Hydatidiform mole diagnostics using circulating gestational trophoblasts isolated from maternal blood

Sunde, Lone; Singh, Ripudaman; Ravn, Katarina; Schelde, Palle; Hansen, Estrid Staehr; Uldbjerg, Niels; Niemann, Isa; Hatt, Lotte

Published in:
Molecular Genetics & Genomic Medicine

DOI (link to publication from Publisher):
10.1002/mgg3.1565

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

Received: 1 August 2020 | Revised: 18 October 2020 | Accepted: 29 October 2020

DOI: 10.1002/mgg3.1565

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

Hydatidiform mole diagnostics using circulating gestational trophoblasts isolated from maternal blood

Lone Sunde[1,2] | **Ripudaman Singh**[3] | **Katarina Ravn**[3] | **Palle Schelde**[3] | **Estrid Stæhr Hansen**[4] | **Niels Uldbjerg**[5] | **Isa Niemann**[5] | **Lotte Hatt**[3]

[1]Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark

[2]Department of Biomedicine, Aarhus University, Aarhus, Denmark

[3]ARCEDI Biotech ApS, Vejle, Denmark

[4]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark

[5]Department of Women's Disease and Birth, Aarhus University Hospital, Aarhus, Denmark

Correspondence
Lone Sunde, Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark.
Email: l.sunde@rn.dk

Funding information
Dagmar Marshalls Fond, Grant/Award Number: 500020; Overlæge Johan Boserup og Lise Boserups Legat, Grant/Award Number: 20795-24; Civilingeniør Frode V. Nyegaard og Hustrus Fond; Health Research Foundation of Central Denmark Region; Riisfort Fonden; Karen A Tolstrups Fond, Grant/Award Number: 300.106-012; Agnes and Poul Friis fond; Etatsraad C. G. Filtenborg & hustru Marie Filtenborgs Studielegat, Grant/Award Number: 70283; Fabrikant Einar Willumsens Mindelegat, Grant/Award Number: 6000073; Grosserer Valdemar Foersom og Hustru Thyra Foersoms Fond, Grant/Award Number: 41452-001

Abstract

Background: In gestational trophoblastic disease, the prognosis is related to the genetic constitution. In some cases, taking a biopsy is contraindicated.

Methods: In a pregnant woman, ultrasound scanning suggested hydatidiform mole. To explore if the genetic constitution can be established without taking a biopsy (or terminating the pregnancy), cell-free DNA and circulating gestational trophoblasts were isolated from maternal blood before evacuation of the uterus. The evacuated tissue showed the morphology of a complete hydatidiform mole. Without prior whole-genome amplification, short tandem repeat analysis of 24 DNA markers was performed on the samples, and on DNA isolated from evacuated tissue, and from the blood of the patient and her partner.

Results: Identical genetic results were obtained in each of three circulating gestational trophoblasts and the evacuated tissue, showing that this conceptus had a diploid androgenetic nuclear genome. In contrast, analysis of cell-free DNA was less informative and less specific due to the inherent presence of cell-free DNA from the patient.

Conclusion: Our results show that it is possible to isolate and analyze circulating gestational trophoblasts originating in a pregnancy without maternal nuclear genome. For diagnosing gestational trophoblastic diseases, genotyping circulating gestational trophoblasts appears to be superior to analysis of cell-free DNA.

KEYWORDS

androgenetic, cell-free nucleic acids, circulating neoplasm cells, other circulating cells, diploidy, genotyping techniques, gestational trophoblastic disease, hydatidiform mole, liquid biopsy

1 | INTRODUCTION

Most hydatidiform moles (HMs) are diploid with both genome sets originating from the father (parental type: PP), or triploid with two genome sets from the father and one from the mother (parental type: PPM). Most of the HMs with the parental type PP show homozygosity in all loci (P1P1), whereas approximately 15% show heterozygosity in some loci (P1P2). HM impose an increased risk of gestational trophoblastic neoplasia (GTN) (Niemann et al., 2007).

Correct diagnosis of gestational trophoblastic diseases is the prerequisite for optimal prognostics and treatment. The risk of GTN is higher after a diploid HM than after a triploid HM (Scholz et al., 2015); and among the diploid HMs, those with the parental type P1P2 seem to have the highest risk (Khawajkie et al., 2020). Furthermore, more intensive treatment is recommended for trophoblastic neoplasia originating in a non-HM pregnancy compared to trophoblastic neoplasia originating in a HM (Ngan et al., 2018).

In some cases of gestational trophoblastic disease, it is undesirable to take a biopsy. In case of trophoblastic neoplasia, the risk of bleeding is high. Similarly, in case of multiple pregnancy including a HM, it would be attractive to know the genetic constitution of the molar part of the pregnancy without performing invasive sampling due to the risk of abortion associated with the procedure. Recently it has been documented that the genetic constitution of trophoblastic neoplasms can be determined by analyzing DNA circulating in the maternal blood (cell-free DNA, cfDNA). However, the interpretation of results from such analyses is hampered by the inherent presence of maternal DNA in the sample (Kristiansen et al., 2016; Openshaw et al., 2015). Here, we demonstrate that it is also possible to determine the genetic constitution of a HM by analyzing a blood sample from the patient, and that analyzing trophoblasts originating from the HM and circulating in maternal blood (circulating Gestational Trophoblasts, cGTs) may be superior to analyzing cfDNA.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

The project was approved by the Danish Data Protection Agency (number: 2003-41-3231) and by The Committee for Ethics in Science of Central Denmark Region (number: 1-10-72-370-13). The patient and her partner gave their written informed consent.

In a 28-year-old G1P0 woman, routine ultrasound examination in gestational week 13 + 5 disclosed a 12 × 10 × 8 cm mass in the uterus suspected of being a hydatidiform mole. Histopathologic examination disclosed morphologic findings characteristic for a complete HM.

Immediately before evacuation of the uterus, 30-ml blood was drawn from the patient in three tubes (Streck Cell-Free DNA BCT®). Trophoblasts were isolated as described previously (Hatt et al., 2014; Hatt et al., 2014). Briefly, Streck tubes were centrifuged. Plasma was removed for analysis of cfDNA. The buffy coat and the red blood cell fraction from the three tubes were pooled, the cells were fixed in paraformaldehyde and red blood cells were lysed. cGTs were enriched using Miltenyi's Magnetic Activated Cell Sorting (MACS) and stained with a pool of cytokeratin antibodies. The enriched and stained cGTs were isolated individually.

The evacuated HM tissue was inspected using a dissection microscope (x25) and manually freed from maternal tissue. For karyotyping, cells were cultivated from approximately 15 mg HM tissue. Metaphases were prepared and stained with quinacrine mustard using standard techniques.

DNA was prepared from ethylenediaminetetraacetic acid (EDTA) blood from the patient and her partner, and from HM tissue, respectively, using standard techniques. Prior to STR analysis, cGTs were lysed using PrepGEM Universal (ZyGEM). No whole-genome amplification was performed.

The origin of the genome in the HM tissue, the individual cGTs, and cfDNA was investigated by Short Tandem Repeat (STR) analysis using the GlobalFiler™ PCR amplification kit (Thermo Fischer Scientific) that targets 24 loci spread across the human nuclear genome. The 24-multiplex PCR was run according to the manufacturer's recommendations with minor changes in thermal cycle number to accommodate the low DNA input from cGTs and cfDNA. Capillary electrophoresis was performed using an ABI 3500 genetic analyzer. Data were analyzed using GeneMapper ID-X software (Thermo Fischer Scientific).

The STR profiles of the HM tissue, the cGTs, and the cfDNA were compared with the corresponding STR profiles of the patient (i.e., the mother) and her partner (i.e., the father).

3 | RESULTS

Chromosome analysis of the HM tissue showed the karyotype 46,XX. The results of STR analysis are illustrated in Figure 1. The observations in all 24 loci are given in Table 1 and Figure A1.

In the HM tissue, the PCR product from the *AMEL* locus was specific for the X chromosome and not for the Y chromosome, and no product was observed for the two other Y chromosomal loci analyzed, in accordance with the observation of no Y chromosome by karyotyping. In each of the 21 autosomal loci, only one allele was observed. Comparing the results with the results for the mother and the father disclosed

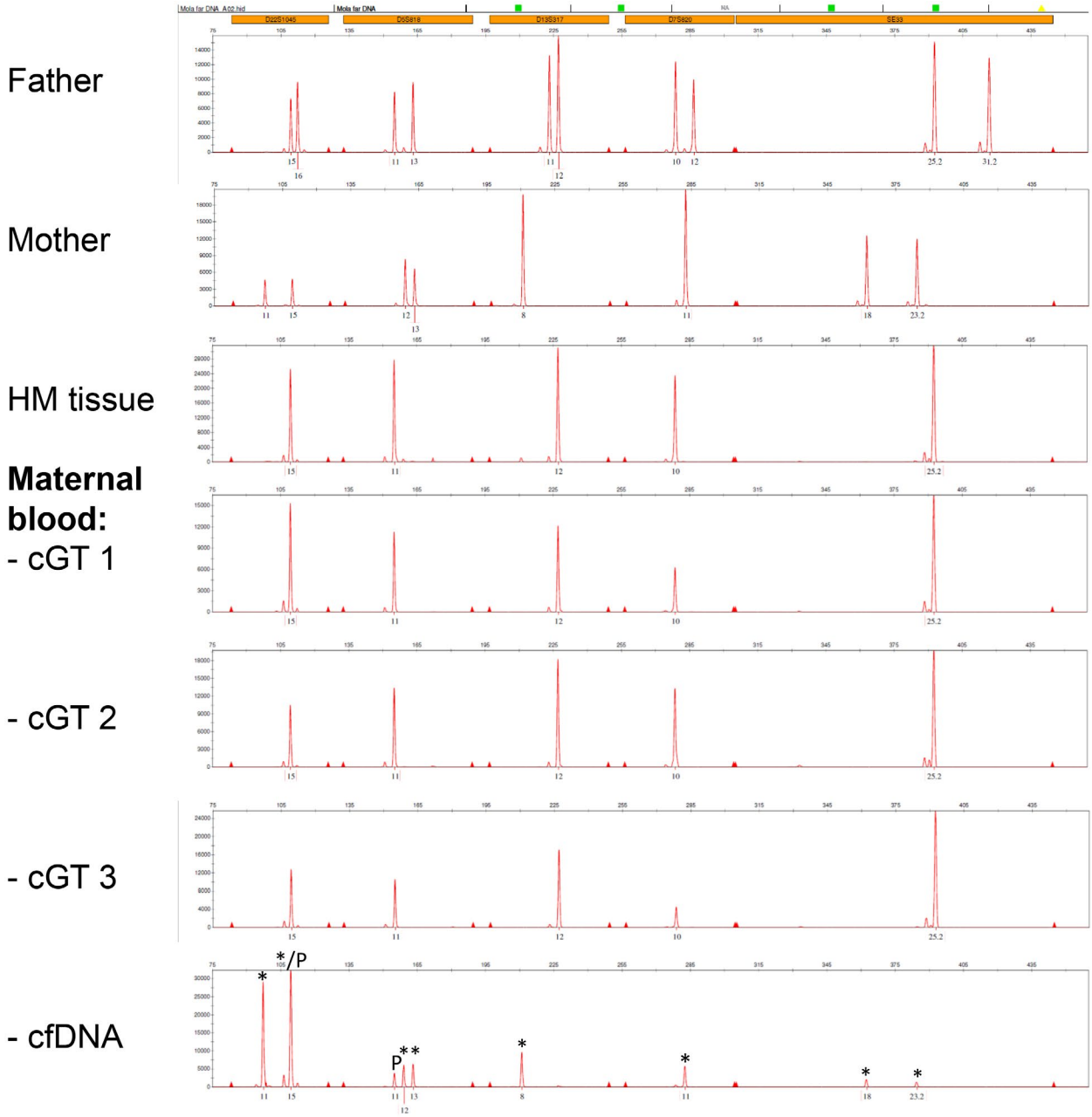

FIGURE 1 Analysis of the STR markers in autosomal loci D22S1045, D5S818, D13S317, D7S820, and SE33.HM tissue: DNA from evacuated hydatidiform mole. cGTs 1, 2, and 3: DNA from three gestational trophoblasts circulating in maternal blood. cfDNA: Cell free DNA circulating in maternal blood. P: Allele identical with allele in the father, only; consistent with the allele being inherited from the father. *: Allele identical with allele in the mother, consistent with the sample being contaminated with DNA from the mother. */P: Allele identical with an allele present both in the mother and the father.

that for all loci, the allele in the HM was identical with an allele in the father, and for 17 loci the allele in the HM was not present in DNA from the mother, indicating that the HM had a homozygous androgenetic nuclear genome (parental type P1P1).

For all of the three cGTs, the electropherograms for each locus showed an allele identical with the allele identified in HM tissue and no other signals. Although the peak heights were varying, these alleles were clearly identifiable in all cases (Figure 1 and Figure A1).

For the cfDNA, the electropherograms for all loci showed peaks indicating the same alleles as identified in the mother. For 10/21 autosomal loci, an additional peak indicating an allele only present in the father was seen, and for further five less informative loci, the results were consistent with a paternal allele being present. In six

TABLE 1 Alleles observed by DNA marker analysis using the GlobalFiler™ kit

DNA marker	Chromosomal location	Father (=partner)	Mother (=patient)	HM tissue	cGT 1	cGT 2	cGT 3	cfDNA[1]
D3S1358	3p21.31	16	15.17	16	16	16	16	?,15,(16),17
vWA	12p13.31	14.17	14.18	14	14	14	14	14,18
D16S539	16q24.1	9.12	10.11	12	12	12	12	10,11,(12)
CSF1PO	5q33.3-34	10.11	12.13	10	10	10	10	12,13
TPOX	2p23-2per	9.11	8	11	11	11	11	8, (11)
Y indel	Yq11.221	2	NS	NS	NS	NS	NS	NS
AMEL	X: p22.1-22.3 Y: p11.2	X,Y	X	X	X	X	X	X
D8S1179	8q24.13	11.12	10.13	11	11	11	11	10,(11),13
D21S11	21q11.2-q21	29	30,32.2	29	29	29	29	(29),30,32.2
D18S51	18q21.33	13.19	15.18	13	13	13	13	(13),15,18
DYS391	Yq11.21	10	NS	NS	NS	NS	NS	NS
D2S441	2p14	11.14	11.14	14	14	14	14	(11),?,14
D19S433	19q12	12.16	13.14	16	16	16	16	13,14,(16)
TH01	11p15.5	9.3	9.3	9.3	9.3	9.3	9.3	9.3
FGA	4q28	22.25	21.23	25	25	25	25	21,23,(25)
D22S1045	22q12.3	15.16	11.15	15	15	15	15	11,15
D5S818	5q21-31	11.13	12.13	11	11	11	11	(11),12,13
D13S317	13q22-31	11.12	8	12	12	12	12	8
D7S820	7q11.21-22	10.12	11	10	10	10	10	11
SE33	6q14	25.2,31.2	18,23.2	25.2	25.2	25.2	25.2	18,23.2
D10S1248	10q26.3	14.15	13.15	14	14	14	14	13,(14),15
D1S1656	1q42.2	12,15.3	16.17	12	12	12	12	16,17
D12S391	12p13.2	17	17.23	17	17	17	17	17,23
D2S1338	2q35	20.24	17.19	24	24	24	24	17,19

Note:: 1: Brackets indicate that the peak had a lower height. Allele underlined is identical with an allele in the father.

Abbreviations: ?, A peak with a position and shape suggesting an artifact; NS, No signal.

autosomal loci, no signal corresponding to a paternal allele was observed.

4 | DISCUSSION

In a woman with a diploid androgenetic HM, we were able to identify the parental origin of the nuclear genome in the HM by analyzing cGTs, without performing whole-genome amplification.

The main limitation in making genetic diagnoses on cGTs is the small number of cells analyzed. In case of mosaicism (e.g., one cell line having the parental type PP and the other having the normal parental type, PM), cells from one cell line, only, may be captured, and thus a PP cell line may be overlooked. Similarly, in case of twinning, one conceptus being a HM, trophoblasts from the non-molar placenta, only, may be captured. Therefore, the result interpretation should take the morphology (e.g., obtained by ultrasound) into consideration. If a HM is suspected and the parental type PM, only, is identified in cGTs, one should consider the more rare diagnoses, such as multiple pregnancy, mosaicism, and even the rare HMs with the parental type PM, which are seen in women with biallelic inactivation of *NLRP7* or *KHDC3L*. Another limitation of the method is that only 1–2 polymorphic loci on each of 20 chromosomes are analyzed; therefore, this method cannot detect all types of unusual karyotype.

Compared to analyzing the parental origin in cfDNA, one major advantage of analyzing cGTs is the absence of maternal DNA, simplifying the interpretation of the results significantly. In addition, the number of loci showing a signal representing the gestation was correspondingly higher.

It is important to correctly differentiate between triploid and diploid HMs, as the diploid HMs with an androgenetic (PP) cell line impose a high risk of gestational trophoblastic disease (Niemann et al., 2007). By far most triploid HMs show the parental type P1P2M, that is, heterozygosity for paternal alleles (Scholz et al., 2015), and thus a triploid HM

is expected to show three alleles in several loci. However, in many HMs showing mosaicism including a PP cell line, for example, parental type P1P1/P2M or P1P2/PxM, analysis of DNA from a tissue sample disclose one maternal and two paternal alleles in many loci (Sunde et al., 2011). Thus, in a marker analysis performed on DNA prepared from tissue, with such a mixture of cells, discrimination between a triploid HM and a diploid HM showing mosaicism is problematic. And it would be correspondingly difficult in cfDNA.

By analysis of DNA markers in individual cells, this problem should be alleviated: In all cells from a triploid HM, some loci should show one maternal and two paternal alleles. In contrast, no cell from a HM showing mosaicism PP/PM should show more than two alleles.

Our data show that the procedure to identify circulating cells from conceptuses with a biparental genome can be used for identifying cells from a HM with nuclear genome exclusively inherited from the father.

We have previously shown that circulating trophoblasts can be isolated from pregnancies in gestational weeks 10–14 for use in cell-based non-invasive prenatal testing (cbNIPT). In a study on 111 pregnancies, we isolated 1–45 (average 12.8) trophoblasts from 30 ml of blood (Kolvraa et al., 2016). The quality of the DNA from these cells allows for a full-genomic DNA analysis and for calling of small copy number variants (Kolvraa et al., 2016; Vestergaard et al., 2017; Vossaert et al., 2019). These circulating cells are expected to be extra-villous trophoblasts (EVTs) migrating from the placental villous to line the spiral arteries in the placenta (Hatt et al., 2014).

This is the first HM analyzed, therefore, we do not know the success rate of analyzing cGTs from HMs. As it has been shown that endovascular EVT invasion is reduced in complete HMs, one could expect that fewer cells might reach the maternal blood (Sebire et al., 2001). However, Moser et al. (2017), have suggested that circulating trophoblasts originate from both invaded uterine arteries, veins, and glands, which could lead to trophoblasts from complete HMs still ending up in the maternal circulation.

Among patients with GTN, Openshaw et al. managed to make a genetic diagnosis by analyzing cfDNA in 12/20 patients (Openshaw et al., 2015). Possibly, analysis of cGTs will allow accurate genetic diagnosis in a higher fraction of women with GTN, where a biopsy is not available. Similarly, this technique may be useful in women with multiple pregnancies including a HM, where taking a biopsy is relatively contraindicated.

5 | CONCLUSION

For determining the genetic constitution in hydatidiform moles and other gestational diseases by analysis of maternal blood, analysis of circulating gestational trophoblasts appears to be superior to analysis of cell-free DNA.

ACKNOWLEDGEMENTS

This work was supported by Agnes and Poul Friis fond; Dagmar Marshalls Fond; Fabrikant Einar Willumsens Mindelegat; Riisfort Fonden, Civilingeniør Frode V. Nyegaard og Hustrus Fond; Karen A Tolstrups Fond; Overlæge Johan Boserup og Lise Boserups Legat; Grosserer Vald. Foersom og hustru Thyra Foresom, født Otto's Fond; Etatsraad C. G. Filtenborg & hustru Marie Filtenborgs Studielegat; and Health Research Foundation of Central Denmark Region. Ripudaman Singh, Katarina Ravn, Palle Schelde, and Lotte Hatt are employed by ARCEDI Biotech ApS, a Danish biotech company involved in the development of cell-based non-invasive prenatal diagnosis. The study was designed using the inventions made in this company. However, neither the company nor any of the funders played a role in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHORS' CONTRIBUTIONS

Lone Sunde conceptualized the project, secured the legal aspects of inclusion of the patients and the genetic analyses, performed the genetic analyses, and drafted and finalized the manuscript. Isa Niemann communicated with the patient and her partner, collected samples, and provided clinical data. Katarina Ravn was involved in planning and execution of the project. Managed the isolation of gestational trophoblasts. Ripudaman Singh and Palle Schelde were involved in planning and execution of the project. Designed the isolation of gestational trophoblasts. Estrid Stæhr Hansen performed the histopathological analyses. Niels Uldbjerg was responsible for study management and legal approval for the project on isolation of gestational cells, and provided expertise and advice. Lotte Hatt designed the STR analysis and analyzed the data. All co-authors were involved in interpretation of the results, and commented and approved the manuscript.

ORCID

Lone Sunde https://orcid.org/0000-0002-8479-165X
Ripudaman Singh https://orcid.org/0000-0003-2657-0661
Katarina Ravn https://orcid.org/0000-0001-5428-8781
Palle Schelde https://orcid.org/0000-0001-8504-0893
Estrid Stæhr Hansen https://orcid.org/0000-0002-7808-908X
Niels Uldbjerg https://orcid.org/0000-0002-6449-6426
Lotte Hatt https://orcid.org/0000-0003-0141-9172

REFERENCES

Hatt, L., Brinch, M., Singh, R., Moller, K., Lauridsen, R. H., Schlutter, J. M., Uldbjerg, N., Christensen, B., & Kolvraa, S. (2014). A new marker set that identifies fetal cells in maternal circulation with high specificity. *Prenatal Diagnosis*, *34*(11), 1066–1072. https://doi.org/10.1002/pd.4429

Hatt, L., Brinch, M., Singh, R., Moller, K., Lauridsen, R. H., Uldbjerg, N., Huppertz, B., Christensen, B., & Kolvraa, S. (2014). Characterization of fetal cells from the maternal circulation by microarray gene expression analysis–could the extravillous trophoblasts be a target for future cell-based non-invasive prenatal diagnosis? *Fetal Diagnosis and Therapy*, *35*(3), 218–227. https://doi.org/10.1159/000356073

Khawajkie, Y., Mechtouf, N., Nguyen, N. M. P., Rahimi, K., Breguet, M., Arseneau, J., Ronnett, B. M., Hoffner, L., Lazure, F., Arnaud, M., Peers, F., Tan, L., Rafea, B. A., Aguinaga, M., Horowitz, N. S., Ao, A., Tan, S. L., Brown, R., Buckett, W., & Slim, R. (2020). Comprehensive analysis of 204 sporadic hydatidiform moles: Revisiting risk factors and their correlations with the molar genotypes. *Modern Pathology*, *33*(5), 880–892. https://doi.org/10.1038/s41379-019-0432-4

Kolvraa, S., Singh, R., Normand, E. A., Qdaisat, S., van den Veyver, I. B., Jackson, L., Hatt, L., Schelde, P., Uldbjerg, N., Vestergaard, E. M., Zhao, L., Chen, R., Shaw, C. A., Breman, A. M., & Beaudet, A. L. (2016). Genome-wide copy number analysis on DNA from fetal cells isolated from the blood of pregnant women. *Prenatal Diagnosis*, *36*(12), 1127–1134. https://doi.org/10.1002/pd.4948

Kristiansen, M. K., Niemann, I., Lindegaard, J. C., Christiansen, M., Joergensen, M. W., Vogel, I., Lildballe, D. L., & Sunde, L. (2016). Cell-free DNA in pregnancy with choriocarcinoma and coexistent live fetus–A case report. *Medicine (United States)*, *95*(37), e4721. https://doi.org/10.1097/MD.0000000000004721

Moser, G., Weiss, G., Sundl, M., Gauster, M., Siwetz, M., Lang-Olip, I., & Huppertz, B. (2017). Extravillous trophoblasts invade more than uterine arteries: evidence for the invasion of uterine veins. *Histochemistry and Cell Biology*, *147*(3), 353–366. https://doi.org/10.1007/s00418-016-1509-5

Ngan, H. Y. S., Seckl, M. J., Berkowitz, R. S., Xiang, Y., Golfier, F., Sekharan, P. K., Lurain, J. R., & Massuger, L. (2018). Update on the diagnosis and management of gestational trophoblastic disease. *International Journal of Gynaecology and Obstetrics*, *143*(Suppl), 79–85. https://doi.org/10.1002/ijgo.12615

Niemann, I., Hansen, E. S., & Sunde, L. (2007). The risk of persistent trophoblastic disease after hydatidiform mole classified by morphology and ploidy. *Gynecologic Oncology*, *104*(2), 411–415. https://doi.org/10.1016/j.ygyno.2006.08.025

Openshaw, M. R., Harvey, R. A., Sebire, N. J., Kaur, B., Sarwar, N., Seckl, M. J., & Fisher, R. A. (2015). Circulating cell free DNA in the diagnosis of trophoblastic tumors. *EBioMedicine*, *4*, 146–152. https://doi.org/10.1016/j.ebiom.2015.12.022

Scholz, N. B., Bolund, L., Nyegaard, M., Faaborg, L., Jørgensen, M. W., Lund, H., Niemann, I., & Sunde, L. (2015). Triploidy-observations in 154 diandric cases. *PLoS One*, *10*(11), e0142545. https://doi.org/10.1371/journal.pone.0142545

Sebire, N. J., Rees, H., Paradinas, F., Fisher, R., Foskett, M., Seckl, M., & Newlands, E. (2001). Extravillus endovascular implantation site trophoblast invasion is abnormal in complete versus partial molar pregnancies. *Placenta*, *22*(8–9), 725–728. https://doi.org/10.1053/plac.2001.0716

Sunde, L., Niemann, I., Hansen, E. S., Hindkjaer, J., Degn, B., Jensen, U. B., & Bolund, L. (2011). Mosaics and moles. *European Journal of Human Genetics*, *19*(10), https://doi.org/10.1038/ejhg.2011.93

Vestergaard, E. M., Singh, R., Schelde, P., Hatt, L., Ravn, K., Christensen, R., Lildballe, D. L., Petersen, O. B., Uldbjerg, N., & Vogel, I. (2017). On the road to replacing invasive testing with cell-based NIPT: Five clinical cases with aneuploidies, microduplication, unbalanced structural rearrangement, or mosaicism. *Prenatal Diagnosis*, *37*(11), 1120–1124. https://doi.org/10.1002/pd.5150

Vossaert, L., Wang, Q., Salman, R., McCombs, A. K., Patel, V., Qu, C., Mancini, M. A., Edwards, D. P., Malovannaya, A., Liu, P., Shaw, C. A., Levy, B., Wapner, R. J., Bi, W., Breman, A. M., Van den Veyver, I. B., & Beaudet, A. L. (2019). Validation studies for single circulating trophoblast genetic testing as a form of noninvasive prenatal diagnosis. *American Journal of Human Genetics*, *105*(6), 1262–1273. https://doi.org/10.1016/j.ajhg.2019.11.004

How to cite this article: Sunde L, Singh R, Ravn K, et al. Hydatidiform mole diagnostics using circulating gestational trophoblasts isolated from maternal blood. *Mol Genet Genomic Med*. 2021;9:e1565. https://doi.org/10.1002/mgg3.1565

APPENDIX A

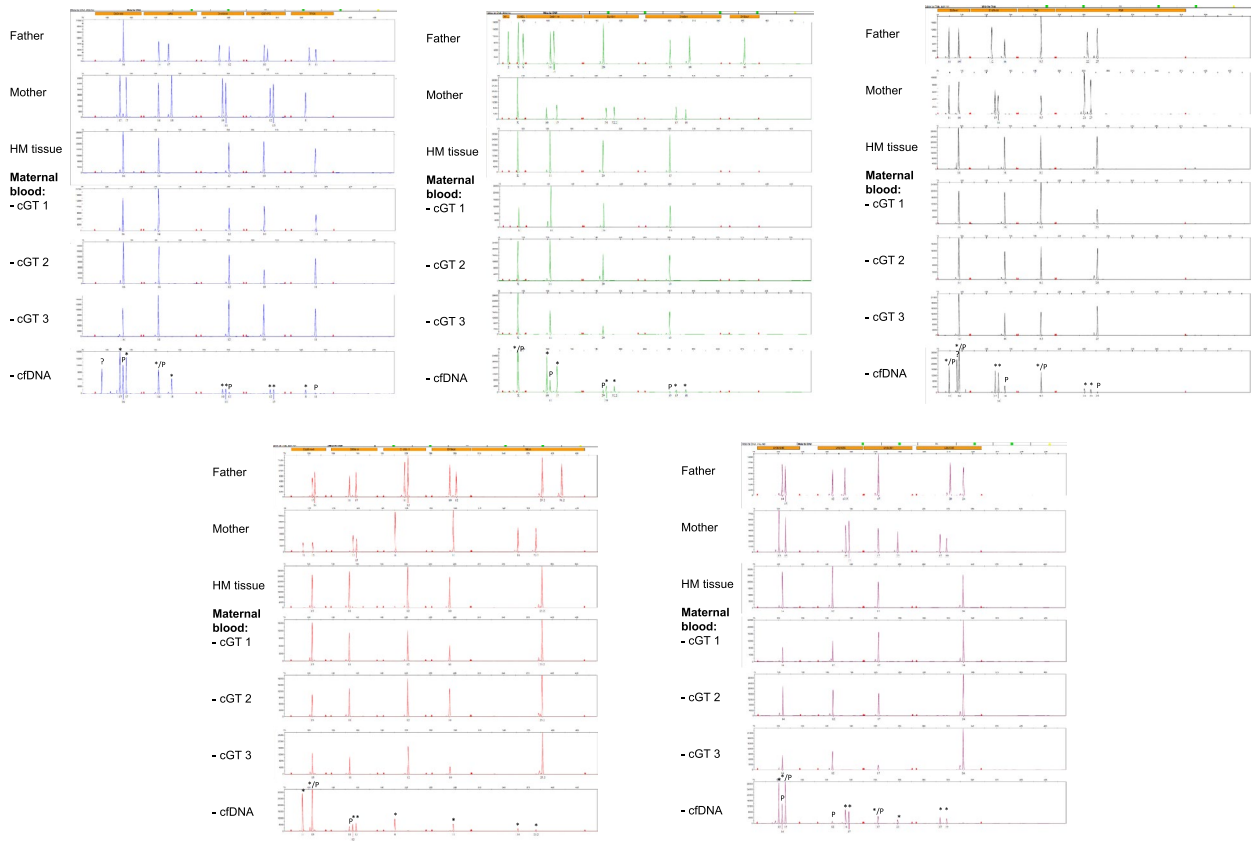

FIGURE A1 DNA marker analysis using the GlobalFiler™ kit. HM tissue: DNA from evacuated hydatidiform mole. cGTs 1, 2, and 3: DNA from three gestational trophoblasts circulating in maternal blood. cfDNA: Cell free DNA circulating in maternal blood. P: Allele identical with allele in the father, only; consistent with the allele being inherited from the father. *: Allele identical with allele in the mother, consistent with the sample being contaminated with DNA from the mother. */P: Allele identical with an allele present both in the mother and the father