, Taiwanese and Iranian population, and thus, demand further studies in different ethnic groups to clarify the effect of the SUMO4 M55V polymorphism in diabetic nephropathy. However, the lack of association between M55V polymorphism of SUMO4 and susceptibility of type 2 diabetes in our study may be due to the insufficient sample size.

More recent, Rudofsky *et al.*^[21,22] have reported no association of the M55V polymorphism in the SUMO4 gene with diabetic nephropathy in 752 Caucasian patients with type 1 and type 2 diabetes. Interestingly, our results confirmed Rudofsky *et al.* studies regarding to this no association.

In summary, this study indicates that the M55V polymorphism of SUMO4 gene is not associated with diabetic nephropathy in Iranian type 2 diabetes patients. In other words, this results show that distribution of SUMO4 genotypes in the Iranian population has common features with the Caucasian populations studied before. Furthermore, this study confirmed our previous study about no association of SUMO4 M55V polymorphism with type 2 diabetes in Iranian population. However, we suppose that further studies using the larger sample sizes are needed to confirm the exact role of SUMO4 M55V polymorphism in diabetic nephropathy and type 2 diabetes in Iranian population.

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