

SUMO4 M55V Polymorphism is associated with diabetic nephropathy in Iranian type 2 diabetes patientsFarhad Shahsavari¹, Abdolreza Kheirollahi², Mehrzad Jafarzadeh^{3*}, Mehdi Hedayati⁴¹. Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran.². Department of Urology, Lorestan University of Medical Sciences, Khorramabad, Iran.³. Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.⁴. Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.mehrzadjafarzadeh@yahoo.com**Abstracts**

Background: We studied the impact of 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 (PCR-RFLP) method for the M55V. **Results:** The frequency of SUMO4 AA, AG, and GG genotypes were 48%, 36%, and 16% in the patient group and 20%, 52%, and 28% in the control group. There was a significant increase in frequency of SUMO4 AA genotype in type 2 diabetes patients with nephropathy compared to type 2 diabetes patients without nephropathy (48% vs 20%, P=0.003). **Discussion:** These findings indicate that SUMO4 M55V Polymorphism is associated with diabetic nephropathy in Iranian type 2 diabetes patients.

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1. Introduction

Many factors may be associated with the pathogenesis of diabetic nephropathy, a leading cause of end-stage 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). Transcription factor of NF- κ B in most 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 (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 (I κ B), 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).

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.

2. Materials and methods**2.1. Patients and Controls**

The patient group consisted of 50 types 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. DNA samples were prepared from peripheral blood leukocytes by the salting-out method.

2.2. Genotyping

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 163 A/G polymorphism. PCR reaction were carried out in a final volume of 25 µl containing 50 ng/µl DNA, 2.5 µl of each primer (Metabion Company, Forward: 5'TGTGAACACGGGGATTGTCG3' and Reverse: 5'TCAGTAGACACCTCCCGTAC3') and 12.5 of 10X 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).

2.3. Statistical analysis

The frequency of SUMO4 genotypes were calculated by direct counting. The Pearson chi-square was performed to assess the association of SUMO4 genotypes with diabetic nephropathy. The statistical test was considered as significant if P value was less than 0.05. 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.

3. Results

The frequency of SUMO4 genotypes in patients and controls are illustrated in Table 1. The frequency of SUMO4 AA, AG, and GG genotypes were 48%, 36%, and 16% in type 2 diabetes patients with nephropathy and 20%, 52%, and 28% in type 2 diabetes patients without nephropathy. The results indicated a significant increase in AA genotype (48% vs 20%, $P=0.003$) and a non-significant decrease in AG (36% vs 52%, $P=0.107$) and GG (16% vs 28%, $P=0.148$) genotypes in type 2 diabetes patients with nephropathy compared to type 2 diabetes patients without nephropathy.

4. 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). In present study we also investigated the association of SUMO4 M55V polymorphism with diabetic nephropathy in Iranian type 2 diabetes patients.

Table 1. The frequency of SUMO4 genotypes in the patient group and the control group.

SUMO4 genotypes	% of patient group (n=50)	% of control group (n=50)
AA	48*	20
AG	36	52
GG	16	28

*Significant difference between the case and the control groups ($P<0.05$)

In summary, this study indicates that the M55V polymorphism of SUMO4 gene is associated with diabetic nephropathy in Iranian type 2 diabetes patients (Table 1). Lin et al (17) have recently reported that the SUMO4 gene M55V variant is associated with severity of diabetic nephropathy in a Taiwanese cohort of 430 patients with type 2 diabetes. Interestingly, our results confirmed Lin et al study regarding to this association. In other words, this results show that distribution of SUMO4 genotypes in the Iranian population has common features with the Asian populations studied before.

On the other hand, 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. In contrast to Rudofsky et al, we report significant association between the SUMO4 M55V polymorphism and diabetic nephropathy in Iranian patients with type 2 diabetes. We believe that the reason for such opposing results may be related to different ethnic groups of the studied cohorts, e.g., Caucasian and Iranian populations. Thus it requires further studies in different ethnic groups to clarify the effect of the SUMO4 M55V polymorphism in diabetic nephropathy.

However the association between M55V polymorphism of SUMO4 and diabetic nephropathy in our study may be due to the insufficient sample size. Thus, we imagine that further studies using the larger sample sizes are needed to confirm the exact role of SUMO4 M55V polymorphism both in diabetic nephropathy and in type 2 diabetes in Iranian population.

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***Corresponding author**

MSc. Mehrzad Jafarzadeh, Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

E-mail: mehrzadjafarzadeh@yahoo.com

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