*Science World Journal Vol. 21(No 1) 2026* https://dx.doi.org/10.4314/swj.v21i1.48
*www.scienceworldjournal.org*
*ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)*
*Published by Faculty of Science, Kaduna State University*



# 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 ratios (ORs) 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 the 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 the 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). The frequency of various other maternal and foetal complications has been 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 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 in 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, contribute 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) were up-regulated in preeclamptic placentas (Ma *et al.*, 2012). Similarly, studies have reported a differential expression of vitamin-D-associated genes in the 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 the 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 the risk of preeclampsia if vitamin D indeed has a role in the disorder. Consequently, the association of genetic polymorphisms in the vitamin D signalling pathway with risk of hypertensive disorders of pregnancy, including preeclampsia, has 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 the association of the CTH gene polymorphism with preeclampsia development remains contradictory. Meanwhile, there is relatively little evidence concerning the relationship between the polymorphism of the GC gene and the 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 the 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.

## MATERIALS AND METHODS

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 that 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 the 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 were 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 fragment length polymorphism (PCR-RFLP) assay employing

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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 the *rs1021737* SNP of the CTH gene; the *rs4588* and *rs7041* SNPs of the GC gene

| Primer sequences | Melting temp./time | Annealing temp./time | Elongation temp./time | No. of cycles |
|---|---|---|---|---|
| **rs1021737**<br>Sense:<br>5'-AGGGCAATCATGACTCATGCATC-3'<br>Antisense:<br>5'-TTGCAAAGGCTCATTGTTGGTCC-3' | Initial: 95$^0$C, 3 hrs.<br>Subsequent: 95$^0$C, 30 mins | 47$^0$C, 30 mins | Initial: 72$^0$C, 1 hr<br>Final: 72$^0$C, 5 hrs | 35 |
| **rs4588 and rs7041**<br>Sense:<br>5'-AAATAATGAGCAAATGAAAGAAGAC-3'<br>Antisense:<br>5'-CAATAACAGCAAAGAAATGAGTAGA-3' | Initial: 94$^0$C, 3 mins.<br>Subsequent: 94$^0$C, 30 s | 55$^0$C, 30 s | Initial: 72$^0$C, 40 mins.<br>Final: 72$^0$C, 7 mins | 35 |

**Restriction Enzyme Digestion of PCR Amplicons**

The PCR amplicons of the CTH gene were digested with EcoRI (FlyCut™, TransGen Biotech, Beijing, China) while the PCR amplicons of the GC gene were digested with HaeIII or StyI (New England BioLabs). The obtained fragments after the restriction enzyme 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'<br>3'…CTTAA↑G…5' | 16 h, 37$^0$C | 20 mins, 65$^0$C | GG (528)<br>GT (528, 429, 99)<br>TT (429, 99) |
| rs4588 | StyI | 5'…C↓CWWGG…3'<br>3'…GGWWC↑C…5' | 8 h, 37$^0$C | 20 mins, 65$^0$C | CC (483)<br>AC (483, 305, 178)<br>AA (305, 178) |
| rs7041 | HaeIII | 5'…GG↓CC…3'<br>3'…CC↑GG…5' | 8 h, 37$^0$C | 20 mins, 80$^0$C | TT (483)<br>GT (483, 297, 186)<br>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 an 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 10 minutes 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 5 minutes. 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.

*Science World Journal Vol. 21(No 1) 2026* https://dx.doi.org/10.4314/swj.v21i1.48
*www.scienceworldjournal.org*
*ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)*
*Published by Faculty of Science, Kaduna State University*

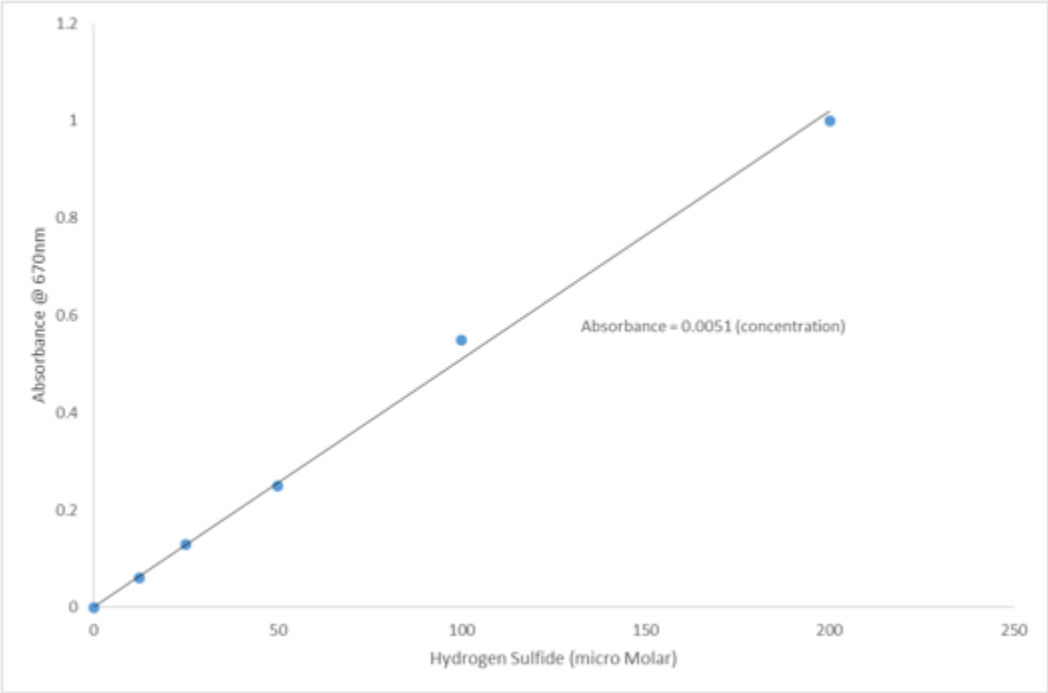


**Figure 1**: Standard curve for plasma $H_2S$ concentration determination

**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 $\chi^2$ (chi-square) test. The 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 were assessed using one-way ANOVA or an 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 significantly 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 gestations*** | | | |
| ***mean±SD*** | 2.60±1.06 | 2.22±1.06 | 0.214 |

| | | | |
|---|---|---|---|
| ***range*** | 1 – 5 | 1 – 6 | |
| ***median*** | 3 | 2 | |
| ***Number of parturitions*** | | | |
| ***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/m$^2$)*** | | | |
| ***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 | |

Values with $p < 0.05$ are 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*** | | ***$\chi^2$ 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

*Science World Journal Vol. 21(No 1) 2026* https://dx.doi.org/10.4314/swj.v21i1.48
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*ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)*
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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 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 ($\chi^2$ = 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 ($\chi^2$ = 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* | | *$\chi^2$ test* | *p value* |
|---|---|---|---|---|---|---|
| | *Observed n (%)* | *Expected (%)* | *Observed n (%)* | *Expected n (%)* | | |
| ***GG*** | 4 (50.00) | 50.62 | 33 (50.77) | 50.69 | 0.002 | 0.999 |
| ***TG*** | 1 (12.50) | 12.34 | 8 (12.31) | 12.33 | | |
| ***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) | | 0.003 | 0.959 |
| ***T*** | 7 (43.75) | | 56 (43.08) | | | |
| ***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 ($\chi^2$ = 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 ($\chi^2$ = 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* | | *$\chi^2$ test* | *p value* |
|---|---|---|---|---|---|---|
| | *Observed n (%)* | *Expected (%)* | *Observed n (%)* | *Expected n (%)* | | |
| ***CC*** | 1 (12.5) | 26.25 | 18 (27.69) | 26.00 | 3.774 | 0.152 |
| ***AC*** | 1 (12.5) | 2.50 | 1 (1.54) | 2.77 | | |
| ***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) | | 0.676 | 0.411 |
| ***A*** | 13 (81.25) | | 93 (71.54) | | | |
| ***Total*** | **16 (100.00)** | | **130 (100.00)** | | | |

Values with p < 0.05 are 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 is 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).

*Science World Journal Vol. 21(No 1) 2026* https://dx.doi.org/10.4314/swj.v21i1.48
*www.scienceworldjournal.org*
*ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)*
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**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 the 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 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 the 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 has mainly been on VDR gene polymorphisms (Rezende *et al.*, 2012; Rezavand *et al.*, 2019; Caccamo *et al.*, 2020; Farajian-Mashhadi *et al.*, 2020), with few 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 was found to be most frequent in the gestational hypertension cohort, and resulted in a risk for gestational hypertension by two-fold, 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 the 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 a 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 has 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 of 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 on 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 disagree with existing 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 the 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; other polymorphic variants should have been examined.

**Conclusion**

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

**REFERENCES**

American College of Obstetrics and Gynecologists. (2013). Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. *Obstetrics and Gynecology, 122*(5): 1122.

Ahmad, F. U., Sattar, M. A., Rathore, H. A., Tan, Y. C., Akhtar, S., Jin, O. H., Pei, Y. P., Abdullah, N. A., & Johns, E. J. (2014). Hydrogen sulphide and tempol treatments improve the blood pressure and renal excretory responses in spontaneously hypertensive rats. *Ren Fail, 36*(4): 598-605. doi:10.3109/0886022X.2014.882218

Arora, P., Mochan, S., Gupta, S. K., Rani, N., Kshetrapal, P., Dwivedi, S., Bhatla, N., & Dhingra, R. (2023). Abnormal trophoblast invasion in early onset preeclampsia: involvement of cystathionine β-synthase, specificity protein 1, and miR-22. *bioRxiv*: 2023.2003. 2008.531728.

Azad, M. A. K., Huang, P., Liu, G., Ren, W., Teklebrh, T., Yan, W., Zhou, X., & Yin, Y. (2018). Hyperhomocysteinemia and cardiovascular disease in an animal model. *Amino Acids, 50*(1): 3-9. doi:10.1007/s00726-017-2503-5

Baca, K. M., Govil, M., Zmuda, J. M., Simhan, H. N., Marazita, M. L., & Bodnar, L. M. (2018). Vitamin D metabolic loci and preeclampsia risk in multi-ethnic pregnant women. *Physiol Rep, 6*(2): e13468. doi:10.14814/phy2.13468

Caccamo, D., Cannata, A., Ricca, S., Catalano, L. M., Montalto, A. F., Alibrandi, A., Ercoli, A., & Granese, R. (2020). Role of Vitamin-D Receptor (VDR) single nucleotide polymorphisms in gestational hypertension development: A case-control study. *PLoS One, 15*(11): e0239407. doi:10.1371/journal.pone.0239407

Chang, K. J., Seow, K. M., & Chen, K. H. (2023). Preeclampsia: Recent Advances in Predicting, Preventing, and Managing the Maternal and Fetal Life-Threatening Condition. *Int J Environ Res Public Health, 20*(4): 2994. doi:10.3390/ijerph20042994

Chen, P., Gong, Y., Pu, Y., Wang, Y., Zhou, B., Song, Y., Wang, T., & Zhang, L. (2016). Association between polymorphisms in the IL-27 gene and pre-eclampsia. *Placenta, 37*: 61-64.

Ciarmela, P., Boschi, S., Bloise, E., Marozio, L., Benedetto, C., Castellucci, M., & Petraglia, F. (2010). Polymorphisms of FAS and FAS ligand genes in preeclamptic women. *Eur J Obstet Gynecol Reprod Biol, 148*(2): 144-146. doi:10.1016/j.ejogrb.2009.10.026

Cindrova-Davies, T. (2014). The therapeutic potential of antioxidants, ER chaperones, NO and H2S donors, and statins for treatment of preeclampsia. *Front Pharmacol, 5*: 119. doi:10.3389/fphar.2014.00119

Cirino, G., Szabo, C., & Papapetropoulos, A. (2023). Physiological roles of hydrogen sulfide in mammalian cells, tissues, and organs. *Physiological Reviews, 103*(1): 31-276. doi:10.1152/physrev. 00028.2021

Deepthi, G., Chaithri, P. K., Latha, P., Usha Rani, V., Rahman, P. F., & Jahan, P. (2015). TGFB 1 Functional Gene Polymorphisms (C-509T and T869C) in the Maternal Susceptibility to Pre-eclampsia in South Indian Women. *Scandinavian Journal of Immunology, 82*(4): 390-397.

Di Mauro, G., Musarra, M., Similia, S., Puzzolo, D., Minutoli, L., Morace, C., Di Giovanni, N., & BRANCATI, V. U. (2024). The multiple effects of Vitamin D on chronic diseases. *World Cancer Res J, 11*: e2739.

El-Beshbishy, H. A., Tawfeek, M. A., Al-Azhary, N. M., Mariah, R. A., Habib, F. A., Aljayar, L., & Alahmadi, A. F. (2015). Estrogen Receptor Alpha (ESR1) Gene Polymorphisms in Pre-eclamptic Saudi Patients. *Pak J Med Sci, 31*(4): 880-885. doi:10.12669/pjms.314.7541

Fan, D., Wang, Y., Liu, X., Zhang, A., & Xu, Q. (2017). Polymorphisms in interleukin-6 and interleukin-10 may be associated with the risk of preeclampsia. *Genetics and Molecular Research, 16*(1): 16018588.

Farajian-Mashhadi, F., Eskandari, F., Rezaei, M., Eskandari, F., Najafi, D., Teimoori, B., Moradi-Sharbabak, M., & Salimi, S. (2020). The possible role of maternal and placental vitamin D receptor polymorphisms and haplotypes in the pathogenesis of preeclampsia. *Clin Exp Hypertens, 42*(2): 171-176. doi:10.1080/10641963.2019.1601203

Fogacci, S., Fogacci, F., Banach, M., Michos, E. D., Hernandez, A. V., Lip, G. Y. H., Blaha, M. J., Toth, P. P., Borghi, C., Cicero, A. F. G., Lipid, & Blood Pressure Meta-analysis Collaboration, G. (2020). Vitamin D supplementation and incident preeclampsia: A systematic review and meta-analysis of randomized clinical trials. *Clinical Nutrition, 39*(6): 1742-1752. doi:10.1016/j.clnu.2019.08.015

Gezen-Ak, D., & Dursun, E. (2023). Vitamin D, a secosteroid hormone, and its multifunctional receptor, the vitamin D receptor, in Alzheimer's type neurodegeneration. *J Alzheimers Dis, 95*: 1273-1299.

Ghorbani, Z., Shakiba, M., Rezavand, N., Rahimi, Z., Vaisi-Raygani, A., Rahimi, Z., & Shakiba, E. (2021). Gene variants and haplotypes of Vitamin D biosynthesis, transport, and function in preeclampsia. *Hypertens Pregnancy, 40*(1): 1-8. doi:10.1080/10641955.2020.1849274

Goolamnobee, J. M. B., Haq, G., Sabir, S., Shaikh, S., Auckloo, S. M. A., & Shah, N. (2022). Frequency of Preeclampsia in Obese Women Presenting at Civil Hospital Karachi. *Pakistan Journal of Medical & Health Sciences, 16*(03): 824.

Gurdol, F., Cakmakoglu, B., Dasdemir, S., Isbilen, E., Bekpinar, S., & Isbir, T. (2012). − 765 G→ C and− 1195 A→ G Promoter Variants of the Cyclooxygenase-2 Gene Decrease the Risk for Preeclampsia. *Genetic testing and molecular biomarkers, 16*(5): 435-438.

Haussler, M. R., Livingston, S., Sabir, Z. L., Haussler, C. A., &

Jurutka, P. W. (2021). Vitamin D Receptor Mediates a Myriad of Biological Actions Dependent on Its 1,25-Dihydroxyvitamin D Ligand: Distinct Regulatory Themes Revealed by Induction of Klotho and Fibroblast Growth Factor-23. *JBMR Plus, 5*(1): e10432. doi:10.1002/jbm4.10432

Hortolani, A. C. C., Tanaka, S. C. S. V., Paschoini, M. C., & Balarin, M. A. S. (2018). Investigation of rs1800469 and rs1800468 Polymorphisms of the TGF-β1 Gene in Women with Pre-eclampsia. *Revista Brasileira de Saúde Materno Infantil, 18*(1): 179-185.

Kaleta, K., Janik, K., Rydz, L., Wrobel, M., & Jurkowska, H. (2024). Bridging the Gap in Cancer Research: Sulfur Metabolism of Leukemic Cells with a Focus on L-Cysteine Metabolism and Hydrogen Sulfide-Producing Enzymes. *Biomolecules, 14*(7): 746. doi:10.3390/biom14070746

Khani, M., Amani, D., Taheripanah, R., Sanadgol, N., Feizollahzadeh, S., & Rahmani, Z. (2015). Transforming growth factor beta-1 (TGF-β1) gene single-nucleotide polymorphisms (SNPs) and susceptibility to pre-eclampsia in Iranian women: A case–control study. *Pregnancy Hypertension, 5*(4): 267-272.

Kimura, H., Shibuya, N., & Kimura, Y. (2012). Hydrogen sulfide is a signaling molecule and a cytoprotectant. *Antioxidants & redox signaling, 17*(1): 45-57.

Kong, J., Xu, F., Qu, J., Wang, Y., Gao, M., Yu, H., & Qian, B. (2015). Genetic polymorphisms in the vitamin D pathway in relation to lung cancer risk and survival. *Oncotarget, 6*(4): 2573-2582. doi:10.18632/oncotarget.2951

Kožich, V., Morris, A. A., & Blom, H. J. (2022). Disorders of sulfur amino acid metabolism. In *Inborn metabolic diseases: diagnosis and treatment* (pp. 407-422): Springer.

Kumari, A., Chakrawarty, A., Singh, A., & Singh, R. (2014). Maternofoetal complications and their association with proteinuria in a tertiary care hospital of a developing country. *Journal of Pregnancy, 2014*.

Lafi, Z. M., Irshaid, Y. M., El-Khateeb, M., Ajlouni, K. M., & Hyassat, D. (2015). Association of rs7041 and rs4588 polymorphisms of the vitamin D binding protein and the rs10741657 polymorphism of CYP2R1 with vitamin D status among Jordanian patients. *Genetic testing and molecular biomarkers, 19*(11): 629-636.

Li, F., Jiang, L., Willis-Owen, S. A., Zhang, Y., & Gao, J. (2011). Vitamin D binding protein variants associate with asthma susceptibility in the Chinese Han population. *BMC Med Genet, 12*(1): 103. doi:10.1186/1471-2350-12-103

Li, L., Bhatia, M., Zhu, Y. Z., Zhu, Y. C., Ramnath, R. D., Wang, Z. J., Anuar, F. B. M., Whiteman, M., Salto-Tellez, M., & Moore, P. K. (2005). Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. *The FASEB journal, 19*(9): 1196-1198.

Li, Y., Zhao, Q., Liu, X. L., Wang, L. Y., Lu, X. F., Li, H. F., Chen, S. F., Huang, J. F., & Gu, D. F. (2008). Relationship between cystathionine gamma-lyase gene polymorphism and essential hypertension in the Northern Chinese Han population. *Chinese medical journal, 121*(8): 716-720.

Ma, R., Gu, Y., Zhao, S., Sun, J., Groome, L. J., & Wang, Y. (2012). Expressions of vitamin D metabolic components VDBP, CYP2R1, CYP27B1, CYP24A1, and VDR in placentas from normal and preeclamptic pregnancies. *Am J Physiol Endocrinol Metab, 303*(7): E928-935. doi:10.1152/ajpendo.00279.2012

Macedo, T. C., Montagna, E., Trevisan, C. M., Zaia, V., de Oliveira, R., Barbosa, C. P., Laganà, A. S., & Bianco, B. (2020). Prevalence of preeclampsia and eclampsia in adolescent pregnancy: A systematic review and meta-analysis of 291,247 adolescents worldwide since 1969. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 248*: 177-186.

Malm, G., Lindh, C. H., Hansson, S. R., Kallen, K., Malm, J., & Rylander, L. (2023). Maternal serum vitamin D level in early pregnancy and risk for preeclampsia: A case-control study in Southern Sweden. *PLoS One, 18*(2): e0281234. doi:10.1371/journal.pone.0281234

Marini, E., Rolando, B., Sodano, F., Blua, F., Concina, G., Guglielmo, S., Lazzarato, L., & Chegaev, K. (2023). Comparative study of different H2S donors as vasodilators and attenuators of superoxide-induced endothelial damage. *Antioxidants, 12*(2): 344.

Masoumi, E., Tavakkol-Afshari, J., Nikpoor, A. R., Ghaffari-Nazari, H., Tahaghoghi-hajghorbani, S., & Jalali, S. A. (2016). Relationship between Fas and Fas Ligand gene polymorphisms and pre-eclampsia. *Journal of Obstetrics and Gynaecology Research, 42*(10): 1272-1278.

Medina, G. (2021). The potential role of sulfur amino acids in the acute inflammatory response.

Miceli, G., Basso, M. G., Pintus, C., Pennacchio, A. R., Cocciola, E., Cuffaro, M., Profita, M., Rizzo, G., & Tuttolomondo, A. (2024). Molecular Pathways of Vulnerable Carotid Plaques at Risk of Ischemic Stroke: A Narrative Review. *International Journal of Molecular Sciences, 25*(8): 4351.

Mirzakhani, H., Litonjua, A. A., McElrath, T. F., O'Connor, G., Lee-Parritz, A., Iverson, R., Macones, G., Strunk, R. C., Bacharier, L. B., Zeiger, R., Hollis, B. W., Handy, D. E., Sharma, A., Laranjo, N., Carey, V., Qiu, W., Santolini, M., Liu, S., Chhabra, D., Enquobahrie, D. A., Williams, M. A., Loscalzo, J., & Weiss, S. T. (2016). Early pregnancy vitamin D status and risk of preeclampsia. *Journal of Clinical Investigation, 126*(12): 4702-4715. doi:10.1172/JCI89031

Mok, Y. Y., Atan, M. S., Yoke Ping, C., Zhong Jing, W., Bhatia, M., Moochhala, S., & Moore, P. K. (2004). Role of hydrogen sulphide in haemorrhagic shock in the rat: protective effect of inhibitors of hydrogen sulphide biosynthesis. *British Journal of Pharmacology, 143*(7): 881-889. doi:10.1038/sj.bjp.0706014

Mrozikiewicz, P. M., Bogacz, A., Omielanczyk, M., Wolski, H., Bartkowiak-Wieczorek, J., Grzeskowiak, E., Czerny, B., Drews, K., & Seremak-Mrozikiewicz, A. (2015). The importance of rs1021737 and rs482843 polymorphisms of cystathionine gamma-lyase in the etiology of preeclampsia in the Caucasian population. *Ginekol Pol, 86*(2): 119-125. doi:10.17772/gp/1998

Murthi, P., Yong, H. E., Nguyen, T. P., Ellery, S., Singh, H., Rahman, R., Dickinson, H., Walker, D. W., Davies-Tuck, M., Wallace, E. M., & Ebeling, P. R. (2016). Role of the Placental Vitamin D Receptor in Modulating Feto-Placental Growth in Fetal Growth Restriction and Preeclampsia-Affected Pregnancies. *Front Physiol, 7*: 43. doi:10.3389/fphys.2016.00043

Overton, E., Tobes, D., & Lee, A. (2022). Preeclampsia diagnosis and management. *Best Pract Res Clin Anaesthesiol, 36*(1): 107-121. doi:10.1016/j.bpa.2022.02.003

Ren, R., Gao, M., Fan, P., Liu, X., Liu, R., Ma, L., Chen, Y., Liu, Y.,

& Bai, H. (2015). Association study between-765G> C and-1195G> A functional polymorphisms in the cyclooxygenase 2 gene and risk of preeclampsia. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 32*(2): 245-249.

Rezavand, N., Tabarok, S., Rahimi, Z., Vaisi-Raygani, A., Mohammadi, E., & Rahimi, Z. (2019). The effect of VDR gene polymorphisms and vitamin D level on blood pressure, risk of preeclampsia, gestational age, and body mass index. *Journal of Cellular Biochemistry, 120*(4): 6441-6448. doi:10.1002/jcb.27934

Rezende, V. B., Sandrim, V. C., Palei, A. C., Machado, L., Cavalli, R. C., Duarte, G., & Tanus-Santos, J. E. (2012). Vitamin D receptor polymorphisms in hypertensive disorders of pregnancy. *Molecular Biology Reports, 39*(12): 10903-10906. doi:10.1007/s11033-012-1988-y

Seremak-Mrozikiewicz, A., Bogacz, A., Bartkowiak-Wieczorek, J., Wolski, H., Czerny, B., Gorska-Paukszta, M., & Drews, K. (2015). The importance of MTHFR, MTR, MTRR and CSE expression levels in Caucasian women with preeclampsia. *Eur J Obstet Gynecol Reprod Biol, 188*: 113-117. doi:10.1016/j.ejogrb.2015.03.009

Seremak-Mrozikiewicz, A., Drews, K., Barlik, M., Sieroszewski, P., Grześkowiak, E., & Mrozikiewicz, P. M. (2011). The significance of− 786T> C polymorphism of endothelial NO synthase (eNOS) gene in severe preeclampsia. *Journal of Maternal-Fetal & Neonatal Medicine, 24*(3): 432-436.

Seremak-Mrozikiewicz, A., Dubiel, M., Drews, K., Breborowicz, G., & Mrozikiewicz, P. (2005). 1166C mutation of angiotensin II type 1 receptor gene is correlated with umbilical blood flow velocimetry in women with preeclampsia. *Journal of Maternal-Fetal & Neonatal Medicine, 17*(2): 117-121.

Sun, C., Zhang, Q., Hu, B., & Zhang, K. (2016). Investigation of the association between matrix metalloproteinase-9 genetic polymorphisms and development of pre-eclampsia in Chinese pregnant women. *Genet. Mol. Res, 15*(3).

Tyrmi, J. S., Kaartokallio, T., Lokki, A. I., Jaaskelainen, T., Kortelainen, E., Ruotsalainen, S., Karjalainen, J., Ripatti, S., Kivioja, A., Laisk, T., Kettunen, J., Pouta, A., Kivinen, K., Kajantie, E., Heinonen, S., Kere, J., Laivuori, H., Finnpec Study Group, F. P., & the Estonian Biobank Research, T. (2023). Genetic Risk Factors Associated With Preeclampsia and Hypertensive Disorders of Pregnancy. *JAMA Cardiol, 8*(7): 674-683. doi:10.1001/jamacardio.2023.1312

Wang, K., Ahmad, S., Cai, M., Rennie, J., Fujisawa, T., Crispi, F., Baily, J., Miller, M. R., Cudmore, M., & Hadoke, P. W. (2013). Dysregulation of the hydrogen sulfide (H2S) producing enzyme cystathionine γ-lyase (CSE) contributes to maternal hypertension and placental abnormalities in preeclampsia. *Circulation, 127*(25): 2514-2522.

Wang, Y., Wang, T., Luo, Y., & Jiao, L. (2022). Identification Markers of Carotid Vulnerable Plaques: An Update. *Biomolecules, 12*(9): 1192. doi:10.3390/biom12091192

Witke, W. F., Gibbs, P. E., Zielinski, R., Yang, F., Bowman, B. H., & Dugaiczyk, A. (1993). Complete structure of the human Gc gene: differences and similarities between members of the albumin gene family. *Genomics, 16*(3): 751-754.

Xu, L., Li, S., Liu, Z., Jiang, S., Wang, J., Guo, M., Zhao, X., Song, W., & Liu, S. (2018). The NLRP3 rs10754558 polymorphism is a risk factor for preeclampsia in a Chinese Han population. *Journal of Maternal-Fetal & Neonatal Medicine*: 1-8.

Yadama, A. P., Maiorino, E., Carey, V. J., McElrath, T. F., Litonjua, A. A., Loscalzo, J., Weiss, S. T., & Mirzakhani, H. (2020). Early-pregnancy transcriptome signatures of preeclampsia: from peripheral blood to placenta. *Sci Rep, 10*(1): 17029. doi:10.1038/s41598-020-74100-1

Youness, R. A., Habashy, D. A., Khater, N., Elsayed, K., Dawoud, A., Hakim, S., Nafea, H., Bourquin, C., Abdel-Kader, R. M., & Gad, M. Z. (2024). Role of Hydrogen Sulfide in Oncological and Non-Oncological Disorders and Its Regulation by Non-Coding RNAs: A Comprehensive Review. *Noncoding RNA, 10*(1): 7. doi:10.3390/ncrna10010007

Yu, C., Ertl, R., Skyfta, E., Akolekar, R., & Nicolaides, K. (2013). Maternal serum vitamin D levels at 11–13 weeks of gestation in preeclampsia. *Journal of human hypertension, 27*(2): 115-118.

Yun, L., Qi, Z., Liu, X.-l., Wang, L.-y., Lu, X.-f., Li, H.-F., Chen, S.-f., Huang, J.-f., & Gu, D.-f. (2008). Relationship between cystathionine γ-lyase gene polymorphism and essential hypertension in the Northern Chinese Han population. *Chinese medical journal, 121*(8): 716-720.

Zhou, W., Yang, Q., Yu, H., Zhang, Q., Zou, Y., Chen, X., Yang, Z., Qu, Y., Tan, R., & Li, L. (2020). Association between an indel polymorphism within CTH and the risk of sudden cardiac death in a Chinese population. *Legal Medicine, 46*: 101736.

Zhu, Y. Z., Wang, Z. J., Ho, P., Loke, Y. Y., Zhu, Y. C., Huang, S. H., Tan, C. S., Whiteman, M., Lu, J., & Moore, P. K. (2007). Hydrogen sulfide and its possible roles in myocardial ischemia in experimental rats. *J Appl Physiol (1985), 102*(1): 261-268. doi:10.1152/japplphysiol.00096.2006