Reproductive BioMedicine Online (2012) **25**, 508–520

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Circulating trophoblastic cells provide genetic diagnosis in 63 fetuses at risk for cystic fibrosis or spinal muscular atrophy

Hussein Mouawia [a,d], Ali Saker [a,e], Jean-Philippe Jais [b], Alexandra Benachi [c], Laurence Bussières [f], Bernard Lacour [g], Jean-Paul Bonnefont [h], René Frydman [c], Joe Leigh Simpson [i], Patrizia Paterlini-Brechot [a,g,*]

[a] *Unité INSERM 807, Université Paris Descartes, Paris, France;* [b] *Biostatistique, EA 4067 Biostatistique, Université Paris Descartes, Faculté de Médecine, AP-HP, Hôpital Necker-Enfants Malades, Paris, France;* [c] *Service de Gynécologie-Obstétrique, Hôpital Antoine Béclère, Clamart, Université Paris Sud France;* [d] ***Current address:*** *Université Libanaise, Faculté des Sciences, Faculté de la Santé Publique, Département de Biologie, Zahlé, Liban;* [e] ***Current address:*** *Damascus University, Faculty of medicine, Department of Embryology and Genetics, Laboratory of Genetic Research, Damas, Syria;* [f] *Unité de Recherche Clinique Paris Ouest, Hôpital Ambroise-Paré AP-HP, Boulogne Billancourt, France;* [g] *Laboratoire de Biochimie A, AP-HP, Hôpital Necker-Enfants Malades, Paris, France;* [h] *Laboratoire de Génétique Médicale, AP-HP, Hôpital Necker-Enfants Malades, Paris, France;* [i] *Florida International University, College of Medicine, Miami, FL, USA*
[*] Corresponding author. *E-mail address:* patriziapaterlini@gmail.com (P Paterlini-Brechot).

Patrizia Paterlini-Brechot, MD, PhD is professor of cell biology and oncology at University Paris Descartes, Paris, France and director at INSERM. In addition to fundamental research studies in her career on hepatitis B and C-related liver carcinogenesis and calcium signalling, she has focused on applied research, targeting the domains of predictive oncology and prenatal diagnosis; her research activity led to the discovery and development of ISET (isolation by size of epithelial tumour/trophoblastic cells) for the characterization of circulating tumour and trophoblastic cells, thus permitting non-invasive cancer diagnostics and non-invasive prenatal diagnosis and leading to high-level publications and numerous awards.

Abstract This study sought to determine whether a reliable non-invasive prenatal diagnosis (NI-PND) of cystic fibrosis (CF) or spinal muscular atrophy (SMA) can be achieved through analysis of circulating fetal trophoblastic cells (CFTC). The kinetics of CFTC circulation were also studied. CFTC were isolated by isolation by size of epithelial tumour/trophoblastic cells at 9–11 weeks of gestation, before chorionic villus sampling (CVS), from the blood of 63 pregnant women at 25% risk for having a child affected by either CF ($n = 32$) or SMA ($n = 31$). Collected cells were laser-microdissected, short tandem repeat-genotyped to determine fetal origin and blindly assessed for mutation analysis. CFTC were independently analysed weekly (4–12 weeks of gestation) in 14 women who achieved pregnancy following IVF. Diagnostic results were compared with those obtained by CVS. All seven CF and seven SMA pregnancies carrying an affected fetus were correctly identified as well as non-affected pregnancies. CFTC provided 100% diagnostic sensitivity (95% CI 76.8–100%) and specificity (95% CI 92.7–100%) in these 63 consecutive pregnancies at risk for CF or SMA. CFTC were found to circulate from 5 weeks of gestation and can be used to develop an early and reliable approach for NI-PND.

KEYWORDS: circulating fetal cells, cystic fibrosis, diagnostic accuracy, ISET, non-invasive prenatal diagnosis, spinal muscular atrophy

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2012.08.002

Introduction

Definitive non-invasive prenatal genetic diagnosis (NI-PND) is a long-standing goal to avoid miscarriage related to invasive prenatal diagnostic procedures (Mujezinovic and Alfirevic, 2007). Initial advances involved recovery of intact fetal cells, usually erythroblasts, from maternal blood (Bianchi et al., 1990; Herzenberg et al., 1979; Holzgreve et al., 1992; Simpson and Elias, 1993). In the National Institute of Child Health and Development collaboration reported by Bianchi et al. (2002), the fluorescence-activated cell sorting and magnetic-activated cell sorting approaches used to isolate circulating fetal erythroid cells resulted in 74% sensitivity in detecting trisomy 21. However, the approach only targeted pregnant women carrying a male fetus and consistently obtaining results has not been possible (Bianchi et al., 2002), thus precluding clinical introduction. Other approaches have since been pursued and current emphasis has shifted to cell-free fetal DNA (Wright and Chitty, 2009). However, the amount of free fetal DNA in plasma is highly variable, with usually a low proportion of fetal free DNA (3.4–6.2%) (Lo et al., 1998). Furthermore, admixture of fetal DNA with the large majority of maternal DNA makes detection of fetal abnormalities technically complex. Thus, the recovery of intact fetal cells still has a unique potential to develop a reliable NI-PND method.

Previous studies has showed proofs of principle for the ability to isolate circulating fetal trophoblastic cells (CFTC) and use them for NI-PND of cystic fibrosis (CF) (Saker et al., 2006) and spinal muscular atrophy (SMA), in mothers with both male and female fetuses (Beroud et al., 2003). CFTC are consistently present in pregnant mothers and do not persist in maternal blood after pregnancy termination (Bianchi et al., 1996; P. Paterlini Brechot, unpublished results), making their use for NI-PND especially attractive.

The present work explores further the potential of CFTC for NI-PND. A prospective, blind validation study was conducted involving a non-invasive test versus chorionic villus sampling (CVS), targeting five or 10 CFTC per mother in consecutive couples at risk of CF or SMA. The kinetics of CFTC circulation were also studied in pregnant women after IVF using precise information of the date of conception. The goal was to assess the possibility of CFTC recovery providing a consistent early NI-PND test. The results show that the CFTC-based test, when compared with the CVS-based test, obtained 100% diagnostic sensitivity and specificity. Furthermore, it was demonstrated that CFTC circulate in maternal blood beginning at 5 weeks of gestation. Having verified the diagnostic accuracy of this method for early and reliable NI-PND of CF and SMA, by analogy the approach can be extended to many other genetic disorders.

Materials and methods

Pregnant women

Blood from two groups of pregnant women were studied. First, a validation study was performed involving 63 pregnant women (mean age 37.9 years) at risk for either CF (mean age 35.3 years), or SMA (mean age 40.5 years). Maternal blood was obtained at 9–11 weeks of gestation prior to CVS and any invasive prenatal genetic procedure. Some couples had a previously affected child. Except for pregnancy terminations, for reasons to be described, pregnancy complications were unusual. Maternal blood samples (20 ml) were collected in EDTA buffer. In each couple, 1 ml of paternal and 1 ml of maternal blood were reserved for parental genotyping. The at-risk groups of 32 CF and 31 SMA women represented a consecutive series recruited in the Necker Enfants Malades Hospital (Paris-France), thus mimicking circumstances under which a definitive NI-PND test would be offered clinically. Data concerning gestational ages are shown in **Tables 1** and **2**. During this study period, no other couples at risk for CF or SMA presented to this clinic.

In 26 of the 32 couples at risk for having a child affected by CF, both parents were F508del carriers; in five couples (nos. 11, 26–28, 31; **Table 1**) one parent was a F508del carrier whereas the other was not a carrier for F508del; in one couple (no. 12; **Table 1**) neither parent had F508del. It is known that other CF mutations existed and could be detected, but testing CFTC for other CF mutations was not necessary to obtain a diagnostic result in this series. In all 31 couples at risk for SMA, each parent carried the heterozygous deletion for SMN1. Thus, the affected fetus in each at-risk pregnancy should be homozygous for the SMA deletion.

Second, a blood sample was obtained weekly from 4 to 12 weeks of gestation from 14 women (nos. 1–14 **Figure 3;** mean age 38.4 years) who conceived by IVF, the goal being to determine the number of CFTC per ml and per gestational age. The 14 women undergoing IVF were recruited in the Antoine Béclère Hospital (Clamart-France). None were at increased risk for having a fetus affected by CF or SMA, or for any other genetic disease.

This study received the approval of both the Ethical Committee of Ile de France XI (approval reference number CCP02001, approval date 11 April 2002), and the local ethics committee. All women participating in this study gave written informed consent.

None of these subjects were among those previously reported by this study group during proof of principle studies (Beroud et al., 2003; Saker et al., 2006).

ISET and identification of CFTC

In ISET (isolation by size of epithelial tumour/trophoblastic cells; Beroud et al., 2003; Saker et al., 2006; Vona et al., 2002), circulating epithelial cells (including trophoblasts) are efficiently recovered on the basis of size, being larger than leukocytes (**Figure 1**). Within 4 h of collection in EDTA, each 10 ml of whole maternal blood is diluted 1:10 using a proprietary buffer that lyses erythrocytes and fixes any nucleated cells (Rarecells, Paris, France). Precisely 10 min thereafter, the solution is passed through a cartridge-containing filter under negative pressure (Rarecells). Cells smaller than 8 μm pass through the filter's calibrated pores, which are 8 μm in diameter. Larger cells remain on the filter, which can be stored at −20°C. Epithelial cells were microdis-