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Original Article

β-T594M epithelial sodium channel gene polymorphism and essential hypertension in individuals of Indo-Aryan ancestry in Northern India

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ABSTRACT

Background: The T594M variant of the β-subunit of the sodium epithelial channel (*ENaC*) gene may contribute to hypertension in individuals of Indo-Aryan origin.
Methods: Present study was performed to assess the role of the *ENaC* gene variant as an independent risk factor for hypertension in subjects of Indo-Aryan ancestry. A total of 150 patients of recently detected essential hypertension and 150 matched controls were genotyped for the T594M polymorphism of the *ENaC* gene by PCR–RFLP method.
Results: β-T594M mutation was found to be non-polymorphic. There was major genotype call in both the groups i.e. cases and controls. Other phenotypic parameters like age, sex and body mass index were also similar among hypertensive patients and controls ($P > 0.05$). Hypertensive patients had significantly higher total cholesterol and triglycerides compared with controls ($P < 0.0001$).
Conclusion: These results do not suggest an important role for the T594M variant of the *ENaC* gene contributing to either the development or severity of hypertension in subjects of Indo-Aryan ancestry.

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1. Introduction

Essential hypertension (EH) is a multifactorial trait with about 30%–60% of the phenotypic variation being attributed to genetic factors.[1] Some genetic causes of human hypertension involve increased renal tubular sodium absorption, either indirectly through excess mineralocorticoid activity or directly as in Liddle's syndrome.[2] This syndrome is caused by mutations of subunits of the epithelial sodium channel (ENaC) that result in increased sodium-channel activity in the distal renal tubule with excess sodium reabsorption leading to high blood pressure and the characteristic suppression of the renin–angiotensin system. The clinical features of Liddle's syndrome overlap with those of some patients with EH. Genes involved in monogenic-mendelian form of hypertension are much easier to map than those involved in multifactorial human hypertension. Despite the rarity of this syndrome, the identification of the corresponding genes may help to clarify the genetics of EH for two reasons: "milder" variants in these same genes may be relatively frequent in the general population and contribute to common EH; and similar physiologic pathways may be relevant to both rare and common forms of hypertension. Gain of function mutations in the β or γ subunit of the *ENaC* gene result in an increase in sodium-channel activity like Liddle's syndrome, mutations which are identified in patients with EH as well.[2–4] Therefore, it is possible that sodium-channel mutations in patients with EH could contribute to the rise in blood pressure by increasing renal tubular sodium reabsorption. The first molecular variant to show an association with hypertension was T594M (rs1799979) in the C-terminus of β-ENaC. Among the polymorphisms identified in the β subunit of ENaC, β-T594M and β-G442V are seen in individuals of African origin.[5,6] T594M is a missense, C/T mutation leading to substitution of threonine by methionine. The physiological significance of the T594M polymorphisms could partly explain the high incidence of salt-sensitive hypertension in African Americans.[5–7] Based on these informations we hypothesized that β-T594M polymorphism of epithelial sodium channel gene, which has a role in sodium balance, may be associated with EH in north Indian patients. To address this question, we screened β-T594M polymorphism of epithelial sodium channel gene in a case–control design and looked for its association with hypertension. This study will help us in understanding the role of b-T594M polymorphism of epithelial sodium channel gene in the pathophysiology of EH in North Indian population, and hence will provide us with better leads in extent of involvement of salt sensitivity in this polulation.

2. Materials and methods

2.1. Study subjects and clinical evaluation

Since the present study was a pilot study to investigate the mentioned polymorphism, age, gender and ethnicity matched hypertensive and control subject (case-control design) of Indo-Aryan ancestry from North India were selected. The study was approved by human ethical committee; written informed consent was obtained from each participant. Patients and controls were recruited from hypertension and general outpatient department, GB Pant Hospital, respectively and some controls were also recruited from blood bank, GB Pant Hospital. The patient's inclusion criteria included: age 25–60 years, diastolic blood pressure (DBP) $\geq$ 90 mm Hg and/or systolic blood pressure (SBP) $\geq$ 140 mm Hg. The blood pressure was measured thrice after every 5 min of rest in the seated position by the same cardiologist. Average of three BP readings was taken. All the patients included were recently diagnosed (<1 month) with EH. The subject with EH was diagnosed as per JNC VII criteria.[8] Patients with target organ damage, secondary form of hypertension, diabetes mellitus, previous or present history suggestive of coronary artery disease, women receiving oral contraceptives or hormone replacement therapy, pregnant women or subjects who weighed greater than 25% of ideal body weight were excluded from the study. Controls recruitment criteria included: age 25–60 years, SBP < 120 mm Hg and DBP < 80 mm Hg, absence of family history of hypertension and any disease medication. Participants who did not give consent or complied with the study protocol were excluded from the study. The sample size comprised of 150 patients and 150 controls.

Family history of hypertension was defined as having father, mother, or siblings with a history of EH. The study was approved by human ethical committee; detailed questionnaire including written informed consent and demographic characteristics were obtained from each participants.

2.2. Biochemical investigations

Plasma samples were used to determine fasting glucose, lipid profile, serum uric acid, serum sodium, potassium, serum creatinine and urinary protein.

2.3. Genetic analysis

Genetic analysis was carried out in CSIR-Institute of Genomics and Integrative Biology, Delhi. DNA was extracted from 10 ml peripheral blood taken from each participant. The modified salting out method was used to extract DNA. Amplification and genotyping of the candidate gene locus was performed by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) method. The rigorous process for initial standardization was done using gradient thermocycler with different temperature and extension periods. For each individual SNP, one pair of sense and antisense primer was designed using DNA STAR software. The concentration of primers, Mg^{2+}, genomic DNA, Taq polymerase and dinucleotides was also varied according to the need. The nonspecific amplification was further reduced by hot start, step down PCR methods. Each step was followed by the proper amplification check on either agarose gel or on a native Polyacrylamide Gel Electrophoresis (PAGE). The exact size was confirmed on a gene scan using ABI prism 377 Genotyper sequencer. The PCR product was further digested by *Nla*III (New England Biolabs) for 2 h and the products were analyzed on a 1.5% agarose gel. The single nucleotide sequence substitution of T for C in the β-T594M variant creates a unique *Nla*III restriction enzyme site. In case of homozygote wild type