microRNA-27a rs895819 is associated with obesity in HIV infected preeclamptic Black South African women on HAART

Niren Ray Maharaj, Prithiksha Ramkaran, Siddharthiya Pillay and Anil Amichund Chuturgoon

Abstract

Background: Preeclampsia (PE) and HIV/AIDS present a major health challenge globally. South Africa has the highest disease burden of both HIV/AIDS and PE in the world. Despite extensive research, the pathophysiology of these conditions is not completely understood, however a genetic predisposition in women may affect susceptibility. MiRNA-27a regulates adipogenesis and glucose metabolism. A single nucleotide polymorphism (SNP) in miRNA-27a (rs895819T > C) has shown to have disparate effects in various populations. This study investigated the frequency of rs895819 in pregnant normotensive and preeclamptic Black South African (SA) women.

Methods: Enrollment into the study included: normotensive (n = 95; 45 HIV+; 80 analysed for rs895819T > C, age range: 16–46 years) and PE patients (n = 98; 45 HIV+; 56 analysed for rs895819T > C, age range: 16–42 years). DNA was isolated from peripheral blood mononuclear cells (PBMC). Genotyping of miRNA-27a rs895819 was detected using a TaqMan® SNP Genotyping assay.

Results: We did not find a significant association of miR-27a polymorphism with PE susceptibility in our data. However, in the subgroup analysis (based in HIV status), the variant genotypes (TC/CC) were associated with higher body mass index (BMI) among PE women (32.57 vs. 29.25, p = 0.064), significantly in the presence of HIV infection (33.47 vs. 27.8, p = 0.005).

Conclusion: The results of this study suggests that miR-27a rs895819 may not be associated with PE susceptibility; however, the miR-27a TC/CC genotype increases susceptibility to elevated BMI in PE, which may be significantly influenced by co-morbid HIV infection among pregnant women on HAART.

Keywords: miR-27a, rs895819, Single nucleotide polymorphism, Preeclampsia, Black South African women, HIV, BMI, HAART

Background

Preeclampsia (PE) is a pregnancy-specific multi-organ syndrome recognized by the new onset of hypertension and proteinuria after 20 weeks of gestation [1]. Globally, PE complicates approximately 2–10% of pregnancies and is associated with 10-15% of direct maternal deaths overall [2]. Perinatal complications include premature delivery, intra-uterine growth restriction, hypoxic neurological lesions and foetal death [3]. The overall risk of PE is further increased by obesity [4] and features of the metabolic syndrome (obesity, hypertension, insulin resistance, impaired glucose tolerance, and dyslipidaemia) occur more commonly in women with PE [5]. Furthermore, PE has also been associated with cardiovascular disease in later life [6].

The pathogenic mechanisms underlying PE remain to be elucidated; however, immune maladaptation, inadequate placental development and trophoblast invasion, placental ischaemia, oxidative stress and thrombosis are all thought to represent key factors in the development of disease [7]. All of these components have genetic factors that may be involved in the pathogenesis of PE [7].

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MicroRNAs (miRNAs, miRs) are small endogenous RNAs that post transcriptionally regulate gene expression and have been shown to have important roles in numerous disease processes [8]. Interestingly, many miRNA-regulated pathways are co-incident with pathophysiological processes related to PE. For instance, miRNAs regulate pathways in adipose tissue that control adipogenesis, insulin resistance and inflammation [8], and regulate endothelial cell function and angiogenesis by regulating pro- and anti-angiogenic activity [9]. They have also been shown to regulate vascular integrity in angiogenesis induced by ischemia [10, 11].

More specifically, evidence shows the involvement of miRNA-27a, a member of the miR-23∼27∼24 cluster in the regulation of many of these processes [12]. MiR-27a promotes angiogenesis by targeting the angiogenesis inhibitor SEMA6A, which controls repulsion of neighboring endothelial cells [9]. It plays an anti-adipogenic role by influencing prohibitin and impairing mitochondrial function [13], is associated with angiogenesis in cardiovascular disease, and endothelial apoptosis in cardiac ischemia [14]. Its role in inflammation is demonstrated by enhanced expression of pro-inflammatory cytokines, such as IL-10 when up-regulated in TLR2- or TLR4-activated macrophages [15]. More recently, a knock down of miR-27a, has been shown to down regulate pro-inflammatory cytokines IL-6 and TNF-α, which are associated with PE [16, 17].

Genetic polymorphisms in miRNA have been shown to affect miRNA expression, maturation or mRNA recognition and may represent an important risk determinant of disease susceptibility [18]. The miR-27a single nucleotide polymorphism (SNP), rs895819, is located in the terminal loop of pre-miR-27a [19]. MicroRNA-27a (miR-27a) targets peroxisome proliferator-activated receptor gamma (PPAR-γ) to prevent the terminal differentiation of adipocytes and negatively regulates lipoprotein lipase in adipocytes [20], thus playing a role in lipid homeostasis. When this homeostasis is dysregulated, as may be in the case of this polymorphism, the resulting increase in maternal adiposity has been linked to increased risk of pre-eclampsia in both nulliparous and multiparous women [21]. Therefore, this study investigated the association of rs895819 with obesity (indicated by body mass index) in PE among Black SA women, who have a high prevalence of PE [22]. Due to the associated high rate of co-morbid HIV infection in this population [23], we included HIV infected women on HAART to identify differential associations.

**Methods**

**Study population and sample collection**

Institutional ethical and hospital regulatory permission was obtained for the study (Biomedical Research Ethics Committee, University of KwaZulu-Natal, South Africa; reference number BE 119/11). After informed consent was obtained, participants were recruited over a 14-month period from July 2013 to September 2014 from the maternity unit at Prince Mshiyeni Memorial Hospital in Durban, South Africa. This hospital is a regional level facility and serves a predominantly semi–urban African population from where the participants were recruited. Normotensive \( n = 95 \), (80 analysed for rs895819T > C), age range: 16–46 years and PE patients \( n = 98 \) (56 analysed for rs895819T > C), age range: 16–42 years were enrolled into the study. Maternal venous blood samples were then taken randomly due to concerns about fasting during pregnant state and possible fetal ramifications. To maintain ethnographic and anthropometric consistency, all patients recruited were of African descent, resident in the same geographical location and of Zulu ethnicity. All patients were non-smokers, non-consumers of alcohol or recreational drugs, and all HIV infected patients were on highly active antiretroviral therapy (HAART viz. tenofovir, emtricitabine, efavirenz) as per the National guidelines [24].

Calcium supplementation was administered routinely to all patients attending the clinic. Women with gestational hypertension, renal disease, diabetes mellitus, chronic hypertension and collagen vascular disease were excluded. PE was defined as a blood pressure \( \geq 140 \text{ mmHg systolic or} \geq 90 \text{ mmHg diastolic} \) on 2 occasions at least 4 h apart after 20 weeks of gestation in a woman with previously normal blood pressure [6]. All patients had proteinuria \( \geq 0.3 \) g/day on urine dipstick testing. Data on all patients was obtained from the institution’s maternity case records and laboratory data from the National Health Laboratory Services® computerised database at the institution. HIV was diagnosed on a rapid test kit. BMI was calculated using the standard formula: mass in kg divided by height in m\(^2\). This is currently used in pregnancy in both clinical and research settings. BMI was taken during pregnancy as the participants were not known to the institution prior to pregnancy. Weight was categorised as: normal weight (BMI: 18-<25), overweight (BMI: 25-<35). Early onset preeclampsia was considered as \( \leq 34 \) weeks of gestation (Tranquilli, 2014). Severe preeclampsia was diagnosed when features included any of the following: systolic blood pressure \( \geq 160 \text{ mmHg} \) or diastolic blood pressure \( \geq 110 \text{ mmHg} \); maternal neurological disorders such as persistent headaches and brisk reflexes, eclampsia, acute pulmonary oedema, proteinuria \( \geq 5 \text{ g/day} \), oliguria \( <500 \text{ cc/day} \), creatinine \( >120 \text{ μmol/L} \), features HELLP syndrome and thrombocytopenia \( <100,000/\text{mm}^3 \), foetal criteria including intrauterine growth retardation, oligohydramnios, or foetal death in utero [25, 26].

**DNA extraction & genotyping**

DNA from PBMCs of 56 PE patients and 80 normotensive subjects was extracted using the Quick-gDNA MiniPrep
kit (Zymo Research, catalogue no. D3006) and FlexiGene DNA kit (Qiagen, catalogue no. 51204) as per the manufacturer’s protocol. DNA was quantified using the Nanodrop2000 spectrophotometer. All samples were standardised to a concentration of 10 ng/µL.

All subjects were genotyped for miR-27a rs895819 using a TaqMan® Pre-designed SNP genotyping assay (Life Technologies, catalogue no. 4351379), following the manufacturer’s protocol. A final reaction mixture consisted of 40× TaqMan® Predesigned genotyping assay, 2× TaqMan® Genotyping Master Mix, nuclease-free water, and a 10 ng genomic DNA template. The experiment was performed using the Applied Biosystems® Viia™ 7 Real-Time PCR System.

The TaqMan Predesigned Genotyping Assay contains two primers for amplifying the sequence of interest, and two TaqMan® minor-groove binding (MGB) probes for detecting alleles. The presence of two probe pairs in each reaction allows genotyping of the two possible alleles at the SNP site in a DNA target sequence. The genotyping assay determines the presence or absence of a SNP based on the change in fluorescence of the dyes associated with the probes. Each probe is labelled with a VIC® dye-labelled probe and FAM™ dye-labelled probe - assigned specifically to either the ancestral or variant allele.

Statistical analysis

Statistical analyses were done using GraphPad prism software (version 5.0). The Hardy–Weinberg equilibrium (HWE) was used to test for deviation of allele/genotype frequency. Allele and genotype frequencies were calculated using the Fisher’s exact test and chi square tests respectively, and the Odds ratio and confidence intervals were determined. To assess difference in the clinical parameters (not grouped according to genotype), the t-test with Welch’s correction or one-way ANOVA tests were used. The correlations between the clinical parameters, grouped per genotype were assessed for PE and normotensive patients, which were also further tested under the HIV positive and negative subsets. The t-test with Welch’s correction and Fisher’s exact test were used for these analyses. A p-value < 0.05 was deemed statistically significant.

Table 1 Clinical characteristics of participants

|                  | PE HIV - (n = 29) | PE HIV + (n = 27) | NT HIV - (n = 42) | NT HIV + (n = 38) | p-value | p-value |
|------------------|-------------------|-------------------|-------------------|-------------------|---------|---------|
| Age (mean ± SEM; years) | 25.03 ± 0.9944 | 29.00 ± 1.563 | 25.00 ± 1.027 | 28.00 ± 1.015 | 0.0423* | 0.6641 |
| Age range (years) | 16–36 | 16–41 | 16–42 | 17–46 | | |
| BMI (n, %) | 21 (74) | 22 (81) | 30 (71) | 33 (87) | | |
| BMI (kg/m²) (mean ± SEM) | 30.86 ± 1.636 | 32.70 ± 1.496 | 29.93 ± 1.343 | 30.46 ± 1.410 | 0.4904 | 0.2783 |

Abbreviations: SEM standard error of the mean, PE preeclampsia, NT normotensive, n total number, p is significant at <0.05
*aComparison amongst all four sub-groups
*bComparison between PE and NT
*indicates a significant difference

Results

The clinical characteristics of all study subjects are shown in Table 1. The study cohort was categorised into 4 groups: (1) HIV-uninfected PE women (PE HIV-), (2) HIV-infected PE women (PE HIV+), (3) HIV-uninfected normotensive women (Normo HIV-) and (4) HIV-infected normotensive women (Normo HIV+). All women were in the third trimester of pregnancy and the mean gestational age was 36.5 weeks. The average duration of HAART was 16.6 and 14.5 weeks in the normotensive and PE groups respectively, however this is not a precise duration of exposure. In accordance with national guidelines and institutional guidelines, ARVs viz. tenofovir, emtricitabine and efavirenz, were commenced from 14 weeks of pregnancy or at first available hospital booking. The effect of ARVs on obesity during pregnancy was challenging to investigate by virtue of these guidelines on clinical practice [27].

The genotype and allele frequencies are shown in Table 2. There was no significant difference in the genotype or allele frequencies when compared between all normotensive and all PE women (p = 0.834, p = 0.806 respectively). The genotype distribution was compatible with the Hardy–Weinberg equilibrium in the study sample (p = 0.972; p = 0.882).

Table 3 represents a sub-analysis of the genotype and allele frequencies among women stratified according to HIV status (i.e. negative (−) or positive (+)). No significant differences were noted in the genotype or allele frequencies among all groups. No significant differences were noted between groups (p = 0.834; 0.806) respectively. Further analysis by group comparison also did not show any significant differences.

Table 4 represents the association between the ancestral and variant genotypes with clinical features in all (a) PE women, and (b) according to HIV status. Of note was the significant association of the variant genotype (TC/CC) with body mass index (BMI) in the PE HIV+ group (27.8 ± 0.69 vs. 33.47 ± 1.66; p = 0.0059). Moreover, a similar but non-significant trend of higher BMI values were also noted among PE HIV- women (29.5 ± 1.45 vs 31.7 ± 2.11; p = 0.388), and among all PE women overall (29.2 vs 32.6, p = 0.064), in association with the TC/CC genotype.

Table 5 shows a sub-analysis of the BMI in all the groups. Among normal pregnancies (normotensive), the
variant genotype was associated with a significantly lower BMI (29.0 vs. 34.3, \(p = 0.047\)), which appears unaffected by HIV infection. In contrast, the variant genotype was associated with a higher BMI among all women with PE (\(p = 0.064\)). This however, was significantly higher in PE women with co-morbid HIV infection on HAART (\(p = 0.005\)). Due to policy guidelines, a cohort of HAART naive women was not available to differentiate the impact of HAART.

Figures 1 and 2 show a graphic analysis of BMI grouped per genotype for all PE patients and NT women. The mean BMI in the ancestral group is 29.25 ± 1.084 compared with 32.57 ± 1.344 (\(p = 0.064\)) in the variant types, demonstrating the elevated BMI is associated with the variant genotype. This observation is in contrast to that observed in the normotensive women that showed a lower BMI in relation to the variant genotype (TT: 34.31 ± 2.258; TC/CC: 29.03 ± 1.019; \(p = 0.0473\)).

Figures 3 and 4 demonstrate the BMI and genotype relationship in PE women with and without co-morbid HIV infection. There was a significant difference in the HIV infected PE group compared with the HIV uninfected group which shows a non-significant increase (27.87 ± 0.6960 vs. 33.47 ± 1.666; \(p = 0.0059\) vs. 29.50 ± 1.458 vs. 31.77 ± 2.111; \(p = 0.3882\)). Figure 5 represents the overall BMI grouped per genotype for all subjects in the cohort.

### Discussion

The morbidity and mortality associated with PE and HIV/AIDS remains a global health concern. In developing countries, and South Africa in particular, hypertensive disorders of pregnancy and HIV/AIDS have remained leading causes of mortality despite sustained interventions [28]. Existing research aimed at better understanding the pathophysiology of PE and the associated complications still remains inconclusive. Contemporary and new studies now extend to include both genetic and epigenetic aspects of PE.

In this study, the findings do not show significant differences in the ancestral and variant genotype and allele frequencies among the groups and in relation to the parameters described. However, of relevance is the observation that the variant TC/CC genotype is associated with higher BMI in the PE women studied, in contrast to lower BMI values in normotensive pregnancies. In the normotensive women, the presence of HIV infection is associated with a decrease in BMI in the variant group, however in PE; it is associated with a significant increase among carriers of the variant genotypes. A relationship of susceptibility to increased BMI may therefore exist in women with PE who carry the

### Table 2

| Genotype n (%) | NT (n = 80) | PE (n = 56) | \(p\)-value (Odds ratio; 95% CI) |
|----------------|------------|------------|-------------------------------|
| TT             | 18 (23)    | 13 (23)    | 0.834                         |
| TC             | 41 (51)    | 26 (46)    |                               |
| CC             | 21 (26)    | 17 (31)    |                               |

| Allele n (%) | T          | C          |
|--------------|------------|------------|
| T            | 77 (48)    | 83 (52)    |
| C            | 52 (46)    | 60 (54)    |

HWE \(p\)-value 0.972 0.882

### Table 3

| Genotypes n (%) | NT HIV- (n = 42) | NT HIV+ (n = 38) | PE HIV- (n = 29) | PE HIV+ (n = 27) |
|-----------------|------------------|------------------|------------------|------------------|
| TT              | 8 (19)           | 10 (26)          | 8 (28)           | 5 (19)           |
| TC              | 23 (55)          | 18 (48)          | 11 (38)          | 15 (56)          |
| CC              | 11 (26)          | 10 (26)          | 10 (34)          | 7 (25)           |

| Alleles n (%)   | T          | C          |
|-----------------|------------|------------|
| T               | 39 (46)    | 38 (50)    |
| C               | 45 (54)    | 38 (50)    |

HWE 0.8076 0.9487

Genotypes \(p\)-values

All groups 0.8338 0.9636

PE HIV- vs. PE HIV+ 0.4131

NT HIV- vs. NT HIV+ 0.7113

NT HIV- vs. PE HIV- 0.3737

NT HIV+ vs. PE HIV + 0.7316

Genotypes \(p\)-values

Alleles \(p\)-values (OR; CI)

All groups 1.000 (0.9898; 0.4707–2.082)

PE HIV- vs. PE HIV+ 0.7515 (1.154; 0.6198–2.148)

NT HIV- vs. NT HIV+ 1.0000 (1.005; 0.5137–1.966)

NT HIV- vs. PE HIV- 0.7238 (0.8621; 0.4285–1.734)

Abbreviations: HWE Hardy Weinberg Equilibrium, CI confidence interval, \(p < 0.05\) is statistically significant, T thymine, C cytosine.
variant genotypes. This relationship may be further potentiated by the presence of co-morbid HIV infection (on HAART), through complex differential regulation of miR-27a.

The regulatory activity of miR-27a in adipogenesis has been demonstrated previously [29], the overexpression of miR-27a specifically inhibited adipocyte formation and expression of miR-27a results in blockade of expression of peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer-binding protein (CEBP)-α, the two master regulators of adipogenesis. MiR-27a, has also been shown to inhibit adipogenic differentiation of 3T3-L1 preadipocytes [29]. In animal models, mature adipocytes from obese mice had lower miR-27a expression as compared to lean mice, indicating miR-27a downregulation may be necessary for adipocyte hypertrophy [30]. The regulatory activity of miR-27a in relation to adipogenesis and obesity in PE and HIV infection, is however complex and warrants expansive investigation.

Interestingly, adipose tissue is considered to be hormonally active, producing cytokines [31] that demonstrate the association of obesity with increased inflammation, insulin resistance and oxidative stress [32, 33]. Inflammatory cytokine release from adipose tissue and elevated inflammatory cytokine levels including TNF-α and IL-6 have been associated with obesity [34].

Obesity is a major epidemic in developed countries, and the trend is now extending to developing countries [35]. The prevalence of obese and overweight women (BMI ≥25 kg/m²) in South Africa is estimated to be 69% according to the World Health Organization [36]. Although the relationship of obesity to increase Type 2 diabetes and cardiovascular disease is well recognized, evidence suggests a three-fold increase in the risk of PE associated with obesity [4]. In the United States, it appears that obesity is the leading attributable risk for PE

| Table 4 | Association of rs895819T/C genotypes with clinical parameters in preeclampsia and HIV co-infection |
|---------|------------------------------------------------------------------------------------------------|
| (a) Preeclampsia (n = 56) |                                                                                                      |
| **Variable** | TT vs. TC/CC | p-value |
| BMI (kg/m²) | 29.25 ± 1.084 vs. 32.57 ± 1.344 | 0.0643 |
| EOPE (%) | 31 vs. 42 | 0.5346 |
| Severe PE (%) | 38 vs. 49 | 0.5453 |
| SYS BP (mmHg) | 154.5 ± 3.890 vs. 160.8 ± 2.342 | 0.1810 |
| DIA BP (mmHg) | 100.0 ± 2.614 vs. 105.0 ± 1.692 | 0.1268 |

| (b) | PE HIV- (n = 29) | PE HIV+ (n = 27) |
| **Variable** | TT vs. TC/CC | p-value | TT vs. TC/CC | p-value |
| BMI (kg/m²) | 29.5 ± 1.45 vs. 31.7 ± 2.111 | 0.3882 | 27.9 ± 0.69 vs. 33.47 ± 1.66 | 0.0059* |
| EOPE (%) | 25 vs. 29 | 1.000 | 40 vs. 55 | 0.6483 |
| Severe PE (%) | 38 vs. 38 | 1.000 | 40 vs. 59 | 0.6280 |
| SYS BP (mmHg) | 153 ± 5.91 vs. 161.7 ± 5.1 | 0.2946 | 156 ± 4.986 vs. 163 ± 3.224 | 0.2764 |
| DIA BP (mmHg) | 99 ± 3.51 vs. 102 ± 2.38 | 0.5116 | 102 ± 4.250 vs. 108 ± 2.274 | 0.2507 |

*indicates a significant difference

| Table 5 | Sub-analysis of body mass index and rs895819 genotypes in all groups |
|---------|---------------------------------------------------------------------|
| **Groups** | TT vs. TC/CC | p-value |
| All PE | 29.25 ± 1.084 (8) vs. 32.57 ± 1.344 (34) | 0.0643 |
| PE HIV- | 29.50 ± 1.458 (7) vs. 31.77 ± 2.111 (19) | 0.3882 |
| PE HIV+ | 27.87 ± 0.6960 (3) vs. 33.47 ± 1.666 (19) | 0.0059* |
| All NT | 34.31 ± 2.258 (11) vs. 29.03 ± 1.019 (48) | 0.0473* |
| NT HIV- | 32.14 ± 2.512 (5) vs. 29.48 ± 1.535 (25) | 0.3971 |
| NT HIV+ | 35.51 ± 3.260 (9) vs. 28.56 ± 1.359 (24) | 0.0774 |

*indicates a significant difference

Fig. 1 BMI per genotype for all PE women. The mean BMI in the ancestral group is lower compared with the variant type, demonstrating that elevated BMI is associated with the variant genotype.
[4, 37–39], and in several other populations around the world the relationship of obesity and increased risk of PE has also been reported. Interestingly, increases in BMI in the normal range has also been shown to be associated with an increased risk of PE [4].

The clinical relevance of this study relates to the prevalence of obesity in South Africa, particularly among women and young girls [39, 40] which is progressively increasing [40, 41], however the study is limited by a small sample size, and racial and gender bias which require future large scale population based studies. The exclusion of a HAART naïve group limits comparisons on its effects; however the inclusion of such a group is unethical in current practice. The promotion of weight loss activities in pregnancy is not feasible, therefore effective obesity prevention strategies are needed which incorporate healthy diet and lifestyle messages to those who are at risk for HIV infection and other non-communicable diseases [24] to reduce morbidity and mortality.

Taken together, the study provides an insight into the association of miR-27a and obesity and the association of miR-27a rs895819 polymorphism in relation to BMI, PE and the influence of HIV infection and HAART in pregnant women. Further scientific investigation is required in the long term to unravel the cross regulatory mechanisms that may be involved.
Conclusion
MiR-27a has an important regulatory function in the development of obesity. The functional rs895819 SNP may negatively regulate the adipogenic activity of miR-27a, and possibly increase the susceptibility to obesity in preeclamptic Black South African women on HAART. The data provides new insight into the role of miR-27a polymorphism in the triad of PE, HIV/HAART and obesity, and has potentially important future therapeutic implications. The study limitation for the present study is the small sample size. A follow up study with a larger study cohort may provide further clarification.

Abbreviations
AHT: Antihypertensive drugs; BMI: Body mass index; C: Cytosine; CEBP-α: CCAAT-enhancer-binding protein; CI: Confidence interval; DBP: Diastolic blood pressure; ECLS: Elective caesarean section; EMCS: Emergency caesarean section; EOPE: Early onset preeclampsia; GA: Gestational age; HIV: Hardy–Weinberg equilibrium; MGB: Minor-groove binding; miRNA:mRNA; MOD: Mode of delivery; NORMO: normotensive; NVD: Normal vaginal delivery; OR: Odds ratio; PBMC: Peripheral blood mononuclear cells; PE: Preeclampsia; PPARγ: Peroxisome proliferator-activated receptor gamma; SA: South African; SD: Standard deviation; SNP: Single nucleotide polymorphism; SYBP: Systolic blood pressure; T: Thymine

Acknowledgements
Dr A. Phulukdaree for technical assistance.

Funding
The Medical Education Initiative (Grant number R24TW008863) and College of Health Sciences, University of KwaZulu-Natal.

Availability of data and materials
All data reported in this study is available from Drs NR Maharaj, P Ramkaran and AA Chuturgoon. Also data is available from the Discipline of Medical Biochemistry, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa.

Authors’ contributions
AAC and NRM conceived and designed the study. SP and PR ran the genotyping experiments and analysed the data. All authors contributed to the manuscript and approved the final version.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Institutional ethical and hospital regulatory permission was obtained for the study (Biomedical Research Ethics Committee, University of KwaZulu-Natal, South Africa; reference number BE 119/11). After informed consent was obtained, participants were recruited over a 14-month period from July 2013 to September 2014 from the maternity unit at Prince Mshiyeni Memorial Hospital in Durban, South Africa. This hospital is a regional level facility and serves a predominantly semi-urban African population from where the participants were recruited.

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Received: 21 June 2016 Accepted: 24 November 2016
Published online: 05 December 2016

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