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# Maternal Variants within the Apolipoprotein L1 gene are Associated with Preeclampsia in a South African Cohort of African Ancestry

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## Abstract

**Objective:** Preeclampsia (PE) is a complex pregnancy-specific medical disorder arising from an ischaemic placenta releasing factors causing widespread endothelial damage involving multiple organs systems, such as the renal system. Two variant alleles, termed G1 and G2, of the *APOL1* gene are strongly associated with progressive renal disease and preeclampsia in the recessive or compound heterozygous state. Hence, we investigated the role of maternal APOL1 genotype in the pathogenesis of preeclampsia in South African women of African ancestry.

**Study design:** This case-control study comprised three groups of South African pregnant women of African ancestry attending a regional hospital in Durban, South Africa: mothers experiencing normotensive pregnancies, early onset preeclampsia and late onset preeclampsia

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Declarations of Interest

None

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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underwent APOL1 genotyping. Differences in G1 and G2 allele and genotype frequencies were analysed for the three groups

**Results:** Our study revealed a significant association between the maternal A*POL1* G1 risk allele and early-onset PE development (OR 2.2, p=0.03). Among the EOPE group, 5% [OR(95%CI) 0.94 (0.29–3.12)] of the study population carried two risk alleles, 49% [OR(95%CI) 1.34 (0.77–2.3)] carried at least one risk allele, while 46% of the participants did not carry either risk allele, compared to the normotensive pregnant group, where 52% carried no risk allele, 42% had at least one risk allele and 6% of the women had both risk alleles.

**Conclusion:** Our results suggest that maternal *APOL1* G1 risk allele may contribute to the development of early-onset PE in South African pregnant women of African ancestry either directly or by transmission of a *APOL1* risk allele to the foetus.

### Keywords

Apolipoprotein L1; *APOL1* G1; *APOL1* G2; preeclampsia; South Africa

## 1. Background

Preeclampsia (PE) is a complex human pregnancy specific disorder which accounts for substantial maternal and neonatal morbidity and mortality world-wide [1–2]. The high morbidity and mortality rates are mainly due to the fact that the exact cause of PE is not known making the disorder difficult to predict, identify women that are at risk and manage clinically, once the diagnosis is made. The only cure at the moment is delivery of the foetus and placenta.

Much however is known of the pathophysiology, as PE is thought to be a two stage disease. During the first stage of the disorder inadequate trophoblast invasion of the uterine spiral arterioles (spiral artery maladaptation) leads to a reduction of blood supply to the foeto-placental unit that causes a hypoxic microenvironment [2–3]. The resultant ischaemia leads to the second stage in which there is a release of factors such as apoptotic debris, exosomes, increased production of oxidation radicals, pro-inflammatory cytokines and an imbalance in the angiogenic-antiangiogenic ratio that leads to widespread endothelial damage involving multiple organ systems and resulting in the preeclampsia eclampsia syndrome. The common clinical signs of PE are hypertension, proteinuria, thrombocytopenia, abnormal liver enzymes, renal dysfunction and seizures.

Risk factors of PE include first pregnancy, limited sperm exposure, multifetal pregnancy, previous PE as well as a family history of hypertension [4–8]. A family history of PE is a major risk factor [9].

### 1.1 Apolipoprotein L1 gene polymorphism

Coding variants in the *APOL1* gene, encoding apolipoprotein L1, is prevalent throughout sub-Saharan Africa [10–11]. Although a single *APOL*1 variant is sufficient to confer protection against human African trypanosomiasis, the presence of two risk alleles is associated with renal disease. The G1 and G2 risk variants show a recessive mode

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of inheritance [10,12]. The G1 allele comprises two SNPs [rs73885319; p.S342G and rs60910145; p.I384M] that are in near absolute linkage disequilibrium with each other, but the rs73885319 SNP is sufficient to define the G1 allele [12]. The G2 [rs71785313; p.N388del:Y389del] is a six base pair in-frame deletion resulting in the loss of two amino acids. *APOL1* variants have a strong association with non-diabetic end stage renal disease (ESRD), focal segmental glomerular sclerosis (FSGS), and HIV-1 associated nephropathy (HIVAN) [10,12,13,14].

### 1.2 Study rationale: Apolipoprotein L1 risk variants and preeclampsia

Lan *et al.* (2014) reported that coding variants in the *APOL1* gene [termed G1 and G2] can induce podocyte damage in the form of necrosis and cellular swelling, an occurrence also evident in PE [15–16]. Moreover, impaired renal function is a feature of most cases of PE and there is propensity to a greater risk of developing chronic disease, albuminuria, ESRD and FSGS [16]. Furthermore, increased expression of *APOL1* induced apoptosis and autophagy occurs in FSGS [17]. Recently, Bruggeman *et al.,* (2016) found in a mouse transgenic model that foetal pups expressing either ancestral APOL1 (G0) or variant APOL1-G2 caused a severe form of PE. This was characterised by pregnancy failure in the form of loss of pups and small litter sizes. Additionally, these mice had significant elevations in blood pressure and a large percentage of cases of eclampsia was observed among founder females [18]. Carriage of two APOL1 variant alleles by the foetus, but not the mother, was reported to increase maternal risk of PE by 2-fold in two independent study groups of African Americans [19]. Since PE affects multiple organ systems, including the kidney [20], we investigated the role of maternal APOL1 genotype in the pathogenesis of PE in South African women of African ancestry.

## 2. Methods and Materials

### 2.1 Study population

This case-control study comprised 428 South African pregnant women of African ancestry attending a regional hospital in Durban, South Africa. All study participants signed informed consent and were grouped as normotensive controls (n=184), early-onset preeclampsia (EOPE) (n=149) and late-onset preeclampsia (LOPE) (n=95). Preeclampsia was defined as a systolic blood pressure of ≥ 140mmHg and or a diastolic blood pressure of 90mmHg or greater taken on two occasions at least 4 hours apart in a previously normotensive women and proteinuria (+1 on the urine dipstick analysis or >300mg urine protein concentration in a 24 hour urine specimen). Early-onset preeclampsia was the manifestation of clinical signs prior to 33 weeks ± 6 days of gestation, while late-onset preeclampsia was the onset of signs at ≥ 34 weeks gestation. The exclusion criteria included women with a history of substance abuse and any medical disorder.

Ethics approval was granted by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN) (BCA338/17). This study made use of previously stored samples, for which gatekeeper permission was obtained. DNA isolation from whole blood samples was performed whilst all molecular analysis was completed at

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the Molecular Genetics Epidemiology Section, Frederick National Laboratory for Cancer Research, National Institute of Health (NIH), USA.

### 2.2 Genotyping

Genomic DNA was extracted from whole blood using the Thermo Scientific GeneJET Whole Blood Genomic DNA Purification mini-kit (as per instructions) and stored at −20°C. Three APOL1 SNPs viz., G1: rs73885319A>G (S342G) and G2 rs71785313insertion/deletion (N388Y389/-) were genotyped using Taqman and rs60910145 was genotyped for quality control according to manufacturer's protocol. (Applied Biosystems by Thermo Fisher Scientific: rs73885319; Assay ID-AH20SD1, rs60910145; Assay ID-AHWR1JA and RS71785313; Assay ID-AH1RT7T ) The absence of both the G1 and G2 variant allele defined the ancestral (common) haplotype (G0).

### 2.3 Statistical analysis

The genotype data obtained was analysed using Stata 13 (Stat_CorpLP, College Station, USA), Microsoft Excel (2016) and Graphpad Prism 5.00 for Windows (GraphPad Software, San Diego California USA). To determine statistical significance for the clinical characteristics of the study population a Mann-Whitney test was performed. The Hardy-Weinberg equation (HWE) was used to test for distortions from HWE expectations between groups. Because the study lacked the power to test the association of the infrequent G1 and G2 alleles in the recessive model, we tested for association with EOPE and LOPE using a dominant genetic model for the independent effects of G1 and G2 and for the haplotype model (1 or 2 risk alleles versus 0 risk alleles). A Chi square test was used to compare participants with one or two risk alleles (G1 and or G2) to those with 0 risk alleles. The Fisher's Exact test was also used for the recessive model comparing participants with 2 risk alleles (G1/G1, G2/G2, or G1/G2) genotypes to those of carrying 1 or 0 risk alleles for PE onset and severity. Statistical significance was considered at $p < 0.05$.

## 3. Results

### 3.1 Clinical characteristics

Patient demographics of the study population included maternal age, weight, systolic and diastolic blood pressure, and gestational age (Table 1). Women experiencing EOPE (30 years) were older than either the LOPE group (26 years) or the normotensive group (26 years) (p<0.0001). Women with EOPE (77kg) were heavier than normotensive controls (72 kg) (p<0.0001). Gestational age at delivery was significantly younger for neonates born to EOPE women (28.7 weeks) compared to either LOPE (37.1 weeks) or normotensive women (38.2 weeks) (P<0.0001). Systolic and diastolic blood pressures of the EOPE (158.9 /101.8 mmHg) and LOPE groups (152.5/ 98.0 mmHg) were higher than the normotensive controls (110/67.4mmHg) (p<0.0001).

The initial group of participants comprised 184 normotensive controls and 244 preeclamptics (Table 1). Genotypes were obtained for 99/184 (53%) of the control groups, 119/149 (80%) of the EOPE group and 55/95 (58%) of the LOPE group. The observed proportions of G1 and G2 genotypes are shown in Table 2. The control group allele

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frequency was 12% for the G1 allele and 15% for the G2 allele; very few homozygotes were observed for either G1 or G2.

### 3.2 Genetic Associations in Preeclampsia

Hardy-Weinberg equilibrium tests revealed that the observed frequencies of APOL1 G1 and G2 genotypes in all pregnancy types were not statistically different from expected ($p \geq 0.07$). Without accounting for the presence or absence of a risk allele on the alternative chromosome, we noted an association with the G1 allele with a greater risk of having a pregnancy complicated with early-onset preeclampsia (OR 1.88, p=0.04). There were too few homozygous individuals to test the recessive genetic model. No significant associations were observed for the G2 allele (Table 2).

### 3.3 Haplotype Analysis in Preeclampsia

The data was stratified to include only cases and controls that lacked the G2 allele or the G1 allele to avoid confounding by the alternative risk allele that might be present on the other chromosome. In the haplotype analysis, comparing G1/G0 and G1/G1 to G0/G0, we observed a 2-fold increased risk of PE in women carrying at least one G1 allele (OR 2.2, p=0.03). No significant associations were observed for the independent effects of the G2 allele (Table 3). Both the allele genotype and the stratified haplotype analysis revealed that carriage of the G1 allele was associated with greater risk of EOPE. Interestingly, G1 was associated with a non-significant trend to decreased risk of LOPE (OR<5; p>0.05) in both the G1 genotype and haplotype analyses. There were no significant associations between G1 and LOPE risk or between G2 and early or late onset PE risk. The number of risk alleles was counted for G1 and G2 carried by each woman and compared carriage of one (G1/G0 or G2/G0) or two risk alleles (G1/G1, G1/G2, or G2/G2) to carriage of 0 (G0/G0) risk alleles (Table 3). No significant associations were observed with either early or late onset preeclampsia.

## 4. Discussion

To our knowledge, this is the first study to investigate the relationship between maternal *APOL1* G1 and G2 risk variants in the pathogenesis of PE in a South African group of African ancestry. Our study, comprising 273 PE cases and controls with APOL1 genotypes, revealed a significant association between the maternal A*POL1* G1 risk allele and EOPE. We found no other significant associations with carriage of G2 for EOPE or LOPE nor did we observe an association between G1 and LOPE. We also observed no associations between carriage of high risk APOL1 genotypes, defined as carriage of two risk alleles, and PE. Although not statistically significant, the G1 risk allele was under-represented in women with LOPE. Among the EOPE group, 5% of the study population carried two risk alleles compared to only 2% of the LOPE group and 6% in the normotensive controls, suggesting that APOL1 renal risk allele might be specifically associated with early-onset PE.

The highest frequencies of the *APOL1* risk alleles have been documented in West African nations. Ulasi *et al.* (2013) reported a high frequency of the two *APOL1* risk alleles in the general population of Igbo, Nigeria (23.3%), while the frequency of the two risk alleles

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among patients with chronic kidney disease was found to be 66% [21]. Kasembeli *et al.* (2015) genotyped individuals of African ancestry residing in Johannesburg, South Africa. They reported that in the general population, the G1 and G2 combined allele frequency is 18.4%, with 32% and 2% carrying one and two risk alleles in the general population, respectively. If maternal carriage of 1 or 2 *APOL1* risk alleles is associated with increased risk of early preeclampsia, this would have considerable impact on maternal and foetal morbidity and mortality in sub-Saharan African.

Our results are in keeping with a prior study of 93 pregnancies complicated by PE and 793 healthy normotensive pregnancies, which reported a significant association between foetal, but not maternal, *APOL1* risk genotypes and PE development [19]. The mother of a compound heterozygous or homozygous foetus would be an obligate carrier of at least one transmitted *APOL1* risk allele. Reidy *et al.,* (2018) studied maternal and foetal APOL1 genotypes, as well as the levels of soluble fms-like tyrosine kinase1 (sFlt-1), placental growth factor (PlGF) and endoglin (serum biomarkers of PE) levels, in an African American cohort living in two distinct geographical regions of the United States. These authors found that carriage of two risk alleles by the foetus was associated with a nearly 2-fold increased risk for PE [19]. They also found that foetal high risk genotypes in PE was associated with higher serum sFlt 1 and PIGF levels at birth. A prior study by Robertson *et al.* (2017), focused on prematurity, did not detect either maternal or foetal *APOL1* associations with a composite outcome of gestational hypertension, PE and eclampsia, possibly because of differences in study design and phenotype definition [11]. A two-hit model for APOL1-associated kidney disease has been proposed where APOL1 renal damage occurs only when APOL1 levels reach a critical threshold since most individuals carrying two risk alleles do not develop kidney disease. It is plausible to assume that the presence of the APOL1 G1 and G2 risk variants together with other stressors, including socioeconomic and environmental factors, play a role in gestational complications, such as preeclampsia [11].

A role for APOL1 in the development of PE is consistent with other studies. Bruggeman *et al.* (2016) reported that transgenic mice expressing either the transgene G2 or G0 develop PE. Both G2 and G0 transgenic dams and wildtype dams of foetal transgenic G2 and G0 mice developed PE, possibly because of maternal-foetal antigenic incompatibility. Interestingly, it is postulated that APOL1 has a placental function seeing as both G2 and G0 mice developed PE [18]. It is also reported that the APOL1 renal risk allele G2 compared G0 caused a more severe form of the disease than the reference protein G0, which does not harbour renal risk alleles, possibly due to over-expression of the G0 transgene or foetal-maternal mismatch since mice do not carry an APOL1 ortholog [18]. They further went on to study podocyte function of G0 and G2 in the presence of a known stressor, i.e. HIV, also using a transgenic mouse model [13]. It was discovered that podocyte hypotrophy, a compensatory mechanism to stress, was absent in mice co-expressing HIV and G2 [13]. It is plausible that proteinuria, characteristic of PE, may arise via this mechanism of podocyte damage.

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## 5. Study Limitations

Our study group was composed of only pregnant women, so we were unable to determine if foetal APOL1 genotype contributed to the risk of PE. We also had limited statistical power to detect associations because of the small sample size and the low frequency of the *APOL1* risk variants in our cohort, particularly after grouping participants by early- or late-onset preeclampsia. However, despite our small sample size we were able to observe significant association between carriage of at least one maternal G1 allele with PE. We were unable to detect associations with carriage of two risk alleles because of the very limited number of homozygotes.

## 6. Future Research

We suggest that future studies to assess the role of *APOL1* include mother-foetal pairs with sufficient power to determine the relative contribution of maternal or foetal APOL1 genotype in the development of PE. Longitudinal studies to investigate the long-term impact of *APOL1* foetal and maternal genotypes on maternal and child renal health in pregnancies complicated by PE should be considered.

## 7. Conclusion

This study suggests that maternal *APOL1* G1 risk allele may contribute to the development of early-onset preeclampsia in South African women of African ancestry either directly or by transmission of a *APOL1* G1 risk allele to the foetus. This may be used as a screening tool in clinical practice to identify at risk women and hence, provide adequate antenatal care.

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## Highlights

- Maternal *APOL1* G1 contributes to early-onset preeclampsia in South African women
- There is a 2-fold increased risk of PE in women carrying at least one G1 allele
- This may be used a screening tool in clinical practice

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**Table 1.**

Participant characteristics in early-onset preeclampsia, late-onset preeclampsia, and normotensive pregnant controls groups.

| | **Early-onset preeclampsia cases (n=149)** | **Late-onset preeclampsia Cases (n=95)** | **Normotensive controls (N=184)** | **Controls *vs.* EOPE *p* value** | **Controls *vs.* LOPE *p* value** | **EOPE *vs.* LOPE *p* value** |
|---|---|---|---|---|---|---|
| Maternal Age, years | 30,09 (±6,61) | 26,10 (±6,49) | 26,01(±6,32) | < 0.0001 | 0.97 | < 0.0001 |
| Maternal Weight, kg | 77,38 (±17.06) | 75,99 (±20,43) | 71,75(±16,14) | 0,01 | 0.23 | 0.36 |
| Systolic BP, mmHg | 158,9 (±15.18) | 152,5(±17.03) | 110 (±13.09) | < 0.0001 | < 0.0001 | 0.003 |
| Diastolic BP, mmHg | 101,8(±9,59) | 98,01(±10,11) | 67,43(±8,55) | < 0.0001 | < 0.0001 | 0.03 |
| Gestational Age at Delivery, weeks | 28,71(±3,86) | 37,08(±2,11) | 38.21(±1,61) | < 0.0001 | < 0.0001 | < 0.0001 |

Notes: EOPE, early onset preeclampsia; LOPE, late onset preeclampsia; normotensive pregnant; all values expressed as mean and standard deviation.

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**Table 2.**

Genotype frequencies and odds ratios for maternal *APOL1* G1 and G2 associations with preeclampsia.

| | Normotensive pregnancy Controls | Early + Late-onset preeclampsia | | Early-onset preeclampsia (EOPE) | | Late-onset preeclampsia (LOPE) | |
|---|---|---|---|---|---|---|---|
| **G1: rs7388319A>G** | **Genotype N(%)** | **Genotype N(%)** | **Controls vs All OR (95%CI)** | **Genotype N(%)** | **Controls vs EOPE OR (95%%CI)** | **Genoty pe N(%)** | **Controls vs LOPE OR (95%CI)** |
| **AA** | 76 (78%) | 126 (72%) | 1 (ref) | 77 (65%) | 1 (ref) | 49 (89%) | 1 (ref) |
| **AG** | 21 (21%) | 47 (27%) | 1.35 (0.75 to 2.43) | 41 (34%) | 1.93 (1.04–3.56) * | 6 (11%) | 0.43 (0.17–1.76) |
| **GG** | 1 (1%) | 1 (.6%) | 0.60 (0.04–9.79) | 1 (1%) | 0.99 (0.06–16.07) | 0 | 0.52 (0.02–12.9) |
| **Dominant Model** *1* | — | — | 1.29 (0.72–2.31) | — | 1.88 (1.02–3.45) * | — | 0.43 (0.16–1.12) |
| **Allele** *2* | **Allele N(%)** | **Allele N(%)** | — | **Allele N(%)** | — | **Allele N(%)** | — |
| **A** | 173 (88%) | 299(86%) | 1 (ref) | 195 (82%) | 1 (ref) | 104 (95%) | 1 (ref) |
| **G** | 23 (12%) | 49 (14% | 1.23 (0.73–2.09) | 43 (18%) | 1.6 (0.96–2.86) | 6 (5%) | 0.43 (0.17–1.10) |
| **G2: rs71785313 I>D** | **N(%)** | **N(%)** | **OR (95%CI)** | **N(%)** | **OR (95%CI)** | **N(%)** | **OR (95%CI)** |
| **II** | 70 (71%) | 128 (75%) | 1 (ref) | 92 (78%) | 1 (ref) | 36 (68%) | 1 (ref) |
| **I/D** | 28 (28%) | 42 (25%) | 0.82 (0.47 to 1.44) | 26 (22%) | 0.71 (0.38–1.31) | 16 (30%) | 1.11 (0.53–2.31) |
| **DD** | 1 (1%) | **1 (0.5%)** | 0.55 (0.03–8.88) | 0 (0%) | 0.25 (0.01–6.33) | 1 (2%) | 1.94 (0.1232.00) |
| **Dominant Model** *1* | — | — | 0.54 (0.47–1.41) | — | 0.68 (0.37–1.26) | | 1.14 (0.55–2.34) |
| **Allele model** *2* | Allele N(%) | | | Allele N(%) | | Allele N(%) | |
| **I** | 168 (85%) | 298(87%) | 1 (ref) | 210 (89%) | 1 (ref) | 88 (83%) | 1 (ref) |
| **D** | 30 (15%) | 44(13%) | 0.83 (0.50–1.36) | 26 (11%) | 0.69 (0.40–1.22) | 18 (17%) | 1.15 (0.60–2.17) |

Notes:

* p≤0.04;

*1* dominant model, comparing GG+AG vs AA or DD+DI vs II, with the variant genotypes being the explanatory variable;

*2* Number of chromosomes carrying reference or variant G1 or G2 allele. Odds ratios do not account for the presence of an alternative explanatory variable on the opposing chromosome.

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**Table 3.**

Stratified analysis of the independent odds ratios for maternal APOL1 G1 and G2 risk alleles association with preeclampsia.

| | | Controls vs Early-onset preeclampsia (EOPE) | | Controls vs Late-onset preeclampsia (LOPE) | |
|---|---|---|---|---|---|
| **G1 stratum** | **Normotensive controls N (%)** | **EOPE N(%)** | **OR (95% CI)** | **LOPE N(%)** | **OR (95% CI)** |
| **G0/G0** | 51 (75%) | 53 (58%) | 1 (ref) | 30 (88%) | 1 (ref) |
| G1/G0 or G1/G1 | 17 (25%) | 38 (42%) | 2.15 * (1.08–4.28) | 4 (12%) | 0.4 (0.12–1.30) |
| **G2: Stratum** | **Normotensive controls N (%)** | **Early onset N(%)** | **OR (95% CI)** | **Late onset N(%)** | **OR (95% CI)** |
| **G0/G0** | 51 (68%) | 53 (70%) | 1 (ref) | 30 (68%) | 1(ref) |
| **G2/G0 or G2/G2** | 24 (32%) | 23 (30%) | 0.92 (0.46 to 1.84) | 14 (32%) | 0.99 (0.45 to 2.24) |

Note: To account for the confounding presence of a risk allele on the alternative chromosome, the groups are subset to only individuals not carrying 1 or 2 copies of G2 in the G1 stratum and individuals not carrying 1 or 2 copies of G1 in the G2 stratum;

* $p<0.03$.

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**Table 4.**

Odds ratios for maternal APOL1 carriage of 1 or 2 APOL1 risk alleles with preeclampsia.

| | No. G1 or G2 Risk Alleles | Normotensive Controls | Controls *vs.* Early + Late- onset Preeclampsia | Controls *vs.* EOPE | Contro | ls *vs.* LOPE |
|---|---|---|---|---|---|---|
| | N (%) | N (%) | OR (95% CI) | N (%) OR (95% CI) | N (%) | OR (95% CI) |
| 0 | 51 (52%) | 84 (49%) | 1 (ref) | 54 1 (ref) (46%) | 30 (58%) | 1 (ref) |
| 1 | 41 (42%) | 79 (47%) | 1.17 (0.7–1.95) | 58 1.34 (0.77–2.3) (49%) | 21 (40%) | 0.87 (0.43–1.74) |
| 2 | 6 (6%) | 7 (4%) | 0.70 (0.23–2.23) | 6 0.94 (0.29–3.12) (5%) | 1 (2%) | 0.28 (0.03–2.47) |
| 1 or 2 | 47 (48%) | 86 (50%) | 1.11 (0.68–1.82) | 64 1.29 (0.75–2.20) (54%) | 22 (42%) | 0.80 (0.40–1.57) |

Note: EOPE, early onset preeclampsia; LOPE, Late onset preeclampsia; 0 risk alleles are defined as carriage of no G1 or G2 risk alleles (G0/G0); 1 risk allele is defined as G1/G0 or G2/G0; 2 risk alleles are defined as carriage of G1/G1 or G1/G2 or G2/G2.