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NOS3 gene Glu298Asp polymorphism and severity of disease in patients of ADPKD from North India

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ABSTRACT

Introduction: Genetic modifying factors play a significant role in disease progression in autosomal dominant polycystic kidney disease (ADPKD). Identification of these factors could inform about targets for intervention. The present study investigates the role of Glu298Asp (c.894G > T) polymorphism of NOS3 gene in the progression of disease in ADPKD patients from North India, and compares with the results in studies among other ethnic groups.
Methods: Genotyping for c.894G > T polymorphism was carried out in 123 patients of ADPKD and 100 healthy controls using polymerase chain reaction method. The allelic and genotypic frequencies of patients of ADPKD and healthy controls were analyzed using Chi-Square test. The mean age at onset of ESRD in the patients with PKD1 gene mutations, and in those with hypertension was correlated with G and T alleles of this polymorphism using Student's *t*-test.
Results: Distribution of GG, GT and TT genotypes were 0.73, 0.25 and 0.02 in patients of ADPKD and 0.67, 0.31 and 0.02 in healthy controls, respectively. The differences in the genotype frequencies between patients and controls were not significant ($p = 0.604$). The frequency of mutant allele T of c.894G > T polymorphism did not vary significantly among patients of ADPKD with ESRD and those without ESRD ($p = 0.112$). This polymorphism was also not associated with hypertension ($p = 0.681$) and severity of disease in PKD1-linked patients ($p = 0.582$).
Conclusions: The GT/TT genotype of c.894G > T (Glu298Asp) polymorphism did not have a significant role in modifying the progression of the disease in patients of ADPKD, or those with mutations in PKD1 gene from North India.

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1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited disorders, with a prevalence of 1/500–1/1000 world-wide. About 85% of patients have mutations in PKD1 gene and the rest 15% in PKD2 gene (Rossetti and Harris, 2007). The disorder is characterized by development of multiple, non-uniform cysts in the cortex and medulla of both kidneys. These cysts grow slowly and eventually lead to renal failure. About 50% of the affected patients reach end-stage renal disease (ESRD) by the age of 60 years (Rossetti and Harris, 2007). The presentation and progression of renal phenotype is highly variable in patients of ADPKD, ranging from in-utero presentation of enlarged echogenic polycystic kidneys to adequate renal function till seventh decade (Stefanakis et al., 2008). About 60% patients of ADPKD have hypertension before renal function declines significantly (Ta et al., 2013). This variability in the renal phenotype is observed among families and within members of the same family (Igarashi, 2002; Milutinovic et al., 1992). It may be due to genetic and allelic heterogeneity, genetic modifiers or environmental factors (Fick et al., 1993; Rossetti and Harris, 2007). Identification of these factors could inform about targets for intervention to delay progress of the disease. Several studies have investigated the effect of polymorphisms of genes such as NOS3, BDKRB1/2, ACE, PKD1, PKD2, DKK3 as potential modulators of progression to ESRD (Devuyst, 2003; Liu et al., 2010; Stefanakis et al., 2008; Tazón-Vega et al., 2007); Stefanakis et al., 2008; Liu et al., 2010. The most studied polymorphism among these is the NOS3 gene, which is located on chromosome 7q35–36, and codes for endothelial nitric oxide synthase. It converts amino acid L.-arginine, in the presence of molecular oxygen, into L-citrulline and nitric oxide. The polymorphism in NOS3 gene results in reduction of nitric oxide, leading to renal impairment. Several different polymorphisms of NOS3 gene [−786T > C (rs2070744), Glu298Asp (rs1799983), IVS4 VNTR 4b/4a] have been studied in patients with ADPKD (Ramanathan et al., 2014), as well as in patients with hypertension (Miyamoto et al., 1998) and coronary

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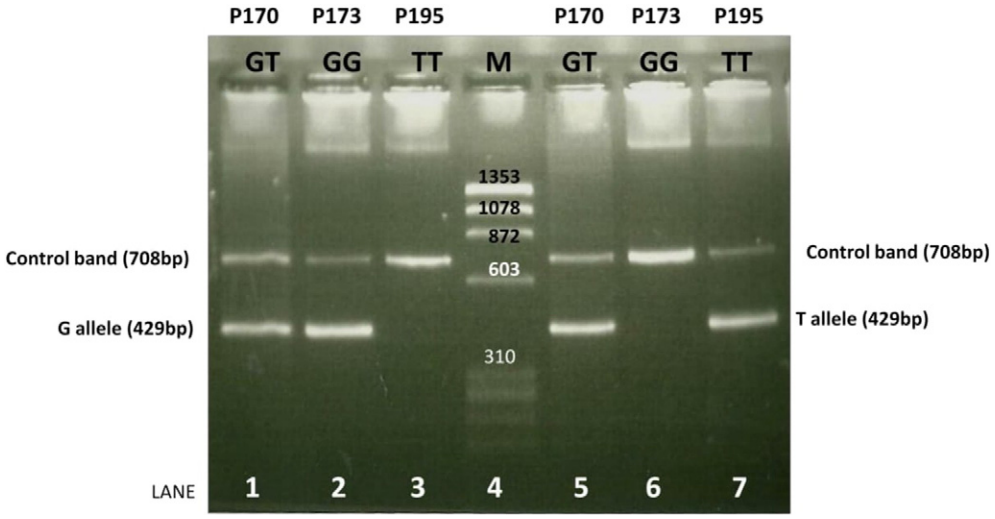

Fig. 1. Lane no 1–3: ARMS PCR for c.894G allele, Lane 4: Marker Ø174/Haem III, Lane nos. 5–7: ARMS PCR for c.894 T allele. Lane 1 & 5: P170-Heterozygous GT; Lane 2 & 6: P173-Homozygous GG and Lane 3 & 7: P195-Homozygous TT. Control band 708 bp and allele specific band 429 bp.

artery disease (Rai et al., 2014). The Glu298Asp polymorphism in NOS3 gene is a commonly studied polymorphism that changes guanine to thymine (c.894G > T) (Joshi et al., 2007). Previous studies have reported that the variant T allele (Asp residue at codon 298) has an increased susceptibility to proteolytic cleavage (Tesauro et al., 2000) and hence, results in reduced enzyme activity (Veldman et al., 2002) and reduced NO production (Wang et al., 2000). This results in renal impairment. A significant association between NOS3 gene polymorphisms and progression to ESRD in patients of ADPKD has been shown in some ethnic groups (Devuyst, 2003; Persu et al., 2002; Stefanakis et al., 2008), while in others no association has been observed (Jung Geon Lee et al., 2002; Walker et al., 2003; Tazón-Vega et al., 2007; Dasar et al., 2012; Ramanathan et al., 2014). Mutations in PKD1 gene and hypertension also act as risk factors for progression of renal disease in patients of ADPKD (Harris and Torres, 2009). Therefore, we investigated the role of (c.894G > T) polymorphism in NOS3 gene in north Indian patients of ADPKD, in modifying the onset of ESRD and as well as with severity of disease in patients linked to PKD1 gene. We also compared the results with studies done in other ethnic groups.

2. Subjects and methods

2.1. Study population

This study consisted of 123 unrelated patients of ADPKD and 100 healthy, unrelated control subjects (without any disease, including renal, enrolled from among those visiting the hospital). The patients of ADPKD were enrolled from Nephrology department of Sir Ganga Ram Hospital, New Delhi and patients referred by nephrologists from neighboring hospitals. The diagnosis was established on the basis of Ravine's diagnostic criteria (Pei et al., 2009; Ravine et al., 1994). The study was approved by the Institution Ethics Committee of Sir Ganga Ram Hospital vide no. EC/10/10/199(A) and informed consent was obtained from all participants.

The mean age of patients in the ADPKD group was 41.54 ± 12.9 years (M:F = 70:53), and the control group was 38.88 ± 7.2 years (M:F = 41:59) (p = 0.067). Mutations in PKD1 gene were detected in 83 (67.5%) of 123 patients that were analyzed for mutations. End stage renal disease (ESRD) was diagnosed when dialysis was initiated. Hypertension was defined as patients with blood pressure ≥ 140/90 mm Hg or those receiving anti-hypertensive therapy (VanDeVoorde and Mitsnefes, 2011).

2.2. Analysis of the ENOS Glu298Asp (c.894G > T) polymorphism

Genomic DNA was extracted from peripheral blood leukocytes by a modified salting out method (Miller et al., 1988). Genotyping of the (c.894G > T) was carried out using the amplification refractory mutation-specific polymerase chain reaction (ARMS-PCR). Following primers were designed using Primer3 online software: Forward-5′-AGGAAACCAGTGGGAGAAGG-3′, and Reverse-5′-GGA TGGAGTGAGAGCCAGAC-3′ (for the control band) and allele-specific primers 5′-GCAGGCCCCAGATGAG-3′ and 5′- TGCAGGCCCCAG ATGAT-3′ for G and T alleles, respectively. DNA was amplified for 30 cycles, each cycle comprising initial denaturation at 95 °C for 35 s, annealing at 58 °C for 40 s, extension at 72 °C for 45 s, followed by a final extension cycle of 7 min at 72 °C. PCR amplification produced a control band of 708 bp and allele specific bands of 429 bp for both G and T alleles. PCR products were separated by electrophoresis on 2% agarose gel, and fragments were stained with ethidium bromide and visualized by ultraviolet transillumination (Fig. 1).

2.3. Analysis of PKD1 and PKD2 genes

Mutations in PKD1 and PKD2 genes were identified by direct Sanger sequencing of DNA of the patients, as per the method of Rossetti et al. (Rossetti et al., 2002).

2.4. Statistical analysis

To test for independent relationships between categorical variables, such as genotype distribution and allelic frequencies, Chi-square (χ^2) test was performed. Odds ratio (OR) was used as a measure of the strength of association between genotypes and allele frequency in

Table 1
Genotype and allelic distribution in patients and controls.

Genotype	Patients (n = 123)	Controls (n = 100)	p-Value
GG	90 (73%)	67 (67%)	
GT	31 (25%)	31 (31%)	0.604
TT	2 (2%)	2 (2%)	
G	211(86%)	165 (82%)	
T	35 (14%)	35 (18%)	0.346

Data expressed as number (%). Determined by χ^2 analysis.

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