Clinical Case Report

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# Cell-free DNA in pregnancy with choriocarcinoma and coexistent live fetus

## A case report

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**Abstract**

**Background:** This case report describes the use of analysis of cell-free DNA in the blood of a patient with a pregnancy with one live fetus and a choriocarcinoma diagnosed at 22 weeks of gestation.

**Results:** The result of the analysis of 16 microsatellite loci on 14 chromosomes in the cell-free DNA in plasma was consistent with the result of the analysis of a tumor biopsy indicating biparental diploid origin of the genome. The DNA markers were discordant with the markers of the placenta indicating two separate conceptions.

**Conclusion:** Our results indicate that analysis of cell-free DNA in plasma allows determination of the origin of a choriocarcinoma without tissue biopsy, even in the presence of a co-existent pregnancy.

**Abbreviations:** cfDNA = cell-free DNA, CT = computed tomography, GTN = gestational trophoblastic neoplasia, hCG = human chorionic gonadotropin.

**Keywords:** cell-free DNA, choriocarcinoma, coexistent pregnancy, noninvasive diagnostics, trophoblastic neoplasia

### 1. Introduction

Trophoblastic diseases encompass a wide range of disorders from benign hydatidiform mole to malignant choriocarcinoma. Choriocarcinoma is seen in 1 of 50,000 deliveries.[1] Choriocarcinoma in a pregnancy with a coexistent live fetus is rare.[2]

Trophoblastic tumors are highly vascular and biopsy therefore implies a risk of life-threatening hemorrhage.[1] Consequently, the origin of these tumors is often estimated from information on the preceding pregnancy. However, a gestational trophoblastic neoplasia (GTN) can present several years after the termination of the pregnancy causing the disease.[3] The optimal treatment and the prognosis differ for GTN after a molar pregnancy compared to both neoplasia after a nonmolar pregnancy and to non-GTN. In addition, the time interval between the pregnancy and the diagnosis of GTN influences the optimal treatment and the prognosis.[3–8]

Gestational DNA can be detected in cell-free DNA (cfDNA) from maternal blood.[9] Likewise, tumor DNA has been detected in cfDNA from patients with cancer.[10] Analysis of cfDNA from patients with trophoblastic disease has only recently been explored and shows potential to improve the diagnosis and treatment for this group of patients.[11]

### 2. Case history

A 33-year-old woman, secundigravida, with 1 normal delivery of a live female infant 15 years earlier was admitted to the department of gynecology at 22 weeks of gestation with a bleeding tumor in the liver, lung metastases, a tumor in the placenta, and human chorionic gonadotropin (hCG) levels higher than expected.

Her pregnancy was the result of a third attempt with ovulation induction and intrauterine insemination. During the first trimester, the patient suffered from severe hyperemesis, which was managed by nasogastric feeding. In the second trimester, she presented with vaginal bleeding and was admitted to the hospital several times. An ultrasound scan at 19 weeks of gestation showed a normal fetus and a solid mass in the placenta measuring 5 cm × 6 cm, which was thought to be a uterine fibroid. At 21 weeks of gestation, the patient experienced severe upper abdominal pain. A computed tomography (CT) scan revealed a 9 cm hemorrhagic tumor in the liver and suggested metastases in the lungs. The level of hCG in serum was 200,000 IU/L. An intrapartum choriocarcinoma was thus suspected and due to severe pain, bleeding, and general fatigue the patient underwent hysterectomy at $22^{+3}$ weeks. The female fetus was live born but died within an hour. Between the placenta and the uterine wall, a tumor sized 6 cm × 7 cm × 7 cm was found.

The placenta and the uterus were sent for histopathologic examination. The histomorphologic appearance of the placenta

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was normal. At gross examination of the uterus, nodules of grayish tumor masses infiltrating the endometrium intermixed with necrosis and hemorrhage were found. Microscopically, the tumor displayed a classic biphasic pattern of alternating layers of mononucleated trophoblastic cells and syncytiotrophoblastic cells as well as areas with extensive and hemorrhage. Thus, histopathology diagnosed a gestational choriocarcinoma.

The patient was first treated with 1 course of chemotherapy with Methotrexate (2.5 mg 4 times a day orally, day 1–5) and Actinomycin-D (0.5 mg IV a day, day 1–5) with no response. Hereafter the patient received 4 courses of Bleomycin (30,000 IU IV day 2, 9, and 16), Etoposide (100 mg/m$^2$ IV a day, day 1–5), and Cisplatin (20 mg/m$^2$ IV a day, day 1–5) (BEP) resulting in a complete response. During the first year of follow-up, the patient has shown no sign of relapse. Total follow-up will be conducted for 5 years.

## 3. Materials and methods

### 3.1. Sample collection for DNA extraction

A sample from the macroscopically normal part of the placenta was collected without fixation.

From the choriocarcinoma, tissue was microdissected from formalin-fixed paraffin-embedded tissue.

Lithium heparin and EDTA blood samples were collected from the patient and her husband. The samples from the patient were collected during the hysterectomy.

### 3.2. Karyotyping

Karyotyping was performed by analyzing 10 Q-banded metaphases from cultured cells, using standard methods.

### 3.3. DNA extraction

DNA was extracted from the tissue samples using Maxwell LEV blood kit according to the instruction of the manufacturer (Promega, Madison, USA) with the exception of lysis being performed overnight.

DNA was extracted from peripheral blood leukocytes (from EDTA stabilized whole blood) using Chemagic MSN I according to the instruction of the manufacturer (Chemagen, Baesweiler, Germany).

For isolation of cfDNA from plasma, plasma was collected by double centrifugation of EDTA stabilized whole blood (centrifuged 1600 rcf for 10 minutes, plasma fraction transferred to a new tube, and centrifuged 14,000 rcf for 10 minutes). Automated DNA extraction from 1 mL plasma was performed with MagNa Pure Compact (Roche Applied Science, Basel, Switzerland) using the MagNA Pure Compact Nucleic Acid Isolation Kit I—Large Volume; DNA was eluted in 50 μL.

### 3.4. DNA marker analysis

All DNA samples were analyzed using the AmpFlSTR Identifier kit that analyzes 16 microsatellite loci on 14 chromosomes, and capillary electrophoresis (3500 × L) according to the instructions of the manufacturer (ThermoFisherScientific/Life Technologies, California, USA). Data were analyzed using GeneMarker v2.6.3 (Softgenetics, Pennsylvania, USA).

The patient gave informed consent to this study.

## 4. Results

Chromosome analysis of the normal placenta disclosed the karyotype 46,XX. The results of DNA marker analyses are illustrated in Figs. 1 to 3 and summarized in Table 1.

In the DNA marker analysis of the normal placenta, we identified 1 to 2 alleles in every locus. By comparing with the alleles in the patient and her husband, the pattern in all loci was consistent with a normal diploid biparental origin of the genome.

For the DNA from the choriocarcinoma, the results for all loci included substantial signals identical with the signals for the DNA of the patient, indicating admixture of maternal cells. In

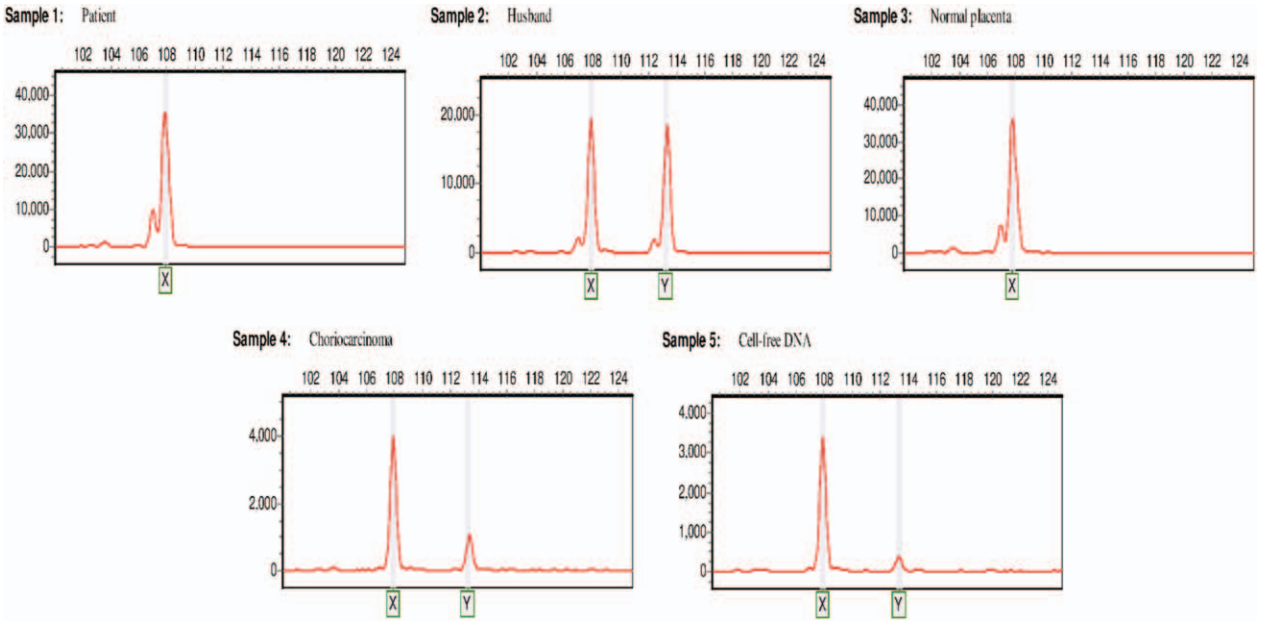


**Figure 1.** Results for the marker AMEL located on both chromosome X and Y. The X-axis represents DNA fragment size in base pairs. The Y-axis shows fluorescence intensity. The marker shows a signal on the chromosome Y in only the husband, the choriocarcinoma, and the cfDNA.

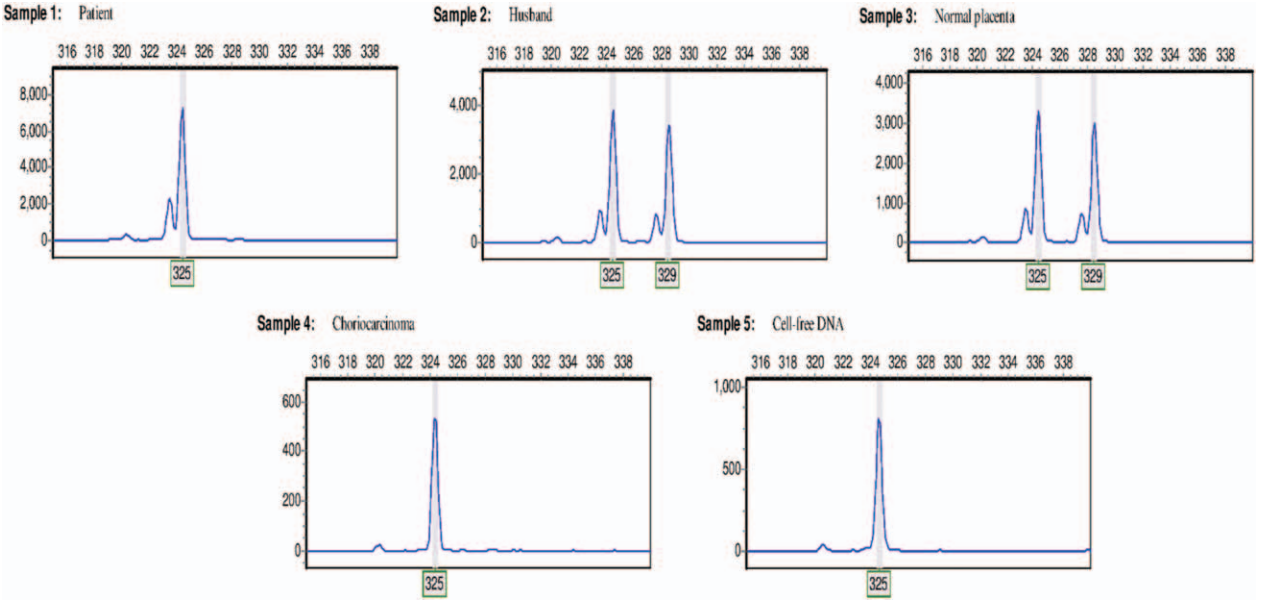


**Figure 2.** Results for the marker CSFP1O located on chromosome 5. The X-axis represents DNA fragment size in base pairs. The Y-axis shows fluorescence intensity. The result for the husband displays that he is heterozygous, whereas the result for the patient shows that she is homozygous for an allele identical with one of the alleles in her husband. The result for the placenta shows 1 allele that could only be paternal, and 1 allele that could be either maternal or paternal. The results for the choriocarcinoma and the cfDNA are identical and show homozygosity for an allele that could be either maternal or paternal, indicating that the placenta and the choriocarcinoma originated in separate conceptions.

addition, for all of 7 informative loci, the results indicated the presence of cells with a biparental, male genome inherited from the patient and her husband. In cfDNA, the results for all loci were comparable to the results from the choriocarcinoma.

Due to the presence of signals identical with those of the patient, we cannot completely exclude polyploidy or mosaicism. However, the intensity of the fluorescence signal for all 16 loci was consistent with biparental diploidy with

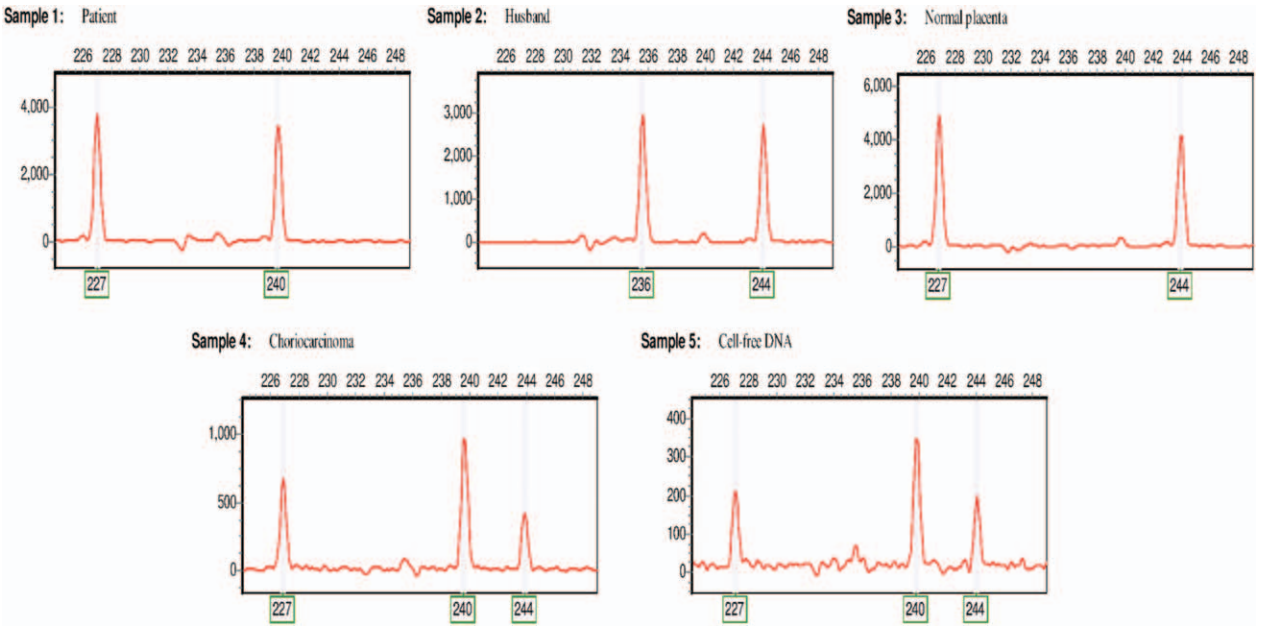


**Figure 3.** Results for the marker FGA located on chromosome 4. The X-axis represents DNA fragment size in base pairs. The Y-axis shows fluorescence intensity. The results for both the husband and the patient show heterozygosity. The patient and the husband do not have identical alleles. The result for the placenta shows 1 allele identical with an allele in the husband and 1 allele identical with an allele in the patient. The results for the choriocarcinoma and the cfDNA are identical. Signals indicating both alleles in the patient are present. However, the fluorescence intensity for 240 base pairs-allele is higher than expected and an allele identical with 1 allele in the husband is present, indicating a mixture of DNA from the patient and from a diploid biparental cell population genetically different from the diploid biparental cell population in the normal placenta.

**Table 1**

**Results of the analysis of 16 microsatellite loci on 14 chromosomes in the husband, the patient, the normal placenta, the choriocarcinoma, and the cfDNA.**

| Marker | Chromosome localization | Husband | Patient | Normal placenta | Choriocarcinoma | Cell-free DNA |
|---|---|---|---|---|---|---|
| AMEL | X: p22.1–22.3; Y: p11.2 | XY | X | **X** | **X**/Y | **X**/Y |
| CSF1PO | 5q33.3-34 | 325/329 | 325 | 325/329 | 325 | 325 |
| D13S317 | 13q22-31 | 232/236 | 236 | 232/236 | 232/**236** | 232/**236** |
| D16S539 | 16q24-qter | 278 | 278/282 | 278/282 | **278**/282 | **278**/282 |
| D18S51 | 18q21.3 | 290/298 | 290/294 | 290 | **290**/294 | **290**/294 |
| D19S433 | 19q12-13.1 | 114/118 | 118/130 | 118/130 | 114/**118**/130 | 114/**118**/130 |
| D21S11 | 21q11.2-q21 | 210/224 | 206/210 | 206/210 | 206/**210** | 206/**210** |
| D2S1338 | 2q35-37.1 | 316/349 | 316/341 | 341/349 | 316/341/349 | 316/341/349 |
| D3S1358 | 3p | 129/133 | 129/133 | 129/133 | 129/**133** | 129/**133** |
| D5S818 | 5q21-31 | 148/161 | 148/157 | 148/157 | **148**/157/161 | **148**/157/161 |
| D7S820 | 7q11.21-22 | 266/270 | 266/281 | 270/281 | **266**/270/281 | **266**/270/281 |
| D8S1179 | 8 | 147 | 147/151 | 147/151 | **147**/151 | **147**/151 |
| FGA | 4q28 | 236/244 | 227/240 | 227/244 | 227/**240**/244 | 227/**240**/244 |
| TH01 | 11p15.5 | 180 | 172/188 | 180/188 | 172/180/**188** | 172/180/**188** |
| TPOX | 2p23-2per | 233 | 233 | 233 | 233 | 233 |
| vWA | 12p12-pter | 176/184 | 184/188 | 184 | 184/188 | 184/188 |

Numbers are DNA fragment size in base pairs and numbers in bold indicate a relatively high intensity of the fluorescence signal.

maternal contamination. Thus, the choriocarcinoma most likely originated in a diploid biparental conceptus.

In 9 loci, the choriocarcinoma displayed at least 1 allele not present in the normal placenta. In 1 locus, the normal placenta displayed an allele not present in the choriocarcinoma, indicating that the choriocarcinoma and the placenta originated from 2 separate conceptions. The discordances included both paternal and maternal alleles.

The female child of the patient born 15 years before the present pregnancy was not analyzed as the marker analyses indicated the presence of a Y-chromosome in the choriocarcinoma.

## 5. Discussion

Treatment and prognosis differ between gestational and non-GTN, and between gestational trophoblastic neoplasia with biparental and androgenetic genomes. Further, the time interval from the pregnancy causing the disease is an important prognostic factor.[8] Thus it is relevant to genotype these tumors. However, biopsy is often avoided due to the risk of hemorrhage and thus avoided.

Our observations illustrate that it is possible to determine the origin of the genome in a choriocarcinoma by analyzing cfDNA in the blood of the patient.

The interpretation of the results of the analysis of cfDNA is hampered by the contamination by maternal DNA. Thus, the origin of the genome in the neoplastic cells is estimated based on fluorescence intensity in the informative loci. In this case, we could not fully exclude polyploidy or mosaicism.

Triploidy and tetraploidy are both observed in gestational trophoblastic disease and can give rise to skewed fluorescence intensities. However, polyploid molar pregnancies generally show heterozygosity for paternal alleles.[12,13] Although we only had few informative loci, we did not observe heterozygosity for paternal markers making polyploidy unlikely. Increasing the number of loci analyzed could increase the validity of this conclusion.

Mosaicism between a diploid androgenetic cell population and a diploid biparental cell population is observed in trophoblastic disease. In analysis of cfDNA, the inherent maternal contamination complicates identification of this genetic constitution, particularly in the mosaics where the paternal genome is identical in the 2 cell lines. Such rare cases may be overlooked no matter how many loci are analyzed.[14]

In the present case, the results of analysis of the choriocarcinoma also indicated significant contamination with maternal cells, suggesting that the validity of analysis of cfDNA may not differ significantly from the validity of analysis of DNA from a biopsy from a trophoblastic tumor.

Identification of markers not present in the patient indicates that a tumor is of gestational origin and biparental diploidy indicates that the tumor most likely did not originate from a molar pregnancy. The prognosis is less favorable for a nonmolar gestational trophoblastic tumor than for a tumor of molar origin. As such, incorrectly concluding that a tumor is of nonmolar origin by overlooking polyploidy or mosaicism would mean a more intensive treatment than might be necessary. Incorrectly concluding that a tumor is of molar origin by overlooking biparental diploidy would imply a risk of inadequate treatment. Fortunately, this risk is low.

Only few cases of choriocarcinoma in a pregnancy with a coexistent live fetus have been reported.[2,15] In many of these cases, the origin of the choriocarcinoma has not been explored. The majority of these women had been pregnant before, making it tempting to assume that if the choriocarcinoma did not originate from the present pregnancy it most likely originated from (one of) the prior known pregnancies. We found that the choriocarcinoma showed a signal on the Y-chromosome, excluding that it originated from the only recognized previous pregnancy, as this was female. Thus, the patient most likely has undergone an unknown male pregnancy before the present pregnancy or the present pregnancy may have been a dizygotic twin pregnancy consisting of 1 conceptus that turned into a choriocarcinoma and a normal pregnancy with a fetus.

Openshaw et al[11] were able to identify DNA originating in the GTN in all of 9 patients with a serum hCG of 66,861 IU/L or above. Consistently the serum hCG in our patient was above 200,000 IU/L.

Interestingly, the analysis of the cfDNA only showed signals from the choriocarcinoma, whereas signals from the normal pregnancy were not observed. It is possible that the amount of DNA from the choriocarcinoma was much higher than the

amount of DNA from the normal pregnancy. It is also possible that the DNA from the choriocarcinoma was more stable than the DNA from the normal pregnancy.

We conclude that analysis of cfDNA in patients with trophoblastic neoplasia is beneficial in order to provide optimal treatment and prognosis, even in patients with a coexisting pregnancy.

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