ORIGINAL ARTICLE

T594M mutation of the epithelial sodium channel beta subunit: No association with essential hypertension in Sudanese hypertensive

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ABSTRACT

Background Hypertension is a multi-factorial disease, results from interplay between genetic and environmental factors. The epithelial sodium channel gene (SCNN1β) was known to regulate sodium absorption in the distal renal tubule. The T594M mutation of this gene results in an excess sodium reabsorption leading to high blood pressure. This study aimed to demonstrate a possible association between T594M C/T mutation of the SCNN1β (rs1799979) with essential hypertension in our population.

Method 179 hypertensive and 100 normotensives were enrolled in this study. T594M mutation was detected by RFLP-PCR using NlaIII restriction enzyme.

Results T594M mutation was found to be non-polymorphic.; both hypertensive and normotensives have the CC wild-type pattern and only one hypertensive carries the mutant heterozygote CT. A significant gender difference was observed with three folds of females increased risk (OR=2.79); 92% of the cases were above 40 years old, with significant difference when compared to the controls (p = 0.01); 41.3% and 26% of the hypertensives developed diabetes and hyperlipidaemia, respectively.

Conclusion This study did not show any association between the T594M mutation of the SCNN1β gene with essential hypertension in the examined subjects, but also showed the rarity of the T594M mutation in our population.

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INTRODUCTION

Hypertension is one of the primary risk factors for cardiovascular diseases and the leading causes of death worldwide¹. The incidence of hypertension continues to accelerate globally. It is known to affect over a billion people worldwide, causing disability and death to over 10 million all over the globe²,³. With the adoption of new westernized lifestyle, coupled with an inadequate and/or insufficient disease control; the developing countries, including Sudan, have received the highest burden of hypertension⁴. Hypertension is a multifactorial disease. It occurs as a result of a complex interplay of environmental risk factors with multiple genetic factors. Although the non-genetic, behavioral risks are important risk factors for hypertension, the genetic factor is the most important one; it gives an insight to the mechanism of the disease and paves the road for better control, prevention and treatment⁵. The previous linkage or association studies have documented the role of certain candidate genes with hypertension predisposition. Of these genes are the renin angiotensin aldosterone system, NEDD protein and epithelial sodium channel⁶,⁷.

Resistant hypertension is a global issue with only 33% of hypertensive adults had controlled blood
pressure. The resistance is due to medication non adherence, medication cost and patient genotype. Africans were found to excrete less sodium than their white counterparts; this is explained by the differences of their genetic components. This phenomenon is known as salt sensitive hypertension which is a common phenotype in black people. It is explained by sodium reabsorption in the kidney; thereby several previous studies have focused on the role of the kidneys in hypertension. The epithelial sodium channel was known to play critical role in this phenotype as it has very important role in maintaining sodium balance. Moreover, drug target for reducing blood pressure among hypertensive; gave it the name amiloride sensitive sodium channel. Four subunits; α, β, γ, and δ of epithelial sodium channel were previously discovered. Several mutations in channel subunits were associated with an inherited form of hypertension. Of these mutations is the T594M (C/T) missense single nucleotide mutation in β subunit of the last exon (exon 13 of the sodium-channel of Chromosome 16); which leads to the substitution of threonine [ACG] by methionine [ATG]) at amino acid 594, causes the synthesis of a protein with an altered amino acid sequence. The subunit of the sodium-channel is a potential target site for phosphorylation by protein kinase C (PKC) which down-regulates sodium channels. The T594M variant was known to increase sodium channel activity in the distal renal tubule by making the channels to become insensitive to negative regulation by PKC, which leads to an excess tubular sodium reabsorption leading to high blood pressure. The β-T594M mutation of the sodium channel was found in people with hypertension of African descent, and it is suspected to play role in salt sensitivity phenotype, however, the results were partly conflicting.

In Sudan, to the best of our knowledge, there was only one genetic study of hypertension targeting the association of Nitric Oxide Synthase 3 with hypertension. Given the fact that hypertension is a polygenic disease; this reflects an increasing need to understand the underlying genetic mechanisms of the disease. Therefore, this study aimed to demonstrate a possible association of the T594M of the sodium channel (rs1799979) with essential hypertension among the genetically diverse Sudanese population.

**METHODS**

This is a cross sectional hospital based study, conducted to assess the prevalence of the genetic variant of sodium channel β subunit (T594M mutation) (rs1799979), and to determine a possible co-occurrence of this mutation with essential hypertension in Sudanese population. Patients with essential hypertension and normotensive of ages ranging from 20-80 years are included. The newly diagnosed patients and the normotensive controls have been confirmed with or without hypertension by an expert physician; according to the criteria of the International Society of Hypertension [systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg]. Patients with hypertension secondary to other diseases such as diabetes were excluded.

A total of 179 of the confirmed hypertensive patients and 100 normotensive control samples were included in this study. Verbal consent was obtained from each participant prior to enrollment and after explaining the purpose of the study. The demographic data was obtained using a well-designed questionnaire. 3 ml of venous blood sample from both cases and controls were collected in EDTA container, blood was centrifuged and plasma aspirated into another container.

**DNA extraction**

Genomic DNA was extracted using guanidine chloride extraction method and the extracted DNA was stored at -20° C until used. Nanodrop photometry was used to test the quantity and the quality of the extracted DNA. Amplification of the sequence containing the T594M mutation of the sodium channel was done using the primer sequences as follow: Sense primer 5’-TCGACTTTGTGTGGATCACC -3’. Antisense primer 5’- GCATCACCCCTACTGTCAGA -3’.
The PCR was conducted using master mix ready to use from iNtRON Biotechnology Company, Inc. following the manufacture instructions with final volume 20 µl. PCR programmed to amplify the target sequence as follow: initial denaturation at 95°C for 2 min. 35 cycles of denaturation at 94°C for 30 sec, followed by annealing at 59°C for 30 sec, then extension at 72°C for 30 sec. Final extension at 72 for 5 min, followed by storing temperature at 4°C for 5 min.

**Restriction fragment length polymorphism**

The Biolab. Digest® Nla III restriction enzyme recognizes the 5’…..CATG…..3’

Three microlitres of the PCR product was added to 2U of NlaIII restriction enzyme and one µl of digestion buffer and the volume was completed to 20 µl, incubated for overnight at 37°C. The mixture was then loaded into 2% agarose gel, electrophoresed and the result visualized in a gel documentation system. The genotypes were scored as follows: The presence of one band sized 283 base pair (bp) indicates a homozygous wild type, three bands (283-149-134) in case of heterozygous, and two bands (149,134) in case of homozygous mutant.

Statistical analysis was done using the statistical package for social sciences (SPSS) software version 21. P-value < 0.05 was taken as significant. Odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of the association.

**RESULTS**

The study included 179 hypertensive patients and 100 normotensive controls. The age of hypertensive patients ranged from 20 to 80 years, and that of controls ranged from 20 to 90 years; 92% of the cases were aged more than 40 years with significant difference when compared to the controls (p = 0.01), this age group has a 2.5-fold increased risk of hypertension. A significant gender difference was observed with three-fold increase of females (P=0.001, OR=2.79). Large scale of the hypertensives within less than ten years of disease duration, they developed diabetes (41.3%) and hyperlipidaemia (26.2%), with less frequency of stroke (5.1%) and kidney disease (3.9%) (Table 1).

The genotypes of the T594M mutation of the sodium channel was shown in Figures 2 and 3. The CC wild-type genotype was frequently observed among both cases and controls (99.4% and 100%, respectively). The CT genotype was observed in only one hypertensive patient (Table 2). The difference between the two groups was not statistically significant.

### Table 1. The demographic data of the hypertensive cases (n=179) compared to the normotensives (n=100)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Case N (%)</th>
<th>Control N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>14 (7.8)</td>
<td>18 (18)</td>
<td>P=0.01</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>165 (92.2)</td>
<td>82 (82)</td>
<td>OR=2.58</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>53 (29.6)</td>
<td>54 (54)</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Female</td>
<td>126 (70.4)</td>
<td>46 (46)</td>
<td>OR=2.79</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>74 (41.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>105 (58.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9 (5.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>169 (94.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (3.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>172 (96.1)</td>
<td></td>
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</tr>
</tbody>
</table>
DISCUSSION

Hypertension “the silent killer” is a public health problem worldwide as it raises the risk of heart disease and can shorten people’s life expectancy. The magnitude of hypertension continues to accelerate globally. In Sudan, the prevalence of hypertension in Sudanese population rose from 7.5% in 1985 to 18.2% in 2002. The complexity of the disease that involves an interaction of diverse environmental conditions with multiple genetic factors; has encouraged the research to identify the underlying genetic mechanisms of the disease. Based on the fact that T594M sodium channel β-
subunit missense mutation may increase sodium-channel activity and could raise blood pressure in affected people by increasing renal tubular sodium reabsorption, this study was undertaken to determine the frequency of carriers of this mutation and to examine whether any relationship exists between this mutation and the susceptibility to hypertension.

The result revealed that T594M mutation of the \textit{SCNN1\beta} (rs1799979) was found to be non-polymorphic. Only one of the 179 examined hypertensive has a mutation whereas none of the controls had the T594M mutation. Therefore, the finding of one case harboring one copy of the mutant allele of the T594M mutation did not give good insight about the association of mutation and hypertension in Sudanese, which is in agreement with study conducted in black hypertensive from South African, in Indian ethnic group and two studies in Japanese population\textsuperscript{24-27}. On the other hand, this result is inconsistent with the finding of previous studies conducted in black people of African origin that suggest a possible role of the T594M variant to the susceptibility to high blood in African population\textsuperscript{28,29}.

Despite the causal effect of this mutation as it changes the amino acid leading to excess tubular sodium reabsorption; however, the rarity and the lack of the association with hypertension in the examined subjects could be explained by:

1. The sodium channel might be affected by additional mutation/s within the \textit{SCNN1\beta} gene which works in an integrated pattern to cause the disease.

2. The heterogeneity of the enrolled subjects, who are belonging to diverse ethnic groups, necessitates a large sample size to obtain a sufficient statistical power to detect genetic risk factors.

CONCLUSIONS AND RECOMMENDATIONS

- The T594M sodium channel \(\beta\)-subunit variant does not appear to play any significant contributory role in the susceptibility to hypertension in the examined subjects.

- Hypertension is a polygenic disease with more than a single gene act in synergism to contribute to the disease warranted further investigation which will help develop patient-specific treatment.

- Because of the heterogeneity of the examined subjects, this data could be considered as a pilot study that encourage us to focus later on a homogeneous ethnic group that has a relatively uniform genetic background or less complex, and expectedly sharing environmental and behavioral risks, thereby small sample size enabling to obtain a sufficient statistical power for the detection of genetic risks.

Acknowledgements

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REFERENCES


4. Bushara SO, Noor SK, Elmadhoun WM, Sulaiman AA, Ahmed MH. Undiagnosed hypertension in a rural community in Sudan and association with some features of the metabolic


