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# Presence of the methylenetetrahydrofolate reductase gene polymorphism MTHFR C677T in molar tissue but not maternal blood predicts failure of methotrexate treatment for low-risk gestational trophoblastic neoplasia

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ABSTRACT

Gestational trophoblastic neoplasia (GTN) is a rare tumor, and its genomic constitution is different from the maternal genome because of its gestational origin. Methotrexate (MTX) is a standard chemotherapeutic agent for low-risk GTN. An association between polymorphisms of the methylenetetrahydrofolate reductase (*MTHFR*) gene and MTX treatment outcome has been reported in various diseases. Thus, we examined the association between clinical outcome and *MTHFR* polymorphisms in both tumor and blood DNA of low-risk GTN patients.

*MTHFR* C677T (rs1801133) and A1298C (rs1801131) were genotyped using high-resolution melting assays in 62 Japanese low-risk GTN patients and in 52 antecedent molar tissues. We compared the genotypes of *MTHFR* polymorphisms with the clinical outcome of 5-day MTX treatment.

Twenty-five patients entered remission and 37 patients developed drug resistance or adverse effects that necessitated a drug change. The *MTHFR* 677T allele in molar tissue was significantly related to the need for drug change ($P$=0.006; odds ratio [OR], 3.13; 95% confidence interval [CI], 1.31–7.49), in contrast to *MTHFR* 1298C ($P$=0.18; OR, 0.63; 95% CI, 0.32–1.25). The *MTHFR* 677T and 1298C alleles obtained from patients' blood DNA were not related to MTX treatment outcome ($P$=0.49; OR 1.31; 95% CI, 0.61–2.91 and $P$=0.10; OR 0.52; 95% CI, 0.22–1.15, respectively).

These data demonstrate for the first time that the genotype of *MTHFR* 677TT in molar tissue is associated with ineffective MTX treatment in Japanese low-risk GTN patients.

## 1. Introduction

Methylenetetrahydrofolate reductase (*MTHFR*) is an important rate-limiting enzyme in folate metabolism. It catalyzes the irreversible conversion of 5,10-methylentetrahydrofolate to 5-methyltetrahydrofolate, which is required for protein synthesis and nucleic acid methylation (Rosenberg et al., 2002). The *MTHFR* polymorphisms C677T (rs1801133; NM_005957.4:c.665C > T; NP_005948.3:p.A222V) and A1298C (rs1801131; NM_005957.4:c.1286A > C; NP_005948.3:p.E429A) are the functional single nucleotide polymorphisms (SNPs) with amino acid changes and decrease the activity of the enzyme (Frosst et al., 1995; van der Put et al., 1998; Weisberg et al., 1998). Thus, the relationships between *MTHFR* polymorphisms and the development of cancer have been investigated in many studies, especially of breast and colon cancer, because dietary folate intake was correlated with an increase or decrease in cancer risk (Kumar et al., 2015; Pooja et al., 2015; Rai, 2015).

Methotrexate (MTX) is used as chemotherapeutic or immunosuppressive agent, depending on clinical settings (Lawrie et al., 2016; Davila-Fajardo et al., 2013; Ulrich et al., 2001); a high dose of MTX is used to treat malignant lymphoma, leukemia, and sarcoma (Lopez-Lopez et al., 2013; Seidemann et al., 2006; Windsor et al., 2012), and a low dose is used to treat rheumatoid arthritis (Hughes et al., 2006). The primary target of MTX is the folate metabolic cycle. A number of studies have suggested a relationship between *MTHFR* polymorphisms and toxicity or efficacy of MTX treatments, although the results are controversial (Lasecka et al., 2011; Seidemann et al., 2006; Ulrich et al., 2001).

One key use of MTX is the treatment of gestational trophoblastic neoplasia (GTN), a rare type of tumor, which arises from villous or placental cells and has a different genetic origin from that of the host patient (Fig. 1). Almost all antecedent pregnancy in GTN is complete hydatidiform mole, namely an androgenetic mole that develops from an abnormal conceptus carrying only paternally derived genomes (Seckl et al., 2010). GTN is divided into low-risk and high-risk cases (FIGO Oncology

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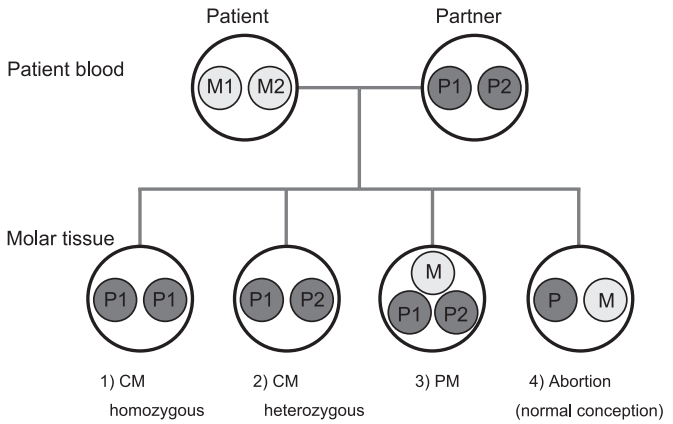


**Fig. 1.** Schematic representation of the cytogenetic constitution of molar tissue. (1) Complete moles (CM) are androgenetic and principally homozygous. (2) Relatively few are heterozygous. (3) Partial moles (PM) are triploid (diandric monogyny). (4) Normal conceptuses are biparental diploid. M, M1, M2, maternal haploid genome. P, P1, P2, paternal haploid genome.

Committee, 2002). Low-risk GTN is sensitive to single-agent chemotherapy with MTX. However, almost half of the patients fail to enter remission with MTX treatment (Matsui et al., 2005).

Considering that GTN is sensitive to MTX, and that the genotypes of tumor and host cells are independent, we reasoned that low-risk GTN might be a useful model to study the pharmacogenomics of folic acid metabolism. Most hydatidiform moles are of homozygous androgenetic origin, which enables us to analyze more easily the relationship between *MTHFR* polymorphisms and clinical outcome of MTX treatment. In addition, MTX is used as a single agent to treat low-risk GTN, without a co-administration of other drugs. Thus, we considered that it could be beneficial to isolate and evaluate the effect of MTX itself on *MTHFR* polymorphisms in our study, as most other studies used multiple agents other than MTX alone (Chiusolo et al., 2012; Seidemann et al., 2006; Windsor et al., 2012).

To elucidate the clinical significance of *MTHFR* polymorphisms in MTX treatment for low-risk GTN, we conducted an association study of two representative *MTHFR* polymorphisms, C677T and A1298C.

## 2. Material and methods

### 2.1. Patients

The study was approved by the Institutional Ethical Committee at the Graduate School of Medicine, Chiba University (No. 291). Between 2007 and 2016 at Chiba University Hospital, 77 Japanese patients were diagnosed with low-risk GTN according to the FIGO 2000 criteria (FIGO Oncology Committee, 2002). Five patients were excluded as they had received other first-line chemotherapy regimens. Seventy-two patients received a 5-day MTX regimen as the primary treatment. Four patients were excluded from the analysis because they had undergone a hysterectomy before or during chemotherapy. It was not possible to collect blood samples from six of the patients because they had moved away. In total, 62 patients were enrolled in this study; all gave written informed consent.

### 2.2. Treatment protocols and evaluation of clinical outcome of MTX therapy

Patients treated with MTX received 20 mg intramuscularly each day for 5 days. This treatment was repeated every 14 days as long as the patient did not show severe toxic effects (Matsui et al., 2005). We did not permit the patients to take a dietary supplement of folic acid or vitamin B during MTX treatment because the effect of MTX could be attenuated.

A second-line regimen was used when the drug induced severe toxicity. In drug rash cases, we stopped MTX and changed to the second-line regimen. Patients who developed oral mucositis discontinued the MTX treatment when they could not eat solid food anymore (grade 3 on Criteria for Adverse Events Version 4.0: http://evs.nci.nih.gov/ftp1/CTCAE/About.html). We changed the drug if liver enzymes elevated to the level of CTCAE grade 2.

Chemotherapy response was monitored by determining serum hCG level twice a week. Primary remission was defined as serum hCG level within the normal range for six months at least. Drug resistance was diagnosed when serum hCG level stopped decreasing over two courses or increased again.

### 2.3. Samples and DNA extraction

Genomic DNA was extracted from a 200-μl blood sample from 62 patients by using a QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Fresh molar tissues were collected when we evacuated the index molar pregnancies in our hospital ($n$ =33). Genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen). Since the patients in 29 cases had been evacuated in other clinics or hospitals, we could not obtain fresh tissues. Instead, in 19 cases, we used formalin-fixed paraffin-embedded blocks of molar tissues and a micro-dissection method to prepare the DNA. Genomic DNA was extracted using a QIAamp DNA Micro Kit (Qiagen) from formalin-fixed paraffin-embedded blocks. Finally, we were able to obtain 52 usable molar tissue samples for the analysis.

### 2.4. Short tandem repeat polymorphism analysis

To confirm the cytogenetic diagnosis and evaluate the contamination of maternal decidua or blood with molar tissue, we compared the short tandem repeat (STR) polymorphic patterns of a patient's blood and molar tissues using the PowerPlex 16 HS system (Promega, Madison, WI, USA) as described previously (Baasanjav et al., 2010). Antecedent pregnancies were diagnosed as androgenetic heterozygous mole, homozygous mole, biparental triploid, or biparental diploid (Fig. 1).

### 2.5. MTHFR genotyping

We genotyped the two *MTHFR* SNPs, C677T (rs1801133) and A1298C (rs1801131), using high-resolution melting (HRM) analysis based on modification of a previously published procedure (Kristensen and Dobrovic, 2008). Real-time PCR was performed using a LightCycler® Nano Instrument (Roche Diagnostics, Basel, Switzerland). The PCR mixtures consisted of genomic DNA (0.5 ng), 1× LightCycler® 480 High Resolution Melting Master (Roche Diagnostics), 2.5 mM $MgCl_2$, and 200 nM of each forward and reverse primer in a total volume of 10 μl. The primer sequences were as follows: forward 5′-GCACTTGAAGGAGAAGGTGTCTG-3′ and reverse 5′-AGCTGCGTGATGATGAAATCG-3′ in *MTHFR* C677T, and forward 5′-GGGGAGGAGCTGACCAGTGA-3′ and reverse 5′-GAGGTAAAGAACCAAGACTTCAAAGACAC-3′ in *MTHFR* A1298C. The thermocycling conditions used for both primer pairs were as follows: 95 °C for 10 min; 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 25 s, extension at 72 °C for 15 s, then hold at 95 °C for 60 s and 40 °C for 60 s, and finished by HRM procedure from 60 °C to 95 °C at 0.05 °C/s. All data were analyzed using LightCycler NanoSoftware 1.1 (Roche Diagnostics) in HRM analysis mode. Genotyping with HRM analysis was performed independently three times.

### 2.6. Calculation of hCG half-life during MTX treatment

The half-life of serum hCG during MTX treatment was calculated