Next-generation genome sequencing of a matched normal-tumor pair from a patient with intractable gestational choriocarcinoma: A case report

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Abstract. Gestational choriocarcinoma is a gestational trophoblastic neoplasia (GTN) originating from trophoblastic cells with abnormal proliferation. Although chemotherapy is effective for treating this cancer, when patients develop chemoresistance, personalized treatment, such as the use of drugs matching their genomes, is required. The present report describes a case of intractable gestational choriocarcinoma identified using a next-generation sequencing (NGS)-based tumor panel. A 51-year-old woman was diagnosed with gestational choriocarcinoma via pathological and short tandem repeat analyses. The patient did not achieve remission despite many regimens of chemotherapy, including high-dose therapy with autologous peripheral blood stem cell transplantation. To identify drugs tailored to this particular choriocarcinoma, NGS was performed on the tumor of the patient, and the tumor genome was compared with that of the patient's blood sample using the NCC Oncopanel System. Consequently, 245 single nucleotide variants (SNVs) with a mean SNV allele frequency of 63.1% were identified. This high frequency was because the genome of the gestational choriocarcinoma contained part of the genome of the partner. Therefore, our experience of the present intractable case of choriocarcinoma suggested that matched normal-tumor pair analysis is not appropriate for treatment decisions in GTN cases. When using an NGS-based tumor panel to assess choriocarcinoma, researchers must consider whether the genomic DNA of the patient and their partner are involved in the GTN.

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Introduction

Gestational choriocarcinoma is a type of gestational trophoblastic neoplasia (GTN) that originates from trophoblasts and can develop from a normal pregnancy, miscarriage, or molar pregnancy. Its estimated incidence in Japan is 1.9-5.5 cases per 100,000 live births (1). Non-gestational choriocarcinoma shows the same morphological pattern as that of the gestational form but originates mostly from germ cells in the ovary and is rarer and associated with worse outcomes. Short tandem repeat analysis using microsatellite markers is useful for distinguishing gestational choriocarcinoma from non-gestational choriocarcinoma (2).

Chemotherapy is effective for treating gestational choriocarcinoma. However, patients with multiple metastases or metastases to sites other than the lungs often do not achieve complete remission (3). When the cancer develops chemoresistance, more tailored therapies are required, such as drugs selected based on the specific cancer genome. The OncoGuide™ NCC Oncopanel System (Sysmex Corporation) (4) is a next-generation sequencing (NGS)-based tumor panel that is covered by health insurance in Japan. This panel facilitates the identification of variants of 114 cancer-related genes through matched normal-tumor pair analysis. Here, we report a case of intractable gestational choriocarcinoma identified using this system.

Case report

A 51-year-old Japanese woman was diagnosed with choriocarcinoma with metastases to the lung, spleen, and lymph nodes. Histopathological examination of the uterine biopsy showed a two-cell pattern of choriocarcinoma, consisting of syncytiotrophoblastic cells and cytotrophoblastic cells. She had experienced six pregnancies, and the last pregnancy ended in spontaneous abortion approximately 4 years prior. The patient was treated with etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine (EMA/CO)

and had suspected drug-induced pneumonia after the third course (Fig. 1). Therefore, she could not continue EMA-CO therapy although it was effective. The regimen was modified, but the new regimen proved ineffective. She was then referred to our institution for further treatment. We performed a drug-induced lymphocyte stimulation test and found that the anticancer drugs etoposide, etoposide, methotrexate, actinomycin , cyclophosphamide, and vincristine did not induce the allergy. Thus, we concluded that her pneumonia was induced by infection and that we could use these anticancer drugs. After obtaining written informed consent from the patient and her partner, we performed short tandem repeat analysis of DNA extracted from the oral mucosal cells of the patient and her partner and from the paraffin-embedded sections of the micro-dissected tumor, as previously described (5). This study was approved by the Ethics Committee of Nagoya University Graduate School of Medicine. Tumor analysis revealed gestational choriocarcinoma of both maternal and paternal origins (Table I). The patient underwent four types of chemotherapy regimens and was then treated with high-dose ifosfamide, carboplatin, and etoposide (ICE), along with autologous peripheral blood stem cell transplantation (2,6,7).

After three courses of high-dose ICE, we performed total hysterectomy and bilateral adnexectomy to reduce the total choriocarcinoma volume. The patient was administered two courses of mini-ICE after the operation, but multiple metastases were found in the brain. She was thus treated with whole-brain radiotherapy (20 Gy); etoposide, cisplatin, methotrexate, and actinomycin D (EP-EMA) chemotherapy; and radiotherapy for the bone metastases.

To identify drugs appropriate for treating the choriocarcinoma in this case, we utilized the NCC Oncopanel System to compare the uterine choriocarcinoma DNA with the patient's germline DNA extracted from peripheral blood. Microdissection was performed to obtain the choriocarcinoma tissue from formaldehyde-fixed and paraffin-embedded tissue sections (10 μm thickness). The samples were prepared and analyzed as previously reported (4). NCC Oncopanel test revealed 245 single-nucleotide variants (SNVs). Compared with the usual allele frequency of SNVs in matched normal-tumor pair analysis of ≤30%, the mean SNV allele frequency of the patient was more than double, at 63.1%. Initially, experimental errors such as sample misidentification were suspected; however, we eventually concluded that part of the gestational choriocarcinoma DNA was derived from the partner of the patient, whereby the SNV burden was increased.

The tumor DNA contained 19 variants in 13 of the 114 cancer-related genes (Table II). The *GNAQ* p.T96S and *TP53* p.R213P variants were considered to be pathogenic variants; the remaining 17 variants are frequent in the Japanese population. There are no targeted therapies for these two pathogenic variants. After nine courses of EP-EMA, the patient was unable to undergo chemotherapy because of pancytopenia and febrile neutropenia. She was treated for 20 months but ultimately died of choriocarcinoma 7 months after the operation.

Table I. Short tandem repeat analysis of DNA from the tumor, patient and her partner.

Marker	Maternal	Paternal	Tumor
D8S1179	10,14	13,13	10,13
D21S11	30,31	30,30	30
D7S820	11,12	9,12	9,12
CFS1PO	10,11	10,10	10
D3S1358	16,16	16,17	16
TH01	6,6	6,9	6
D13S317	11,12	11,11	11,12
D16S539	11,11	9,9	9,11
D2S1338	17,20	23	-
D19S433	13,13	13,15.2	13
vWA	17,19	16,16	16,17,19
TPOX	8,11	8,11	11
D18S51	14,18	14,17	17,18
Amerogenin	X,X	X,Y	X,X
D5S818	10,12	9,11	10,11,12
FGA	23,26	23,24	23,24,26

The tumor contained maternal and paternal alleles, suggesting that it was gestational.

Discussion

This is the first study using the NCC Oncopanel System test for gestational choriocarcinoma. The test was performed to seek appropriate drugs for the intractable choriocarcinoma, but no drug matched the tumor genome. A hospital-based prospective study using the NCC Oncopanel System test showed that only 13.4% of the patients were eligible for targeted drug therapies based on the sequencing results, and this result is similar to that obtained using another cancer-gene panel (11%) (8). The relatively low likelihood of identifying a targeted therapy should be explained to patients before applying a gene-panel test. Additional genome-matched clinical trials are required to determine the applications that these tests would suit the most.

The results of the NCC Oncopanel test in our case indicate two limitations to using NGS-based tumor-profiling multiplex gene panels for GTN patients. First, panel tests for tumor and matched non-tumor samples, such as the NCC Oncopanel test, show a high SNV burden in the genomic DNA from GTNs, and such results may be misinterpreted as 'tumor-derived' variants. The NCC Oncopanel test is inappropriate for tumors like GTNs containing the DNA of other persons. Tumor-profiling gene-testing using only tumor samples should be used for GTNs. Second, the patient, her partner, and/or their children might be the source of pathogenic variants or secondary genetic findings in the tumor DNA of GTNs. A case of choriocarcinoma in a woman whose partner had a genomic *TP53* variant leading to Li-Fraumeni syndrome has been reported, wherein the *TP53* variant was detected in her tumor but not in her germline DNA (9). Since there are ethical issues associated with genomic screening in GTN cases, informed consent should be obtained from patients and their partners, and specific ethical guidelines should be laid down for tumor-panel testing of patients with GTN.

In the present case, two pathogenic variants (*GNAQ* p.T96S and *TP53* p.R213P) were identified in the tumor DNA. The

Table II. Genomic findings for the tumor and blood of the patient, obtained using the NCC Oncopanel System Test.

Gene name	Mutation allele frequency	Amino acid change	dbSNP	HGVD allele frequency
BARD1	66.3	R24S	rs1048108	0.350
SETD2	68.0	M1080I	rs76208147	0.143
ROS1	64.9	S2229C	rs619203	0.145
ROS1	67.9	K2228Q	rs529156	0.146
ROS1	68.4	D2213N	rs529038	0.151
GNAQ	12.8	T96S	rs777679970	Not detected
TP53	61.7	R213P	rs587778720	Not detected
BRCA1	66.5	S1613G	rs1799966	0.331
BRCA1	62.8	K1183R	rs16942	0.329
BRCA1	63.0	E1038G	rs16941	0.329
BRCA1	70.4	R871L	rs799917	0.331
FGFR4	74.9	G388R	rs351855	0.414
NOTCH2	69.0	R1260H	rs75423398	0.070
PRKCI	73.0	R327R	rs55683301	0.061
ESR1	78.5	P146Q	rs17847065	0.047
PTCH1	70.1	R893H	rs138154222	0.019
BRCA2	62.8	M784V	rs11571653	0.095
CREBBP	64.6	L551I	rs61753381	0.032
BRCA1	51.0	Y856H	rs80356892	0.009

SNP allele frequency of *GNAQ* and *TP53* were not detected in the Japanese database, HGVD. These data demonstrated that *GNAQ* and *TP53* may be pathogenic variants. dbSNP, database of single nucleotide polymorphism; HGVD, human genetic variation database.

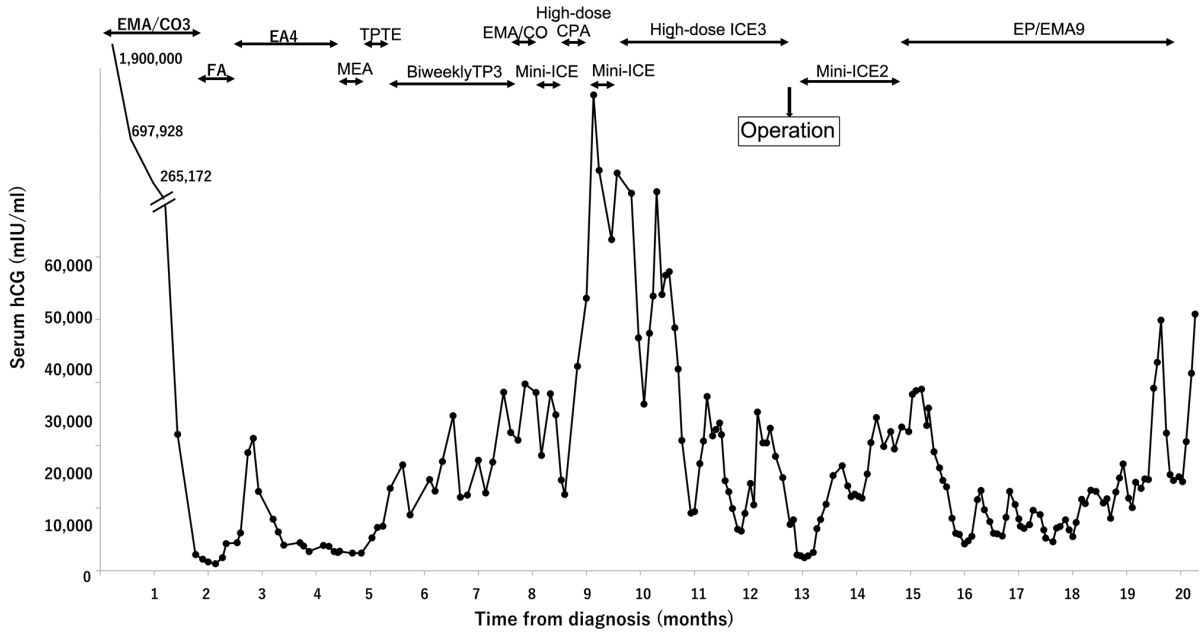

Figure 1. Changes in the serum hCG level of the patient and the treatment progress of choriocarcinoma. EMA/CO, etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine; FA, fluorouracil and actinomycin D; EA, etoposide and actinomycin D; MEA, methotrexate, etoposide, and actinomycin D; TPTE, paclitaxel, cisplatin, and etoposide; biweekly TP, biweekly paclitaxel and cisplatin; ICE, ifosfamide, carboplatin, and etoposide; CPA, cyclophosphamide; EP/EMA, etoposide, cisplatin, methotrexate, and actinomycin D; hCG, human chorionic gonadotropin.

sequence report from the NCC Oncopanel revealed a 12.8% variant allele frequency for *GNAQ* p.T96S, classifying this allele as a somatic variant. The variant allele frequency of *TP53* p.R213P was 61.7%, indicating that this allele might have been a germline variant, but the DNA of the patient's blood did not have it. We realized that this allele might have been a true somatic variant in her tumor or a germline variant in the partner, one of the children of the patient, or the lost pregnancy, because the

tumor was a gestational choriocarcinoma. Genetic counseling sessions were conducted with the partner of the patient to discuss our findings for this variant and its association with Li-Fraumeni syndrome. Upon the request of the partner, we checked the existence of *TP53* p.R213P variants with only his blood but not her children's blood. We found that he did not have this variant.

We here report a case of intractable gestational choriocarcinoma resistant to numerous chemotherapies, including high-dose ICE with peripheral stem cell rescue. It is suggested that it is difficult for choriocarcinoma patients to achieve complete remission when the second chemotherapy regimen fails and multiple metastases exist (3). High-dose chemotherapy with stem cell rescue and anti-programmed cell death-1 (PD-1) antibody therapy might be an option for intractable choriocarcinoma (2,7,10). The effectiveness of anti-PD-1 antibody therapy for intractable GTN patients has recently been reported (10). Our patient was not eligible for this treatment because her choriocarcinoma did not show a high microsatellite instability status, which is required for health-insurance coverage in Japan. Clinical trials of anti-PD-1 antibody therapy for intractable GTN are needed, as this therapy has been shown to be effective for patients with GTN with unknown microsatellite instability statuses (11).

In conclusion, our experience of an intractable choriocarcinoma case screened with the NCC Oncopanel System suggests that matched normal-tumor pair analysis is not appropriate for GTN. When using an NGS-based tumor panel to assess choriocarcinoma, researchers must consider whether the patient's and partner's genomic DNA is involved in the GTN.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KNii, EY, SM, HH, MMorik, HK and FK designed the study. KNii wrote the final manuscript. SM, HH, MMorik, MH and MMorit analyzed and interpreted the data, and wrote the outline of the manuscript regarding genetic analysis. MMorik, MH and MMorit were responsible for genetic counseling. KNis, YO, EW and TY analyzed and interpreted the data of STR analysis. KNii, KNis, YO, EW, HK and FK were involved in the treatment of patients as attending physicians and provided important advice for decision making. EY, TY, HK and FK critically revised the paper for important intellectual content. KNii and EY confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from the study subjects prior to the collection of all the biological samples according to the regulations set out by the Ethics Committee at Nagoya University.

Patient consent for publication

Written informed consent was obtained from the partner of the patient for the publication of these data.

Competing interests

The authors declare that they have no competing interests.

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