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A high-risk gestational trophoblastic neoplasia derived from a complete hydatidiform mole with coexisting fetus identified by short tandem repeats analysis: A case report

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ABSTRACT

A complete hydatidiform mole coexisting with a fetus (CHMCF) is a rare form of twin pregnancy. High-risk gestational trophoblastic neoplasia (GTN) can occur after a CHMCF pregnancy, although the frequency is low. In cases of GTN, the clinical diagnosis and that based on the International Federation of Gynecology and Obstetrics (FIGO) scoring system can differ. This case report concerns a patient with a choriocarcinoma that was initially diagnosed and treated as a low-risk stage III GTN following a live birth from a CHMCF pregnancy. We used short tandem repeat (STR) analysis to identify the causative pregnancy as the patient's earlier complete hydatidiform mole. Clinicians should anticipate a high-risk GTN when treating persistent trophoblastic disease (PTD) in patients with a non-typical course.

1. Introduction

Complete hydatidiform mole coexisting with a fetus (CHMCF) is a rare form of twin pregnancy, with a rate of one in 10,000 to 100,000 pregnancies [1,2]. Although an increased risk of perinatal complications and persistent trophoblastic disease (PTD) exists, there are also reports of live births [3–5].

In previous reports, PTD after a CHMCF pregnancy is often manifested as a low-risk gestational trophoblastic neoplasia (GTN), as diagnosed through the International Federation of Gynecology and Obstetrics (FIGO) scoring system [4–7].

Analysis of short tandem repeats (STR), which vary from individual to individual, is used to test human identity by analyzing the polymerase chain reaction (PCR) amplification product of a specific region of deoxyribonucleic acid (DNA) [8]. In atypical GTN cases where the FIGO scoring system and clinical practice present conflicting diagnoses, STR analysis can be used to identify the causative pregnancy [9–13].

In this paper, we describe a STR analysis to identify the causative pregnancy of a choriocarcinoma after a CHMCF.

2. Case Presentation

A 40-year-old Japanese woman (gravida 1, para 1) became pregnant by in vitro fertilization with embryo transfer of two blastocysts. At the age of 37 she had presented with a history of cervical conization for cervical intraepithelial neoplasia 3. She was referred to a general hospital in the 10th week of gestation after transvaginal ultrasonography detected a possible twin pregnancy with a live fetus and a hydatidiform mole located outside the gestational sac.

Transvaginal ultrasonography revealed a fetus and a mass measuring $109 \times 85 \times 64$ mm with a multivesicular pattern in the uterus; her serum human chorionic gonadotropin (hCG) level was 367,297 mIU/mL. Magnetic resonance imaging of the uterine cavity at 12 weeks revealed a honeycomb-shaped T2W1 hyperintensity region on the right side and a normal fetus and placenta on the left side, which suggested CHMCF.

She chose to continue the pregnancy with an understanding of the perinatal risks of CHMCF. As the pregnancy progressed, the patient's serum hCG was measured every two to four weeks, and a search for metastatic lesions was performed by chest computed tomography (CT) every two to three months.

Amniocentesis at week 16 was performed at the patient's request and revealed a normal karyotype. At week 23, mild preeclampsia was

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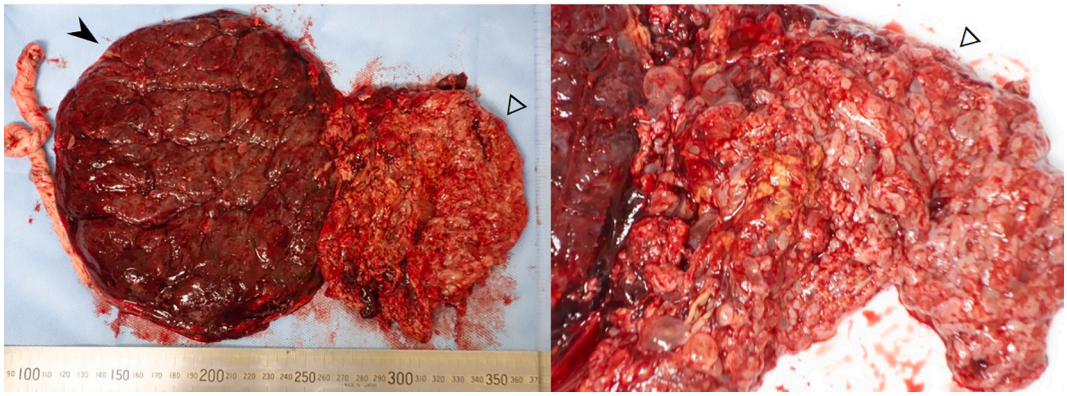

Fig. 1. A gross image of the placenta and complete hydatidiform mole delivered after the birth.
Normal placenta (➤) and edematous swollen chorionic villi (▷).

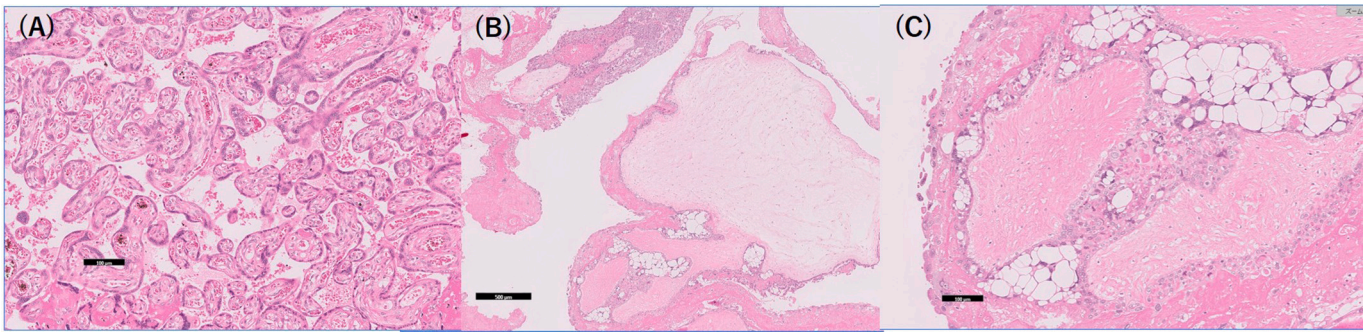

Fig. 2. Microscopic findings of the placenta and complete hydatidiform mole.
(A) Normal placenta equivalent to the 37th week of gestation (×100); (B) (C) complete hydatidiform mole (×10, ×100)

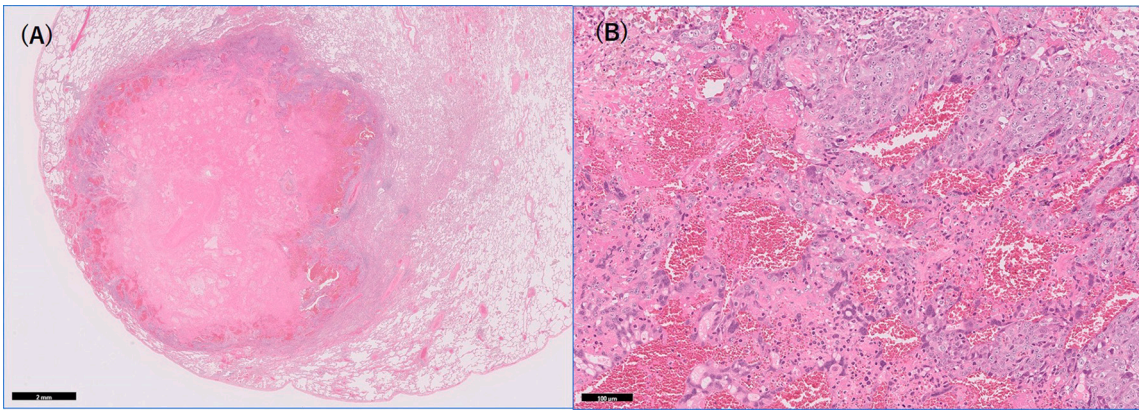

Fig. 3. Histopathological findings of metastatic choriocarcinoma.
(A) Hemorrhage and necrosis can be seen inside the mass (×3). (B) Atypical syncytiotrophoblasts, cytotrophoblasts, and intermediate syncytiotrophoblasts proliferated in sheets with hemorrhage and necrosis (×100).

detected, and blood pressure management was begun. A chest CT at week 34 revealed a 5 mm nodule in the left lung, but the pregnancy was continued, with careful monitoring, and without an elevated serum hCG level that would suggest GTN.

Labor was induced at 37 weeks, and a live male infant weighing 2864 g was delivered by vacuum extraction; his Apgar score was 8/9, and no abnormalities were observed. The delivered placenta exhibited edematous swollen chorionic villi (Fig. 1). A diagnosis of CHMCF was made after a histopathological examination revealed a normal placenta and an androgenesis complete hydatidiform mole (immunohistochemical staining for p57Kip2 and TSSC3 was negative) (Fig. 2).

Endometrial histology at one month postpartum showed no remaining cells from the complete hydatidiform mole; however, serum hCG initially decreased from 10^4 mIU/mL to 10^2 mIU/mL, but it did not normalize, and then it at four months after delivery it had increased. Because the patient's serum hCG was more than three times normal, and she presented with a residual lung nodule, she was diagnosed with PTD.

Since the index pregnancy was a CHMCF, the FIGO scoring system determined the PTD to be a low-risk stage III GTN, and single-agent chemotherapy (methotrexate) was initiated. However, the serum hCG continued to increase, and the lung nodule grew after three cycles of methotrexate. The lung nodule was refractory to methotrexate, and a

Table 1
STR analysis results for blood and tissues.

	Normal placenta		CHM		Choriocarcinoma			Maternal blood		Paternal blood	
Marker	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 1	Allele 2
TH01	7	9.3	7		6	7	9.3	6	9.3	7	8
D21S11	29		30		29	30		29	30	29	30
D5S818	12	13	11		11	13		13		11	12
D13S317	8	12	8		8	10	12	10	12	8	10
D7S820	10	12	8		8	10		8	10	8	12
D16S539	9	10	9		9	10		9	10	8	9
CSF1PO	10	13	11		7	11	13	7	13	10	11
AMEL	X	Y	X		X			X		X	Y
vWA	14	17	14		14	15	17	15	17	14	17
TPOX	8	9	8		8	9		8	9	8	9

Maternal and paternal alleles were detected in the normal placenta. Only paternal alleles were detected in the complete hydatidiform mole. The same alleles as complete hydatidiform mole were detected in choriocarcinoma. Some of the detected maternal alleles were thought to be due to a mixture of maternal normal lung tissue.
CHM: complete hydatidiform mole.

video-assisted thoracic surgery lung tumor resection was performed for histopathological determination.

The resected tumor was solid, hemorrhagic, and necrosis was noted inside; choriocarcinoma was diagnosed after histopathology showed atypical syncytiotrophoblasts, cytotrophoblasts, intermediate trophoblasts mixed in the margin of the tumor, and sheet-like growth (Fig. 3).

After the diagnosis of a high-risk GTN, the patient's chemotherapy was changed to a multidrug combination regimen (EMA/CO: etoposide, methotrexate, actinomycin-D, cyclophosphamide, and vincristine). After the first two cycles of EMA/CO, the patient's serum hCG normalized; after five cycles, she was declared to be in remission.

At 10 months after the chemotherapy treatment, no increase was found in serum hCG, and no recurrence of GTN was observed on chest and abdominal CT. According to the FIGO scoring system, this was a case of undiagnosed GTN. However, we used STR analysis on formalin-fixed, paraffin-embedded (FFPE) DNA samples of choriocarcinoma to confirm the GTN and identify the causative pregnancy.

This study was performed with signed informed consent from the patient.

We separated the placenta and complete hydatidiform mole, and blood was collected from the patient (the mother) and the father for examination. DNA extraction and STR analysis were performed by Takara Bio, Inc. (Kusatsu, Shiga, Japan), and STR analysis was performed on 10 loci (D21S11, TH01, TPOX, vWA, amelogenin, CSF1PO, D16S539, D7S820, D13S317, D5S818) using Promega's GenePrint 10 System (Madison, WI, US) (Table 1). For FFPE samples, genomic DNA was extracted using the Nucleo Spin DNA FFPE XS kit (Takara Bio, Inc.), and a blood-sample of genomic DNA was extracted using the Nucleo Spin Blood kit.

The DNA quality was tested by absorption quantification by NanoDrop (Thermo Fisher Scientific, Waltham, MA, US) and fluorescence quantification by Qubit dsDNA BR Assay Kit (Lumiprobe, Hunt Valley, Maryland, US). The extracted DNA was diluted to 10 ng/uL with sterile water, and 1 uL was used for PCR (For DNA less than 10 ng/uL, 1 uL of undiluted solution was used for PCR). Human genomic DNA (2800 M Control DNA) that came with the kit was used for a positive control, and sterile water was used for a negative control.

PCR was performed with a 5 x Master Mix 5 μL at 96 °C for 1 min; 5 x Primer Pair Mix 5 μL at 94 °C for 10 s, 59 °C for 1 min, 72 °C for 30 s (30 times); template DNA,1 μL at 60 °C for 10 min; and 14 μL of Otsuka distilled water at 4 °C. The sequence was mixed with 2 μL of the PCR product with size standard ILS600 and Hi-Di Formamide (Thermo Fisher Scientific), followed by heat denaturation and ice cooling; electrophoresis was performed with the Applied Biosystem 3730xl DNA Analyzer (Foster City, CA, US).

Since the peak derived from the FFPE was remarkably low in the macromolecular locus, the template DNA at the time of PCR was changed to 2 uL of the undiluted solution, and PCR and sequencing were again performed. The waveform data that were obtained were analyzed by Gene Mapper software ver. 5.0 (Life Technologies, Carlsbad, CA, US), and peak detection and peak sizing were performed. On STR analysis, alleles derived from the father and mother were detected in all loci in the placenta. The complete hydatidiform mole was a haploid DNA type derived from the father.

Although the choriocarcinoma contained alleles from both the father and mother, it matched the DNA type of the father and the complete hydatidiform mole; it exhibited no other alleles (TH01 type 8, D5S818 type 12, D7S820 type 12, D16S539 type 8, and CSF1PO type 10).

Given this result, the complete hydatidiform mole was judged to be the causative pregnancy of the choriocarcinoma and not the normal pregnancy of the mother's first child.

The maternal allele found in the choriocarcinoma was thought to be sourced to the maternal lung tissue around the tumor.

3. Discussion

The FIGO scoring system for GTN takes into account risk factors such as maternal age, index pregnancy, length of time after an index pregnancy, hCG levels, and site of metastases; a FIGO diagnosis has a sensitivity of 90% or more [14]. GTN occurs more frequently after a CHMCF pregnancy than after a normal pregnancy [4–7].

The FIGO score tends to be low when GTN is diagnosed after CHMCF because the index pregnancy is a complete hydatidiform mole. However, causative pregnancies of GTN are not always the index pregnancy [15], and a discrepancy may exist between the FIGO score and clinical findings in some cases.

Drug resistance is also considered a risk factor in the World Health Organization (WHO) risk score as used by FIGO. However, administration of multiple regimens takes time, and adverse events are also a concern. In this case, the removal of the lung tumor made it possible to make an early histopathological diagnosis, and, further, the responsible pregnancy could be identified by STR analysis.

Sebire et al. reported that only four cases of GTN occurring in 77 cases of CHMCF pregnancies (5.2%) required multidrug combination chemotherapy [5], suggesting CHMCF does not present a high risk for GTN. However, previous case reports describe diagnoses of high-risk GTN after CHMCF or cases requiring multidrug combination chemotherapy for GTN (presumed to be high-risk GTN) [16–24]. In six of 12 cases (50%), including the present case, single-agent chemotherapy was given as the initial chemotherapy for PTD.

This is because the FIGO scoring system mistakenly suggests a diagnosis of PTD rather than high-risk GTN after a case of CHMCF, especially in pregnancies with few metastases. In many cases, the CHMCF pregnancies were terminated at the first trimester due to tumor

Table 2
Twelve cases of CHMCF diagnosed with sequential high-risk GTN required multi-agent chemotherapy after a CHMCF pregnancy.

case No.	Authors	Years	Age	G-P	Methods of pregnancy	Gestational age of termination (week)	Live birth	Maximum HCG(mIU/mL)	Metastatic sites (quantity)	Chemotherapy①	Chemotherapy②	Chemotherapy③	Prognosis	Remarks
1	Adachi et al.	1992	27	0–0	CC	24	×	256,000 (urine)	Lung (single)	MAC④	PEP④		NED	Neonatal death
2	Miller et al.	1992	27	1–0	Not reported	16	×	645,456 (βHCG)	Lung (single)	MTX①	ActD①	EMA/C①	NED	Induced abortion due to severe hyperemesis gravidarum
3	Steller et al.	1994	37	5–4	Not reported	Not reported	×	2,460,000 (βHCG)	Vagina, lung	EMA④			NED	Cesarean hysterectomy
4			28	2–0	Not reported	Not reported	×	500,000 (βHCG)	Lung	ActD②	EMA③		NED	
5			36	2–1	Not reported	Not reported	×	955,000 (βHCG)	Lung	MTX③	EMA⑦	VBP③	NED	Fetal death
6	T Yamada et al.	2008	33	2–0	ICSI	16	×	774,840	No	MTX②	EMA/CO⑤		NED	Induced abortion due to tumor growth
7	HH Peng et al.	2014	34	4–0	Not reported	37	○	310,277 (βHCG)	Lung (single)	MTX③	EMA/CO④		NED	
8	S Couto et al.	2015	33	3–0	IVF	8	×	336,731	Lung (2)	EMA/CO⑤			NED	Induced abortion at the request of the patient
9	I Nobuhara et al.	2018	42	0–0	IVF	10	×	647,000	Lung (>30)	EMA/CO⑪			NED	Hysterectomy after chemotherapy
10	Y Maeda et al.	2018	31	2–2	Not reported	31	○	156,800	Lung, multiple	EMA/CO③	EP/EMA③		NED	Cesarean hysterectomy, newborn was treated for TGA type III
11	D Odera et al.	2020	34	1–0	Not reported	23	×	900,000	Vagina, lung (2)	EP②	EMA/CO⑦		NED	Neonatal death
12	Present case		40	1–1	IVF	37	○	367,297	Lung (single)	MTX③	EMA/CO⑤		NED	

CC: clomiphene citrate, ICSI: intracytoplasmic sperm injection, IVF: in vitro fertilization, MAC: methotrexate, actinomycin-D, chlorambucil, MTX: methotrexate, EMA: etoposide, methotrexate, actinomycin-D, ActD: actinomycin-D, EMA/CO: methotrexate, etoposide, actinomycin-D, cyclophosphamide, vincristine, EP: methotrexate, cisplatin, PEP: peplomycin, VBP: vinblastine, cisplatin, NED: no evidence of disease, TGA: transposition of the great arteries.

growth, bleeding, and morning sickness; however, CHMCF may present a high risk for GTN regardless of whether the pregnancy is continued or not.

Since STR analysis can be used to identify unique individuals [8], it can recognize causative pregnancies and be used in atypical GTN cases that might be incorrectly diagnosed by the FIGO scoring system. STR analysis can be added to the review of FIGO risk factors and better determine the management and treatment methods for this rare disease [9–13].

To our knowledge, only one report exists in which the causative pregnancy of choriocarcinoma after CHMCF was identified by STR analysis [23]. In the STR analysis of the present case, the DNA of choriocarcinoma matched the haploid of the complete hydatidiform mole. Only the father's DNA had been detected in the complete hydatidiform mole and the choriocarcinoma; therefore, the causative pregnancy for the choriocarcinoma was determined to be the complete hydatidiform mole.

We determined that the CHMCF pregnancy was the source of the choriocarcinoma, which is similar to a case reported by Maeda et al. [23]. Although the patient's prognosis was good with the multidrug combination chemotherapy shown in Table 2, choriocarcinoma can metastasize to the brain and may have a significant impact on the patient's quality of life and fertility.

Our patient received in vitro fertilization with embryo transfer of two blastocysts, and many cases have been reported of CHMCF pregnancies occurring after multiple embryo transfers with assisted reproductive technology. Nine of 72 cases (13%) in a report by Lin et al. occurred after the use of assisted reproductive technology [25]. A high rate of perinatal complications can occur, and the birth rate is approximately 50% [26]; pregnancy management should be decided after informing patients about the possibility of high-risk GTN.

There are several limitations to this study. First, since FFPE samples were used for both CHMCF and choriocarcinoma DNA analysis, the DNA could have been degraded, and the locus on the polymer side (CSF1PO, TPOX) would not have been detected [27]. To eliminate this limitation, a method is required for more appropriately extracting DNA from FFPE samples and preserving and managing these tissues so they can be used for STR analysis.

The second limitation is possible contamination from maternal tissues. Since CHMCF and choriocarcinoma are delivered and removed from the mother, avoiding contamination from the mother's blood or organs is necessary. Performing STR analysis after removing the maternal tissue or extracting only the target tissue is desirable, but this can be difficult.

If the highest peak value is 100% in a particular STR locus and the other allelic peak is in an imbalanced state, in the range of 15% to 70%, there is the possibility of trace contamination; different tissues may be present if three or more similar alleles are found in multiple loci [28–31].

For a more accurate identification of the pregnancy that leads to the choriocarcinoma, STR analysis of the first child's DNA and STR reanalysis of choriocarcinoma tissue that does not contain maternal tissue are suggested. However, this line of investigation was abandoned in this study because collecting a sample from the first child was difficult.

4. Conclusion

CHMCF pregnancies present a high risk of PTD that can manifest as GTN, regardless of whether or not the pregnancy continued to delivery. In PTD cases with an atypical course, clinicians should consider that a high-risk GTN may be present, and proper management and treatment depend on a correct diagnosis.

Contributors

Yusuke Taira contributed to patient care and drafted the manuscript.

Yuko Shimoji contributed to the review and editing of the manuscript.

Tadaharu Nakasone contributed to the review and editing of the manuscript.

Yoshihisa Arakaki contributed to the review and editing of the manuscript.

Tomoko Nakamoto contributed to the review and editing of the manuscript.

Tadatsugu Kinjo contributed to obstetrical management, and to the review and editing of the manuscript.

Wataru Kudaka contributed to the review and editing of the manuscript.

Keiko Mekaru contributed to the review and editing of the manuscript.

Yoichi Aoki contributed to the review and editing of the manuscript.

All authors contributed equally to the creation of this case report.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this case report.

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Patient Consent

Obtained.

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