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# Placental heme receptor LRP1 correlates with the heme exporter FLVCR1 and neonatal iron status

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## Abstract

LDL receptor-related protein 1 (LRP 1) is a transmembrane receptor highly expressed in human placenta. It was recently found to be the receptor for heme and its plasma binding protein hemopexin and is integral to systemic heme clearance. Little is known about systemic concentrations of hemopexin during pregnancy and whether maternal hemopexin and placental LRP1 contributes to fetal iron homeostasis during pregnancy. We hypothesized that placental LRP1 would be up-regulated in maternal/neonatal iron insufficiency and would be related to maternal circulating hemopexin. Placental LRP1 expression was assessed in 57 pregnant adolescents (14–18 y) in relation to maternal and cord blood iron status indicators [hemoglobin, serum ferritin, transferrin receptor], the iron regulatory hormone hepcidin and serum hemopexin. Hemopexin at mid-gestation correlated positively with hemoglobin at mid-gestation ($r = 0.35$, $P = 0.02$) and hemopexin at delivery correlated positively with cord hepcidin ($r = 0.37$, $P = 0.005$). Placental LRP1 protein expression was significantly higher in women who exhibited greater decreases in serum hemopexin from mid-gestation to term ($r = 0.28$, $P = 0.04$). Significant associations were also found between placental LRP1 protein with cord hepcidin ($r = -0.29$, $P = 0.03$) and placental heme exporter FLVCR1 ($r = 0.34$, $P = 0.03$). Our data are consistent with a role for placental heme iron utilization in supporting fetal iron demands.

## Introduction

The placenta serves as the sole conduit for the transfer of maternal nutrients to the fetus across pregnancy. It is believed that most, if not all, of the iron (Fe) delivered to the placenta

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CC and KOO designed and performed the research, analyzed the data, and wrote the manuscript; EKP and EMC and RG conducted the research and assisted with participant recruitment. MW performed hepcidin analyses.

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is supplied as non-heme Fe. It is unclear whether plasma heme obtained from either intravascular red blood cell (RBC) catabolism (Tolosano *et al.* 2010), or from macrophage heme export (Yang *et al.* 2010), might also be utilized to support fetal Fe demands.

Hemopexin (Hx) is a plasma protein with high affinity for heme and contributes to systemic Fe homeostasis by delivery heme to the liver for storage (Tolosano *et al.* 2010). Decreases in serum Hx reflect a discharge of heme into the circulation and Hx is commonly measured along with the plasma hemoglobin (Hb) scavenge protein, haptoglobin (Hp), to assess the severity of intravascular hemolysis (Delanghe & Langlois 2001). Few data are available on Hx concentrations in pregnant women and it is unclear whether Hx is related to Fe status.

The LDL receptor-related protein 1 (LRP1) is a trans-membrane protein expressed in a variety of cell types including macrophages, hepatocytes, and neurons (Moestrup *et al.* 1992). Of interest, LRP1 is highly expressed in human placenta (Jensen *et al.* 1988, Jensen *et al.* 1989) and has been suggested to play a role in placental lipid transport (Gafvels *et al.* 1992). Recently, Hvidberg et al. identified LRP1 as the receptor for the Hx-heme complex and revealed a novel role of LRP1 in systemic heme recycling (Hvidberg *et al.* 2005). These authors suggested that the high expression of LRP1 in the human placenta may enable Fe recovered from maternal circulating heme to be delivered to the fetus (Hvidberg *et al.* 2005) but no studies have examined this hypothesis.

Fe efflux from the placenta to the fetus is thought to be mediated by the only known mammalian cellular non-heme Fe exporter, ferroportin (FPN) (Donovan *et al.* 2000, McKie *et al.* 2000). Consistent with its role in Fe export, FPN is localized to the basolateral side of human placental syncytiotrophoblast (Bastin *et al.* 2006). FPN is under tight control by the hormone hepcidin, which binds and induces degradation of FPN protein and thus limits cellular Fe export. Whether this FPN-hepcidin model in macrophages applies to the human placenta and whether placental FPN is related to expression of heme Fe transporters remain unknown.

The objectives of this study were: 1) to evaluate placental expression of the heme transporter LRP1 and the Fe export protein FPN in pregnant adolescents delivering at term and 2) to investigate the relationships between placental LRP1 and FPN with placental heme Fe exporter FLVCR1, maternal and neonatal Fe status, and changes in maternal Hx across gestation.

## Material and methods

### Subjects

Pregnant adolescents in this study were enrolled in a larger prospective study addressing the relationship between maternal and fetal mineral status. All participants were recruited from the Rochester Adolescent Maternity Program in Rochester, NY, USA between 2007 and 2011. Characteristics of this study cohort have been described elsewhere (Young *et al.* 2011, Young *et al.* 2012a) and data on placental expression of Fe and vitamin D-related proteins have been published (Young *et al.* 2010, Jaacks *et al.* 2011, O'Brien *et al.* 2014). The Institutional Review Boards at Cornell University and the University of Rochester approved

all study procedures and written informed consent was obtained from all participants. A maternal blood sample was taken at mid-gestation (~26 wks) and at delivery (~40 wks) at which time cord blood and placental tissue were also obtained. Placental tissue used in this study was from a subset (n = 57) of the 113 teens who delivered term infants.

## Biochemical analysis

Blood Hb was measured using a Cell Dyn 4000 hematology analyzer (Abbott Laboratories, Abbott Park, IL, USA). Serum was separated and stored at −80°C prior to analysis. Serum Hx and Hp were determined by sandwich ELISAs from Genway Biotech (Genway Biotech, San Diego, CA, USA) and ALPCO (ALPCO Diagnostics, Salem, NH, USA), respectively. Serum Hp lower than the normal range (0.3–2.0 g/L) was considered indicative of hemolysis (Delanghe & Langlois 2001). Serum ferritin (SF) and serum soluble transferrin receptor (sTfR) were measured using commercially available ELISA kits (Ramco Laboratories Inc., Stafford, TX, USA). Total body iron (TBI) was calculated from SF and sTfR concentrations using the equation developed by (Cook *et al.* 2003): TBI (mg/kg) = − [$\log_{10}$ (sTfR/SF) − 2.8229]/0.1207. Maternal ID was defined as either SF < 12 μg/L, sTfR > 8.5 mg/L, or TBI< 0 mg/kg (Akesson *et al.* 1998, Mei *et al.* 2011). Because the Hb distribution for African Americans is shifted to the left of that for the Caucasians, we adjusted the Hb cutoffs downward by 0.8 g/dL to define anemia in African American teens as recommended by IOM for black populations (IOM 1993). Specifically, anemia in whites was defined as Hb < 10.5 g/dL in the second trimester and Hb < 11.0 g/dL in the third trimester and anemia in blacks was defined as Hb < 9.7 g/dL in the second and Hb < 0.8 g/dL in the third trimester. Neonatal anemia was defined when cord blood Hb was < 13.0 g/dL (Nathan & Oski 1993). C-reactive protein (CRP) was measured using Immulite 2000 immunoassay system (Seimens Diagnostics, Los Angeles, CA). Intrinsic LifeSciences (La Jolla, CA, USA) measured serum hepcidin using a competitive ELISA specific for the mature peptide. The lower limit for detection of this assay is 5 μg/L. Serum hepcidin concentration below the limit of detection was given a value of 2.5 μg/L for data analysis purposes as previously described for this variable (Young *et al.* 2012b).

## Placental sample collection

Placentas were collected immediately after delivery. Placental weight and dimensions were recorded. Multiple (4–5) placental samples were collected from different quadrants of the placenta, the maternal and fetal membranes removed and the samples then each cut into quarters and then randomly distributed into aliquots. These aliquots were either flash frozen (for western blot analyses) or placed into RNAlater (Ambion, Austin, TX, USA) and kept at −80°C. Placental protein lysates were prepared as previously described (Young *et al.* 2010, Jaacks *et al.* 2011) and protein concentrations of lysates were determined using Bio-Rad dye reagent (Bio-Rad, Hercules, CA, USA). Lysates were diluted in SDS-PAGE sample buffer and stored at −80°C until analysis.

### Placental tissue Fe content

Total tissue Fe content of the placental tissue was determined using atomic absorption spectrophotometry as detailed before (Jaacks *et al.* 2011) and expressed as μg Fe/g placental dry weight.

### Real-time quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR)

Expression of placental *LRP1* mRNA was undertaken in 42 placentas with quality RNA. Total RNA was extracted from the placental tissue samples using the RNeasy Microarray Tissue Mini Kit (Qiagen, Valencia, CA, USA). The extracted RNA was quantified and verified for integrity by the Experion automated electrophoresis system (Bio-Rad, Hercules, CA, USA). RNA purity was checked by the ratio of absorbance at 260 and 280 nm on a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). A total of 1 μg RNA was reverse-transcribed into cDNA with a transcriptor cDNA synthesis kit (Roche Applied Sciences, Indianapolis, IN, USA). All qRT-PCR reactions were set up in a 10 μL reaction mixture containing 2 μL of the cDNA template, 5 μL of SYBR Green I Master reaction mix, and 0.7 μM of primers and run in triplicate in 384-well plates on a LightCycler 480 instrument (Roche Applied Sciences, Indianapolis, IN, USA). The cycling conditions included an initial denaturation step at 95°C for 5 min, followed by 45 cycles at 95°C for 10 sec, annealing at 60°C for 10 sec, and extension at 72°C for 10 sec. Specificity of amplifications was verified by melt curve analysis after PCR cycles. A single peak was consistently observed in all samples. Relative *LRP1* mRNA expression was normalized to *β-actin* and compared to that obtained from a control placenta sample using the 2-ΔΔCt method using the following equations: ΔCt = Ct(*LRP1*) − Ct(*β-actin*); ΔΔCt = ΔCt(sample) − ΔCt(control placenta); and Fold Change = $2^{-\Delta\Delta Ct}$. The primers were designed using the ProbeFinder software from Roche Applied Science using the NCBI sequence ID (NM_002332 for *LRP1* and NM_001101 for *β-actin*) and purchased from Integrated DNA Technologies (Coralville, IA, USA). Sequences of the primer-pairs were as follows: *LRP1*, Forward: 5′ – *GAT GAG ACA CAC GCC AATC TG* - 3′, Reverse: 5′-*CGG CAC TGG AAC TCA TCA* – 3′; *β-actin*, Forward: 5′– *CCA ACC GCG AGA AGA TGA* –3′, Reverse: 5′ *CCA GAG GCG TAC AGG GAT AG* – 3′.

### Western blot analysis

Western blot was performed to determine placental protein expression of LRP1 and FPN. Placental lysates were separated by SDS-PAGE using a triple-wide electrophoresis unit (CBS Scientific, Del Mar, CA, USA) and electro-transferred to polyvinylidenedifluoride membranes (Millipore, Billerica, MA, USA). Membranes were blocked in Odyssey Blocking Buffer (Li-Cor, Lincoln, NB, USA) for 1 hr and incubated overnight at 4°C with antibodies against the following targets: mouse anti-LRP1 (1:3000 dilution; ab28320, Abcam, Cambridge, MA, USA), rabbit anti-FPN (1:500 dilution; MTP-11A, Alpha Diagnostics, San Antonio, USA), and β-actin (rabbit anti β-actin: 1:5000 dilution: Abcam, Cambridge, MA, USA or mouse anti β-actin: 1:2000 dilution: Santa Cruz Biotechnology, Santa Cruz, CA, USA). Blots were then incubated with appropriate fluorescence-coupled secondary antibodies (Li-Cor, Lincoln, NE, USA) for 1 hr at room temperature. After washing, fluorescence of the protein bands was quantified by with the Odyssey IR imaging

system (Li-Cor, Lincoln, NE, USA). A band at 85 kDa corresponding to the LRP1 β-chain was observed in human placenta and rat liver (Supplemental Fig. 1). A band of a lower molecular weight was evident in the placental lysates and may represent differential phosphorylation of the LRP1 β-chain (May *et al.* 2003), as similar patterns have been observed in human placenta (Kristensen *et al.* 1990). The combined fluorescence of the upper and lower bands and that of the upper band was highly correlated ($r = 0.96$) thus the latter was used for data analysis. The use of combined signal of the upper and lower band did not change study findings. A control placenta sample was loaded in the left, middle, and right part of the gel and the CV for the 3 samples was 2.6% indicating minimal influence of the sample location on band intensity. A representative image of FPN western blot is shown in Supplemental Fig. 2.

Detailed western blotting methods for the determination of placental FLVCR1 expression in this cohort were previously published (Jaacks *et al.* 2011). Existing data on placental FLVCR1 were used in the present study to explore its relationship with placental LRP1 and FPN.

### Statistical analysis

Non-normally distributed variables were transformed to achieve normality prior to data analysis. Paired t-tests were used to compare serum Fe status indicators and Hx between mid-gestation and delivery. Pearson's correlations were used to examine associations between expression of placental LRP1, FPN, and FLVCR1, and serum Fe status indicators. Multivariate analysis was used to study associations while controlling for possible confounders. Multiple linear regression was used to model predictors of placental LRP1 expression. P values $< 0.05$ were considered significant. Data were reported as the means ± SDs unless otherwise stated.

## Results

### Subject characteristics

General characteristics of the study participants are presented in Table 1. Average age at enrollment was 17 y and the majority of adolescents were African American and non-Hispanic. Approximately 19.3% of the adolescents were obese prior to pregnancy and 61.4% gained more than the IOM gestational weight gain recommendations. Of the 57 adolescents, five delivered by Caesarean section. Eleven infants were born small for gestational age and two were large for gestational age.

### Maternal Fe status and Hx concentrations across pregnancy

Suboptimal Fe status in this adolescent cohort was evident (Table 2). Prevalence of anemia was 4.3% at mid-gestation and increased significantly to 10.2 % at term. Serum Hx decreased significantly by 9.9% from mid-gestation to delivery ($p = 0.005$). Mid-gestation Hx was positively associated with maternal Hb ($r = 0.35$, $p = 0.02$) and CRP ($r = 0.30$, $p = 0.03$) at mid-gestation and with maternal hepcidin ($r = 0.29$, $p = 0.03$) at delivery. Delivery Hx correlated with maternal hepcidin ($r = 0.28$, $p = 0.04$) at mid-gestation.

Both mid-gestation Hx ($r = 0.50$, $p = 0.002$) and delivery Hx ($r = 0.52$, $p = 0.002$) correlated strongly with cord Hb and remained positive predictors of cord Hb after controlling for maternal Hb at the respective blood sampling point. Higher maternal Hx at delivery was related to better neonatal Fe status as evidenced by its association with high cord hepcidin ($r = 0.37$, $p = 0.005$) and low cord sTfR ($r = -0.27$, $p = 0.046$). There was a non-significant positive correlation between the decrease in Hx from mid-gestation to delivery and the time elapsed between the two blood sampling points ($r = 0.26$, $p = 0.06$). Greater decreases in Hx across gestation were seen in women with lower mid-gestation hepcidin ($r = -0.27$, $p = 0.046$) and in neonates with lower hepcidin ($r = -0.30$, $p = 0.03$). These relationships remained significant after controlling for time interval between the mid-gestation and delivery measures. Using all the variables obtained, a multivariate model that included weeks elapsed between the biochemical assessments made at mid-gestation and delivery, maternal hepcidin at mid-gestation, and maternal TBI at delivery was found to capture 23.3% of the variation in changes in Hx from mid-gestation to delivery ($p = 0.004$).

Serum Hp was assessed as an additional indicator of hemolysis in maternal mid-gestation blood samples. Serum Hp did not correlate with Fe status indexes in the maternal or neonatal circulation. Four of the 57 teens had low Hp levels (< 0.3 g/L) suggestive of hemolysis but there were no differences in Fe status indicators or hepcidin between these 4 teens and the rest of the cohort. Correlation analysis revealed significant positive associations between serum Hp with serum CRP ($r = 0.31$, $p = 0.02$) and Hx ($r = 0.33$, $p = 0.01$) at mid-gestation.

### Neonatal Fe Status and Hx concentrations

Neonatal hematological data are presented in Table 2. Nearly 1/3 of neonates were anemic at birth (cord Hb < 13 g/dL). Neonatal serum Hx was 74% lower than ($p < 0.0001$), and moderately correlated with maternal Hx at mid-gestation ($r = 0.29$, $p = 0.046$) but not at delivery. Cord Hx was not related to cord Fe status indicators or hepcidin.

### Associations between placental LRP1 and FPN with Fe status and Hx concentrations

Expression of LRP1 and FPN in the placenta was not related to maternal race, ethnicity, gestational age at delivery, delivery mode (vaginal delivery, C-section), placental weight, or infant weight.

Placental LRP1 protein expression correlated positively with suboptimal neonatal Fe status as indicated by low cord hepcidin ($r = -0.29$, $p = 0.03$; Fig. 1A) and high cord sTfR ($r = 0.36$, $p = 0.006$; Fig. 1B). Greater decreases in Hx from mid-gestation to delivery were associated with higher placental LRP1 protein expression ($r = 0.28$, $p = 0.04$; Fig. 1C). In the 41 adolescents with data on placental FLVCR1 protein, there was a positive correlation between placental LRP1 and FLVCR1 ($r = 0.34$, $p = 0.03$; Fig. 1D). Placental *LRP1* mRNA was not significantly correlated with LRP1 protein ($p = 0.14$). Greater *LRP1* mRNA tended to be associated with higher placental weight ($r = 0.28$, $p = 0.08$) and birth weight ($r = 0.26$, $p = 0.09$). None of the maternal or neonatal Fe status variables were significantly associated with placental LRP1 mRNA.

Placental FPN protein expression was insignificantly negatively correlated with maternal serum hepcidin ($r = -0.23$, $p = 0.08$) and TBI at mid-gestation ($r = -0.23$, $p = 0.09$). No significant relationship was found between placental FPN with neonatal hepcidin or other Fe status indicators. Similarly, there were no significant relationships between placental FPN and neonatal hepcidin when data were examined in adolescents who were ID or non-ID. Placental Fe content was positively correlated with placental FPN protein expression ($r = 0.37$, $p = 0.01$). Neither LRP1 nor FLVCR1 was correlated with FPN protein expression in the placenta.

## Discussion

During late pregnancy, Fe flux across the placenta reaches a maximum of 3–8 mg per day (Viteri 1994), a magnitude several times higher than the amount absorbed from the diet. The high Fe transport capacity of the placenta is partly explained by the abundant presence of TfR on the syncytiotrophoblast (Loh *et al.* 1980) and it is believed that transferrin-bound inorganic Fe is the predominant form of Fe utilized by the placenta to support fetal requirements (van Dijk 1988, McArdle *et al.* 2011). Recently, attention has been focused on the presence of multiple heme transport proteins in the human placenta (Cao & O'Brien 2013), which suggests that this organ may be an alternate site of heme clearance and/or utilization. In a group of healthy pregnant adolescents, we assessed maternal and neonatal serum Hx in relation to placental expression of the heme-Hx receptor LRP1, the heme exporter FLVCR1, and the non-heme Fe exporter FPN. We documented for the first time a significant positive correlation between two heme transporters LRP1 and FLVCR1 in the human placenta. In addition, placental LRP1 protein expression was higher in neonates with suboptimal Fe status and in women who experienced greater decreases in serum Hx from mid-gestation to delivery. Finally, we report novel associations between maternal Hx with neonatal Hb and hepcidin, adding to the biological plausibility of a role for plasma heme in maintaining Fe homeostasis during pregnancy.

Circulating Hx and liver LRP1 facilitate plasma heme clearance and protect against heme-associated oxidative damage. The importance of Hx in the resolution of hemolysis is evident from knockout studies showing that mice lacking Hx are more sensitive to both induced hemolysis (Tolosano *et al.* 1999) and heme overload (Vinchi *et al.* 2008). In addition to systemic heme scavenging, Hx and LRP1 may mediate localized heme clearance in the brain during cerebral hemorrhage (Li *et al.* 2009). Functional analyses have demonstrated the Hx-heme binding activity of LRP1 purified from human placenta (Hvidberg *et al.* 2005), but few studies have examined the potential role of placenta as a site of Hx-heme clearance. In our study, placental protein expression of LRP1 was positively associated with that of FLVCR1, a plasma membrane heme exporter essential for erythroid cell survival and macrophage heme Fe recycling (Keel *et al.* 2008). It is possible that placental heme uptake (mediated by LRP1) and heme export (mediated by FLVCR1) are coordinately regulated to allow for sequestration of circulating heme while preventing accumulation of intracellular heme as depicted in Fig. 2.

Little is known about the cellular regulation of LRP1 expression by systemic Fe status. Studies in mouse hepatoma cells have found that Fe deprivation doubled the number of

LRP1 and increased heme uptake, indicating that LRP1 may be negatively regulated by Fe availability (Smith & Ledford 1988). Consistent with this reciprocal Fe regulation of LRP1 in the liver, we found that high placental LRP1 was associated with suboptimal neonatal Fe status, which may represent a compensatory mechanism to increase placental heme uptake during Fe insufficiency in support of fetal demands.

Knockout experiments and localization studies provide convincing evidence that FPN is the protein that mediates Fe exit from placenta to the fetal circulation (Donovan *et al.* 2000, Donovan *et al.* 2005, Bastin *et al.* 2006). At this time it is unclear whether levels of FPN protein in the placenta are controlled by hepcidin in a similar way to that found in macrophages. Our study, and previous research in rodents (Gambling *et al.* 2001, Gambling *et al.* 2009, Cornock *et al.* 2013) and humans (Li *et al.* 2008), suggests that placental FPN protein is not regulated by maternal or neonatal Fe status. These data support the notion that FPN-mediated Fe export may not be the rate-limiting step for placental Fe transfer (Gambling *et al.* 2001). However, it still may be possible that changes in FPN subcellular location may inhibit Fe export without increased proteolytic degradation.

The Hx concentrations observed in maternal and neonatal circulation were within the ranges previously established for healthy adults (Delanghe & Langlois 2001) and term infants (Kanakoudi *et al.* 1995). Similar to previous reports (Muller-Eberhard *et al.* 1968, Gitlin & Biasucci 1969), cord Hx concentrations were 74% lower than those observed in maternal circulation, a phenomenon that has been suggested to be a consequence of low fetal Hx production (Lundh *et al.* 1970).

Kinetic studies demonstrated that extracellular Hx enhances heme export from human macrophages, supporting an emerging role of Hx in systemic heme trafficking (Yang *et al.* 2010). It has been suggested that the high serum concentration of Hx, which is comparable to that of the non-heme Fe transporter transferrin, is supportive of the importance of Hx in macrophage heme recycling under physiological conditions (Keel *et al.* 2008). Consistent with a role of Hx in systemic Fe balance in pregnancy, we observed associations between maternal Hx with cord Hb and hepcidin. Few studies have examined possible relationships between Hx and Fe status. In diabetic patients and healthy volunteers (n = 213), serum transferrin was a weak predictor of serum Hx while serum tri-acylglyerol and the diabetic state accounted for the majority of the variation (Van Campenhout *et al.* 2006). It is possible that the positive association between maternal Hx with maternal and cord Hb is representative of a greater capacity of maternal heme recycling and transport in support of fetal erythropoiesis.

The major strength of this study is the concurrent measurement of placental expression of LRP1 and FLVCR1 protein expression with serum Hx in a group of pregnant adolescents with well-characterized Fe status. Due to the observational nature of this study, we could not determine cellular processes underlying the associations between LRP1, Hx, and Fe status and mechanistic studies are needed to assess the heme transport activity of placental LRP1 and determine whether this process is responsive to changes in maternal/neonatal Fe status.

In summary, this study extends the current discussion of heme utilization to the placenta by documenting a relationship between placental LRP1 with maternal and neonatal Fe status in pregnant adolescents with term delivery. Because of the observational nature of this study, further research is needed to elucidate the mechanisms underlying these associations and examine the significance of placental heme scavenging in supporting fetal Fe demands.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations used

| | |
|---|---|
| **CRP** | C reactive protein |
| **Fe** | iron |
| **FLVCR** | feline leukemia virus C receptor |
| **FPN** | ferroportin |
| **Hb** | hemoglobin |
| **Hp** | haptoglobin |
| **Hx** | hemopexin |
| **ID** | iron deficiency |
| **LRP1** | receptor-related protein 1 |
| **RBC** | red blood cells |
| **SF** | serum ferritin |
| **sTfR** | soluble transferrin receptor |
| **TBI** | total body iron |

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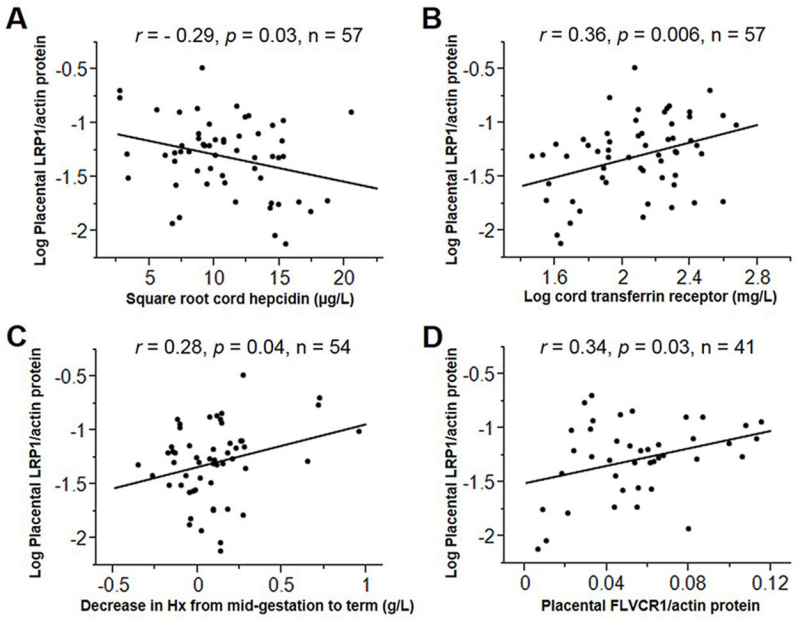


**Fig. 1.**

Significant correlates of placental LRP1 protein expression in 57 healthy pregnant adolescents. Placental LRP1 protein expression was assessed as a ratio between LRP1 and β-actin and log-transformed as required for regression analysis. Placental LRP1 protein expression increased in neonates with high Fe demand as indicated by its inverse correlation with cord hepcidin (Panel A) and positive correlation with cord sTfR (Panel B). Placental LRP1 protein expression correlated positively with the decrease in Hx from mid-gestation to term (Panel C) and with placental protein expression of the heme exporter FLVCR1 (Panel D).

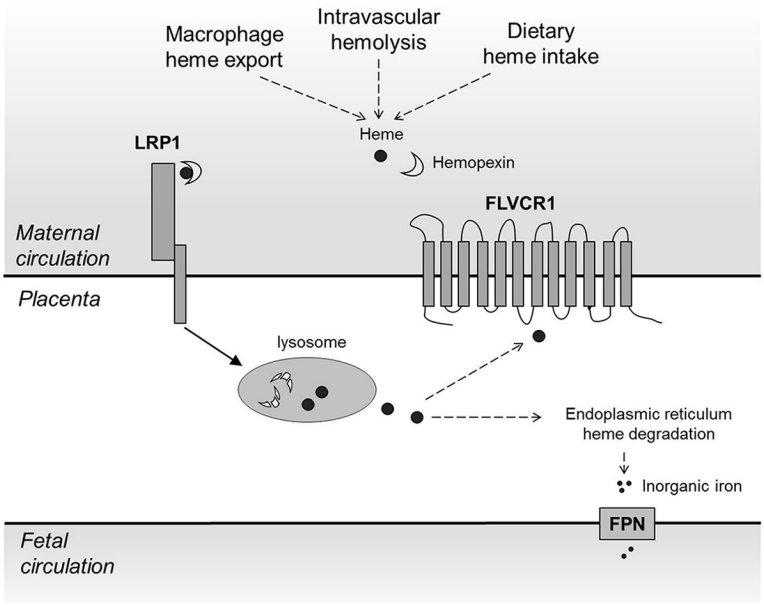


**Fig. 2.**

Proposed pathway of heme Fe utilization in the placenta. Maternal plasma heme may be sourced from heme export from macrophages, oxidation of hemoglobin released during intravascular hemolysis or dietary heme intake. Free heme binds with hemopexin and the complex is taken up by the placental syncytiotrophoblast via LRP1-mediated endocytosis. Upon lysosomal degradation of hemopexin, free heme may be either 1) exported out into the maternal circulation by FLVCR1 or 2) converted to inorganic Fe by heme oxygenase in the endoplasmic reticulum and exported to the fetal circulation by FPN. The figure was modified from the schematic depicting the LRP1-mediated heme uptake pathway by Hvidberg et al. (Hvidberg *et al.* 2005)

**Table 1**

Characteristics of study participants (n = 57)

| Characteristics | Mean ± SD (Range) |
|---|---|
| Age at enrollment (y) | 17.1 ± 1.1 (14.0–18.7) |
| Gestational age at mid-gestation blood draw (wk) | 25.8 ± 4.0 (20.1–37.2) |
| Gestational age at delivery (wk) | 40.0 ± 1.0 (37.9–41.7) |
| Parity ≥ 1 (%) | 11 |
| Pre-pregnancy BMI (kg/m$^2$) | 25.1 ± 6.0 (17.2–43.5) |
| Gestational weight gain (kg) | 17.6 ± 7.7 (−2.1–43.2) |
| Race (%) | |
| African American | 61 |
| Caucasian | 37 |
| Native American | 2 |
| Ethnicity (%) | |
| Hispanic | 25 |
| Non-Hispanic | 75 |
| Delivery mode (%) | |
| Vaginal delivery | 91 |
| Cesarean section | 9 |
| Placental weight (kg) | 0.61 ± 0.11 (0.35–0.86) |
| Birth weight (kg) | 3.30 ± 0.45 (2.50–4.71) |
| Infant gender (%) | |
| Male | 60 |
| Female | 40 |

## Table 2

Iron status indicators in 57 pregnant adolescents and their neonates[1]

| Variable | Mid-Gestation | Delivery | Cord Blood | P value* |
|---|---|---|---|---|
| Hb (g/dL) | 11.4 ± 0.8 (47) [a] | 11.8 ± 1.3 (49) [a] | 13.7 ± 2.7 (36) [b] | < 0.0001 |
| SF (μg/L) | 22.2 ± 19.1 (57) [a] | 22.6 ± 12.9 (57) [a] | 133.0 ± 79.4 (57) [b] | < 0.0001 |
| Serum sTfR (mg/L) | 5.6 ± 5.9 (57) [a] | 5.4 ± 2.7 (57) [a] | 8.3 ± 2.5 (57) [b] | < 0.0001 |
| TBI (mg/kg) | 3.2 ± 3.8 (57) [a] | 3.6 ± 3.6 (57) [a] | 8.0 ± 2.6 (57) [b] | < 0.0001 |
| Serum hepcidin (μg/L) | 28.0 ± 19.9 (57) [a] | 36.2 ± 28.4 (57) [a] | 131.8 ± 88.8 (57) [b] | < 0.0001 |
| Serum Hx (g/L) | 1.0 ± 0.2 (57) [a] | 0.9 ± 0.3 (54) [b] | 0.3 ± 0.2 (47) [c] | < 0.0001 |
| Serum Hp (g/L) | 0.9 ± 0.5 (57) | ND | ND | |
| Serum CRP (mg/L) | 6.6 ± 6.9 (55) [a] | ND | 2.2 ± 10.8 (44) [b] | < 0.0001 |

[1] Data are presented as Mean ± SD (sample size). Hb, hemoglobin; SF, serum ferritin; serum sTfR, serum soluble transferrin receptor; TBI, total body iron; Hx, hemopexin; Hp, haptoglobin; CRP, C-reactive protein; ND, not determined. Sample sizes are different due to missing assay (Hb) or sample availability (serum Hx and CRP);

* Indicators were compared between groups using a linear regression model with Group (mid-gestation, delivery, and cord) as a fixed effect and Subject as a random effect. Values with different subscripts ([a, b, c]) are significantly different