ORIGINAL ARTICLE

SNP in KCNQ1 Gene is Associated with Susceptibility to Diabetic Nephropathy in Subjects with Type 2 Diabetes in India

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Abstract

Objective: Diabetic nephropathy (DN) remains the most common cause of end stage renal disease (ESRD) as the burden of diabetes increases worldwide. Only 25 to 40% of patients with type 2 diabetes mellitus (T2DM) develop diabetic nephropathy irrespective of glycemic control so there should be a specific genetic basis for the development of diabetic nephropathy.

Method: We have collected venous blood samples from 50 cases (Diabetic nephropathy) and 20 controls (T2DM without nephropathy) diagnosed by spot urine albumin creatinine ratio (ACR). DNA was isolated from processed samples. PCR study and sequencing was done to detect polymorphism of rs2237897 in KCNQ1 gene.

Result: Statistically significant difference was found when the allelic frequencies between the two groups were compared (p=0.03), with the C allele having a 2.4 fold higher risk of having diabetic nephropathy (risk ratio, RR)= 1.16, 95%CI of RR = 1.01 to 1.3, Odds Ratio (OR) =2.4; 95% CI of OR =1.06 to 4.6). Chi-square analysis showed a significant difference in genotype frequency of rs2237897 ($\chi 2$ = 4.63, p=0.03) in Diabetic nephropathy subjects, compared with that of controls.

Conclusion: This study suggested that, *KCNQ1* being an established type 2 diabetes gene, genetic variation in this gene may contribute to susceptibility to diabetic nephropathy and the C allele is the risk allele for diabetic nephropathy, which is different from Japanese population where the T allele was the risk allele.

Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM are caused by a complex interaction of genetics and environmental factors.[1]

Complications from diabetes can be classified as microvascular or macrovascular. Microvascular complications include nervous system damage (neuropathy), renal system damage (nephropathy) and eye damage (retinopathy).[2]

Diabetic nephropathy is an increasingly growing complication of diabetes that occurs in 20% to 40% of all diabetics. In the entire world, DN is the primary single cause of end-stage Renal disease (ESRD). Both type 1 and type 2 diabetes can lead to nephropathy with a higher propensity to develop into type 1 diabetes, but the number of cases of DN are more in type 2 due to the high prevalence of type 2 diabetes.[3]

Diabetic nephropathy is a chronic disorder typically characterized by progressive albuminuria and a decline in renal function. Based on the levels of urine albumin excretion, in a didactic manner, DN has two phases: incipient nephropathy or the microalbuminuria phase and clinical nephropathy or the proteinuria phase. Microalbuminuria is considered a risk factor for DN progression.[4]

Many environmental factors have been established as contributing to the development of DN while the role of others has yet to be clearly understood.[5]

Research has focused on seeking potential genetic alterations associated with CKD and ESRD. In fact, genetic evidence has been found in case-control association and linkage studies, and more recently using genome-wide scan (GWS). These studies support the assumption that onset, progression, and severity of DN can be in part attributed to genetic factors.[6]

The studies of Japanese and East Asian population also showed that in addition to *KCNQ1* being an established type 2 diabetes gene, genetic variation in this gene may contribute to susceptibility to diabetic nephropathy.[7,8]

In epithelial tissues from organs such as lung, stomach, cochlea, intestine, and kidney, where salt and water transport is crucial for proper function, a lack of functional *KCNQ1* channel expression has been found to have severe implications.[9,10]

In the kidney, *KCNQ1* has been shown to assemble with KCNE1, the β subunit of the potassium channel, forming a potassium channel complex localized to the brush border of the mid to late proximal tubule.[11,12] Moreover, it has been shown to play a role in the Na+ secretion at the proximal tubule by maintaining a driving force for Na+ transport across the membrane.[13]

These observations suggest the possibility that *KCNQ1* may be a candidate for conferring susceptibility to diabetic nephropathy. SNPtyping of DNA samples of the subjects showed that among the reported

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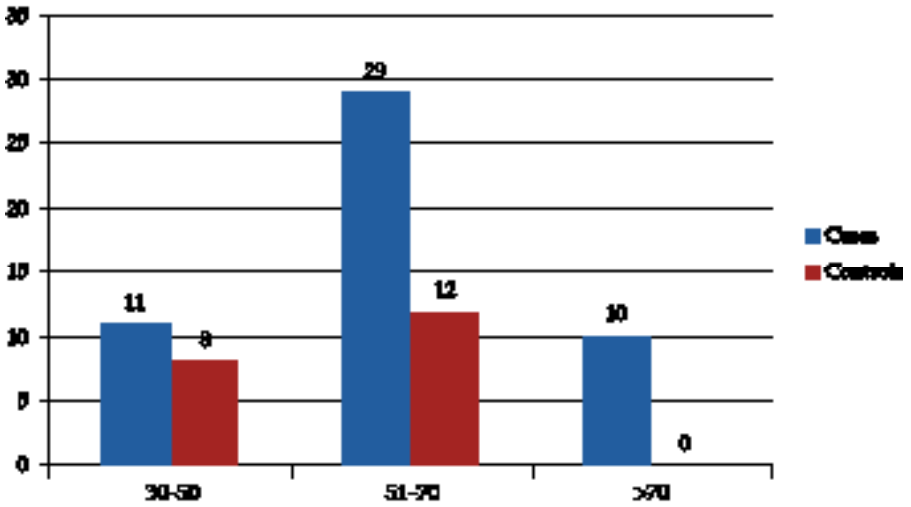

Fig. 1: Age-wise distribution of Diabetic nephropathy patients and controls

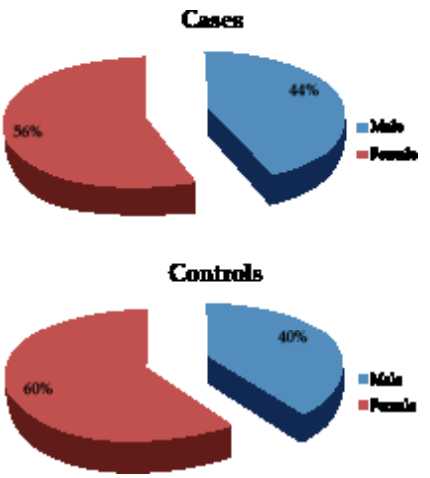

Fig. 2: Sex-wise distribution of Diabetic nephropathy patients and controls

SNPs, rs2237897 was most commonly associated with DN in Japanese and Chinese study so we choose this site for our study.

Aims and Objectives

In this study, our aim is to find out the association of single nucleotide polymorphisms (SNPs) of rs2237897 within a *KCNQ1* gene with diabetic nephropathy subjects with type 2 diabetes in Indian population.

Material and Methods

This study was conducted jointly by a tertiary care hospital and Genetic center. Blood samples of 50 cases (Diabetic nephropathy) and 20 controls (T2DM without nephropathy) were collected for analysis. Among 50 samples, DNA was isolated from 39 only as rest 11 samples are lysed.

Informed consent was taken beforehand, procedure and motive of the study were explained to the patients. Ethical committee approval was taken for study.

Patients with type 2 diabetes mellitus of age more than 30 years with Microalbuminuria or Macroalbuminuria included as cases and without diabetic nephropathy as controls. Patients with chronic kidney disease of etiology other than diabetes, pregnant females, patients having co-morbid condition except hypertension were excluded from the study.

Each patient was subjected to detailed history and complete general physical examination. Detailed history about age, sex, weight, duration of diabetes were noted and spot urine Albumin/creatinine ratio was done to detect microalbuminuria.

Genomic DNA was extracted from venous blood, drawn from subjects, by the Guanidine Thiocyanate (GTC) procedure (Sambrook et al 1989 and Hammond et al. 1996) and dissolved in water. Polymerase chain reaction (PCR) primers were designed for Restriction fragment length polymorphism (RFLP) analysis of rs2237897 using National Center for Biotechnology Information (NCBI) primers and subsequent restriction digestion with appropriate restriction enzymes was carried out using a standard protocol to genotype the polymorphic sites. (Kapur et al., 2007).

Genotype distributions were examined for a significant departure from Hardy–Weinberg equilibrium by v2-test. A trend test was performed to determine any increase in risk with an increase in the number of risk allele (Schoojans 1993). The risk of rs2237897 polymorphism was calculated by odds ratios (ORs) and 95% confidence intervals (CIs), for genotypes in both control and case groups. An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. In statistics, a confidence interval (CI) is a type of interval estimation, computed from the statistics of the observed data, that might contain the true value of an unknown population parameter. The interval has an associated confidence level that, loosely speaking, quantifies the level of confidence that the parameter lies in the interval. The chi-square test was used for comparison of genotype frequencies in the studied groups; A chi-square statistic is one way to show a relationship between two categorical variables. In statistics, there are two types of variables: numerical (countable) variables and non-numerical (categorical) variables. The chi-squared statistic is a single number that tells you how much difference exists between your observed counts and the counts you would expect if there were no relationship at all in the population. Fisher's exact test is a statistical significance test used in the analysis of contingency tables. Although in practice it is employed when sample sizes are small, it is valid for all sample sizes. Fisher's exact test was used for comparison of allelic frequencies in this study.

Results

The Figure 1 shows that both the cases and the control groups were more or less similar with reference to distribution of the study population, according to age most of the patients both in control and study group were in the age group of 51-70 yrs (58% of cases, 60% of controls). The mean age in diabetic nephropathy cases was 60.54±11.02 years while the mean age in controls was 57.75±7.73 years.

Figure 2 shows that out of total 50 cases, 22 (56%) were females and 28 (44%) were males. Male and female ratio in diabetic nephropathy patients was (1:1.27).

The genotype distribution and allele frequencies of the polymorphic site rs2237897 were studied in the groups. The genotype frequencies of rs2237897 for CC, CT and TT are 0.77, 0.20 and 0.03 for cases and 0.55, 0.4, and 0.05 for controls, respectively (Table 1, Figure 3).

The allelic frequencies of rs2237897 for C and T in two different groups were 75% and 25%, respectively in controls, and 87% and 13%, respectively in Diabetic nephropathy subjects, with the C allele being more frequent in Diabetic nephropathy patients than in the control population (Table 2, Figure 4).

The highly significant association in the frequency of the C allele between cases and control subjects, giving an odds ratio of 2.4, (CI 95%, 1.1 to 5.4) was found in the Diabetic nephropathy group. Chi-square analysis showed a significant difference in genotype frequency of rs2237897 ($\chi 2 = 4.67$, p=0.03) in Diabetic nephropathy subjects, compared with that of controls (Table 1, Figure 3).

Table 1: Genotypic frequency of rs2237897 Polymorphism in diabetic nephropathy patients

	N	CC	CT	TT
Cases	39	30 (0.77)	8 (0.20)	1* (0.03)
Control	20	11 (0.55)	8 (0.4)	1* (0.05)

Chi-square = 4.67 with 2 degrees of freedom; P = 0.03

Table 2: Allelic frequency of rs2237897 Polymorphism in Diabetic nephropathy patients

	N	2N	C	T
Cases	39	78	68 (0.87)	10 (0.13)
Control	20	40	30 (0.75)	10 (0.25)

Chi-square = 4.68 with 1 degree of freedom; P = 0.03

A statistically significant difference was also found when the allelic frequencies between the two groups were compared (Chi-square = 4.68, p=0.03) with the C allele having a 2.4 fold higher risk of having Diabetic Nephropathy (risk ratio, RR) = 1.4, 95% CI of RR = 1.1 to 1.9, Odds Ratio (OR) = 2.4; 95% CI of OR = 1.1 to 5.4) (Table 2, Figure 4).

Discussion

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM are caused by a complex interaction of genetics and environmental factors. A large proportion of people with diabetes will develop microvascular complications. Diabetic nephropathy is a chronic disorder typically characterized by progressive albuminuria and a decline in renal function.

Research has focused to find out potential genetic alterations associated with Chronic Kidney Disease and ESRD in type 2 diabetic patients. In fact, genetic evidence has been found in case-control association and linkage studies, and more recently using genome-wide scan (GWS). These studies support the assumption that onset, progression, and severity of DN can be in part attributed to genetic factors.[6]

To identify the role of variants in candidate genes for contribution to diabetic nephropathy risk, usually compares the frequency of the variant between cases and controls, i.e. Whether the variant is associated with the disease. Association studies offer a potentially powerful approach to identifying genetic variants that influence susceptibility to disease.

The KCNQ1 gene is associated with type 2 diabetes and the expression of *KCNQ1* could be observed in the human kidney. In the kidney, KCNQ1 has been shown to assemble with KCNE1, the β subunit of the potassium channel, forming a potassium channel complex localized to the brush border of the mid to late proximal tubule,[12,13] moreover, it has been shown to play a role in the Na^+ secretion at the proximal tubule by maintaining a driving force for Na^+ transport across the membrane.[13]

These observations suggest the possibility that variants in *the KCNQ1 gene* may be a candidate for conferring susceptibility to diabetic nephropathy. To test this hypothesis, we focused on *KCNQ1* as a candidate gene for diabetic nephropathy and investigating the association between the single nucleotide polymorphisms (SNPs) within *KCNQ1* and diabetic nephropathy in type 2 diabetes.

The genotype distribution and allele frequencies of the polymorphic site in the groups studied are shown in Tables 1and2. The genotype frequency of rs2237897 for CC, CT and TT are 0.77, 0.20 and 0.03 for cases and 0.55, 0.4, and 0. 05 for controls, respectively (Table 1, Figure 3).

The highly significant association in the frequency of the C allele between cases and control subjects, giving an odds ratio of 2.4, (CI 95%, 1.1 to 5.4) was found in the Diabetic nephropathy group (Table 2).

Comparison of genotype frequencies showed a statistically significant difference between the studied groups (p=0.03).

In a similar study done by Ohshige *et al*[7] (2010) in Japan, genotyped 33 SNPs in *KCNQ1* using 754 type 2 diabetic patients with overt nephropathy and 558 control subjects (an initial study),

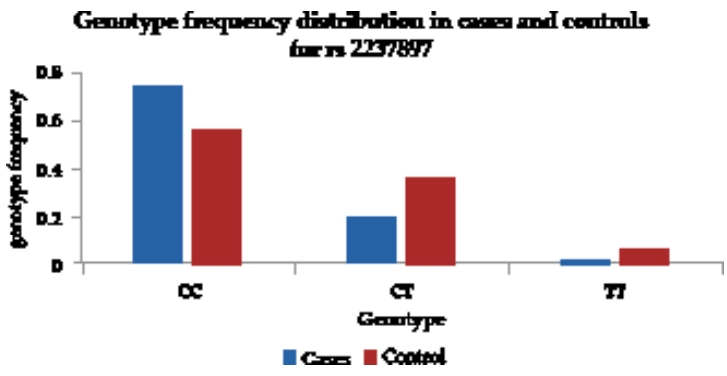

Fig. 3: Genotype distribution of rs2237897

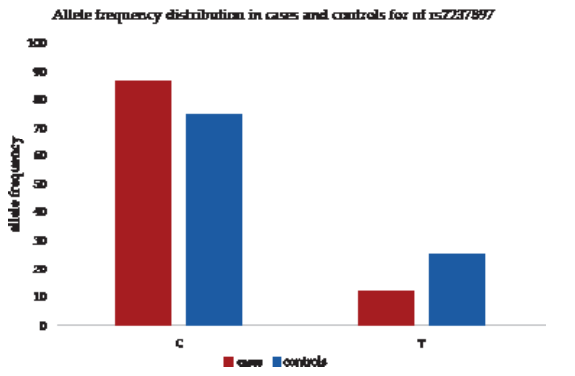

Fig. 4: Allelic distribution of rs2237897

and we further examined the association of a candidate SNP using three other independent Japanese populations and found that Combined analysis by a meta- analysis revealed that the T allele of rs2237897 was significantly associated with susceptibility to diabetic nephropathy in Japanese subjects with type 2 diabetes (odds ratio 1.22 [95% CI 1.10-1.34], P = 3.1 x 10 (-4), corrected P = 0.01).

A study done by X.L. Lim *et al*,[8] 2012 included a total of 752 Chinese patients with type 2 diabetes. Albuminuria was determined by ACR using spot urine samples, and renal function was approximated using estimated GFR. Genotyping was performed using invader and Taqman assays as appropriate. Multivariate regression analyses were used to analyze the associations between SNPs and renal traits.

Significant associations were detected between rs2283228 and macroalbuminuria ($p < 0.001$, corrected $p < 0.01$), as well as log (e) ACR ($p = 0.004$, corrected $p = 0.036$) after multiple hypothesis testing and adjustment for potential confounding. A trend of increasing OR was observed with increasing severity of diabetic nephropathy (low and high microalbuminuria, macroalbuminuria). rs2237897, previously implicated in the earlier Japanese study, was also associated with macroalbuminuria, but this finding did not remain significant after correction for multiple testing. Meta-analyses of the Chinese and Japanese studies revealed both SNPs to be significantly associated with microalbuminuria.

In our study done in India, we also found that the frequencies of rs2237897 were consistently higher in the nephropathy groups than in the control groups and the frequency of C allele is higher, so in our study C allele was the risk allele for diabetic nephropathy, which is different from the T allele of Japanese study. This difference will be most probably due to different ethnicity, race and genetic composition of different geographical area.

Conclusions

Together with the previous Japanese study and East Asians study, our findings also support the hypothesis that, in addition to *KCNQ1* being an established type 2 diabetes gene, genetic variation in this gene may contribute to susceptibility to diabetic nephropathy and *KCNQ1* may be a good candidate marker for diabetic nephropathy in the future, but this is a small study so larger studies will be required to establish as a marker of DN.

Competing Interests

The authors of this study have no personal or financial conflicting interests that bias the work of this study.

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