SUMO4 163G>A variation is associated with nephropathy in type 2 diabetes in Indian population

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Background and Objectives

- ❖ Diabetic nephropathy- leading cause of end stage renal disease
- ❖ 25% to 40% of patients with diabetes develop diabetic nephropathy
- ❖ In India diabetic nephropathy is the commonest (44%) cause of ESRD.
- ❖ The pathogenesis of DN appears to be multifactorial
- ❖Genetic susceptibility plays an important role in the pathogenesis DN
- ❖ Single nucleotide polymorphisms (SNPs) are considered to be useful markers to identify genetic variants that may confer susceptibility to etiologically complex diseases
- ❖ SUMO4 (Small ubiquitin-related modifier 4) mRNA was recently found to be mainly expressed in the kidney
- ❖Substitution of methionine with valine at codon 55 (M55V) of SUMO4 may induce higher nuclear factor-κB activity which is known to mediate the development of diabetic nephropathy
- ❖ **This study was designed to investigate the association of SUMO4 163G>A variation with nephropathy in type 2 diabetes mellitus (T2DM).**

Subjects and Methods

Study design

This case–control study carried out at PGIMER Chandigarh. Study was approved by the institute Ethics Committee and written informed consent was taken.

Patient selection

•A total of 417 type 2 diabetic subjects:216 without nephropathy (DM) and 201 with nephropathy (DN)

•Diabetic nephropathy (DN) group: (a) eGFR<6oml/min (b) proteinuria 500mg/day

•Type 2 diabetes (DM): a) duration of onset ≤ 5 years
b) negative for dipstick urinary protein
c) urinary albumin <150 mg/day

Polymorphisms selected for the study

Gene	Polymorphism	Position	Effect
SUMO4	163 G>A (M55V)	6q25, at 163 nucleotide position in coding region	Enhances protein stability and modulates subcellular localization

Genetic analysis

•Genomic DNA isolated from peripheral blood leucocytes using standard phenol-chloroform method

•Polymorphism analyzed using specific primer pairs by PCR-RFLP

Primer pairs & Restriction Enzymes used for genotyping

Polymorphism	Primers	Restriction enzyme	Restriction digestion product (base pair)
163 G>A (M55V)	F: 5'-ATTGTGAACCACGGGGATTGTTA-3' R: 5'-CAGCGTTCTGGAGTAATAAAGAAG-3'	Mse1	AA:161,21 GA:181,161,21 GG:181

Statistical analysis

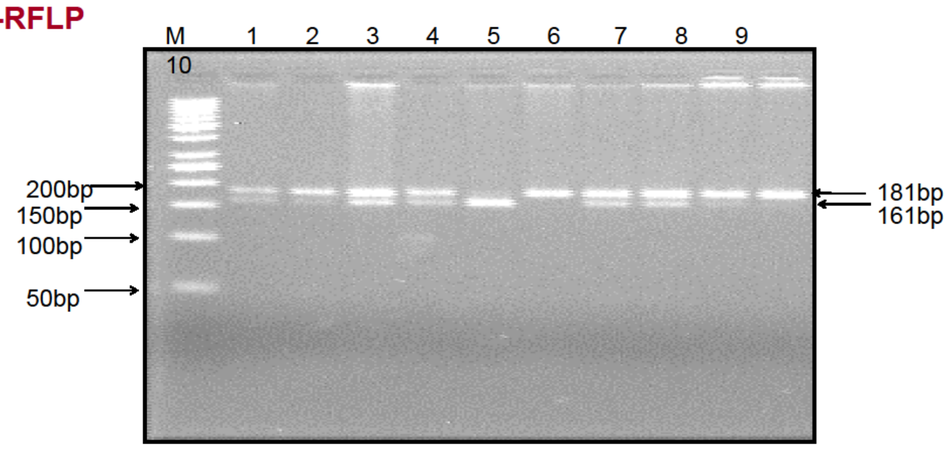
Data are expressed as the means±SD. Continuous and nominal clinical variables were compared with t-test and χ2 test in DM and DN groups. Parameter with skewed distribution were analysed with Mann–Whitney U test. Hardy Weinberg equilibrium (HWE) was tested for each SNP using the data obtained by genotyping healthy controls. Allelic and genotypic associations of SNPs were evaluated by Pearson's χ2 test and odds ratio (OR) and 95% confidence intervals (CI). One-Way ANOVA was used for more than two variables.

Results

Baseline characteristics of the patients in the two groups

Parameters	DM (N=216)	DN(N=201)	P value
Age (years)	55.7±10.0	56.7±8.8	0.2
Male/female	123/92	140/62	0.006
Body mass index (Kg/m2)	26.27±4.08	25.48±4.02	0.06
Duration of diabetes (years)	9.7±6.37	13.81±7.01	<0.0001
SBP (mmHg)	134.63±19.27	142.67±22.04	<0.0001
DBP (mmHg)	82.55±11.25	86.49±11.88	0.002
Fasting blood sugar	129.9±61	139±52.5	0.1
Post pandrial blood sugar	191.8±71.5	205±74.5	0.7
Serum creatinine (mg/dl)	1.0±0.28	1.55±0.97	<0.0001
Total cholesterol (mg/dl)	169.9±42.5	183.6±55.5	0.004
HDL (mg/dl)	45.9±11.7	45.1±14.7	0.5
LDL (mg/dl)	95.06±35.40	102.27±44.87	0.08
Triglyceride (mg/dl)	152.5±73.15	176.0±97.4	0.05
HbA1c (%)	7.50±1.5	8.0±1.9	0.014
Neuropathy	104	135	0.0001
Ratinopathy	59	139	<0.0001
CAD	39	41	0.6
Hypertension	141	163	<0.0001

PCR-RFLP



M: 50 bp ladder ;1,2,3,4,7,8,9:GA ;5:AA ;6,10: GG

Genotype and Allele frequencies of the genetic variation in Sumo4 in DM and DN group

Gene/ polymorphism	Genotype	Diabetic without Nephropathy (DM) N = 216 (%)	Diabetic with Nephropathy (DN) N = 201	Odd ratio (95%CI)	P value
SUMO4 (163 G>A)	GG	42(19.4)	59 (29.4)	1.72 (1.09-2.7)	0.01 (GG Vs AA+GA)
	GA	103(47.7)	89(44.3)		
	AA	71(32.9)	53(26.3)		
Allele					
SUMO4 (163 G>A)	G	187(45.4)	207(51.5)	1.4 (1.1-1.8)	0.017 (G Vs A)
	A	245(59.5)	195(48.5)		

Clinical parameters in diabetic subjects according to SUMO4 163 G>A genotypes

Parameter	AA Genotype	GA Genotype	GG Genotype	P value
Age (years)	54.6±8.8	56.8±9.5	56.8±8.9	0.08
Male/female (No)	77/47	123/69	64/37	0.94
Duration of diabetes (years)	10.50±6.52	12.34±7.44	11.95±6.5	0.068
Blood Sugar Fasting (mg/dl)	1.35E2±49.31	1.35E2±62.41	1.33E2±57.71	0.953
Blood Sugar Post-prandial (mg/dl)	1.98E2±75.53	1.97E2±69.46	1.98E2±77.87	0.990
Body mass index (Kg/m2)	25.80±4.28	26.07±4.39	25.64±3.30	0.565
Serum Creatinine (mg/dl)	1.23±0.78	1.22±0.67	1.36±0.92	0.336
Systolic Blood Pressure (mmHg)	98.4±37.3	88.9±31.5	140.29±20.56	0.363
Diastolic Blood Pressure(mmHg)	83.42±10.61	84.95±11.46	84.19±11.32	0.501
Total Cholesterol (mg/dl)	176.51±57.16	174.29±44.54	177.68±49.19	0.707
Triglyceride(mg/dl)	153.72±79.96	163.44±85.59	176.72±93.70	0.162
HBA1(%)	8.09±2.04	7.71±1.63	7.62±1.39	0.131

Conclusions

- The duration of diabetes was higher in DN compared to DM (p=0.001) and prevalence of retinopathy, neuropathy and hypertension was higher in DN.
- GA & AA genotype was higher in DM as compared to DN. GG genotype was significantly more frequent in DN as compared to DM (p=0.018, OR=1.72,(1.1-2.7)).
- G allele was more frequent in DN compared to DM (p=0.017, OR=1.4,(1.1-1.8)). GG+GA genotype was associated with duration of diabetes (p=0.01).

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Diabetes (complications & therapy)

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