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Original Research Article

Human AGT mutations in Pre-Eclamptic Women in Calabar, Nigeria

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Abstract

Preeclampsia is a multifactorial disorder that is influenced by many factors that include genes, race and parity. The Renin angiotensin aldosterone system (RAAS) is vital in the regulation of blood pressure and has been implicated in the pathophysiology of preeclampsia among certain populations but such studies have been not reported in Nigeria. Angiotensinogen is the precursor molecule and a very important component of the RAAS. This pilot study investigated mutations in exon 2 of the angiotensinogen gene in preeclamptic women in Calabar, Nigeria. 19 preeclamptic and 20 normotensive pregnant women were recruited into the study from the University of Calabar Teaching Hospital, Calabar. Ethical approval and informed consent was obtained from the Ethics research committee and the women. Clinical variables were obtained and analyzed using a two tailed independent sample t-test, 3mls of blood was also collected from all the women. DNA was extracted, PCRs performed and the products were sequenced. Multiple sequence alignment was performed for all the sequenced products. This study observed a deletion of guanine at position 26 in all the 19 preeclamptic women and 5 tranversion mutations 4T>G, 911C>G, 1C>A, 795G>T, 912C>A in 17(89.5), 16(84.2), 4(21.1), 1(5.3) and 1(5.3) preeclamptic women respectively but not in the normotensive women. These results will serve as baseline information for subsequent molecular studies into the pathogenesis of preeclampsia among women in Calabar.

Keywords: Angiotensinogen, Preeclamptic, Normotensive, women, Mutations, Calabar.

INTRODUCTION

Hypertensive disorders of pregnancy are important causes of severe morbidity, long term disability and death among both mothers and their babies. In Africa and Asia, nearly one tenth of all maternal deaths are associated with hypertensive disorders of pregnancy and among this disorders, preeclampsia is a major contributor to maternal, perinatal mortality and morbidity complicating 2% -8% of pregnancies [1]. Preeclampsia is a disorder of pregnancy characterized by the onset of high blood pressure (systolic blood pressure higher than 140mmHg and/or diastolic blood pressure higher than 90mmHg) and often a significant amount of protein in the urine usually after 20 weeks of pregnancy [2]. Preeclampsia is associated with long term outcomes for both the mother and the baby, if left untreated may result in seizures at which point it is known as eclampsia [3, 4]. Preeclampsia is a multifactorial disorder with familial tendency which has both strong genetic and environmental component. It is influenced by race, parity, health status of the placenta, diet, body size, obesity, history of hypertension, an increase in age and diabetes mellitus amongst others [5, 3]. Many genes have been investigated in association with preeclampsia, these genes include angiotensinogen (AGT), STOX1, STOX2, syncytin envelope gene, MTHER(677T), AVCR2A gene, GO210A mutation of factor II (prothrombin gene), the VEGF TT-460 SNP genotype, ACE 11D polymorphism, BCLL polymorphism of the GR gene and polymorphism of EPHX gene [6-11]. However no such studies on preeclampsia have been documented in Calabar. Thus, this study seeks to investigate mutations of the human AGT gene and its involvement in the pathogenesis of the preeclampsia in Calabar women, Nigeria.

METHODOLOGY

Ethical approval was obtained in accordance with the Helskini declaration. Full approval was granted for the research from the Health Research Ethics Committee (HREC), University of Calabar Teaching Hospital (UCTH), Calabar. The Research protocols of the Ethics Committee were strictly adhered to. A total of thirty nine (39) participants were recruited into the research, comprising nineteen (19) preeclampsia patients and twenty (20) normotensive volunteer pregnant women as controls. All the women were

attending ante natal care at the Department of Obstetrics and Gynaecology, University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State. About 2mls of blood from patients and controls were collected into EDTA bottles and stored in deep freezer (20^0C) in the Obstetrics and Gynaecology Department for onward transportation to the Biotechnology Laboratory Unit of Animal Science and Genetics Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Genomic DNA was extracted from whole blood using Quick-gDNA^{TM}MiniPrep kit (Zymo Research, USA), PCR amplification, and purification of amplicons were carried out at Biotechnology Laboratory Unit of Animal Science and Genetics Department, Federal University of Agriculture, Abeokuta,Ogun State, Nigeria. The sequencing of amplicons was carried in STABVIDA Laboratory, Quinta de torre, Portugal. Age, weight, height, body mass index (BMI), Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) readings were measured by the nurses at the clinic, urine samples were also collected from each pregnant women and sent to the laboratory for proteinuria analysis. These results were collated for all participants.

A sets of primers was selected to amplify the AGT gene on exon 2. The first forward was 5^1GGTGACAGGGAATATTGAG 3^1 and the reverse primer was 5^1 CTAAGTCCTAGGGCCAGAGC 3^1. These primers were designed at STABVIDA Laboratory, Quinta de torre, Portugal. The PCR was performed for 35 cycles. The temperature for the initial denaturation of DNA was 95^{o}C for one minute, annealing at 71^{o}C for one minute and extension at 72^{o}C for another one minute. The final extension at 72^{o}C for 7 minutes. After amplification, the products were sequenced. Continuous variables were compared between the preeclamptic group and the normotensive group using a two tailed independent *t*-test. The nucleotide sequence of exon 2 of the AGT gene were decoded from the chromatogram using ChromasPro software (www.technelysium.com.au).Multiple alignment was carried out using Clustal W in MEGA 6.06 [12] excluding all the gaps.

RESULTS

The exon 2 of the angiotensinogen gene of 19 preeclamptic women and 20 normotensive women was amplified and sequenced for mutations. Table-1 displays the continuous variables measured for the sample population. The mean age for the preeclamptic women was 31.63 ± 8.09 and 27.3 ± 6.07 for the normotensive women. The mean body mass index was 22.60 ± 2.09.48 and 23.99 ± 2.63 for preeclamptic women and normotensive women respectively. Also the mean SBP and DBP readings were 152.26 ± 3.14 and 92.63 ± 1.98 for preeclamptic women, 113.50 ± 7.96 and 73 ± 7.33 for normotensive women respectively. No protein was observed in the urine of the normotensive women while the mean protein in the preeclamptic women was 1.73 ± 0.45. Analysis using the 2 tailed t- test showed no significant differences between the means of the preeclamptic women and normotensive women in all the continuous variables measured except for the SBP and DBP values where significant differences were observed between patient and control means. Mutations observed include non – synonymous tranversions where a purine base was changed to a pyrimidine base. A 4T>C mutation (substitution of thymine by cytosine for at position 4 was observed in 17(89.5%) patients that resulted in an amino acid substitution of leucine for tryptophan at position 2 (p.L2W) Fig-1. Another substitution of cytosine by guanine at position 911 (911C>G) was observed in 16(84.2%) patients resulting in an amino acid substitution of alanine by glycine at position 304 (p.A304G) Fig-2. A substitution of adenine for cytosine at position 1 was observed in 4(21.1%) patients with a resultant amino acid substitution of proline for threonine at position 1 (p.P1T). The last 2 transversion mutations were observed in a patient each, the g. 795G>T and g. 912C>A that lead to amino acid substitutions of glutamin by histidine at position 265(p.Q265H) and alanine by glycine at position 304(p.A304G) respectively. In silico analysis also revealed a deletion of guanine at position 26 (26delG) in all the 19(100%) preeclamptic women, Fig-3. Table-2 summarizes the mutations observed.

Table-1: Continuous variables measured in the sample population

Variables	Mean±SD in Preeclamptic women	Mean±SD in Normotensive women	t. value	p. value	<0.5
Age (yrs)	31.63 ± 8.09	27.3 ± 6.07	1.9	0.65	N.S
Body weight (kg)	64.75 ± 5.08	66.75 ± 5.88	-1.13	0.27	N.S
Height (m^2)	1.44 ± 0.10	1.40 ± 0.15	0.95	0.35	N.S
BMI (kg/m^2)	22.60 ± 2.09.48	23.99 ± 2.63	-1.84	0.07	N.S
SBP (mmHg)	152.26 ± 3.14	113.50 ± 7.96	19.79	<0.00001	S
DBP (mmHg)	92.63 ± 1.98	73 ± 7.33	11.28	<0.0001	S
Proteinuria (mg/dl)	1.73 ± 0.45	0			

Kg: kilograms, m^2: meters squared, mmHg: millimeter per mercury,
S.D: Standard Deviation, mg/dl: milligrams per deciliter,
SBP: systolic blood pressure, DBP: diastolic blood pressure
BMI: body mass index; N.S: Not Significant; S: Significant

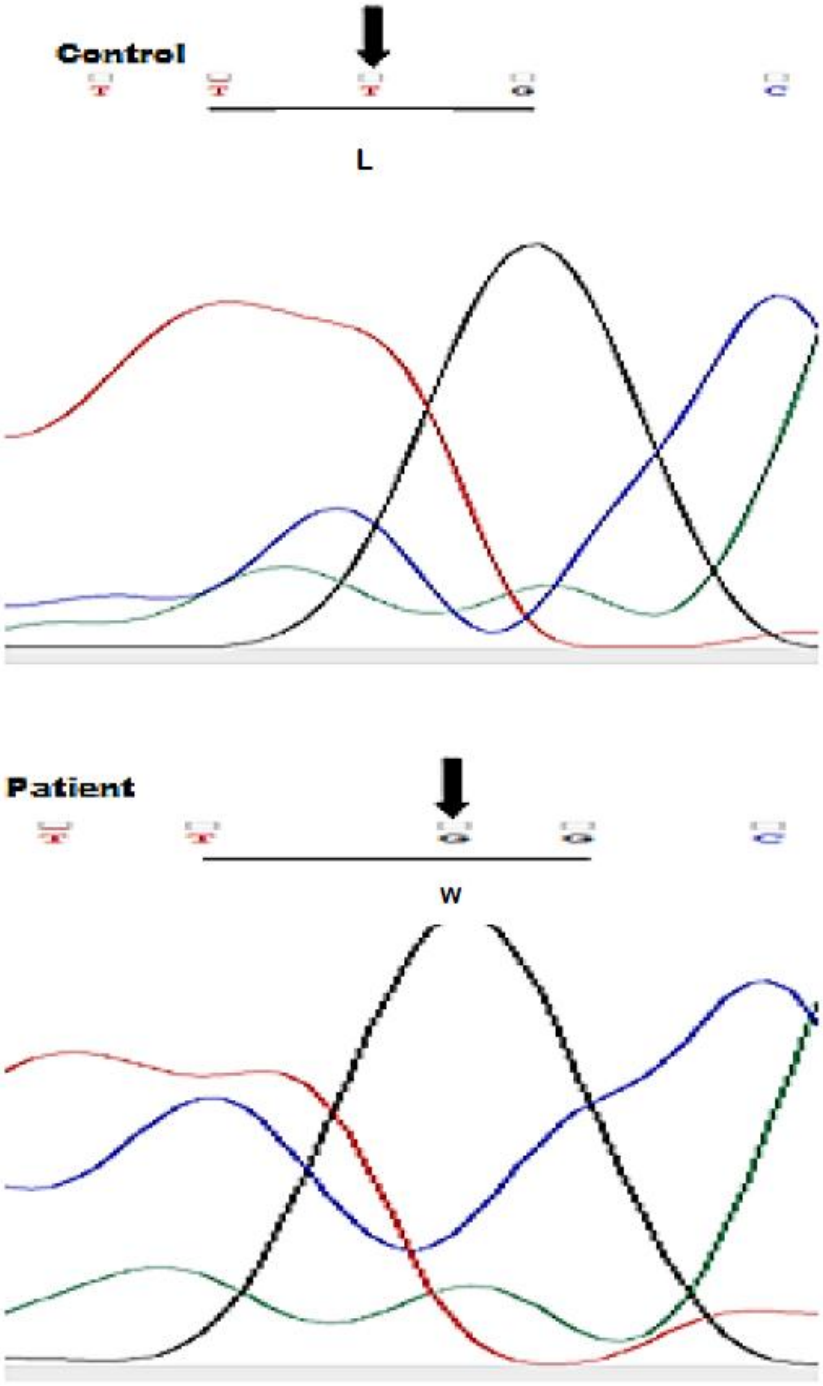

Fig 1: Chromatogram showing missense mutation of AGT gene on exon 2

Legend: upper panel display normal case (control), Lower panel display patient with mutation. The arrow indicate the T>G transversion. The encoded amino acid at codon 2 (underline) as indicated.TTG encoded leucine while TGG encodes tryptophan

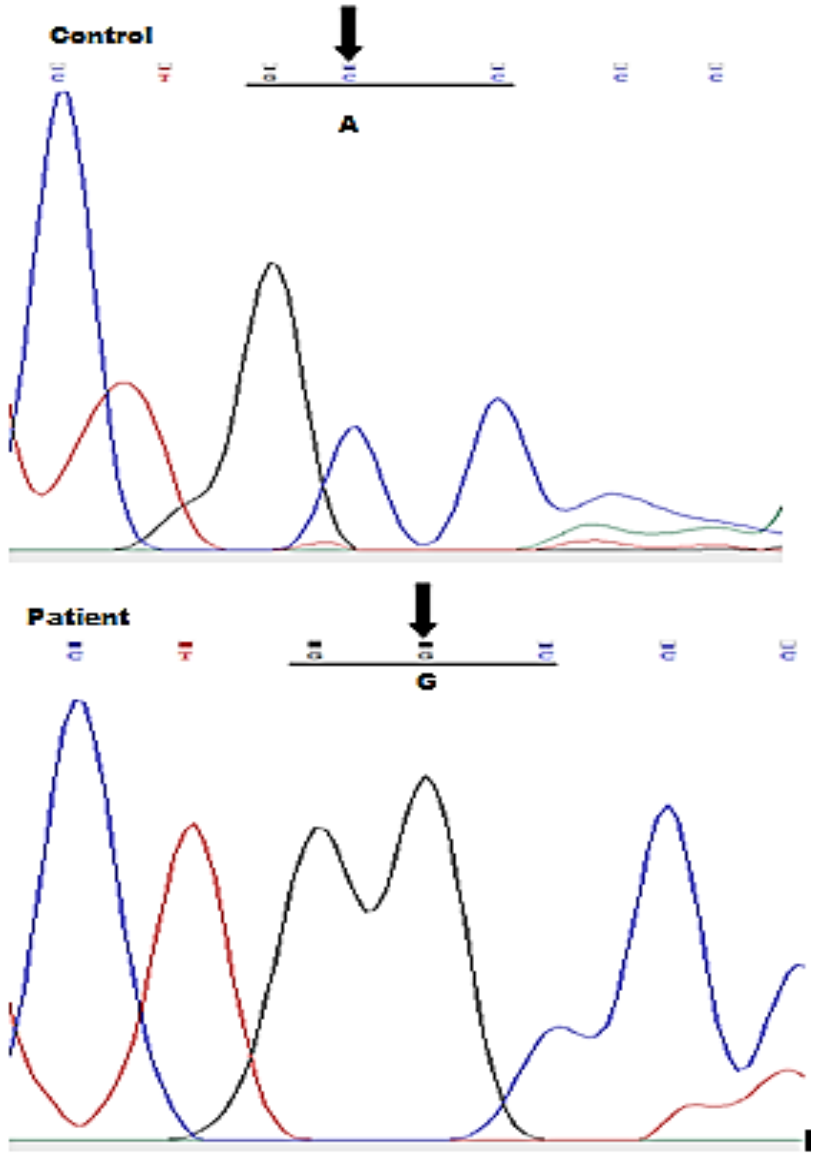

Fig-2: Chromatogram showing missense mutation of AGT gene on exon 2

Legend: upper panel display normal case (control), Lower panel display patient with mutation.
The arrow indicate the C>G transversion.
The encoded amino acid at codon 304 (underline) as indicated. GCC encoded alanine while GGC encodes glycine

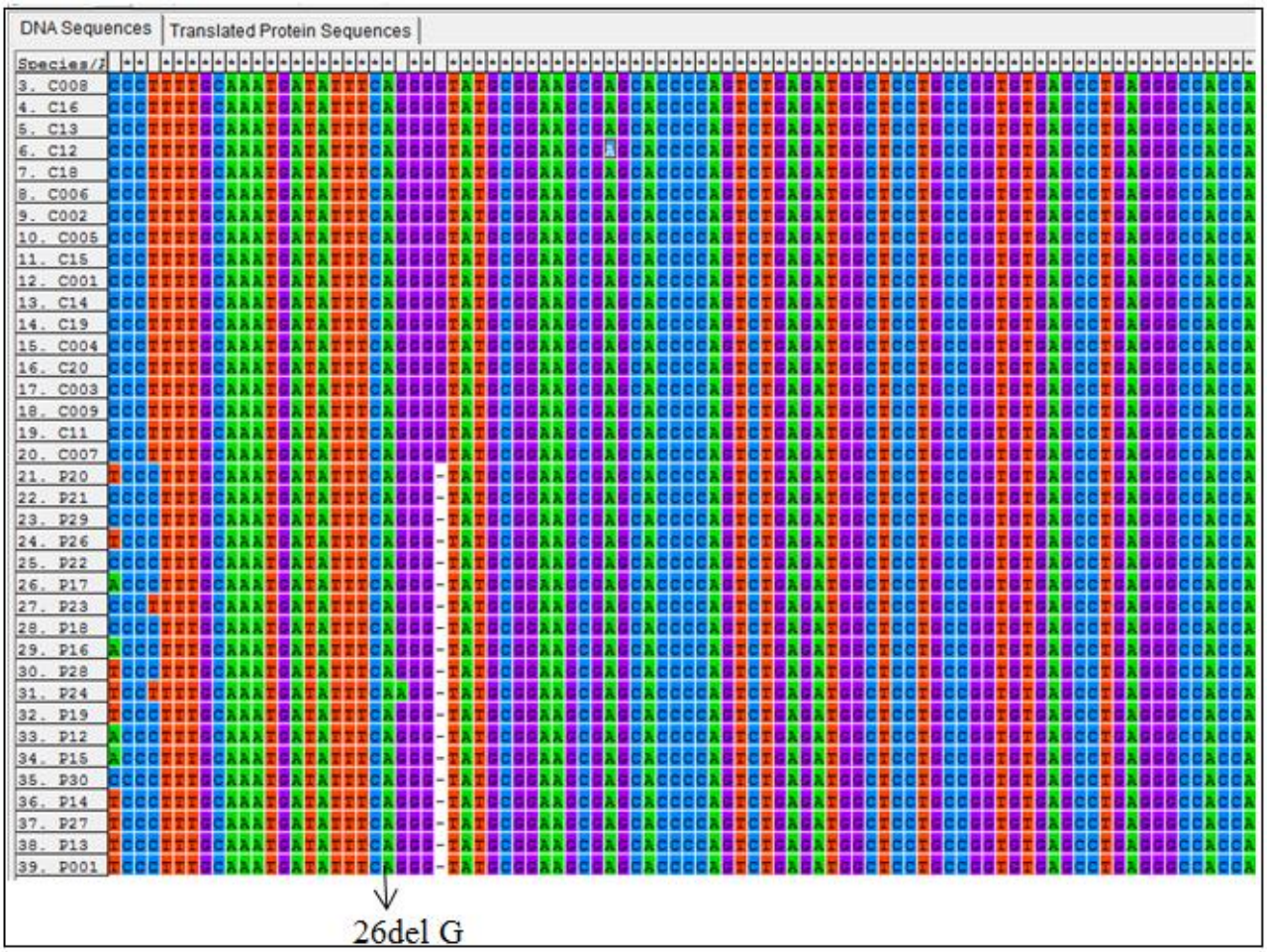

Fig-3: Legend: Deletion of guanine at position 26 in the coding region of the preeclamptic patients but this was absent in the control subjects

Table-2: Summary of Human AGT Mutations in exon 2 in preeclamptic women

S/No	Mutations	Frequency	Amino acid Substitution	Type
1	26del G	19(100)	Coding region	
2	4T>G	17(89.5)	Leu 2 Trp	Tranversion and Non Synonymous
3	911C>G	16(84.2)	Ala 304 Gly	Tranversion and Non Synonymous
4	1C>A	4(21.1)	Pro 1 Thro	Tranversion and Non Synonymous
5	795G>T	1(5.3)	Gln 265 His	Tranversion and Non Synonymous
6	912C>A	1(5.3)	Ala 304 Gly	Tranversion and Non Synonymous

DISCUSSIONS

This case control study showed significant differences in the mean of values of the blood pressure readings and this is expected as preeclamptic women usually have higher blood pressure than the normal pregnant women [3]. The mean age of 31.63 ± 8.09 for our preeclamptic women was similar to that documented by Zitouni *et al.*, [13] who reported a similar mean age of 31.3 years in Tunisian women. But a lower mean age of 28.87 years was also reported among preclamptic patients in Egypt [14].The body mass index was normal for our preeclamptic women, other studies documented a higher mean BMI of patients indicating obesity [14, 13]. In silico analysis revealed a deletion of guanine at position 23 in all the 19 preeclamptic women and 5 tranversion mutations 4T>G, 911C>G, 1C>A, 795G>T, 912C>A in 17(89.5), 16(84.2), 4(21.1), 1(5.3) and 1(5.3) preeclamptic women respectively but not in the normotensive women. Insertion/deletions are thought to contribute significantly to the genetic diversity between species [15]. Tranversion mutations on the other hand are believed to affect gene expression by disrupting the binding of transcription factors and other DNA binding proteins. Tranversions alter the amino acid sequence of proteins causing large changes in the DNA backbone [16]. Most studies on the AGT gene in literature document researches into the M235T allele, the T174M allele polymorphism among preeclamptic women, hypertensives, hyperoxaluria type 1 patients, Coronary heart disease patients amongst others [13, 17, 18, 19], little information is found in literature on the Missense,

Insertion/deletion mutations in the human AGT genes. Due to the small sample size, the results observed in this study will need to be assessed in larger number of preeclamptic women in Calabar for definite associations to be reached between these mutations and preeclampsia for our population.

CONCLUSION

This study observed mutations in exon 2 of the human AGT gene in preeclamptic women in Calabar. A deletion of Guanine at position 26 in the coding region and 5 non synonymous, transversion mutations (4T>G, 911C>G, 1C>A, 795G>T, 912C>A) were observed in these women but were absent in the normotensive pregnant women.

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