Original Research *Highlight Article*

Different morphological and gene expression profile in placentas of the same sickle cell anemia patient in pregnancies of opposite outcomes

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Impact statement

Environmentally induced changes in placental morphological and molecular phenotypes may provide relevant insight towards pathophysiology of diseases. The rare opportunity to evaluate the same patient, with sickle cell anemia (SCA), in two different pregnancies, of opposite outcomes (one early onset severe preeclampsia (PE) and the other mostly non-complicated) can prove such concept. In addition, the comparison to other conditions of known placental and vascular/inflammatory involvement strengthens such findings. Our results suggest that the clinical association between SCA and PE can be supported by common pathophysiological mechanisms, but that pathways involving response to copper and triglyceride metabolism may be important drivers of the pathophysiology of PE. Future studies using in a larger number of samples should confirm these findings and explore pathways involved in the pathophysiology of PE and its relationship with SCA.

Abstract

Environmentally induced changes in placental morphological and molecular phenotypes may provide relevant insight towards pathophysiology of diseases. Sickle cell disease (SCD) is a common inherited hemoglobin disorder characterized by chronic hemolytic anemia and vaso-occlusive crisis. SCD leads to higher morbidity and mortality, especially during pregnancy, with increased risk of preeclampsia (PE). To compare clinical findings, placental morphology, and gene expression in villous placental tissue using next generation sequencing. We included five cases. Two placentas from the same woman with homozygous SCD that had been pregnant twice and had different maternal and fetal outcomes (one early onset PE/eclampsia and a mostly non-complicated pregnancy); an early onset PE, a fetal growth restriction and a term, non-complicated pregnancy. Sixty-four differentially expressed genes were observed in the SCD+PE case, in comparison with the placenta from the SCD without PE, based on fold change. Among these genes, 59 were upregulated and 5 were downregulated. Enrichment analysis indicated two significant biological processes: response to copper ion (CYP1A1, AOC1, AQP1, and ATP5D) and triglyceride-rich lipoprotein particle clearance (GPIHBP1, APOC1, and APOE). The rare opportunity to evaluate the same patient in two different pregnancies, of opposing outcomes, and compare to other conditions of known placental and vascular/inflammatory involvement, may further the understanding of the pathophysiology of PE in SCD. Our results suggest that the clinical association between SCD and PE may be supported by common pathophysiological mechanisms, but that pathways involving response to copper and triglyceride metabolism could be important drivers of PE pathophysiology.

Keywords: Placenta, sickle cell disease, gene expression, preeclampsia, and pregnancy

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Introduction

Sickle cell disease (SCD) is one of the most common inherited diseases worldwide,[1] affecting around 300,000 newborns every year.[2–4] Birth rates of SCD vary according to the location; in the Americas, it is about 0.49 per 1000 live births.[5] The *HBB* Glu6Val genetic mutation causes the production of abnormal ß globin chains forming hemoglobin S

(HbS), and homozygosity for this mutation has the most severe clinical presentation.[1,2,6] HbS polymerizes when exposed to lower oxygen levels, making erythrocytes rigid and sickle shaped. These changes also cause increased cell adhesion to the endothelium, triggering its activation and the production of proinflammatory cytokines and chemokines.[7] Sickled red blood cells have a shorter lifespan, leading to chronic hemolytic anemia, and increased cell adhesiveness is the pathophysiological basis for the characteristic vaso-occlusive crises,[6,7] with higher morbidity[2,8,9] and mortality[8,10] with detrimental impact on quality of life.

SCD is especially challenging during pregnancy as it increases maternal and fetal morbidity and mortality.[2] Maternal complications include worsening anemia and a higher susceptibility to pain crises, acute chest syndrome, and infections.[1,11] SCD also doubles the risk of preeclampsia (PE) and increases the risk of eclampsia in homozygous patients by five times.[8] Fetal complications include higher risk of growth restriction, fetal demise, low birth weight, and prematurity.[8,10,12,13]

The placenta plays an important role in the severity of fetal complications, but the mechanisms are still not fully understood. Lower placental oxygen levels (due to anemia and sickling episodes) may lead to chronic inflammation and a hypoxic environment.[7] This proinflammatory environment could support the vaso-occlusive episodes and tissue necrosis,[14,15] which can further decrease the placental function and contribute to fetal growth restriction, PE and prematurity.[7]

The study presents a case of a patient with homozygous SCD that was pregnant twice and had different maternal and fetal outcomes. To further characterize the differences between her complicated and uncomplicated pregnancies, we compared clinical findings, placental morphology, and gene expression in placental tissue using next generation sequencing. The molecular findings were also compared with non-SCD cases without complications, with PE, and with fetal growth restriction (FGR). Our data emphasize the intra-individual heterogeneity of pregnancy in SCD, and suggest that studying placental abnormalities may help explain the some aspects pathophysiology of obstetric complication in SCD.

Materials and methods

Study group

This study was approved by the local Ethics Board (approval number 5404/2015). Pregnant women were recruited from the high-risk outpatient clinic and maternity of the University of Campinas (UNICAMP), and all samples and clinical data were collected upon written informed consent. This study included one woman with SCD (HbSS), one woman with PE, one woman that delivered a baby with FGR, and one woman with non-complicated pregnancy as a control. All included cases delivered by cesarean section, without labor. Characteristics of the patients included in this study are shown in Table 1. The selected conditions were chosen due to their placental findings and pathophysiology of diseases related to endothelial and inflammatory state.

Hemoglobinopathy diagnosis (HbSS) was confirmed by clinical data and hemoglobin electrophoresis (high performance liquid chromatography, Variant II, Bio-Rad, USA). PE was defined as a blood pressure higher than 140/90 mmHg, with proteinuria of more than 0.3 g in a 24-h sample.[16] FGR was defined as birth weight below the 10th percentile of that anticipated for the given gestational age.[17]

Placental tissue collection and RNA extraction

Placentas (stored at $+4^{\circ}$C) were sampled within 3 h after caesarean section. Placental villous tissue samples were randomly selected from the central areas close to the umbilical cord, as previously described.[18] Before collection, the placentas were weighed and photographed. The samples were collected (≈200 mg) in three different regions avoiding areas of visible infarcts or calcifications; they were then separated into three equal size pieces and washed with phosphate saline buffer to remove residual

Table 1. Maternal and perinatal outcomes of the same sickel cell anemia patient in two different pregnancies.

	SCA 1st pregnancy	SCA 2nd pregnancy
Maternal age	19	21
First medical appointment	10 weeks	11 weeks
Hb level at first trimester	8.4 mg/dL	7.2 mg/dL
GA at hospital admission (weeks)/ medical condition	13/ pain crisis 17/ pain crisis 20/ respiratory infection 28/ acute chest syndrome/ worsening anemia 33/ fetal growth restriction	24/ pneumonia 33/ pain crisis 35/ pain crisis 36/ worsening anemia
Prophylactic blood transfusion[a]	No	Yes (28 weeks)
Mode of delivery	C-section	C-section
GA at delivery	34 weeks	36 weeks
Newborn weight	1910 g	2585 g
5-min apgar score	10	10
Neonatal outcome	Neonatal death	Alive
Postpartum contraceptive method	Oral contraceptive	Oral contraceptive

[a]Prophylactic blood transfusion became a routine in the prenatal care since September 2011 and aims to keep Hb level above 9.0 mg/dL to reduce maternal and fetal complications and reduce overall HbS.
SCA: sickle cell anemia; Hb: hemoglobin; GA: gestational age; g: grams.

maternal blood. Immediately after collection, the tissues were frozen in liquid nitrogen and stored at –80°C until the RNA extraction. All samples were collected by the same person and using identical protocol. Total RNA was extracted using TRIzol Reagent (Ambion), followed by the RNeasy mini kit (Qiagen). Purity level and concentration of isolated total RNA were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific), and RNA integrity was determined using the Bioanalyzer 2100 system (Agilent Technologies).

RNA sequencing

RNA-seq was performed on the five placenta samples included in the study. RNA sequencing (RNA-Seq) has been employed in placental villous tissue to describe the comprehensive transcriptome of two placentas, from different pregnancies (two years apart) from one woman with SCD. For comparison, placental villous tissue from one FGR, one preeclamptic, and one control pregnancy (without complications), all with HbAA genotype, was also sequenced. Library preparation and RNA-Seq were performed by Life Sciences Core Facility (LaCTAD). Complementary DNA libraries were prepared using Illumina TruSeq RNA Sample Preparation Kit according to the manufacturer's protocol (Illumina). Paired-end 100 bp high output sequencing was performed on Illumina HiSeq2500. The raw sequencing data were evaluated by FastQC version 0.11.5 for quality control. Reads were aligned to human genome assembly (GRCh38.88) using STAR 2.5.

Differential gene expression analysis

Differential gene expression analysis of the RNA-Seq data was performed with the R package DESeq2 and edgeR. The differentially expressed genes (DEGs) list was restricted to genes showing a fold change more than fourfold difference. Heatmaps were constructed through of a web tool, ClustVis,[19] using Pearson's correlation coefficient.

Histology

The placental samples were fixed in buffered formalin for 24 h, and further paraffin-embedded for light microscopy. Five-micrometer-thick histological sections were obtained, deparaffinized in xylene, rehydrated in a series of ethanol, and washed in phosphate-buffered saline (PBS), stained for hematoxylin and eosin (H&E) in order to provide morphology examination. Images were acquired using a Zeiss Axiophot2 microscope with a color Olympus DP72 digital camera using Olympus cellSens Standard 1.14 software.

Results

Case description

We present a unique opportunity to analyze placentas from a same patient, from pregnancies two years apart (Table 1). A 19-year-old Caucasian female with sickle cell anemia (SCA), previously on regular follow-up at the Hematology clinic with no indication for hydroxyurea or chronic transfusions. Her last blood transfusion had been over six months before she got pregnant. Her first admission was at 13 weeks for pain crisis. Additional admissions occurred, including acute chest syndrome/worsening anemia (Hb 5.0 g/dL). At 33 weeks' gestation, an ultrasound revealed a growth restricted fetus with oligohydramnios and the patient was readmitted for fetal surveillance. At 34 weeks, she began with headache and epigastric pain, associated with high blood pressure. Due to the diagnosis of PE with severe features, treatment with intravenous magnesium sulfate was started and a C-section was subsequently performed due to worsening symptoms. During anesthesia, the patient had a seizure (eclampsia). After delivery, she was admitted at the intensive care unit (ICU). The newborn weighed 1910 g, with a 5-min Apgar score of 10, but died of pneumonia at 24 days of age. She began combined oral contraceptives postpartum but returned to our high-risk antenatal care at 11 weeks' gestation two years later for another unplanned pregnancy (Table 1). Due to her history of eclampsia, aspirin and calcium supplementation were prescribed.

A chronic transfusion program was started at 28 weeks; however, she needed additional transfusions in the setting of pain crisis. The ultrasound showed adequate fetal growth throughout pregnancy. She gave birth to a healthy newborn weighing 2585 g at 36 weeks. C-section was indicated due to worsening anemia, an unfavorable cervix, and patient's choice not to undergo induction. Both mother and child were discharged on the third day postpartum.

The SCA patient (cases 1 and 2) and the other cases are also described in Table 2. Case 3 is a control; a term pregnancy with no complications. Case 4 is an early onset PE with no underlying disease, a primigravida with normal hemoglobin electrophoresis that underwent a C-section at 33 weeks' gestation due to severe features of PE. Number 5 is a case of FGR with no other background (no chronic disease and not a smoker) that delivered at 34 weeks' gestation, by C-section, due to fetal distress. Low birth weight was confirmed postpartum (1640 g).

Placental histology

In the gross examination of the placentas from the same patient with SCA, it is possible to observe the maternal (Figure 1(a) and (c)) and fetal (Figure 1(b) and (d)) faces of each placenta. Note the extensive microcalcifications (Figure 1(a)), and velamentous insertion of the umbilical cord of the first gestation placenta (Figure 1(b)), a prognostic adverse finding.

Histological examination showed that the first pregnancy placenta has more pronounced perivillous fibrin deposition, an expected feature in SCA placentas, and hypermature villi, an adaptation to chronic uteroplacental hypoxia, while the second gestation placenta showed abundant tertiary villi, and (Figure 2) sickle-cell erythrocytes in the intervillous space, a common feature in SCD.

Table 2. Socio-demographic characteristics, maternal and perinatal outcomes of cases included in this study.

Case number	Socio demographic characteristics	Obstetric history	Maternal outcomes	Fetal outcomes
1-SCA 1st pregnancy	Ethnicity: Non white Maternal age (years): 19	Parity: 0 GA at delivery (weeks): 34 Placental weight (g): 410	Eclampsia	5-min Apgar score: 10 Birth weight (g): 1910 Neonatal ICU: yes
2-SCA 2nd pregnancy	Ethnicity: Non white Maternal age (years): 21	Parity: 1 GA at delivery (weeks): 36 Placental weight (g): 540	Uneventful	5-min Apgar score: 10 Birth weight (g): 2585 Neonatal ICU: no
3-Control	Ethnicity: White Maternal age (years): 21	Parity: 1 GA at delivery (weeks): 38 Placental weight (g): 570	Uneventful	5-min Apgar score: 10 Birth weight (g): 3160 Neonatal ICU: no
4-PE	Ethnicity: White Maternal age (years): 20	Parity: 0 GA at delivery (weeks): 33 Placental weight (g): 430	PE-severe features	5-min Apgar score: 8 Birth weight (g): 2115 Neonatal ICU: no
5-FGR	Ethnicity: White Maternal age (years): 37	Parity: 0 GA at delivery (weeks): 35 Placental weight (g): 515	Uneventful	5-min Apgar score: 9 Birth weight (g): 1640 Neonatal ICU: yes

SCA: sickle cell anemia; PE: preeclampsia; FGR: fetal growth restriction; GA: gestational age; ND: not determined; ICU: intensive care unit; g: grams.

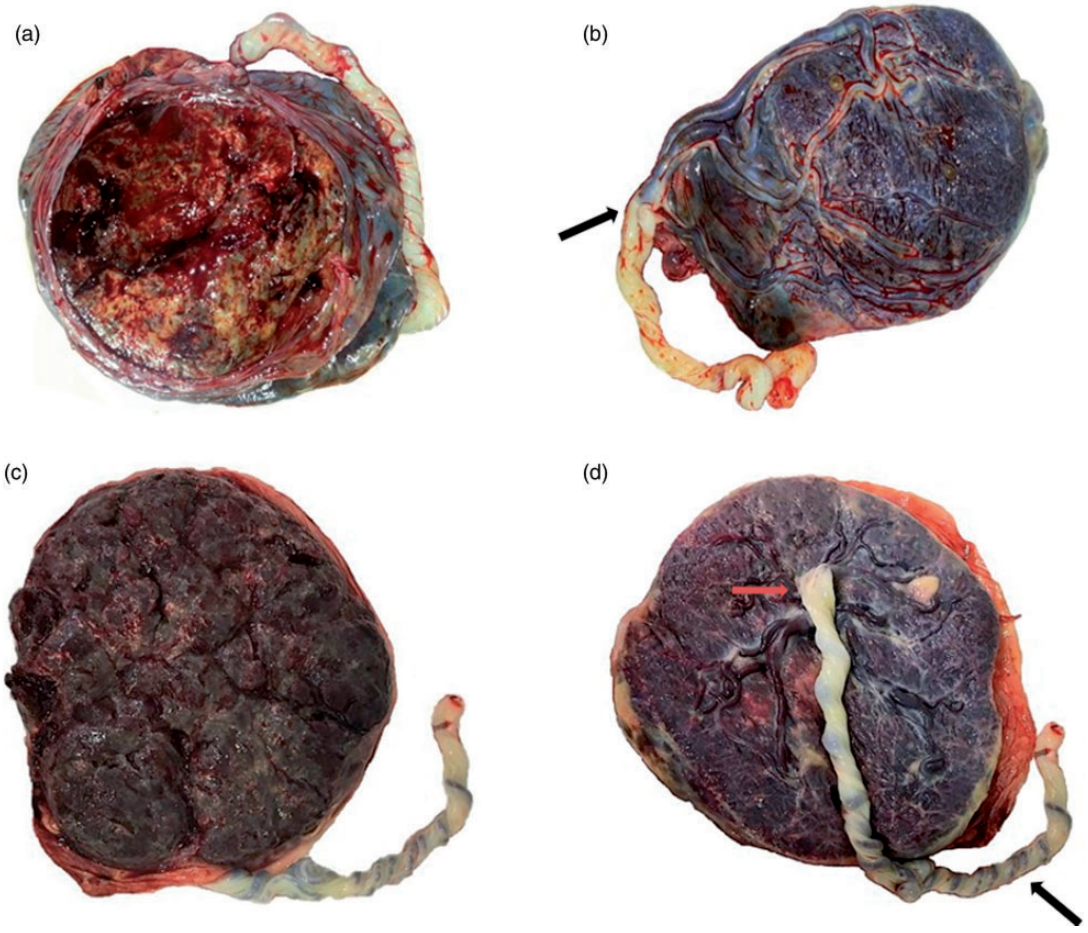

Figure 1. Macroscopic views of the placentas from the same patient with SCA in two different pregnancies. Placental gross examination from the same patient with SCA. Maternal (panel A) and fetal (panel B) sides of the placenta from a pregnancy complicated by severe preeclampsia. Maternal (panel C) and fetal (panel D) sides of the placenta from a pregnancy without preeclampsia. (a) extensive fibrin deposition and calcifications in about 40% of the maternal surface (low half of the figure), (b) umbilical cord with abnormal cord insertion (velamentous, black arrow), (c) well defined cotyledons, no gross abnormalities, and (d) umbilical cord with paracentral insertion (red arrow) and slight hypercoiling (black arrow). (A color version of this figure is available in the online journal.)

Overview of sequencing data of RNA-Seq analysis

After filtering, each individual transcriptome yielded a mean of 44 million 101-base pair paired-end reads (range: 26.17–58.86 million). GC content was approximately 48% for each sample. A minimum of 89% of the bases reached a Q score of 30 (Q30) and at least 68% of the sequence reads

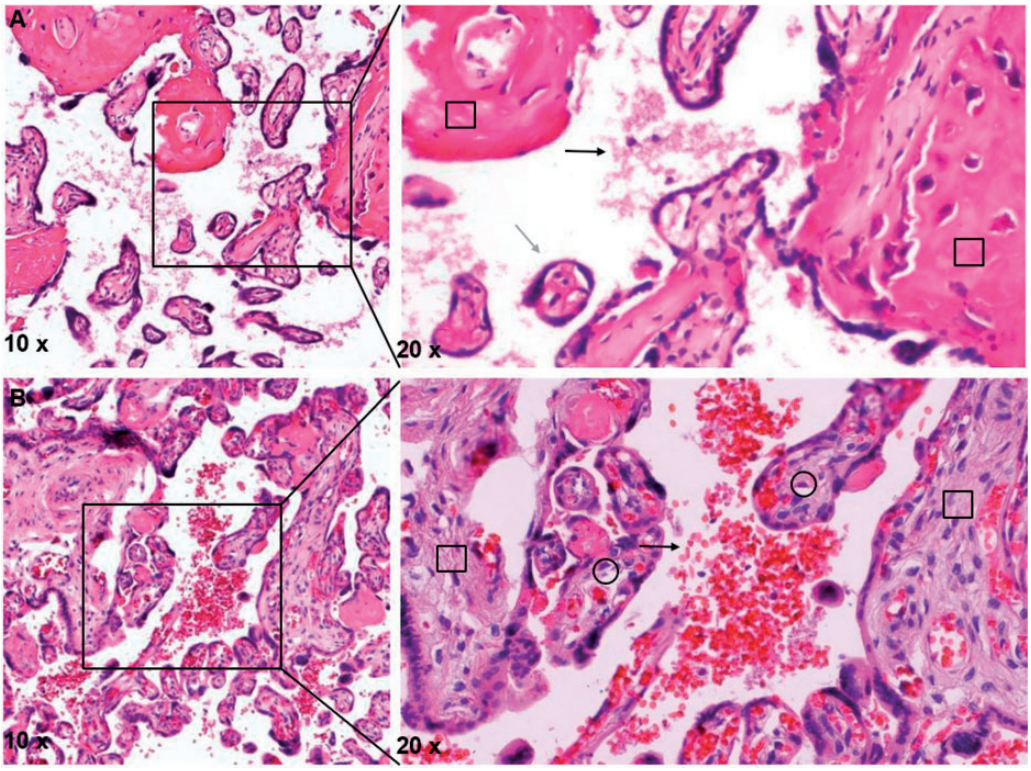

Figure 2. Placental histopathology assessed by H&E staining. Panel A represents the SCA patient's first pregnancy and Panel B represents the second pregnancy, with the placenta seen in 10× magnification and further details in 20× magnification, with arrows in the most relevant findings. (a) First pregnancy placenta features: hypermature villi (gray arrow), hemolysis (black arrow), and accentuated perivillous fibrin deposition (square). (b) Second pregnancy placenta features: showing intermediate (square) and tertiary villi (circle) and sickle-cell erythrocytes (black arrow). (A color version of this figure is available in the online journal.)

Table 3. Differentially expressed genes identified by comparing the placenta from the same sickle cell anemia patient in her first pregnancy complicated with preeclampsia and fetal growth restriction (SCA+PE+FGR) with the placenta from her second pregnancy not complicated (SCA).

Gene	SCA+PE+FGR vs. SCA fold change
PSG9	−5.48
NAA11	−4.85
CPXM2	−4.30
USP6	−4.15
PSG4	−4.12
LAMA3	4.00
HSPB1	4.00
FBXL15	4.00
ACTG2	4.00
AQP1	4.00
ATP5D	4.00
DKK1	4.00
GPIHBP1	4.00
IRF7	4.00
NDUFS7	4.00
PRG2	4.00
ROMO1	4.00
WDR18	4.00
KRT17	4.02
B3GNT7	4.02
PRKY	4.05
CAPG	4.05
ODF3B	4.05
IL1R2	4.07
CDKN1C	4.08
KRT14	4.09
SCAND1	4.14
CST6	4.14
COMP	4.16
MRPL12	4.18
HN1	4.18
PPP1R14A	4.21
COX5B	4.26
FSTL3	4.28
NOS2	4.29
CLDN5	4.29
SOX18	4.33
GKN1	4.34
ALKBH7	4.34
CKB	4.35
TSPO	4.38
HLA-DRB5	4.39
NME3	4.44
SYT8	4.45
ADAMDEC1	4.51
AOC1	4.52
APOC1	4.53
APOE	4.55

(continued)

Table 3. Continued

(continued)

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Gene	SCA+PE+FGR vs. SCA fold change
VASN	4.55
HBM	4.59
CEBPD	4.60
PPDPF	4.67
B3GNT3	4.81
CRLF1	4.87
HLA-G	4.89
RAMP3	4.89
NOG	4.99
CCDC85B	5.04
SELENOM	5.11
CYP1A1	5.18
ASCL2	5.24
CFD	5.28
NOTUM	5.64
TAC3	5.84

were uniquely aligned to the human genome, with adequate read mapping evenness across the transcripts of all samples.

Identification of DEGs

Based on fold change (with a threshold above fourfold difference), 64 DEGs were identified from the comparison between the two placentas from the same patient, with and without complication by PE. Among these genes, 59 were upregulated and five were downregulated (Table 3). A graphic overview of the differential status of gene expressions is represented by the heatmap on Figure 3. The identified genes were analyzed according to Gene Ontology categories under biological process enrichment using WebGestalt (WEB-based GEneSeTAnaLysis Toolkit). The two most significant biological processes identified were response to copper (FDR < 0.006 for genes *CYP1A1*, *AOC1*, *AQP1,* and *ATP5D*) and triglyceride-rich lipoprotein particle clearance (FDR < 0.007 for genes *GPIHBP1*, *APOC1,* and *APOE*).

Discussion

Environmentally induced changes in placental morphological and molecular phenotypes may provide relevant insight towards pathophysiology of diseases.[20] The rare opportunity to evaluate the same patient, with SCD, in two different pregnancies, with opposite outcomes can prove such concept. And the comparison with other conditions of known placental involvement strengthens such findings.

Pregnancy in patients with SCA is known to have a higher risk for PE and FGR, complications associated with significant placental abnormalities. To the best of our knowledge, this is the first report of a rare opportunity to compare different placentas from the same patient in different pregnancies and outcomes.

The first pregnancy represents a typical situation of the high risk involving an SCA patient during pregnancy, with several hospital admissions, severe preterm PE, and eclampsia. In comparison, her second pregnancy was almost uneventful. We believe close surveillance, prophylaxis for PE, and programmed blood transfusions contributed significantly to a more favorable outcome, with no major complications. It appears important to decrease HbS during gestation in women with SCD, decreasing hemolysis and vaso-occlusion, and preventing their effects on the placenta, so blood transfusion may be of more benefit if started during the second trimester, or even earlier during the first trimester of gestation.[10]

The morphology of the placentas helps to exemplify the effect of PE on the placental architecture. In addition to this, the molecular analysis allowed us to uncover significant differences in gene expression with a paired analysis between placentas from the same patient, and to further explore the comparison with healthy control and placentas from patients with complicated pregnancies without SCA. We observed similarities in genetic expression among placentas with PE, FGR, and SCA with PE. These data agree with a previous study showing a common gene expression profile between PE and FGR.[21] Impaired deep placentation has been linked with a spectrum of complications during pregnancy including PE and FGR.[22] A possible interpretation might be that PE, and a subset of FGR are in fact manifestations of the same disorder caused by failure in trophoblast invasion leading to an abnormally shallow vasculature and impaired remodeling of the spiral arteries, and that the remaining differences depend on the degree of severity.[23]

Unlike in PE and FGR, placentas from women with SCD have seldom been studied. In our previous analysis,[10] we demonstrated that there is change in the expression of some genes associated with the inflammatory response, suggesting that the chronic inflammatory state in SCD can affect the placental physiology. Since both SCD and PE may impair the adequate development of the placenta and lead to maternal–fetal complications, the clinical association between SCD and PE may be explained by both diseases sharing a common genetic signature, with dysregulation of genes involved in inflammatory response and vascular endothelium dysfunction. In addition, increased free heme is a common factor among PE, FGR, and SCD and may be one of the responsible for changes in placental physiology.

Intravascular hemolysis, common in sickle cell patients, is a recurrent process that causes chronic inflammation. The release of inflammatory mediators and the recruitment of leukocytes and erythrocytes to the endothelium consequently result in endothelial dysfunction and vaso-occlusion, mainly in the microcirculation.[24] This process occurs most frequently during gestation, leading to the release of heme. On the other hand, in PE and FGR, the levels of free HbF and heme were found increased in the fetoplacental and maternal circulation.[25,26] The heme is considered a "damaging molecular pattern" (DAMP), in the circulation. DAMPs may activate multiple inflammatory pathways, including endothelial activation and injury.[27] According to Prophet *et al*.,[28] since SCD mothers already have ongoing processes that damage the maternal vascular endothelium, it could explain the likelihood of PE in patients with SCD. Besides being identified as a DAMP by the immune system, a study by Liu *et al*.[29] indicated

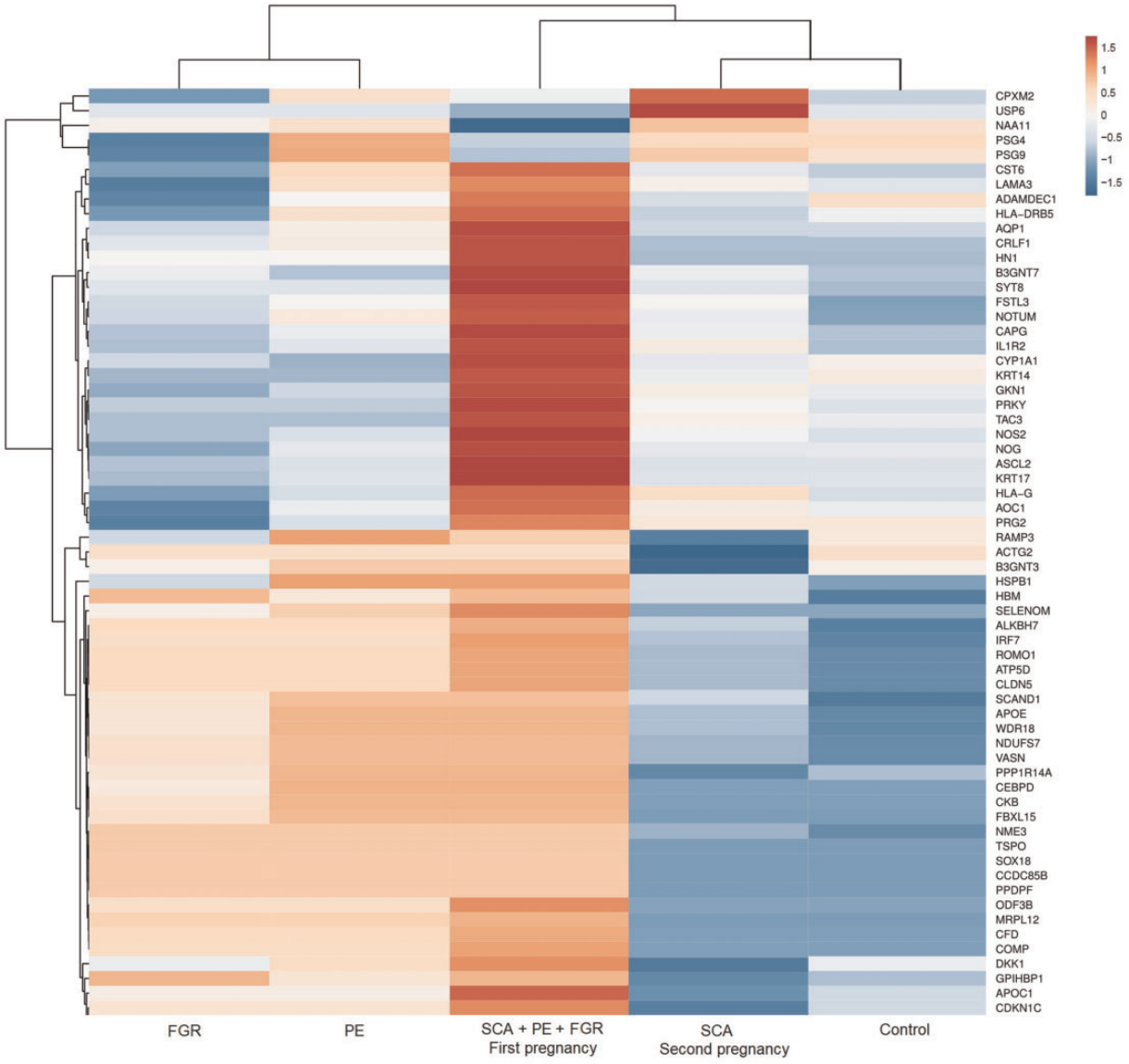

Figure 3. Heatmap generated from 64 genes with the greatest difference in expression between the placental samples from first pregnancy (SCA+PE+FGR) and second pregnancy (SCA). For comparison were also included placentas samples from patients with fetal growth restriction (FGR, $n = 1$), preeclampsia (PE, $n = 1$), and one healthy control. The figure represents hierarchically clustering the gene expression values of 64 genes. Up-regulated expressions are marked in red, down-regulations are colored in blue; white reflects no difference in expression levels. (A color version of this figure is available in the online journal.)

heme as an inhibitory agent of fusion of trophoblasts, that is, cytotrophoblasts cannot fuse to form the syncytiotrophoblasts. Because of this failure to differentiate cytotrophoblasts, the placenta may be impaired to perform nutrient and oxygen exchange, thus impairing fetal growth. Therefore, heme increase may be one of the factors involved in the higher risk PE in sickle cell patients. Nevertheless, not all patients with SCD will develop PE, and the case studied allowed us to investigate whether there is a genetic expression profile more associated with PE in the context of SCD. Nevertheless, not all patients with SCD will develop PE, and the case studied allowed us to investigate whether there is a genetic expression profile more associated with PE in the context of SCD.

Among the DEGs observed to be most up-regulated in the SCA placenta with PE in comparison with all other placentas, *HLA-G, TAC3, AOC1, B3GNT7, IL1R2,* and *PRG2* deserve particular attention, because these genes are specific to extravillous trophoblasts (EVTs), or highly expressed in these cells.[30] This suggests that the development of PE in SCA may be associated with an increase in the synthesis of EVT-derived proteins, and how this relates to impaired autophagy in EVT involved in poor placentation in PE is unclear.[31]

Increased expression of *HLA-DRB5* in SCA-associated PE is remarkable because the physiological lack of HLA major histocompatibility complex class II expression on trophoblasts is meant to prevent alloreactivity of maternal T cells against paternal antigens and allow successful tolerance. Aberrant expression of HLA-DR in PE has been very recently reported[32] and our results confirm that placental up-regulation of *HLA-DRB5* seems to be associated with

development of PE in SCA. This may be worthy of investigation as to what mechanisms elicit the expression of HLA-DR in the placenta that could explain the development of PE. Furthermore, there is evidence that associates certain HLA-DRB haplotypes with the appearance of either allo- or autoantibodies against red blood cell antigens in SCD patients, thus implying that abnormal HLA-DR expression may support the existence of a dysregulated immune system in SCD that could favor PE.[33]

Interestingly, five genes (*CPXM2*, *USP6*, *NAA11*, *PSG4*, and *PSG9*) were downregulated in SCA combined with PE as well as in FGR. Of these genes, two are pregnancy-specific glycoproteins (PSGs) secreted by the placental syncytiotrophoblast.[34] Low PSG levels are associated with adverse pregnancy outcomes including FGR, PE, and spontaneous abortion, suggesting the importance of PSGs for a successful pregnancy.[35–37] To further elucidate the role of PSGs, Jones *et al.*[38] showed that PSG9 is important to the induction of immune tolerance during pregnancy. This suggests again that in pregnant patients with SCD, the immune tolerance may be decreased with a consequent attack on the placenta favoring the development of PE.

One of the most significant biological processes identified in association with PE included genes involved in the response to copper (*CYP1A1*, *AOC1*, *AQP1,* and *ATP5D*). This may relate to previous descriptions of the association of increased copper levels with occurrence and severity of PE[39] and with some populations of SCD patients,[40] although the exact mechanism by which levels of this metal increase in these diseases is unknown.

From these genes, *AQP1* encodes an aquaporin, which functions as a molecular water channel protein. Previous studies have also demonstrated *AQP1* expression in human and mice trophoblast cells[41] and this protein has been described to have roles in normal pregnancy, fetal growth, and homeostasis of amniotic fluid volume.[42] Dysregulated expression of *AQP1* is also associated with placental abnormalities[43] and PE.[44] An increased expression of *AQP1* in our case of SCA complicated with PE may be involved in the dysregulation of the amniotic fluid production and development of oligohydramnios.[45]

Up-regulation of genes involved in triglyceride-rich lipoprotein particles clearance were *GPIHBP1*, *APOC1*, and *APOE* and of the cytochrome P450 family 1 subfamily A member 1 (*CYP1A1*) gene suggest a defense mechanism of the trophoblasts against triglycerides in PE.[46,47] An association between hemolytic markers and triglyceride levels has been shown in SCD patients,[48] so the observed gene expression profile may recapitulate the severity of hemolysis and its effects on the placenta.

As a limitation, pairing of the patients was imperfect, with some significant differences in age and parity, although ethnicity, smoking status, and type of delivery were similar across samples. The placental samples used here included a mixture of cells, so we cannot determine which particular cells in the placenta are up- or downregulating the specific genes investigated. Of course it is a preliminary result and would be necessary to study a large number of patients, including some confirmatory studies with proteins. The strength of this study is the unique opportunity of exploring how clinical management during pregnancy can affect maternal/perinatal outcomes and placental development in an SCA patient.

Taken together, our results suggest that the clinical association between SCD and PE may be supported by common pathophysiological mechanisms, but that pathways involving response to copper and triglyceride metabolism may be important drivers of the pathophysiology of PE. How and to what extent the clinical interventions currently being used to manage pregnant SCD patients affect these pathways remain to be determined. Future studies using placenta RNA-seq in a larger number of samples should confirm these findings and explore important pathways involved in the pathophysiology of PE and its relationship with SCD.

Authors' contributions: Conceived and designed the experiments: LCB, COF, MBM, and MLC. Performed the experiments: LCB, and AA. Analyzed the data: MLC, AA, LCB, COF, and BBS. Wrote the paper: KYF, LCB, COF, FFC, and MLC.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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