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Polymorphisms within vitamin D binding protein gene within a Preeclamptic South African population

Yeshnee Naidoo[a], Jagidesa Moodley[b], Veron Ramsuran[c], and Thajasvarie Naicker[d]

[a]Discipline of Optics and Imaging, Women's Health and HIV Research Group, Durban, South Africa; [b]KZN Research Innovation and Sequencing Platform, University of KwaZulu-Natal, Durban, South Africa; [c]KwaZulu-Natal Research and Innovation Sequencing Platform, University of KwaZulu-Natal Nelson R Mandela School of Medicine, Durban, South Africa; [d]Department of Optics and Imaging, University of KwaZulu-Natal, Durban, South Africa

ABSTRACT

Objectives: The vitamin D binding protein encoded by the *GC* gene contains two single nucleotide polymorphisms (rs4588 and rs7041) that have been associated with disease outcome, these include periodontitis coronary heart disease and hypertension. In pregnancy, these SNPs influence vitamin D metabolism that could result in hypertensive disorders such as PE. The etiology of PE, still remains elusive. The aim of this study was to evaluate the distribution of rs4588 and rs7041 within the *GC* gene among PE and normotensive pregnant women, residing in Durban, KwaZulu-Natal, South Africa.

Study design: Our study consisted of n = 600 participants (normotensive (n = 246, N); early onset PE (n = 167, EOPE); and late-onset PE (n = 246, LOPE)). We extracted DNA from whole blood and genotyped for rs4588 and rs7041 SNPs using the TaqMan assay.

Results: Regardless of HIV status, we observed the rs4588 (CC genotype) more frequently in PE (EOPE+LOPE) compared to the normotensive participants with an OD ratio of 0.74 (95% CI, 0.35–1.5; p < 0.001). We report a significant difference in the frequency of rs7041 (GT genotype) in the EOPE group compared to the normotensive group with an OD ratio of 11.48 (95% CI, 2.6–103.7; $p < 0.001$). The rs7041 GT genotype had a higher frequency in the EOPE compared to the LOPE group, with an OD ratio of 15.15 (95% CI, 2.3–639.2; $p < 0.001$).

Conclusion: This is the first study to describe the prevalence of SNPs of the rs4588 and rs7041 within the *GC* gene in women with PE within the high HIV endemic area of KZN, South Africa. Notably, a significant association of the rs7041 (TT genotype) and rs4588 (CC genotype) occurred at a higher frequency in PE compared to the normotensive cohort. Future studies will examine the functional effect of the *GC* region in relation to pregnancy and vitamin D deficiency.

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Introduction

Preeclampsia (PE) is a disorder of widespread vascular endothelial malfunction and vasospasm that occurs after 20 weeks of gestation and presents as late as 4–6 week postpartum. It is clinically defined by hypertension and proteinuria (1). The prevalence of PE is estimated to be 4,6% globally (2).

Preeclampsia may be sub-typed into early-onset (before 34 weeks of gestation; EOPE) and late-onset (after 34 weeks of gestation; LOPE) (3). Deficient cytotrophoblast invasion, with a failure of normal spiral arterial remodeling, characterizes EOPE. Consequentially, the reduced uteroplacental blood flow contributes to an imbalance of angiogenic factors and oxidative stress retarding fetal growth (4,5), while LOPE is associated with increased maternal susceptibility to atherosis of the placenta (6).

Moodley et al. (2017) reported that 12% of all Black African primigravidae at a regional hospital in Durban, South Africa (SA) had PE (7). In addition, the annual Saving Mothers' Report (2017) indicates that PE accounts for 14.8% of maternal deaths and a considerable proportion of stillbirths and neonatal deaths in South Africa. Moreover, many large epidemiological and family studies demonstrate a genetic contribution to PE susceptibility (8,9).

In normal pregnancy, vitamin D is implicated in the regulation of key target genes associated with implantation of the embryo, trophoblast invasion and implantation immunotolerance (10). However, PE is associated with a downregulation of vitamin D with concomitant adverse maternal and fetal outcomes (9). Nilsson et al., 2004 noted if vitamin D deficiency increases the risk of PE, then known functional sequence polymorphisms may

CONTACT Yeshnee Naidoo 204003110@stu.ukzn.ac.za Optics and Imaging Centre, Doris Duke Medical Research Institute, College of Health Sciences, University of KwaZulu-Natal, South Africa

predispose to PE development (11). Several studies have also shown that GC, CYP27B1 and VDR are integral genes to vitamin D metabolism with allelic variants associated with chronic disease (12–14).

The vitamin D binding protein (VDBP) is encoded by a highly polymorphic gene GC which is located on chromosome 4q13 and has 13 exons encoding 474 amino acids (15). Two common (rs4588 and rs7041) positioned at codons 416 and 420 in exon 11 are associated with 25 (OH) D deficiencies. The rs4588 polymorphism substitutes nucleic acid A for C, which results in a nonsynonymous substitution altering the amino acid from lysine to threonine, while the rs7041 polymorphism substitutes G to T, which also results in a nonsynonymous change from glutamate to aspartate. The amino acid changes result in variant proteins that differ in their affinity for vitamin D (16).

Studies have shown African Americans display lower 25(OH) D levels compared to European Americans (EA) (17,18). Genetic traits of a T variant of rs7041 are associated with lower vitamin D binding protein in both races, but lower 25(OH) D levels occur only in African Americans. The A variant in rs4588 is associated with higher vitamin D binding protein in both races but with lower 25(OH) D levels in European Americans (17).

The active form of vitamin D (1.25-dihydroxyvitamin D3) controls the transcription and function of genes connected to placental invasion, normal implantation, and angiogenesis (10). The *GC* and *VDR* genes involved in the vitamin D pathway play an important role in vitamin D metabolism. Allelic variants in these genes have been associated with chronic disease risk (12–14,19). However, studies in PE have been limited to only a few targeted SNPs in the *VDR* gene (20,21). Studies have suggested that variants in the GC region affect the proteins affinity to 25(OH) D which controls the bioavailability of free 25(OH) D, thereby decreasing the concentration of 25(OH) D. These genetic variations may increase the risk of PE due to vitamin D deficiency (22). Vitamin D deficiency as mentioned above is responsible for chronic diseases such as diabetes, cancer, cardiac disease, and autoimmune disorders (23).

The molecular mechanisms of how alterations in the vitamin D pathway associates with PE remains unknown. Various studies have examined the role of vitamin D deficiency leading to PE. Chan et al.,2015 demonstrated that vitamin D supplementation was associated with an increase in extravillous trophoblast invasion (EVT). Therefore, dysregulated bioactivity of vitamin D in uteroplacental tissue in combination with attenuated EVT could cause inadequate placentation suggesting that vitamin D deficiency could increase the risk of PE development (24). Pro-inflammatory cytokines are elevated during PE, Diaz et al. using cultured trophoblastic cells mimicked a pro-inflammatory micro-environment with consequential induction of elevated cytokines. However, they demonstrated a downregulation of these pro-inflammatory cytokines (TNF α, IL-6 and IFN) in the presence of calcitriol (active form of vitamin D) (25).

In this study, we evaluate rs4588 and rs7041 polymorphisms within a South African Black female population with PE.

Materials and methods

Ethics: Institutional (UKZN BREC) ethical approval and patient consent were obtained for this study (BCA 338/17).

Sample size was determined in consultation with the institutional biostatistician, using the Cohen's effect (26)

Study Site: Participants were recruited from a regional hospital in Durban, South Africa.

Study Population: Participants (n = 600) were grouped into the following classifications: normotensive (n = 246; N), early onset (n = 167; EOPE) and late onset (n = 187; LOPE) PE. Only Black South African women >18 years of Zulu ethnicity (self-reported) were included in the study. Inclusion criterion for normotensive was a blood pressure of 120/80 mmHg obtained at least 2 h apart with the absence of proteinuria. Preeclampsia was defined as a new onset of hypertension (BP systolic ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg) at or after 20 week gestation (27). Early-onset PE was defined as preeclampsia occurring <34 weeks of gestation whilst LOPE had a gestational age >34 weeks (28). Exclusion criteria for preeclampsia were chorioamnionitis, polycystic ovarian syndrome, chronic hypertension, eclampsia and placental-related complications, Intrauterine death, abruptio placentae, gestational diabetes, chronic renal disease, systemic lupus erythematous, antiphospholipid antibody syndrome, thyroid disease, cardiac disease and chronic asthma requiring medication during pregnancy, and epilepsy.

Each group was further stratified by HIV status. Venous blood was collected and stored at −80°C until genomic DNA extraction.

Genomic DNA extractions: Genomic DNA was extracted from 200 µl of whole blood using the QIAamp genomic DNA blood 250 mini kit (QIAGEN Sciences; Maryland, USA) according to manufacturer's instruction.

Genotyping: Genotyping reactions were carried out using the TaqMan assay (ABI; Foster City, CA) for SNPs of rs7041 and rs4588. Real-time polymerase

PCR reactions were performed on the light cycler 480 (Roche Diagnostics, Germany).

The assay mixture (including unlabeled PCR primers, FAM™ and VIC® dye-labeled TaqMan MGB probes) was designed by the manufacturer (Applied Biosystems). The reaction contained 50 ng of genomic DNA, 10 µl of 2X TaqMan™ Genotyping Master Mix, 0.5 µl of 40x assay mix adjusted with Milli-Q H_2O to a total volume of 20 µl. The PCR conditions consisted of an initial step at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 60 sec in a 96-well plate that included negative and positive controls to ensure genotyping accuracy.

Statistical analysis: Descriptive statistics was performed using the Stata V13.1 statistical package. Frequencies and percentages were used for categorical data. The numeric data were not normally distributed, so medians were used. Each subgroup was tested for the Hardy–Weinberg Equilibrium and a *p* value was reported. The distribution of alleles in each sub-group was compared using a Chi-square or Fisher's exact test.

Results

Demographic data: Six hundred females were recruited in the study; these were then categorized according to HIV status (Table 1).

The clinical characteristics are documented for all study groups and are outlined in Tables 1 and 2. The median age was significantly different amongst study groups irrespective of HIV status (N *vs* EOPE *vs* LOPE; 25 yrs *vs* 30 yrs vs 26 yrs, $p < 0.0001$). As expected, the median gestational age was lower in the EOPE compared to the LOPE group (29 weeks *vs* 37 weeks, $p < 0.001$).

As per selection criteria, the median diastolic and systolic blood pressure differed amongst the study groups ($p < 0.0001$). Notably, both the systolic and the diastolic were higher in the EOPE (155/101 mmHg) compared to LOPE (148/97) groups. Gravidity ($p < 0.0459$), parity ($p < 0.9578$) and maternal weight were similar amongst the groups.

Each study group was further stratified by age into <20, 21–24 and 25–41 years (Table 3). We observed 31.2% of the study population had PE in the <20-year-old ($p < 0.001$). Similarly, when compared to the control group 37% had PE in the 21-24-year-old group. The EOPE group delivered at ≤33 weeks whilst the LOPE group between 34 and 42 weeks of gestation, $p < 0.001$ (Table 3). Urinary protein was observed in the PE groups (EOPE and LOPE), Table 3. Only 0.4% of the normotensive group had proteinuria ($1^+ - 2^+$). Forty-one percent of the EOPE group displayed 2^+-3^+proteinuria.

Table 1. Shows the study groups according to HIV status. There was no significant difference among the groups with regard to HIV status.

	HIV pos		HIV neg			
Groups	*n*	%	*n*	%	Total	P-VALUE
Control	117	47.6	129	52.4	246	0.4
PE early onset	85	45.2	103	54.8	188	
PE late onset	68	41.0	98	59.0	166	
Total	270	45	330	55.0	600	

Genotyping results

The rs4588 and rs7041 SNP were in agreement with Hardy–Weinberg equilibrium (p =0.6) (29). We calculated the frequencies of genotypes and alleles in the study population and associated these with PE and HIV status.

*rs 4588*a total of 582 samples were genotyped; the remainder 18 had poor sample integrity. Regardless of HIV status, we report that the frequency (92,4%) of the CC genotype was predominant compared to AA and AC in the PE with an OD ratio of 0.74 (95% CI, 0.35–1.5; $p < 0.001$) compared to the normotensive group (Table 4). The frequency of the CC genotype among the HIV positive participants (PE vs normotensive) was high (94,1% vs 98%), respectively, with an OD ratio of 0.28 (95% CI, 0.03–1.4) albeit non-significantly ($p < 0.121$; Table 4).

rs 7041 – a total of 594 samples were genotyped; the remainder 6 could not due to poor sample integrity. The

Table 2. Showing characteristics of participants.

	Normotensive		Early-onset PE		Late-onset PE		
	n = 246		*n* = 188		*n* = 166		
	Median	IQR	Median	IQR	Median	IQR	*p* Value
Age	25	(21–31)	30	(24–35)	26	(21–33)	0.0001
Gestational age	38	(37–39)	29	(26–32)	37	(36–38)	0.0001
Systolic BP	110	(101–118	155	(147–165)	148	(143–156)	0.0001
Diastolic BP	68	(62–72)	101	(94–107)	97	(93–102)	0.0001
Gravidity	2	(1–3)	2	(1–3)	2	(1–3)	0.0459
Paritity	1	(0–2)	1	(0–2)	1	(0–2)	0.9578
Maternal weight	68.5	(60–81)	74.6	(67–92)	74.3	(65–91)	0.0001

Table 3. Showing variables among participants.

	Normotensive (*n*= 246)		Early-onset PE (*n* = 188)		Late-onset PE (*n* = 166)			
	n	%	*n*	%	*n*	%	Total	*p* Value
Age group								
<20 yrs	50	20.3%	19	10.1%	35	21.1%	104	<0.001
21–24	69	28.0%	30	16.0%	35	21.1%	134	
25–41	127	51.6%	139	73.9%	96	57.8%	362	
GEST ages GP								
< = 33 wks	2	0.8%	188	100.0%	0	0.0%	190	<0.001
34–42 wks	244	99.2%	0	0.0%	166	100.0%	410	
Urine protein								
0	245	99.6%	0	0.0%	0	0.0%	245	0.1
1	1	0.4%	111	59.0%	110	66.3%	222	
2	0	0.0%	49	26.1%	42	25.3%	91	
3	0	0.0%	28	14.9%	12	7.2%	40	
4	0	0.0%	0	0.0%	2	1.2%	2	
Sex child								
Male	43	51.8%	12	26.7%	25	43.1%	80	0.5
Female	40	48.2%	16	35.6%	33	56.9%	89	
Total	83	100.0%	28	62.2%	58	100.0%	169	

Table 4. Genotype frequency of rs4588.

	PE total *n* = 340		Normotensive *n* = 242			
RS4588	*N*	%	*N*	%	OR(95%CI)	*p* Value
AA	**4**	**1.1%**	**0**	**0.0%**	**N/A**	**<0.001**
AC	**22**	**6.4%**	**14**	**5.7%**	1.13(0.5–2.4)	
CC	314	92.4%	228	94.2%	0.74(0.35–1.5)	0.18
A	30	4.4%	14	2.9%	1.55(0.8–3.2)	
C	650	95.6%	470	97.1%		
	Total PE HIV **p**ositive ***n*** = 152		Normotensive HIV positive ***n*** = 117			
AA	**0**	**0**	**0**	**0**	**N/A**	
AC	**9**	**5.9%**	**2**	**1.7%**	**3.62(0.7–34.9)**	**0.121**
CC	143	94.1%	115	98.3%	0.28(0.03–1.4)	0.125
A	9	3%	2	1%	3.54(0.7–33.9)	
C	295	97%	232	99%		
	Total PE HIV **n**egative ***n*** = 188		Normotensive HIV **n**egative ***n*** = 125			
AA	4	2.1%	0	0.0%	N/A	<0.001
AC	**13**	**6.9%**	**12**	**9.6%**	**0.70(0.03–1.7)**	
CC	171	91.0%	113	90.4%	1.07(0.4–2.50)	0.667
A	21	6%	12	5%	1.17(0.5–2.7)	
C	335	94%	238	95%		
	Early-onset PE ***n*** = 187		Normotensive ***n*** = 246			
AA	**0**	**0**	**0**	**0**	**N/A**	
AC	**12**	**6.4%**	**14**	**5.6%**	1.12(0.5–2.7)	**0.79**
CC	175	93.1%	228	92.6%	0.90(0.4–2.2)	0.789
A	12	3.2%	14	2.9%	1.17(0.5–2.7)	
C	362	96.8%	470	97.1%		
	Late-onset PE ***n*** = 153		Normotensive ***n*** = 246			
AA	4	2.6%	0	0.0%	N/A	0.045
AC	**10**	**6.5%**	**14**	**5.8%**	**1.14(0.4–2.8)**	
CC	139	90.8%	228	92.6%	0.61(0.3–1.4)	0.038
A	18	5.9%	14	2.1%	2.10(0.9–4.6)	
C	288	94.1%	470	97.1%		
	Early-onset PE ***n*** = 187		Late-onset PE ***n*** = 153			
AA	0	0.0%	4	2.6%	N/A	0.109
AC	**12**	**6.4%**	**10**	**6.5%**	**0.98(0.4–2.6)**	
CC	175	93.6%	139	90.8%	1.47(0.6–0.6)	0.091
A	12	3%	18	6%	2.10(0.9–4.6)	
C	362	97%	288	94%		

genotype GG was not observed within the study population. The frequency of the GT genotype was higher between the PE *vs* normotensive group (4.9% *vs* 0.8%), with an OD ratio of 6.27 (95% CI 1.4–53.6; $p = 0.004$). A significant difference was noted between the EOPE compared to the normotensive group. The GT genotype occurred at a higher frequency (8.6% *vs* 0.8%) with an OD of 11.48 (95% CI 2.6–103.7; $p < 0.001$). Similarly, the G allele was significantly higher in EOPE compared to normotensive (4.3% *vs* 0.4%) with an OD ratio of 11.01 (95% CI2.6–99.1; $p < 0.001$; Table 5).

The GT genotype was likewise predominant in the EOPE compared to the LOPE group (8.6% *vs* 0.6%) with an OD ratio of 15.15 (95% CI 2.3–639.2; $p < 0.001$),

Table 5. Genotype frequency of rs7041.

	PE total *n* = 348		Normotensive *n* = 246			
rs7041	N	%	*n*	%	OR (95%CI)	*p* Value
GT	17	4.9%	2	0.8%	6.27(1.4–56.3)	0,004
TT	331	95.1%	244	99.2%	0.16(0.02–0.68)	0.005
G	17	2%	2	0.4%	6.13(1.4–54.9)	
T	679	98%	490	100%		
	Total PE HIV **p**ositive ***n*** = 153		Normotensive HIV **p**ositive ***n*** = 117			
GT	7	4.6%	0	0%	N/A	0.02
TT	146	95.4%	117	100%	N/A	0.02
G	7	2%	0	0%	N/A	
T	299	98%	234	100%		
	Total PE HIV **n**egative ***n*** = 195		Normotensive HIV **n**egative ***n*** = 129			
GT	10	5.1%	2	1.6%	3.43(0.7–32.6)	0,134
TT	185	94.9%	127	98.4%	0.29(0.03–1.4)	0.098
G	10	2.6%	2	1%	3.37(0.7–31.8)	
T	380	97.4%	256	99%		
	Early-onset PE ***n*** = 186		Normotensive ***n*** = 246			
GT	16	8.6%	2	0.8%	**11.48(2.6–103.7)**	**<0.001**
TT	170	91.4%	244	99.2%	**0.09(0.01–0.38)**	**<0.001**
G	16	4.3%	2	0.4%	**11.01(2.6–99.1)**	
T	356	95.7%	490	99.6%		
	Late-onset PE ***n***= 162		Normotensive ***n*** = 246			
GT	1	0.6%	2	0.8%	0.76(0.01–14.7)	0,821
TT	161	99.4%	244	99.2%	1.32(0.07–78.3)	0.9
G	1	0.3%	2	0.4%	0.76(0.01–14.6)	
T	323	99.7%	490	99.6%		
	Early-onset PE ***n*** = 186		Late-onset PE ***n*** = 162			
GT	16	8.6%	1	0.6%	**15.15(2.3–639.2)**	**<0.001**
TT	170	91.4%	161	99.4%	**0.07(0.01–0.4)**	**<0.001**
G	16	4%	1	0%	**14.52(2.2–610)**	
T	356	96%	323	100%		

while the G allele occurred in 4% of the EOPE population compared to 0% in the LOPE group (Table 5).

Discussion

Summary

The demographic data of study participants were assessed with patients' recorded HIV status (either HIV positive or HIV negative). There was no association between HIV status and PE among the study participants.

Distinguishing between EOPE and LOPE is a modern concept which is becoming an improved indicator of disease incidence. In our study, the incidence of EOPE was greater compared to LOPE (31.3% vs 27.6%). Clinical characteristics like systolic and diastolic blood pressure were significantly higher in the EOPE group suggesting increased maternal total vascular resistance which strengthens abnormal placentation as the probable cause for early-onset subtype.

In our study, we assessed the genetic variability of the VDBP gene in PE and normotensive pregnant women. The GC region (rs4588 and rs7041) was of interest due to prior polymorphisms being associated with vitamin D deficiency among the African American population (16).

Our results showed that the frequency of the genotypes of rs4588 was greater in PE compared to normotensive irrespective of HIV status ($p < 0.001$) in an African population of Zulu origin. Interestingly, for rs4588 we report that the CC and the AC genotypes occurred at a higher frequency in PE compared to the normotensive group ($p < 0.001$). Our results suggest an association with PE despite small numbers; however, these results need to be validated in a larger cohort.

Strengths and limitations

Major strengths of our study included a large number of African cases of PE. Previous studies did not report on the prevalence of these SNPs in South Africa, our study was the first to perform this. This study forms the basis of other research avenues investigating PE development with regard to the role of vitamin D pathways during pregnancy.

Vitamin D supplementation, diet, and sunlight exposure that influence the amount of pre-vitamin D in the body were not measured. Our findings may not be generalizable to other racial groups. Our study also lacked data on fetal genotype and the ability to study maternal-fetal genotype interaction, which may be important to adverse birth outcomes.

Comparison with existing literature

In our study, the difference between HIV infections among normotensive vs EOPE vs LOPE was minimal (47.6% vs 45.2% vs 41.). Kalumba et al. 2013 described a lower rate of HIV-infection among women with pre-eclampsia when

compared with women without pre-eclampsia in South Africa. Our finding is corroborated by a study conducted by Wimalasundera et al. (2002) which showed that the rate of PE in HIV-infected women was not different from that in uninfected pregnant women (4.2% vs. 5.6%, respectively). Several studies show no difference in the rates of PE between HIV-uninfected and HIV-infected women (30,31). Prevalence data on HIV and PE are scarce, and other studies have shown that HIV-infected pregnant women receiving HAART were more prone to developing PE. However, we did not assess the treatment regimen of patients. The role of HIV infection in the development of PE is controversial. A study conducted in the US showed that the rate of PE remained relatively stable regardless of receiving HAART during pregnancy (32). However, other studies have reported that HIV-infected pregnant women receiving HAART have an increased risk of PE with adverse fetal outcomes (33,34). Nonetheless, due to the complexity of PE and the immunological aspects of HIV, further studies should be considered to assess immune characteristics among HIV-infected women receiving HAART with and without PE.

Our study concentrated on the known characteristics associated with EOPE and LOPE, other factors previously considered include birth weight, placental characteristics and umbilical-cord vessel morphology (35–37). Both subtypes of PE enhance inflammatory responses resulting in oxidative stress on vessels and tissues. Both umbilical-cord and fetal vasculature share the same embryonic origin which are derived from intra- and extra-embryonic mesoderm layers; the umbilical-cord vessels can be used as a model to investigate non-accessible fetal vessels to reflect new-born vascular health (36). These factors can be considered for new studies that evaluate early features of disturbed cardiovascular development in the new-borns.

The age distribution in our study showed a similar pattern to previous findings that hypertensive complications of pregnancy are also common among females younger than 20 years (38,39). The median age of EOPE was 30 years which were more mature than LOPE 26 years ($p < 0.001$).

Powe et al., 2013 observed the prevalence of the rs4588 and rs7041 SNP among African and White Americans. In our study, genotypes of rs7041 showed that GT, TT, and alleles G and T were predominant among the HIV positive PE vs normotensive HIV positive group. We report that GT, TT genotypes and G and T alleles occurred at a higher frequency in EOPE compared to LOPE ($p < 0.001$). African Americans carried the T allele of rs7041 at a higher frequency; in addition, this allele was associated with decreased levels of 25-hydroxyvitamin D levels (16). These results corroborate our findings with regards to the frequency of the T allele (rs7041), in participants of African ethnicity.

The primary carrier of vitamin D is VDBP as it is responsible for 85-90% of total circulating 25-hydroxyvitamin D. Genetic polymorphism in the VDBP produce variant proteins that differ in the affinity for VD resulting in vitamin D deficiency (40). Cell culture studies have observed other functions of VD receptor (VDR) that influence the development of PE by maintaining an inflammatory response, promoting placental maladaptation and by endothelial repair (41–43). Cell culture studies reported that, through a VDR-mediated mechanisms, $1,25(OH)_2D_3$ suppresses renin transcription, which is a vital regulator of blood pressure (44). Poor GC -25(OH)D binding reduces the serum concentration of 25(OH)D and other vitamin D metabolites; therefore, genetic variation in the gene may increase the risk of PE development via vitamin D deficiency (45). In our study, we genotyped common SNPs in the *GC* region encoding.

To date, there is a paucity of data that correlate vitamin-D-related genes with risk of PE development. Gene coverage has been limited to only three variants in *VDR* (20,21). Our study focused on the GC region coded by rs4588 and rs7041; these SNPs were studied for their specific role in vitamin D deficiency. Therefore, our study is novel as we report the prevalence of these SNPs in relation to PE development. In pregnancy, vitamin D deficiency will lead to high blood pressure. Recently, Wang et al. 2010 reported that polymorphisms in the GC gene influence vitamin D status and genetic variation identifies individuals of European descent who have substantially elevated risk of vitamin D insufficiency.

Implications of research and practice

The results of our study advocate that a higher frequency of CC genotype (rs4588) and the GT (rs741) associates with PE development. Our results show that the PE population has a higher prevalence to one of the genotypes in each SNP examined, i.e., rs7041, TT genotype was prevalent among the PE arm vs the normotensive arm and rs4588 the CC genotype was prevalent among the PE group vs normotensive group, irrespective of HIV status. These results are corroborated by Speeckaert and Fu L et al. who showed that the T allele was more common than the G allele in an African American population (46,47). In fact, there is evidence corroborating an unequal distribution of these polymorphisms in different populations (23). Therefore, studies examining the possible association between VDR polymorphisms and hypertensive disorders of pregnancy are warranted in other ethnic groups.

Conclusion

In conclusion, our findings indicate that genetic polymorphisms may exert effects in pregnancy, and therefore may have an association with PE development. Further research is required to validate these SNPs, and we would need to identify the functionality of *GC* SNPs, to test interactions and associations between 25 (OH) D and these allelic variants, and to identify if SNPs in other vitamin-D-related genes have a relationship with PE risk. Our findings suggest the polymorphisms examined here may be responsible for hypertensive disorders of pregnancy. There is, however variable prevalence of PE across different countries, and these differences could reflect genetic variances among those living in different geographic regions.

Future considerations

Future research should focus on larger pregnancy cohorts with racial grouping, separated by clinical subtype to advance the field. Quantifying vitamin D metabolites and PE biomarkers along with 25(OH) D longitudinally during gestation will help to elucidate pathways linking vitamin D subsets of PE that may be receptive to vitamin D treatment.

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Competing of interest

The authors declare they have no competing of interests.

Disclosure statement

No potential conflict of interest was reported by the authors.

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