Cell-free fetal DNA in the maternal circulation does not stem from the transplacental passage of fetal erythroblasts

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Fetal cells, specifically fetal erythroblasts, as well as cell-free fetal DNA are present in the maternal circulation. Both are currently being investigated as a means for the non-invasive risk-free analysis of fetal genetic traits. The origin of this cell-free fetal DNA in the maternal circulation is currently unclear. Since numerous fetal erythroblasts have been demonstrated to exhibit an apoptotic phenotype in the form of fragmented nuclear DNA, it has been proposed that such trafficking fetal cells may be a possible source. This hypothesis is supported by reports of elevated numbers of fetal erythroblast and cell-free fetal DNA concentrations in pregnancies affected by pre-eclampsia or polyhydramnios. To address this question, we have examined fetal erythroblast numbers and cell-free DNA concentrations in the same maternal blood samples. Our study, performed on both normal and pathologically-affected pregnancies, indicates that no correlation exists between these two fetal cellular and molecular species. This is most evident in pregnancies affected by onset of preterm labour, where significant elevations in cell-free fetal DNA concentrations were detected without any concomitant elevation in fetal erythroblast numbers. Our data therefore suggest that an alternative cell type is the source of cell-free fetal DNA. Furthermore, it appears that the release of cell-free fetal DNA from this cell type is affected by pathological placental conditions which are not associated with an increase in fetal cell trafficking.

Key words: fetal cells/fetal DNA/maternal blood

Introduction

Two main strategies have emerged in the pursuit to develop a non-invasive and hence risk-free method for prenatal diagnosis of fetal genetic traits: the enrichment of fetal cells from the maternal circulation and, more recently, the analysis of cell-free fetal DNA in maternal plasma or serum (Bianchi and Lo, 2001; Holzgreve and Hahn, 2001). For the cell-based strategy, the fetal erythroblast has emerged as the cell of choice for most researchers. This is largely due to its abundance in the early fetal circulation, high density expression of novel antigens suitable for enrichment and identification, and its short life span which precludes obtaining any fetal cells persisting from previous pregnancies (Bianchi and Lo, 2001; Holzgreve and Hahn, 2001).

Proof of principle studies have indicated that enriched fetal erythroblasts can be examined by fluorescence in-situ hybridization (FISH) for the common aneuploidies (13, 18, 21, X and Y) and by single cell PCR (SC-PCR) for the presence of Mendelian disorders (Bianchi *et al.*, 1992; Elias *et al.*, 1992; Holzgreve *et al.*, 1992; Cheung *et al.*, 1996; Troeger *et al.*, 1999). The efficacy of such an approach is currently being investigated by a large scale study conducted under the auspices of the National Institute for Child Health and Development. Preliminary data from this study have indicated that fetal aneuploidies can be detected by this technology with a higher degree of specificity and lower false positive rate than current non-invasive methods, such as serum screening or ultrasound (de la Cruz *et al.*, 1998; Bianchi *et al.*, 1999).

By extending upon the observation that tumour-derived cell-free

DNA can be detected in the circulation of cancer patients (Chen *et al.*, 1996), Lo and colleagues reported the presence of male cell-free fetal DNA in the serum and plasma of pregnant women (Lo *et al.*, 1997). Recent large scale studies have indicated that fetal genetic loci, such as the Y chromosome or the fetal RhD gene, can be detected by these means with sensitivities of >95 and 100% specificity, which is considerably higher than can be currently attained by the analysis of fetal cells (Sekizawa *et al.*, 2001; Zhong *et al.*, 2001a).

An unexpected scientific spin-off of this research is that it has yielded novel insights into the underlying biology of certain pregnancy-related disorders (Holzgreve and Hahn, 1999). In this regard, in 1994 our group made the novel observation that erythroblast numbers are elevated in pregnancies affected by pre-eclampsia (Ganshirt *et al.*, 1994a). By the use of FISH, we were later able to confirm that a significant proportion of these cells was of fetal origin and that fetomaternal cell traffic was indeed elevated in pre-eclampsia (Holzgreve *et al.*, 1998). In follow-up studies using prospectively collected samples, we and others were furthermore able to show that this disturbance occurred before the onset of symptoms in those pregnancies which subsequently developed pre-eclampsia (Al-Mufti *et al.*, 2000; Holzgreve *et al.*, 2001).

An advantage of studies performed on cell-free fetal DNA is that the quantity of this material can be reliably and reproducibly quantified by the use of sensitive quantitative real-time PCR technology (Lo *et al.*, 1998). By these means, increased concentrations of cell-free fetal DNA have been noted in pregnancies at risk of or with fulminate

pre-eclampsia (Lo *et al.*, 1999b; Leung *et al.*, 2001; Zhong *et al.*, 2001b,c, 2002). In addition, we have observed a very high number of fetal erythroblasts and high concentrations of cell-free fetal DNA in a polyhydramnic pregnancy (Zhong *et al.*, 2000c). Of further interest is that increments in these two fetal parameters have also been observed in pregnancies with certain aneuploid fetuses, in particular those with trisomy 21 (Bianchi *et al.*, 1997; Lo *et al.*, 1999a; Zhong *et al.*, 2000a).

These varied independent results have suggested that some form of inter-relationship may exist between the presence of fetal erythroblasts, or fetal cell trafficking in general, and the release of cell-free fetal DNA into the maternal circulation (Bianchi and Lo, 2001). This hypothesis is further supported by data indicating that a significant proportion of fetal erythroblasts exhibit an apoptotic phenotype in the form of fragmented nuclear DNA (Sekizawa *et al.*, 2000; Hristoskova *et al.*, 2001) and that apoptotic fetal cell remnants are detectable in the plasma of pregnant women (van Wijk *et al.*, 2000). We have now addressed this hypothesis by examining the levels of fetal erythroblasts and cell-free DNA levels in the same maternal blood samples from different pregnancy conditions.

Materials and methods

Ethical approval for this study was obtained from our institutional review committee. Written informed consent was obtained in all instances. The gestational age was confirmed by first trimester ultrasound in all cases. As we quantified the number of male cells or amount of cell-free fetal DNA via the Y chromosome, we have only studied women pregnant with singleton male fetuses. Several parts of this study have relied on the re-analysis of data regarding the number of fetal erythroblasts in relation to cell-free DNA levels in the same sample. Although these data have been obtained from previous unrelated studies, they have not been viewed in this context before. These parts have been annotated accordingly. Four groups of pregnant women were examined in an independent manner.

Groups of pregnant women

Normal pregnant women in the first or second trimester of pregnancy
All blood samples (15 ml on average) were drawn immediately prior to an invasive procedure. The samples were processed within 24 h. The average gestational age at the time of blood sampling was 14 ± 4 weeks. In this study we examined 36 normal maternal blood samples by a combination of XY FISH on enriched fetal cells and real-time PCR on the plasma DNA. We have previously examined fetal erythroblasts by SC-PCR for the RhD gene and the Y chromosome-specific SRY locus in maternal blood samples from 19 RhD pregnant women (Troeger *et al.*, 1999). In a parallel study, we had examined the accuracy of a real-time PCR assay in determining these two fetal loci from the cell-free fetal DNA (Zhong *et al.*, 2001a). We have now examined whether a quantitative relationship exists between these two parameters.

Pregnant women with manifest pre-eclampsia

Pre-eclampsia was defined by a blood pressure of 140/90 mmHg in two determinations 4 h apart or by a diastolic blood pressure of 110 mmHg and an associated proteinuria of 300 mg/24 h after 20 weeks gestation (Holzgreve *et al.*, 1998). In this study, we examined blood samples from seven pregnant women with manifest pre-eclampsia and from 17 matched controls. The mean gestational ages of the subjects were 30 ± 1 weeks (X.Y.Zhong, W.Holzgreve and S.Hahn, unpublished data).

Pregnant women at risk for pre-eclampsia

This part of our examination was again performed by re-analysing data regarding fetal erythroblast numbers and cell-free DNA concentrations that had been obtained previously. Details of this study cohort have been published previously (Holzgreve *et al.*, 2001; Zhong *et al.*, 2001c, 2002).

Pregnant women at risk for preterm labour

Preterm contractions were defined as four or more contractions/20 min according to the Canadian preterm labour investigators' group (Goldenberg *et al.*, 2001). In this study, 31 pregnant women between 20 ± 0 and 33 ± 6 weeks of gestation with preterm contractions were examined. Our study

included 13 gestationally matched controls who all delivered normally at term (Hoesli, Holzgreve and Hahn, unpublished data; Zhong, Holzgreve and Hahn, unpublished data).

Quantification of fetal erythroblasts

Erythroblasts were enriched according to our established protocol using miniMacs magnetic separation (Miltenyi Biotec, Bergisch Gladbach, Germany) (Hahn *et al.*, 1999; Troeger *et al.*, 1999). Positively enriched cells were analysed by XY chromosome-specific FISH analysis (Hahn *et al.*, 1999) or by SC-PCR for the presence of the Y chromosome and/or fetal RhD (Troeger *et al.*, 1999; Zhong *et al.*, 2000c).

We have previously shown that a significant proportion (up to 50%) of the erythroblasts in the maternal circulation are of fetal origin, both by the use of FISH and SC-PCR (Holzgreve *et al.*, 1998; Hahn *et al.*, 1999; Troeger *et al.*, 1999; Zhong *et al.*, 2000c).

Quantitative analysis of cell-free fetal DNA

Plasma was separated by centrifugation and stored at -80°C as described previously (Hahn *et al.*, 2000; Zhong *et al.*, 2000a,b,c). Quantitation of the concentration of male fetal DNA was performed using a well established Y chromosome-specific TaqMan real-time PCR. (Zhong *et al.*, 2000a,b,c). The concentrations are expressed as copies per ml maternal plasma.

Data analysis

The data were analysed by Excel software (Microsoft, Redmond, WA, USA) and are represented by a scatter diagram wherein the number of enriched fetal cells have been plotted against the quantity of fetal DNA determined in the same samples. As the data are not parametric, the significance of the correlation between the two parameters was analysed using Spearman Rank analysis (SPSS Statistical Software Package for Windows).

Results

Normal pregnancies

Our initial intention was to compare the number of male fetal erythroblasts with the concentration of male cell-free fetal DNA in the maternal circulation of normal healthy pregnancies. In the first part of this examination, we used FISH for the X and Y chromosomes to quantify the number of fetal erythroblasts following enrichment from 36 pregnancies with male singleton fetuses; XY-positive cells could be detected in 22 of 36 samples, corresponding to a sensitivity of 61.1%. Our analysis of cell-free fetal DNA by use of real-time TaqMan PCR indicated that male fetal DNA could be detected and quantified in the plasma samples of 34 out of 36 women bearing male fetuses (sensitivity = 94.4%). These values correspond well with the results obtained in previous large scale studies (Hahn *et al.*, 1999; Zhong *et al.*, 2001a).

Due to the extreme scarcity of fetal erythroblasts in the maternal circulation, being in the order of 1 in 10^6 – 10^7 maternal nucleated cells, their frequency is low even after enrichment (Holzgreve and Hahn, 2001). Consequently, we were only able to detect between one and five XY-positive cells amongst the 1000 nucleated cells scored by FISH analysis following enrichment. On the other hand, in those instances where circulatory fetal DNA was detectable, the concentration of cell-free fetal DNA ranged between 28.8 and 424.7 copies per ml maternal plasma, as determined using a Y chromosome-specific real-time PCR assay.

With the determination of these two parameters we could now address our original question and examine whether any correlation exists between the number of enumerated fetal erythroblasts and the level of cell-free fetal DNA. To our surprise, this analysis indicated that no correlation exists between these two parameters (Table I; Figure 1).

As we had previously observed that the analysis of fetal erythroblasts was potentially more efficient by SC-PCR than by FISH

Table I. Lack of correlation between fetal erythroblast numbers and cell-free fetal DNA concentrations in normal 1st and 2nd trimester pregnancies

Study	Fetal erythroblasts	Concentration of fetal DNA/ml maternal plasma	Correlation	P-value
FISH analysis	1 (0–5)	70.12 (28.7–424.7)	–0.22	0.20
SC-PCR analysis	3 (0–22)	58.93 (28.3–176.7)	0.28	0.27

SC-PCR = single cell PCR.
Fetal erythroblasts were either identified by FISH and enumerated per 1000 nucleated cells, or by SC-PCR and enumerated per 10 ml maternal blood. Concentration of fetal DNA is in copies/ml maternal plasma. Values are indicated as median with range in brackets. Correlation was determined using Spearman Rank analysis, which was considered significant at $P < 0.05$.

Figure 1. Lack of correlation between circulating fetal DNA concentrations and erythroblast numbers in the maternal circulation of normal pregnancies. (A) Fetal erythroblasts studied by FISH; (B) Fetal erythroblasts analysed by single cell PCR. There was no significant correlation between these two parameters in either instance ($r = -0.22$, $P = 0.2$ and $r = 0.28$, $P = 0.27$ respectively).

(Garvin *et al.*, 1998; Troeger *et al.* 1999), we next examined the quantitative relationship between the number of fetal erythroblasts identified by SC-PCR with the comparable quantity of cell-free fetal DNA. This analysis was performed on data we had previously obtained regarding the examination of individual micro-manipulated fetal erythroblasts by SC-PCR for the RhD and SRY loci (Troeger *et al.*, 1999), and the parallel evaluation of a real-time PCR assay in determining these fetal genetic traits on cell-free fetal DNA (Zhong

et al., 2001a). Even though we were able to detect almost twice as many fetal erythroblasts in these samples, no correlation was found to exist with the corresponding concentration of cell-free DNA (Table I; Figure 1B). In this regard it is worth noting that even though we are able to detect more fetal erythroblasts by SC-PCR than by FISH, a very good concordance between these two techniques is achieved in cell recycling studies whereby fetal cells are first analysed by FISH and then subsequently by PCR (Sekizawa *et al.*, 1999).

Pregnancies affected by pre-eclampsia

As we and others have previously observed significant increases in fetal cell trafficking as well as release of cell-free fetal DNA in pregnancies affected by pre-eclampsia (Holzgreve *et al.*, 1998; Lo *et al.*, 1999b; Zhong *et al.*, 2001c), we examined these two parameters under these conditions. For this analysis we made use of a recently performed study in which the numbers of fetal erythroblasts were determined by SC-PCR (Zhong, Holzgreve and Hahn, unpublished data); we were again able to confirm that pre-eclampsia is associated with a significant increase in the trafficking of fetal erythroblasts into the maternal periphery compared with that in matched controls (median of 10 versus 0 per ml maternal blood). The simultaneous assessment of the fetal DNA concentrations in these samples indicated that the levels of cell-free fetal DNA were also elevated in those pregnancies affected by pre-eclampsia compared with matched control pregnancies (median of 933.9 versus 179.3 copies per ml maternal plasma) (Zhong, Holzgreve and Hahn, unpublished data). In our assessment of the possible relationship between these two parameters we could, however, determine no correlation between fetal erythroblasts numbers and cell-free fetal DNA levels in either the pre-eclamptic or normotensive pregnancies (Figure 2).

Pregnancies at risk for pre-eclampsia

We and others have previously observed that fetal cell trafficking (Al-Mufti *et al.*, 2000; Holzgreve *et al.*, 2001) and cell-free fetal DNA (Leung *et al.*, 2001; Zhong *et al.*, 2001c, 2002) in maternal blood are already elevated at 20 weeks of pregnancy in those pregnancies which later develop pre-eclampsia. These results imply that these parameters may be able to serve as novel markers to predict the disorder (Holzgreve and Hahn, 1999). We have now examined whether the release of cell-free fetal DNA into maternal blood is associated with the observations we had previously made regarding increased levels in fetal cell trafficking (Holzgreve *et al.*, 2001) and elevated fetal DNA concentrations (Zhong *et al.*, 2001c, 2002). Although our previous observations indicated an increase in erythroblast numbers (~6-fold) and cell-free DNA (~3-fold) in those pregnancies which subsequently developed pre-eclampsia (Holzgreve *et al.*, 2001; Zhong *et al.*, 2001c, 2002), our re-assessment of their corresponding quantities indicated that no correlation exists between these two parameters under such conditions (Figure 3).

Figure 2. Lack of correlation between circulating fetal DNA concentrations and erythroblast numbers in the maternal circulation of pregnancies with manifest pre-eclampsia ($n = 7$, $r = 0.02$, $P = 0.97$) or matched controls ($n = 17$, $r = -0.01$, $P = 0.67$). Erythroblasts were identified morphologically and subsequently characterized by single cell PCR. Values are indicative of total erythroblast numbers per 10 ml maternal blood. Concentration of fetal DNA is in copies/ml maternal plasma. Correlation was determined using Spearman Rank analysis, which was considered significant at $P < 0.05$.

Figure 3. Lack of correlation between circulating fetal DNA concentrations and erythroblast numbers in the maternal circulation of pregnancies which subsequently developed pre-eclampsia ($r = 0.23$, $P = 0.55$) or in those pregnancies with normal outcome ($r = 0.02$, $P = 0.89$). Erythroblasts were identified morphologically and enumerated per 10 ml maternal blood. Concentration of fetal DNA is in copies/ml maternal plasma. Correlation was determined using Spearman Rank analysis, which was considered significant at $P < 0.05$.

Pregnancies affected by preterm labour

It has recently been reported that preterm labour is associated with an increased release of cell-free fetal DNA into the maternal circulation (Leung *et al.*, 1998). For this purpose we have recently examined fetal erythroblast (Hoesli *et al.*, unpublished data) and cell-free fetal DNA levels (Zhong, Holzgreve and Hahn, unpublished data) under these conditions. We have now determined whether a correlation exists between these two parameters under these conditions.

A striking feature of this study was that we determined that there was no increment in erythroblast numbers in pregnancies with preterm contractions when compared with the control group (median = 0 erythroblasts per ml maternal blood in both instances; $P = 0.8$) (Hoesli *et al.*, unpublished data). Single cell PCR analysis of the few erythroblasts we were able to retrieve indicated that 48% were of fetal origin. On the other hand, we observed significant elevations in cell-free DNA concentrations in pregnancies with preterm labour when compared with the control group (median concentration of 251.5 versus 91.6 copies per ml maternal plasma; $P = 0.003$) (Zhong, Holzgreve and Hahn, unpublished data).

This result therefore indicates that cell-free fetal DNA levels can be elevated in the absence of elevations in the traffic of fetal erythroblasts, a feature which is graphically represented in Figure 4.

Discussion

The novel discoveries of cell-free fetal DNA (Lo *et al.*, 1997), fetal cell remnants (van Wijk *et al.*, 2000) and fetal erythroblasts with apoptotic character (Sekizawa *et al.*, 2000; Hristoskova *et al.*, 2001) in the maternal circulation have led authors to question the relationship between these molecular and cellular entities (Bianchi and Lo, 2001). In this regard, it has been proposed that cell-free fetal DNA may be derived from the interaction of fetal cells with maternal immune effector cells (Sekizawa *et al.*, 2000). This proposal is based upon an observation that numerous erythroblasts in the maternal circulation exhibit an apoptotic phenotype in the form of nuclear fragmentation as measured by the TUNEL assay (Sekizawa *et al.*, 2000). In a separate study, we have shown that numerous fetal erythroblasts already display such characteristics while still in the fetal circulation (Hristoskova *et al.*, 2001), a feature which is consistent with other observations that erythroblast terminal differentiation and enucleation involves apoptosis-like processes (Zermati *et al.*, 2001). Since erythroblast enucleation has been observed in the periphery in the absence of accessory cells (Marmont, 1998), it is possible that this end stage differentiation step may be a key contributor to the presence of cell-free fetal DNA in the maternal circulation. This hypothesis would be reinforced by observations that elevations in both fetal erythroblasts

Figure 4. Lack of correlation between circulating fetal DNA concentrations and erythroblasts numbers in the maternal circulation of pregnancies affected by onset of preterm contractions. The study included 31 cases with preterm contractions ($r = -0.44$, $P = 0.82$) and 13 matched controls ($r = -0.06$, $P = 0.84$). Erythroblasts were identified morphologically and subsequently characterized by single cell PCR. Values are indicative of total erythroblast numbers per 10 ml maternal blood. Concentration of fetal DNA is in copies/ml maternal plasma. Correlation was determined using Spearman Rank analysis, which was considered significant at $P < 0.05$.

(Holzgreve *et al.*, 1998, 2001) and cell-free fetal DNA (Lo *et al.*, 1999b; Leung *et al.*, 2001; Zhong *et al.*, 2001b,c, 2002) occur in several pregnancy-related pathologies, such as pre-eclampsia.

In this study, we have now examined this issue in greater detail by determining the relationship between fetal erythroblast numbers and cell-free fetal DNA levels in the same maternal blood samples under different pregnancy conditions. In our analysis, we have used different technologies to enumerate the numbers of fetal erythroblasts, namely FISH and SC-PCR, and real-time PCR to quantify the concentration of cell-free DNA. The reason for this technical diversity is that currently no reliable method exists for the quantification of fetal erythroblasts by real-time PCR. Therefore, some small deviation in our results may be attributable to subtle differences in these technologies. Because we have observed very comparable results for either of the techniques used in numerous blinded and prospective studies, we feel confident that the error margin is very small.

Our present study has shown that no correlation exists between fetal erythroblast numbers and cell-free fetal DNA levels in normal pregnancies, or under a variety of pregnancy-related pathologies associated with increased fetal cell traffic or release of cell-free fetal DNA.

The disparity between fetal erythroblast numbers and cell-free DNA concentration was most notable in pregnancies with preterm contractions, where we observed significant increments in cell-free fetal DNA levels without any discernible increase in fetal erythroblast numbers. Therefore, our data strongly suggest that another cell type is responsible for the release of cell-free fetal DNA into the maternal circulation. Our data further imply, provided that fetal erythroblasts can be used as a marker for fetal cell traffic across the placenta in general, that the cell type responsible for the release of cell-free fetal DNA is affected by conditions which do not alter fetal cell traffic. In this manner our results may help elucidate fundamental differences between various pregnancy-related pathologies such as pre-eclampsia and preterm labour. Although we have not determined the source of cell-free fetal DNA and it is possible that the source may vary depending on the type of pregnancy related disorder, our data do, however, favour hypotheses which propose that cell-free fetal DNA is derived from the placenta and is not the result of fetal cell trafficking (Lo, 2000). Observations which support a placental component include the very rapid disappearance of cell-free DNA from the maternal circulation following delivery (Lo *et al.*, 1999c), conditions under which fetal erythroblasts are still very readily detectable for several

days post-delivery (Ganshirt *et al.*, 1994b). Indeed, it was the similarity between the placenta and tumours (Lo, 2000) that first led to the original investigations of whether cell-free DNA existed in the maternal circulation, in that: (i) the placenta and tumours are large tissues in direct contact with the peripheral circulation; (ii) both have a very high degree of cell division and cell turnover; (iii) both express high levels of certain proto-oncogenes; and (iv) tumours are known to release cell-free DNA into the circulation of cancer patients.

Other observations favouring placental origin are that cell-free fetal DNA levels are in general several orders of magnitude greater than fetal cell levels (Lo, 2000). Furthermore, cell-free fetal DNA concentrations are not increased by processing conditions which would lead to the demise of fetal cells present in the maternal blood sample being processed, for instance by the preparation of serum which involves blood clotting (Chui *et al.*, 2001; Hahn *et al.*, 2001). These experiments have also demonstrated that most of the cell-free fetal DNA in maternal plasma exists in a distinct acellular form and very little is present in the form of apoptotic cell remnants (Chui *et al.*, 2001; Hahn *et al.*, 2001). Furthermore, there is no significant increase in the amount of cell-free fetal DNA in serum samples, conditions under which the fetal cells present would be expected to die and release their DNA during the clotting process. This is in strong contrast to the total cell-free DNA concentrations, which are significantly increased in serum samples.

Although it is unclear by which means cell-free DNA is released from the placenta, one attractive hypothesis is that it is mediated by the shedding of cell fragments and excess nuclei from the syncytiotrophoblast as it is constantly replenished by the underlying differentiating cytotrophoblast (Huppertz *et al.*, 2001). It has been estimated that the amount of cellular material released by this process into the maternal circulation is in the order of several grams per day (Huppertz *et al.*, 2001). Since the half life of cell-free fetal DNA in the maternal circulation has been estimated to be in the order of 15 min (Lo *et al.*, 1999c), it becomes clear that prodigious quantities of input material would be required to facilitate a high level steady-state concentration (Lo, 2000).

It is likely that the true source of cell-free DNA in the maternal circulation will remain a scientifically contentious issue (Bianchi and Lo, 2001), which will require many more studies to be resolved. The best approach will probably be to examine cell-free fetal DNA levels with fetal genes which are epigenetically modified in different fetal tissues (Poon *et al.*, 2002).

Our data do, however, indicate that fetal erythroblasts are not involved to a significant degree in the generation of this material. Furthermore, our study provides new evidence of discrete differences between pre-eclampsia and preterm labour, perhaps at a placental level, in that preterm contractions are only associated with release of cell-free fetal DNA whereas both fetal cell traffic and cell-free DNA release are perturbed in pre-eclampsia. Therefore, further exploration of these phenomena may aid in our understanding of these intriguing disorders.

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