Science World Journal Vol. 20(No 2) 2025 https://dx.doi.org/10.4314/swj.v20i2.50
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

INVESTIGATION OF THE ASSOCIATION OF CYSTATHIONINE GAMMA-LYASE (CTH) AND VITAMIN D-BINDING PROTEIN (GC) GENES POLYMORPHISMS WITH PREECLAMPSIA IN SOME PREGNANT WOMEN ATTENDING PRENATAL CARE AT A GENERAL HOSPITAL IN LAGOS NIGERIA

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ABSTRACT
Preeclampsia is associated with high maternal and foetal morbidity and mortality. Studies have demonstrated that polymorphisms in the genes that regulate vascular dynamics may play vital roles in the development of chronic hypertension and preeclampsia. This study aimed to evaluate the relationship between polymorphisms in the *rs1021737G>T* of the cystathionine gamma-lyase (CTH), and *rs7041G>T* & *rs4588C>A* of the vitamin D-binding protein (GC) genes and the risk of preeclampsia in pregnant Nigerian women. A case-control study was conducted. Blood samples collected from 73 patients (8 cases and 65 controls) were used for DNA genotyping, while 101 patient blood samples (15 cases and 86 controls) were utilised for the plasma H_2S levels evaluation. The examined polymorphisms were determined using the PCR-RFLP method. Unconditional logistic regression analysis reveals no statistically significant difference among the odds ratio (OR) and 95% confidence interval (CI) of the genotypes and alleles for the *rs1021737G>T* of the CTH gene, and *rs7041G>T* & *rs4588C>A* of the GC gene. Furthermore, there were no relationships between studied polymorphisms and selected clinical parameters. The preeclamptic pregnant women showed no statistically significant difference in plasma H_2S level (32.87±15.16 *vs.* 56.29±33.34 µM, p = 0.055) as compared with the control group. This study suggests that examined polymorphisms in the CTH and GC genes are not associated with preeclampsia development in pregnant Nigerian women. Further studies with large sample sizes are needed to confirm these findings.

Keywords: Pre-Eclampsia, Cystathionine gamma-Lyase, Vitamin D-Binding Protein, Polymorphism, Single Nucleotide.

INTRODUCTION

Preeclampsia is a human pregnancy disorder characterized by new-onset hypertension before 20 weeks of gestation and after 20 weeks with systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90 mmHg, which can be detected by the presence of ≥ 0.3g/dl of protein in a urine sample (or urine dipstick protein test of +1) identified at least twice in spot urine tests (ACOG, 2013; Goolamnobee *et al.*, 2022). Preeclampsia in the absence of proteinuria is diagnosed as hypertension in association with thrombocytopenia, impaired liver function, a new development of renal insufficiency, pulmonary oedema, or new-onset cerebral or visual disturbances (Overton *et al.*, 2022). Preeclampsia affects 5–10% of all pregnancies worldwide (Macedo *et al.*, 2020). Its frequency and of various other maternal and foetal complications have been respectively estimated at between 14–53% and 22–92% (Kumari *et al.*, 2014). The associated complications include hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, death, eclampsia, stroke, caesarean section, and placental abruption for the women; complications for the foetuses include stillbirth, neonatal death, and small for gestational age (Overton *et al.*, 2022). Hypertension, proteinuria, edema, and other systemic manifestations of the syndrome of preeclampsia are the direct consequences of maternal endothelial dysfunction (Chang *et al.*, 2023).

Cystathionine γ-lyase (CTH) is a significant hydrogen sulphide (H_2S)-producing enzyme in the cardiovascular system, liver, kidney, pancreas and prostate. Endogenous H_2S plays a vital role in regulating physiological processes such as blood flow, vasodilation, arterial diameter, and leukocyte adhesion (Cirino *et al.*, 2023). H_2S acts as a vasodilator by opening ATP-sensitive K-channels in smooth muscle cells and up-regulates vascular endothelial growth factor. Also, H_2S has the antioxidant capacity by direct scavenging of nitrogen or reactive oxygen species and stimulates angiogenesis (Marini *et al.*, 2023). Furthermore, H_2S has a vital role in regulating the balance between growth and death of cells by inhibition of the CTH/H_2S pathway in the excessive apoptosis of vascular smooth muscle cells (Miceli *et al.*, 2024). Consequently, abnormal function of the CTH/H_2S pathway is associated with the pathogenesis and mechanism of cardiovascular diseases, including atherosclerosis and hypertension (Wang *et al.*, 2022).

Cystathionine γ-lyase is encoded by the CTH gene. The gene is located on the short arm of chromosome 1 and consists of 13 exons and 12 introns. Due to alternative splicing, three isoforms of human CTH arise (Youness *et al.*, 2024). Genetic variations analysis of the CTH gene showed a large number of polymorphisms (Zhou *et al.*, 2020). A decrease of the expression of CTH entails a drop in the level of cysteine, glutathione (GSH), taurine and H_2S in the cells and, more importantly, leads to cystathioninuria. H_2S, endogenously formed by CTH, affects the vasodilation and regulation of blood pressure (Kožich *et al.*, 2022). CTH knockout

mice have decreased levels of H_2S, hypertension, and reduced capacity for vascular endothelium relaxation (Azad *et al.*, 2018; Cirino *et al.*, 2023). Overexpression of the CTH gene in the cells leads to increased production of H_2S (Cirino *et al.*, 2023; Kaleta *et al.*, 2024). Cystathionine β-synthase (CBS) and CTH are down-regulated in several cardiovascular and pulmonary diseases (Arora *et al.*, 2023; Medina, 2021).

Vitamin D is a pleiotropic secosteroid hormone crucial for health and disease prevention (Gezen-Ak & Dursun, 2023). Bioactive vitamin D is synthesized through a series of reactions catalyzed by various enzymes. CYP2R1 and CYP27A1, which are 25-hydroxylases, first convert pro-vitamin D absorbed from diet or produced in the skin following sun exposure to a major circulating form of vitamin D, 25(OH)D. Afterward, CYP27B1 converts 25(OH)D to 1,25-dihydroxyvitamin D [$1,25(OH)_2D_3$] either in the kidney (where it is released into the circulation) or specific target organs. Circulating $1,25(OH)_2D_3$ is degraded by CYP24A1. Both vitamin D metabolites bind to the vitamin D-binding protein (VDBP), also known as group-specific component (GC), which facilitates vitamin D transportation (Kong *et al.*, 2015; Murthi *et al.*, 2016). In target tissues, $1,25(OH)_2D_3$ binds to the vitamin D nuclear receptor (VDR). The complex then forms a heterodimer with the retinoid X receptor (RXR), which binds to vitamin D response elements on multiple genomic loci, some of which are known to regulate blood pressure (Haussler *et al.*, 2021). Various studies have revealed that vitamin D deficiency, as well as disorders in vitamin D signaling pathways, contributes to many chronic diseases, including cancers, cardiovascular diseases, metabolic syndromes, and autoimmune disorders (Di Mauro *et al.*, 2024). Link between low maternal serum vitamin D and increased risk of preeclampsia has been reported in several studies (Fogacci *et al.*, 2020; Malm *et al.*, 2023; Yu *et al.*, 2013). Vitamin D metabolic and signaling components, such as VDBP, 25-hydroxylase (CYP2R1), and vitamin D receptor (VDR) were found to be down-regulated while 1α-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24A1) where up-regulated in preeclamptic placentas (Ma *et al.*, 2012). Similarly, studies have reported a differential expression of vitamin-D associated genes in peripheral blood of women who developed preeclampsia (Mirzakhani *et al.*, 2016; Yadama *et al.*, 2020).

Preeclampsia is now considered as a multifactorial condition with substantial evidence of genetic factors in its causation (Chang *et al.*, 2023; Tyrmi *et al.*, 2023). Findings in recent years have emphasized that polymorphisms of genes encoding for vasodilator factors or disruptions in their metabolism have an essential role in the pathogenesis of preeclampsia (Ahmad *et al.*, 2014; Cindrova-Davies, 2014). In previous studies, there have been mixed reports about the associations between candidate single nucleotide polymorphisms (SNPs) in CTH gene and risk of preeclampsia (Mrozikiewicz *et al.*, 2015; A. Seremak-Mrozikiewicz *et al.*, 2015; Agnieszka Seremak-Mrozikiewicz *et al.*, 2011; Yun *et al.*, 2008). Also, genetic polymorphisms in vitamin D-related genes influencing vitamin D status and proper utilization may affect its biological functions, and thus may influence risk of preeclampsia if vitamin D indeed has a role in the disorder. Consequently, the association of genetic polymorphisms in vitamin D signalling pathway with risk of hypertensive disorders of pregnancy, including preeclampsia, have been investigated (Caccamo *et al.*, 2020; Farajian-Mashhadi *et al.*, 2020; Rezavand *et al.*, 2019; Rezende *et al.*, 2012), pertinently, a few studies found that GC gene polymorphism is associated with the risk of preeclampsia (Baca *et al.*, 2018; Ghorbani *et al.*, 2021).

Despite numerous past research efforts, evidence of association of CTH gene polymorphism with preeclampsia development remains contradictory. Meanwhile, there is relatively little evidence concerning the relationship between the polymorphism of GC gene and risk of preeclampsia development. Moreover, to our knowledge, no previous research has investigated the association of CTH or GC gene polymorphism in the etiology of preeclampsia in pregnant Nigerian women. Hence, this study aims to investigate cystathionine γ-lyase and vitamin D-binding protein genetic polymorphisms in relation to risk of preeclampsia in pregnant Nigerian women, we conducted a case-control study. The selected SNPs in this study include rs1021737 in CTH and rs7041 and rs4588 in GC genes.

SUBJECTS AND METHODOLOGY

Subjects

This study included data and specimens from pregnant women attending prenatal clinics at Ifako-Ijaiye General Hospital in Agege area of Lagos State, Nigeria. It is a case-controlled study which included women diagnosed with preeclampsia as cases; and normotensive pregnant women as controls. Seventy-three (73) subject samples (8 cases and 65 controls) were adopted for the DNA genotyping analysis, while 101 subject samples (15 cases and 86 controls) were utilised for the plasma H_2S assay. Blood samples were obtained from enrolled participants (both cases and controls) for germline DNA genotyping after appropriate informed consent and before starting any new therapy. 4 ml of blood sample was collected in a Lithium-Heparinized bottle with minimal signs of haemolysis. The diagnostic criteria for pre-eclampsia were as follows: systolic pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg. Via administration of a standard questionnaire, demographic and clinical data were obtained from participants including their medical records which were used to clinically annotate the specimens. Blood samples were transported in Ice packs to the Molecular Biology Laboratory of Covenant University (CU), Otta – Ogun State where the DNA extraction and molecular analysis were performed. All samples were processed for recovery within 96 hours. Ethical approval was obtained for this study from the Ethics committee of the Lagos State University Teaching Hospital (LASUTH), Ikeja before the commencement of study.

Deoxyribonucleic acid (DNA) Isolation

Genomic DNA was extracted from whole blood using a DNeasy kit (Qiagen, Inc., Valencia CA) following the manufacturer's procedure. Extracted DNA was then stored at freezing temperature until required for genotyping. Before genotyping, quantification and purity assessment of DNA was performed using a microvolume spectrophotometer (NanoDrop™ 2000, Thermo Scientific™, Waltham, MA USA). An appropriate portion of the DNA was diluted with distilled water to 200µL volume, which corresponds to a DNA concentration of 10ng/µL.

Genotyping

The isolated DNA samples were subjected to PCR analysis to identify the single nucleotide polymorphism of the genes to be studied. The frequencies of the investigated SNPs, rs1021737 in the CTH gene; rs4588 and rs7041 in the GC/VBBP gene were examined by standard polymerase chain reaction-restriction

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fragment length polymorphism (PCR-RFLP) assay employing previously described procedures with slight modifications (F. Li *et al.*, 2011; Y. Li *et al.*, 2008).

PCR amplification of the CTH and GC genes

PCR was carried out in a thermal cycler (C1000 Touch™, Bio-Rad, California, USA). The reaction components comprised 10µL DNA template, 1µL primers, 5µL buffer, 4µL dNTPs, 1µL Taq DNA polymerase and 29µL RNase-free H_2O to make a 50µL mixture. The primers and conditions for the DNA amplification are presented in Table 1 below. After the completion of the PCR reaction, twenty (20) samples were randomly selected for each gene and subjected to agarose gel electrophoresis (PowerPac™, Bio-Rad, California, USA) to ascertain the quality of the amplicons.

Table 1: Primers and amplification conditions for *rs1021737* SNP of the CTH gene; *rs4588* and *rs7041* SNPs of the GC gene

Primer sequences	Melting temp./time	Annealing temp./time	Elongation temp./time	No. of cycles
rs1021737 Sense: 5'-AGGGCAATCATGACTCATGCATC-3' Antisense: 5'-TTGCAAAGGCTCATTGTTGGTCC-3'	Initial: 95°C, 3 hrs. Subsequent: 95°C, 30 mins	47°C, 30 mins	Initial: 72°C, 1 hr Final: 72°C, 5 hrs	35
rs4588 and rs7041 Sense: 5'-AAATAATGAGCAAATGAAAGAAGAC-3' Antisense: 5'-CAATAACAGCAAAGAAATGAGTAGA-3'	Initial: 94°C, 3 mins. Subsequent: 94°C, 30 s	55°C, 30 s	Initial: 72°C, 40 mins. Final: 72°C, 7 mins	35

Restriction Enzyme Digestion of PCR Amplicons

The PCR amplicons of CTH gene were digested with EcoRI (FlyCut™, TransGen Biotech, Beijing, China) while the PCR amplicons of GC gene were digested with HaeIII or StyI (New England BioLabs). The obtained fragments after the restriction enzymes digestion are presented in Table 2. The restriction products were then separated using agarose gel electrophoresis (PowerPac™, Bio-Rad, California, USA) to determine the polymorphic variants of the CTH and GC genes. Products of the electrophoresis were evaluated by visualization under UV light using a Transilluminator (UVP M-26V, BioDoc-It® 220 Imaging System, Upland CA, USA

Table 2: Restriction enzyme and hydrolysis conditions for *rs1021737* SNP of the CTH gene; *rs4588* and *rs7041* SNPs of the GC gene.

SNP	Restriction enzyme	Recognised Sequence	Time/temperature of incubation	Time/temperature of deactivation	Length of fragments (bp)
rs1021737	EcoRI	5'...G↓AATTC...3' 3'...CTTAA↑G...5'	16 h, 37°C	20 mins, 65°C	GG (528) GT (528, 429, 99) TT (429, 99)
rs4588	StyI	5'...C↓CWWGG...3' 3'...GGWWC↑C...5'	8 h, 37°C	20 mins, 65°C	CC (483) AC (483, 305, 178) AA (305, 178)
rs7041	HaeIII	5'...GG↓CC...3' 3'...CC↑GG...5'	8 h, 37°C	20 mins, 80°C	TT (483) GT (483, 297, 186) GG (297, 186)

W = A or T

Measurement of Plasma H_2S Concentration

Plasma hydrogen sulphide (H_2S) concentrations were measured according to previously described procedures (Li *et al.*, 2005; Mok *et al.*, 2004; Zhu *et al.*, 2007) with little modification. Briefly, 75µL of plasma was diluted with 425µL of distilled water and then mixed with 250µL of 1% (w/v) zinc acetate in Eppendorf tube. This is followed by the addition of 133µL of 2,3-Dimercapto-1-propanesulfonic acid (DMPS), then 133µL of Ferric chloride ($FeCl_3$) was added. The reaction mixture was incubated for 10mins at room temperature, which was then followed by the addition of 250µL of 10% (v/v) trichloroacetic acid (TCA) to remove the plasma protein, and then pelleted by centrifugation at 12000rpm for 5min. The absorbance of the resulting solution was then read at 670nm with a spectrophotometer (Beckman DU520, Beckman-Coulter, Woburn MA, USA). Concentration in the solution was calculated against a calibration curve of NaHS (Figure 1). Results show plasma H_2S concentration in micromolar.

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ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
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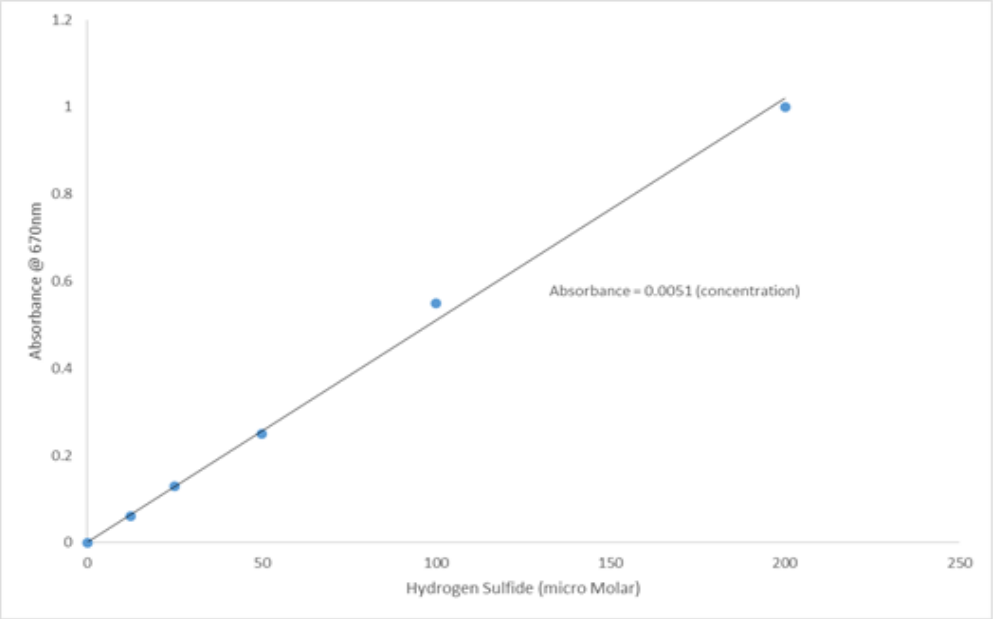

Figure 1: Standard curve for plasma H_2S concentration determination

Statistical Analysis

The statistical analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The frequencies of the alleles and genotypes between cases and controls were compared by the χ^2 (chi-square) test. Association between polymorphisms and preeclampsia was calculated by computing the odds ratio (OR) at 95% confidence intervals (95% CI) from logistic regression analyses. Clinical and biochemical parameters and their relationship with the reported polymorphisms was assessed using one-way ANOVA or independent-sample t-test. The values of $p<0.05$ were considered statistically significant.

RESULTS

Demographic, Clinical and Biochemical Parameters

This study analysed selected demographic and clinical parameters in preeclamptic women and controls (Table 3). Of statistical significance, higher systolic (147.73±16.69 *vs*. 105.32±10.81 mmHg, $p<0.001$) and diastolic (96.73±12.70 *vs*. 66.29±7.96 mmHg, $p<0.001$) blood pressure were observed in the preeclamptic subjects. Furthermore, these women were characterized by significant higher body weight (80.00±17.63 *vs*. 66.99±10.22 kg, $p<0.001$) and higher body mass index (30.25±6.59 *vs*. 25.75±4.07 kg/m^2, $p = 0.001$) during pregnancy as compared with controls. Though the preeclamptic pregnant women exhibit lower plasma H_2S level in comparison with the normotensive control, the difference was not statistically significant (i.e., 32.87±15.16 *vs*. 56.29±33.34 µM, $p = 0.055$).

Table 3: Demographic, clinical and biochemical parameters of patients with preeclampsia and control subjects.

Parameter	*Study group (PE) n = 15*	*Control group n = 72*	*p value*
Age (years)			
mean±SD	33.07±5.29	31.24±5.34	0.230
range	25 – 44	17 – 43	
median	32	31	
Gestational age (weeks)			
mean±SD	25.40±9.38	24.12±5.59	0.483
range	12 – 40	8 – 38	
median	24	24	
Number of gestation			
mean±SD	2.60±1.06	2.22±1.06	0.214
range	1 – 5	1 – 6	
median	3	2	

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Number of parturition			
mean±SD	0.87±0.74	0.85±0.94	0.940
range	0 – 2	0 – 3	
median	1	1	
Body weight (kg)			
mean±SD	80.00±17.63	66.99±10.22	<0.001
range	62 – 123	51 – 100	
median	73	67	
Body Mass Index (kg/m²)			
mean±SD	30.25±6.59	25.75±4.07	0.001
range	21.44 – 43.32	19.96 – 40.31	
median	27.94	24.91	
Systolic blood pressure (mmHg)			
mean±SD	147.73±16.69	105.32±10.81	<0.001
range	127 – 180	74 – 138	
median	145	104	
Diastolic blood pressure (mmHg)			
mean±SD	96.73±12.70	66.29±7.96	<0.001
range	84 – 129	48 – 86	
median	92.00	68.50	
H_2S concentration (µM)			
mean±SD	32.87±15.16	56.29±33.34	0.055
range	18.82 – 65.10	19.41 – 149.41	
median	30.00	44.71	

Value with $p < 0.05$ is considered significant.

Genotype Distributions and Allele Frequencies

The genotype distributions and allele frequencies of *rs1021737G>T* of the CTH gene (Table 4), and *rs7041G>T* (Table 5) and *rs4588C>A* (Table 6) of the GC gene are shown below.

For *rs1021737*, the study group (PE) has 6 (75.00%), 1 (12.50%), and 1 (12.50%) of the GG, GT, and TT genotypes, respectively; whereas the control subjects show 51 (78.46%), 11 (16.92%), and 3 (4.62%) of the GG, GT, and TT genotypes, respectively. The chi-square test indicated no statistical difference in the genotype frequencies of *rs1021737G>T* between the two groups under study ($\chi^2 = 0.904$, $p = 0.636$). In addition, a statistically similar frequency of the G allele (81.25% *vs.* 86.92%) and the mutated T allele (18.75% *vs.* 13.08%) for the *rs1021737* polymorphism was observed in women with PE and controls ($\chi^2 = 0.388$, $p = 0.533$) (Table 4).

Table 4: Frequency of genotypes of *rs1021737G>T* polymorphism of the CTH gene in women with preeclampsia and the control group

Polymorphism rs1021737	*Study group (PE)*		*Control group*		*χ^2 test*	*p value*
	Observed n (%)	*Expected (%)*	*Observed n (%)*	*Expected (%)*		
GG	6 (75.00)	78.48	51 (78.46)	78.03		
GT	1 (12.50)	16.46	11 (16.92)	16.44	0.904	0.636
TT	1 (12.50)	5.06	3 (4.62)	5.53		
Total	**8 (100.00)**	**100.00**	**65 (100.0)**	**100.00**		
G	13 (81.25)		113 (86.92)			
T	3 (18.75)		17 (13.08)		0.388	0.533
Total	**16 (100.00)**		**130 (100.00)**			

Value with $p < 0.05$ is considered significant

For *rs7041G>T* of the GC gene, the study group (PE) has 4 (50.00%), 1 (12.50%) and 3 (37.50%) of the GG, TG, and TT

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www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
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genotypes, respectively. Also, the control subjects contain 33 (50.77%), 8 (12.31%) and 24 (36.92%) of the GG, TG and TT genotypes respectively. The chi-square test indicated no statistical difference in the genotype frequencies of *rs7041G>T* between the study and control groups (χ^2 = 0.002, p = 0.999). Furthermore, a statistically similar frequency of the G allele (56.25% *vs.* 56.92%) and the mutated T allele (43.75% *vs.* 43.08%) for the *rs7041* polymorphism was observed in women with PE and the control (χ^2 = 0.003, p = 0.959) (Table 5).

Table 5: Frequency of genotypes and alleles of *rs7041G>T* polymorphism of the GC gene in women with preeclampsia and the control group

Polymorphism rs7041	*Study group (PE)*		*Control group*		*χ^2 test*	*p value*
	Observed n (%)	*Expected (%)*	*Observed n (%)*	*Expected n (%)*		
GG	4 (50.00)	50.62	33 (50.77)	50.69		
TG	1 (12.50)	12.34	8 (12.31)	12.33	0.002	0.999
TT	3 (37.50)	37.04	24 (36.92)	36.98		
Total	**8 (100.00)**	**100.00**	**65 (100.00)**	**100.00**		
G	9 (56.25)		74 (56.92)			
T	7 (43.75)		56 (43.08)		0.003	0.959
Total	**16 (100.00)**		**130 (100.00)**			

Value with p < 0.05 is considered significant

For *rs4588C>A* of the GC gene, the study group (PE) contains 1 (12.5%), 1 (12.50%) and 6 (75.00%) of the CC, AC, and AA genotypes, respectively. However, in the control group, 18 (27.69%), 1 (1.54%) and 46 (70.77%) of the CC, AC and AA genotypes was observed, respectively. The chi-square test indicated no statistical difference in the genotype frequencies of *rs4588C>A* between the study and control groups (χ^2 = 3.774, p = 0.152). Furthermore, a statistically similar frequency of the C allele (18.75% *vs.* 28.46%) and the mutated A allele (81.25% *vs.* 71.54%) for the *rs4588* polymorphism was observed in women with PE and the control (χ^2 = 0.676, p = 0.411) (Table 6).

Table 6: Frequency of genotypes of *rs4588C>A* polymorphism of the GC gene in women with preeclampsia and the control group

Polymorphism rs4588	*Study group (PE)*		*Control group*		*χ^2 test*	*p value*
	Observed n (%)	*Expected (%)*	*Observed n (%)*	*Expected n (%)*		
CC	1 (12.5)	26.25	18 (27.69)	26.00		
AC	1 (12.5)	2.50	1 (1.54)	2.77	3.774	0.152
AA	6 (75.00)	71.25	46 (70.77)	71.23		
Total	**8 (100.00)**	**100.00**	**65 (100.00)**	**100.00**		
C	3 (18.75)		37 (28.46)			
A	13 (81.25)		93 (71.54)		0.676	0.411
Total	**16 (100.00)**		**130 (100.00)**			

Value with p < 0.05 is considered significant

Relationship between Genotype and Allele Frequency with Preeclampsia

The comparison of the odds ratio (OR) and 95% confidence interval (CI) for each genotype of the *rs1021737* of the CTH gene, *rs7041* and *rs4588* of the GC gene polymorphisms are shown below (Table 7 – 9).

Unconditional logistic regression analysis reveals no statistically significant difference among the OR and 95% CI of the genotypes and alleles for the *rs1021737G>T* polymorphism of the CTH gene. Individuals carrying the GT heterozygote (OR = 0.77, 95% CI = 0.08 – 7.08, p = 0.820) and TT mutated homozygote (OR = 2.83, 95% CI = 0.25 – 31.74, p = 0.398) genotypes show no likelihood of preeclampsia development during pregnancy as compared to those having the GG homozygote wild-type genotype. Similarly, individuals possessing the mutated T allele are at no risk of pre-eclampsia as compared to those having the G allele (OR = 1.53, 95% CI = 0.39 – 5.95, p = 0.536), see (Table 7).

Table 7: Association between CTH gene *rs1021737G>T* genetic polymorphism and development of pre-eclampsia.

Polymorphism rs1021737	OR	95% CI	p value
GG	1.00	Ref.	-
GT	0.77	0.08 – 7.08	0.820
TT	2.83	0.25 – 31.74	0.398
G	1.00	Ref.	-
T	1.53	0.39 – 5.95	0.536

Ref. = Reference; Value with p < 0.05 is considered significant

Also, unconditional logistic regression analysis indicates no statistically significant difference among the OR and 95% CI of the genotypes and alleles for the *rs7041G>T* polymorphism of the GC gene. Individuals carrying the TG heterozygote (OR = 1.03, 95% CI = 0.10 – 10.53, p = 0.979) and TT mutated homozygote (OR = 1.03, 95% CI = 0.21 – 5.04, p = 0.970) genotypes show no likelihood of preeclampsia development during pregnancy as compared to patients with the GG homozygote wild-type genotype. Likewise, individuals possessing the mutated T allele are at no risk of pre-eclampsia as compared to those having the G allele (OR = 1.03, 95% CI = 0.36 – 2.93, p = 0.959), see (Table 8).

Table 8: Association between GC gene *rs7041G>T* genetic polymorphism and development of pre-eclampsia.

Polymorphism rs1021737	OR	95% CI	p value
GG	1.00	Ref.	-
TG	1.03	0.10 – 10.53	0.979
TT	1.03	0.21 – 5.04	0.970
G	1.00	Ref.	-
T	1.03	0.36 – 2.93	0.959

Ref. = Reference; Value with p < 0.05 is considered significant

Furthermore, analysis using unconditional logistic regression reveals no statistically significant difference among the OR and 95% CI of the genotypes and alleles for the rs4588C>A polymorphism of the GC gene. Individuals carrying the AC heterozygote (OR = 18.00, 95% CI = 0.59 – 553.59, p = 0.098) and AA mutated homozygote (OR = 2.35, 95% CI = 0.26 – 20.89, p = 0.444) genotypes show no likelihood of preeclampsia development during pregnancy as compared to patients with the CC homozygote wild-type genotype. Also, individuals possessing the mutated T allele are at no risk of pre-eclampsia as compared to those having the G allele (OR = 1.72, 95% CI = 0.46 – 6.40, p = 0.416), see (Table 9).

Table 9: Association between GC gene *rs4588C>A* genetic polymorphism and development of pre-eclampsia.

Polymorphism rs1021737	OR	95% CI	p value
CC	1.00	Ref.	-
AC	18.00	0.59 – 553.59	0.098
AA	2.35	0.26 – 20.89	0.444
C	1.00	Ref.	-
A	1.72	0.46 – 6.40	0.416

Ref. = Reference; Value with p < 0.05 is considered significant

DISCUSSION

Many epidemiological studies have indicated that various polymorphisms in genes such as estrogen alpha receptor, angiotensin 2 type-1 receptor, endothelial nitric oxide, matrix metalloproteinase-9, NLRP3, transforming growth factor beta-1, cyclooxygenase 2, FAS and FAS ligand, interleukin-27, interleukin-6, interleukin 10, cystathionine gamma-lyase, vitamin D receptor and vitamin D binding protein might play a pivotal role in the pathogenesis of preeclampsia (Agnieszka Seremak-Mrozikiewicz *et al.*, 2005; Ciarmela *et al.*, 2010; Agnieszka Seremak-Mrozikiewicz *et al.*, 2011; Gurdol *et al.*, 2012; Deepthi *et al.*, 2015; El-Beshbishy *et al.*, 2015; Khani *et al.*, 2015; Mrozikiewicz *et al.*, 2015; Ren *et al.*, 2015; Chen *et al.*, 2016; Masoumi *et al.*, 2016; Sun *et al.*, 2016; Fan *et al.*, 2017; Hortolani *et al.*, 2018; Xu *et al.*, 2018; Rezavand *et al.*, 2019; Ghorbani *et al.*, 2021). In this study, we conducted a case-control investigation to determine the possible roles of polymorphisms of *rs1021737* of the CTH gene and rs7041 & rs4588 of the GC gene in the risk of preeclampsia development, and it was observed that the genotypes and alleles of these SNPs have no association with elevated risk of preeclampsia in pregnant women in Lagos, Nigeria. Likewise, the preeclamptic and normotensive subjects show no difference in their endogenous plasma H_2S level.

Pertinently, previous studies have investigated the association between *rs1021737G>T* and *rs482843A>G* polymorphisms of the *CTH* gene and essential hypertension (Li *et al.*, 2008) and preeclampsia (Mrozikiewicz *et al.*, 2015). Li *et al.* (2008) studied the relationship between the CTH gene polymorphisms and essential hypertension in Northern Chinese Han population. They found that the analyzed rs482843 and rs1021737 polymorphisms did not show any impact on the development of essential hypertension among the studied population (Li *et al.*, 2008). Likewise, Mrozikiewicz *et al.* (2015) investigated the possible influence of the rs482843 and rs1021737 polymorphisms of the CTH gene on the development of preeclampsia in the population of Polish pregnant women. They reported that no correlation was observed between genotypes and alleles of the rs1021737 polymorphism and preeclampsia. However, analyses of the rs482843 polymorphism showed a higher frequency of the mutated GG genotype in women with preeclampsia compared to the control group, resulting in the suggestion that the rs482843 polymorphism of the CTH gene predisposes to the occurrence of preeclampsia in Polish pregnant women (Mrozikiewicz *et al.*, 2015). These findings are in concordance with this present study where we also show that no correlation exists between the genotypes and alleles of the *rs1021737* polymorphism of the CTH gene and risk of preeclampsia development in pregnant women in Lagos, Nigeria.

Also, we went further to determine the plasma H_2S concentrations of the subjects, and it is revealed that the preeclamptic pregnant women possess similar plasma H_2S levels as compared to the normotensive pregnant women. Our result disagrees with the findings of a study conducted in the Chinese population (Wang *et al.*, 2013). The authors reported that plasma H_2S levels were significantly reduced in women with preeclampsia, which was simultaneously associated with reduced placental expression of CTH, and they proposed that endogenous H_2S is required for healthy placental vasculature and a decrease in CTH/H_2S activity may contribute to the pathogenesis of preeclampsia (Wang *et al.*, 2013). The observed discrepancy could be as a result of the vast difference in the sample size.

The highly polymorphic GC gene encodes vitamin-D binding protein (VDBP) which serves as the transporter for vitamin D and its metabolites in circulation (Li *et al.*, 2011). Two common functional single nucleotide polymorphisms have been identified in exon 11 of GC. These variants are rs4588 and rs7041 that have been associated with different binding affinity for 25(OH)-D (Li *et al.*, 2011). Reduced binding of 25(OH)-D to VDBP might decrease the 25(OH)-D and other vitamin D metabolite levels. Hence, polymorphism in GC gene might enhance the risk of preeclampsia through increased risk of vitamin D deficiency (Lafi *et al.*, 2015, Baca *et al.*, 2018;). Studies have investigated the effect of genetic polymorphism in vitamin-D signaling and metabolism on susceptibility to pregnancy related hypertensive disorders, however, focus have been, mainly on VDR gene polymorphisms (Rezende *et al.*, 2012; Rezavand *et al.*, 2019; Caccamo *et al.*, 2020; Farajian-Mashhadi *et al.*, 2020), with little reports on the effect of GC gene polymorphisms (Baca *et al.*, 2018; Ghorbani *et al.*, 2021).
In a study, the FF/bB haplotype of the VDR FokI/BsmI genetic variants were found to be most frequent in gestational hypertension cohort, and resulted in risk for gestational hypertension by two folds, and 92% of this gestational cohort, notably presents hypovitaminosis D (Kimura *et al.*, 2012). Likewise, Rezavand *et al.* (2019) reported an association between VDR FokI polymorphism and deficient serum 25(OH)-D level with risk of preeclampsia. However, some comparable studies reported contrary findings. In a study that investigated for possible association between VDR FokI, ApaI and BsmI polymorphisms with preeclampsia or gestational hypertension. Findings from this study show no association between VDR polymorphisms or haplotypes with preeclampsia or gestational hypertension and the authors concluded that genetic variations in VDR do not predispose to hypertensive disorders of pregnancy (Rezende *et al.*, 2012). Similarly, another study evaluated the potential relationship between maternal and placental VDR polymorphisms and the predisposition to preeclampsia in an Iranian population. The authors reported that maternal and placental VDR FokI polymorphism and TABf haplotype were associated with lower preeclampsia risk while no relationship was observed between preeclampsia susceptibility and the maternal and placental VDR BsmI, TaqI and ApaI polymorphisms. Though, the placental TABF haplotype was associated with higher risk of preeclampsia (Farajian-Mashhadi *et al.*, 2020).

Similar to this study, the association between polymorphisms of the GC gene and predisposition to preeclampsia have been investigated (Baca *et al.*, 2018; Ghorbani *et al.*, 2021). The relationship between maternal allelic variants in three vitamin D metabolism genes (VDR, GC and CYP27B1) and risk of preeclampsia was evaluated. According to the authors, meta-analysis identified associations for one intron GC variant (rs843010:1.4) and two variants of the flanking region of GC (rs842991:1.5 and rs16846876:0.75) with risk preeclampsia (Baca *et al.*, 2018). Also, Ghorbani *et al.* (2021) determined the influence of gene variants and haplotypes of vitamin D biosynthesis, transport and function in the risk of preeclampsia. They reported that the CYP27B1, GC rs7041 and VDR ApaI variants were all associated with the risk of preeclampsia (Ghorbani *et al.*, 2021). Contrarily, our findings suggested that there is no relationship between GC rs7041 and rs4588 variants and susceptibility to preeclampsia.
Nevertheless, while the CTH gene rs1021737 polymorphism result from this study is consistent with previous studies, our findings on the GC rs7041 and rs4588 variants disagrees with existence evidence. Therefore, we are cautious in the interpretation of our results as it is not void of limitations. Firstly, this study made use of a small sample size, especially in the case group. Secondly, the GC gene has over 42,000 base pairs (Witke *et al.*, 1993). Hence, this study cannot rule out the possibilities of other GC polymorphisms contributing to predisposition to preeclampsia. Thirdly, we did not measure serum or plasma levels of 25(OH)-D and its derivatives, which are the most relevant functional molecules. Fourthly, the expression level of CTH gene that could have provided pertinent functional insight wasn't determined. Lastly, it is possible that the absence of an association between the rs1021737 SNP and preeclampsia might be because it is a non-functional SNP, hence other polymorphic variants should have been examined.

Conclusion

In summary, the present study suggests that the rs1021737G>T polymorphism of the CTH gene and rs7041G>T and rs4588C>A polymorphisms of the GC gene are not associated with risk of

preeclampsia in pregnant Nigerian women. Comparing the findings from this study with previous reports, we can suggest that *CTH/GC* gene polymorphic variants have a population-dependent role in preeclampsia development. Therefore, replications in different population-backgrounds and further functional studies are required for clarification of the role of the *CTH/GC* gene in the pathogenesis of preeclampsia and other hypertensive disorders of pregnancy. Such studies can provide important information about preeclampsia pathogenesis and in finding a suitable genetic marker that allows for accurate diagnosis and early detection of women at risk of preeclampsia development.

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