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*Original Article*

# ERCC1 C118T associates with response to FOLFOX4 chemotherapy in colorectal cancer patients in Han Chinese

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**Abstract:** Background: Genetic variations influence treatment outcomes in cancer patients treated with chemotherapy. Detection of pharmacogenetic markers associated with treatment response may enable doctor to plan more precise and effective treatment tailoring to individual cancer patients. Methods: A novel oligonucleotide microarray was developed to genotype 13 variations (DPYD*2, DPYD*5, DPYD*9, TYMS 6 bp Ins/Del, UGT1A1*6, UGT1A1*27, UGT1A1*28, GSTP1 Ile105Val, XRCC1 Arg399Gln, MTHFR C677T, MDR1 C3435T/A, MDR1 G2677A/T and ERCC1 C118T). The accuracy of genotypes obtained by microarray was assessed by independent sequencing. 73 patients first diagnosed with colorectal cancer (CRC) were treated with FOLFOX4 chemotherapy. Results: All genotypes were successfully called by microarray, and were consistent with those identified by independent sequencing except two TYMS 6 bp Ins/Del genotypes. Patients with CT or TT genotype exhibited a higher probability of response to treatment than those with CC genotype. No other SNP was found to be associated with treatment response. Furthermore, these SNPs showed no associations with gastrointestinal, hematological or neurological toxicity. Conclusions: ERCC1 C118T may be a predictive marker of treatment response to 5-FU/platinum chemotherapy for CRC. The microarray can significantly facilitate the process of detecting genetic variations and may help doctor plan more effective medication for individual cancer patient.

**Keywords:** Single nucleotide polymorphism, 5-FU, platinum, drug response, ERCC1, molecular biomarker

## Introduction

Malignant tumor has become an important healthy problem, and its mortality rate was 374.1 per 100,000 person-years [1]. Cancer has been become the leading cause of death in Chinese adults [1]. Among them, colorectal cancer (CRC) is the fifth leading cause of cancer death in China and its incidence has been increasing over recent decades [2, 3]. In recent years, chemotherapy is the standard treatment of choice except surgery. Despite the recent development of new therapeutic strategies, the prognosis of CRC patients especially with metastasis remains poor, which is mainly due to interindividual variation in drug response. It has been estimated that genetic variants may account for 20-95% of the interindividual differences in drug response [4]. Therefore, identification of pharmacogenetic markers influencing drug response may enable doctor to plan more precise and effective treatment tailoring to individual cancer patients.

5-FU is one of the most commonly used agents in chemotherapy of cancer alone or combined with other cytotoxic drugs, especially for gastrointestinal and breast cancers [4-6]. However, severe (grade 3/4) toxicity occurred in approximately 30% of patients treated with 5-FU-based chemotherapy. The main reason for the toxicity of 5-FU is the deficiency of the dihydropyrimidine dehydrogenase gene (DPYD) gene, encoding the key rate-limiting enzyme dihydropyrimidine dehydrogenase (DPD) in the catabolism of 5-FU. It has been reported that genetic

DPD deficiency is responsible for about 50% of severe 5-FU- related toxicity [5]. The most common deficient allele is DYPD*9 in Chinese with the frequent of 5-14% [6, 7]. Thymidylate synthase (TYMS) plays an important role in the methylation of deoxyuridine-5'-monophosphate (dUMP) to deoxythymidine-5'-monophosphate (dTMP). The expression of TYMS is inversely associated with 5-FU sensitivity [8]. Recent studies have shown 6 bp indel in the 3'UTR of TYMS have effect on gene expression [9], and therefore affect the responses to 5-FU [10-12]. Furthermore, polymorphisms of methylenetetrahydrofolate reductase (MTHFR) are also reported to be associated with 5-FU response [13].

Platinum-based chemotherapy is the mainstay of treatment for many cancers, including lung, ovarian, breast and gastrointestinal cancer. Platinum drugs exert their cytotoxic effect through the DNA cross linking. There are at least four pathways of DNA repair to operate on specific types of damaged DNA, including base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR) and double-strand-break repair (DDSBR) pathway [14]. Excision repair cross-complementation group 1 (ERCC1) is the rate-limiting enzyme of NER that has been associated with risk for many cancers, including colorectal cancer [15]. It has been demonstrated that high mRNA expression of ERCC1 is predictive of resistance to platinum therapy in several cancers [16]. ERCC1 C118T is a functional single nucleotide polymorphism (SNP) that affects the ERCC1 mRNA stability and level [17, 18]. Recent studies have been shown that C118T is associated with sensitivity to platinum-based chemotherapy in many cancers [19]. Furthermore, polymorphism in GSTP [20], XRCC1 and MDR1 are also reported to be associated with response to platinum-based chemotherapy.

To date, the United States Food and Drug Administration (US FDA) has recommended label change for at least 5 drugs, including 5-FU and irinotecan, to include pharmacogenetic information on treatment outcome [21]. In the present study, we developed a microarray containing 13 polymorphisms, which were reported to be associated with treatment outcomes of anticancer agents. In addition, we evaluated the relationship between these polymorphisms and drug response in CRC patients treated with neoadjuvant FOLFOX4 chemotherapy.

## Materials and methods

### *Patients*

A total of 73 CRC patients and 48 health individuals were recruited from Tongji Hospital between March 2010 and May 2011. All cases were histologically confirmed incident cases of primary CRC. Epidemiologic data were collected by trained interviewers with the specific standardized questionnaire to obtain detailed information on demographic factors, personal habits, medical history, family history of cancer, history of occupational and environmental exposures. Patients known to have family history of cancer or metastasized cancer from other or unknown origins were excluded. All participating individuals were unrelated ethnic Han Chinese. Patients were informed about the project and gave their written consent to participate in the study. This study was approved by the ethical committee of the hospital.

All patients were treated with FOLFOX4 chemotherapy. The chemotherapeutic drugs were administered intravenously, and patients were treated for two to six cycles, with a median of four cycles. Patients' responses to treatment were classified into four categories according to the WHO criteria [22]: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). An objective response was defined by a decrease of at least 50% of initial tumor size. After chemotherapy, patients were classified into two groups as responders (CR + PR) and Non-responders (SD + PD).

### *Genotyping*

According to the manufacturer's protocol, genomic DNA was extracted from the anticoagulation peripheral blood using Flexigene DNA kit (Qiagen, Hilden, Germany) and then stored at -20ºC. Thirteen polymorphisms were shown in **Table 1**. Genotyping was performed by ligation detection reaction (LDR) based microarray method [23]. Primers were designed by software of Primer 3 (http://frodo.wi.mit.edu/primer3/input.htm) and SNP BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed to avoid polymorphic sites in the primer sequences. The sequences of primers were listed in **Table 2**.

**Table 1.** Patient clinicopathologic characteristics

| Characteristics | Patient No. | (%) |
|---|---|---|
| Age | | |
| Median (range) | 59 (24-87) | |
| Gender | | |
| Female | 28 | 61.6 |
| Male | 45 | 38.4 |
| Smoking stastus | | |
| Smoker | 20 | 27.4 |
| Non-smoker | 53 | 72.6 |
| Tumor stage | | |
| I | 11 | 15.1 |
| II | 14 | 19.2 |
| III | 33 | 45.2 |
| IV | 15 | 20.5 |
| Response | | |
| Responser | 48 | 65.8 |
| CR | 23 | 31.5 |
| PR | 25 | 34.3 |
| Non-responser | 25 | 34.2 |
| SD | 12 | 16.4 |
| PD | 13 | 17.8 |

All DNA fragments were co-amplified only in a single multiplex reaction. The reaction was carried out in a total volume of 30 μL containing 0.3 μM of each deoxynucleoside triphosphate, 40 mM Tricine-KOH (pH 8.7), 16 mM KCl, 3.5 mM $MgCl_2$, 3.75 μg/mL BSA, 2 μM of each primer, 60 ng of DNA and 1.2× Titanium polymerase (Clontech, PaloAlto, USA). Cycling conditions were as follows: 95°C for 3 min; followed by 40 cycles of 95°C for 30 s, 61°C for 50 s, 68 for 30 s; followed by 68°C for 5 min. PCR products were purified with 2 U shrimp alkaline phosphatase (Fermentas, Vilnius, Lithuania) and 4 U exonuclease I (Fermentas, Vilnius, Lithuania) at 37°C for 1 h, and then denatured at 95°C for 10 min.

The sequences of primers for LDR were listed in **Table 3**. The ligation reaction was carried out in a volume of 20 μl containing 10 μl of purified PCR product, 500 fmol of LDR primer, 2× Taq DNA ligase buffer, 10 U TaqDNA ligase (New England Biolabs, USA). The LCR was performed

**Table 2.** Primer sequences

| SNP | F/R* | Primer sequence (5→3') | Amplicon size (bp) |
|---|---|---|---|
| DPYD*2 | F | 5'-GACAAATGTTTCCCCCAGAAT-3' | 370 |
| | R | 5'-AGATATGCTGCTTCTGCCTCA-3' | |
| DPYD*5 | F | 5'-AATTCGGATGCTGTGTTGAAG-3' | 157 |
| | R | 5'-ATGGGACAGAAAGGAAGGAAA-3' | |
| DPYD*9 | F | 5'-TTAGCCAGGTGTGGTAGCGTA-3' | 280 |
| | R | 5'-TGCCTTACAATGTGTGGAGTG-3' | |
| TYMS-6bp | F | 5'-CTCAAATCTGAGGGAGCTGAGT-3' | 136 |
| | R | 5'-GCTAATGAGGGCAAAATGGTAG-3' | |
| UGT1A1*28 | F | 5'-TGAACTCCCTGCTACCTTTGTG-3' | 407 |
| | R | 5'-ATGGCACAGGGTACGTCTTCA-3' | |
| UGT1A1*27 | F | 5'-TGATTCTTTCCTGCAGCGTGT-3' | 250 |
| | R | 5'-TGGGCCTAGGGTAATCCTTCA-3' | |
| UGT1A1*6 | F | 5'-TGAACTCCCTGCTACCTTTGTG-3' | 407 |
| | R | 5'-ATGGCACAGGGTACGTCTTCA-3' | |
| GSTP1-G105A | F | 5'-TGCTGTGTGGCAGTCTCTCAT-3' | 94 |
| | R | 5'-GGTTGATGTCCCAGGCAATAA-3' | |
| XRCC1-G399A | F | 5'-CTCCCTTGGTCTCCAACCTCT-3' | 215 |
| | R | 5'-GCTCCTCCAGCCTTTTCTGAT-3' | |
| ERCC1-C118T | F | 5'-GGAAGTGTTCAGGACCACAGG-3' | 462 |
| | R | 5'-CATGCCCAGAGGCTTCTCATA-3' | |
| MTHFR-C677T | F | 5'-TGTGCTGTTGGAAGGTGCAA-3' | 347 |
| | R | 5'-AGTGGGGTGGAGGGAGCTTA-3' | |
| MDR1 A3435C/T | F | 5'-GGATCCAGGGATTAGGACACA-3' | 376 |
| | R | 5'-ACATGCTCCCAGGCTGTTTAT-3' | |
| MDR1 G2677A/T | F | 5'-TCAGCATTCTGAAGTCATGG-3' | 360 |
| | R | 5'-TGCTGTGTGGCAGTCTCTCAT-3' | |

*F: Forwar primer; R: Reverse primer

Table 3. Sequences of LDR primers

| SNP | Primer | Sequence | TM |
|---|---|---|---|
| DPYD*2 | DPYD*2G | 5'-ACTACGCAACACTGAACGGAGGCTGACTTTCCAGACAACG-3' | 58 |
| | DPYD*2A | 5'-TTACGACGCATCTCAAGCACAGGCTGACTTTCCAGACAACA-3' | 57 |
| | DPYD*2P | 5'-TAAGTGTGATTTAACATCTAAAACAA-TAMRA-3' | 52 |
| DPYD*5 | DPYD*5A | 5'-CGAATCAGTCTTGCTCATCGTAGAAATGGCCGGATTGAAGTTTA-3' | 57 |
| | DPYD*5G | 5'-TCAGTCCATCTTGCTACAGGTAGAAATGGCCGGATTGAAGTTTG-3' | 58 |
| | DPYD*5P | 5'-TAAATCCTTTTGGTCTTGCTAGCG-TAMRA-3' | 58 |
| DPYD* | DPYD*9T | 5'-AACGCTTGGTCTAAACTCCCAATTTCTTGGCCGAAGTGGAACA-3' | 58 |
| | DPYD*9C | 5'-CACGTGACGACTACTGTTGATTCTTGGCCGAAGTGGAACG-3' | 58 |
| | DPYD*9P | 5'-CAGAGTTGCATGAGTTTGTGTTC-TAMRA-3' | 58 |
| TYMS 6 bp Ins/ Del | TYMS-6I | 5'-GTAGTCACGACGAACAGCTAGCAACATATAAAACAACTATAACTT-3' | 51 |
| | TYMS-6D | 5'-TTCCACTAGCAGCACGACTTTATTATAGCAACATATAAAACAACTA-3' | 51 |
| | TYMS-6P | 5'-TAAAGTTCATAACCACACTCTACAT-TAMRA-3' | 58 |
| UGT1A1*28 | UGT1A1*28-6 | 5'-GGAAGCAGTTGTCGTTAGCAGTGTATCGATTGGTTTTTGCCATAT-3' | 57 |
| | UGT1A1*28-7 | 5'-AGACTCTCACTGCAAGCTGTGTATCGATTGGTTTTTGCCATATAT-3' | 56 |
| | UGT1A1*28P | 5'-ATATATATATAAGTAGGAGAGGGCG-TAMRA-3' | 57 |
| UGT1A1*6 | UGT1A1*6A | 5'-CCAGATTCAGACTAGCCTACGCCTCGTTGTACATCAGAGACA-3' | 59 |
| | UGT1A1*6G | 5'-CAGTCGCACCGTTCAAGTTTCCTCGTTGTACATCAGAGACG-3' | 59 |
| | UGT1A1*6P | 5'-GAGCATTTTACACCTTGAAGACG-TAMRA-3' | 58 |
| UGT1A1*27 | UGT1A1*27A | 5'-AACGGGATACAGTTCTCGGTCTGAGGCAAGGGTTGCATACT-3' | 59 |
| | UGT1A1*27C | 5'-TTGACGCTACAGGTGACGATTGAGGCAAGGGTTGCATACG-3' | 58 |
| | UGT1A1*27P | 5'-GGGAATAAACCACGTCGCACA-TAMRA-3' | 59 |
| GSTP1 Ile105Val | GSTP1-A | 5'-TAGGCACAAACGTCACAGTCAGGACCTCCGCTGCAAATACA-3' | 58 |
| | GSTP1-G | 5'-TGCACCTCTAAGAACTGACGGGACCTCCGCTGCAAATACG-3' | 60 |
| | GSTP1-105P | 5'-TCTCCCTCATCTACACCAACTAT-TAMRA-3' | 58 |
| XRCC1 Arg399Gln | XRCC1-399A | 5'-CAACGCACTGACCATACCTACGTCGGCGGCTGCCCTCCCA-3' | 66 |
| | XRCC1-399G | 5'-CTGAATCCTCCATCCGTGTTGTCGGCGGCTGCCCTCCCG-3' | 68 |
| | XRCC1-399P | 5'-GAGGTAAGGCCTCACACGCCA-TAMRA-3' | 62 |
| ERCC1 C118T | ERCC1-118C | 5'-GTGTGAAGCAAACTGTAGGCGTACTGAAGTTCGTGCGCAAC-3' | 59 |
| | ERCC1-118T | 5'-AAGTCAACGTAAGCAGCAGCGTACTGAAGTTCGTGCGCAAT-3' | 59 |
| | ERCC1-118P | 5'-GTGCCCTGGGAATTTGGCGA-TAMRA-3' | 60 |
| MTHFR C677T | MTHFR-677T | 5'-AGCGAGCATACGGCAATACTAAGCTGCGTGATGATGAAATCGA-3' | 58 |
| | MTHFR-677C | 5'-GATGATCGCTCTACGTGACAGCTGCGTGATGATGAAATCGG-3' | 59 |
| | MTHFR-677P | 5'-CTCCCGCAGACACCTTCTCGTCAAT-TAMRA-3' | 60 |
| MDR1 C3435T/A | MDR1-3435T | 5'-AGTGGATCTTGTACGTGGACCTCCTTTGCTGCCCTCACA-3' | 60 |
| | MDR1-3435C | 5'-AGGCTTACCGTCTGATTGCTCCTTTGCTGCCCTCACG-3' | 60 |
| | MDR1-3435A | 5'-ACCTGCGAATGTCCATAACGCCTCCTTTGCTGCCCTCACT-3' | 60 |
| | MDR1-3435P | 5'-ATCTCTTCCTGTGACACCACC-TAMRA-3' | 59 |
| MDR1 G2677A/T | MDR1-2677G | 5'-CGACGTCTATAGCATTCAGGAAAGATAAGAAAGAACTAGAAGGTG-3' | 58 |
| | MDR1-2677T | 5'-AGCTCACCATGTACGAACTGAAAGATAAGAAAGAACTAGAAGGTT-3' | 58 |
| | MDR1-2677A | 5'-GTACACGATTCAGAGAGAGGAAAGATAAGAAAGAACTAGAAGGTA-3' | 58 |
| | MDR1-2677P | 5'-CTGGGAAGGTGAGTCAAACTAAAT-TAMRA-3' | 58 |

using 40 cycles of at 94°C for 20 s and 60°C for 2 min.

LDR products were added to a hybridization solution containing 6× SSPE, 5% DMSO, 0.1% Triton X-100 and 1 nM fluorescence control oligonucleotide in a final volume of 25 μL. The hybridization solution was denatured at 95ºC for 10 min and then placed on ice. Twenty μL of hybridization solution was transferred to the

Table 4. Association between SNPs and treatment response to chemotherapy

| SNP | Genotype | Responder | Non-responder | *P* value |
|---|---|---|---|---|
| DPYD*5 | AA | 25 (52.1%) | 11 (44%) | 0.685 |
| | AG | 20 (41.7%) | 13 (52%) | |
| | GG | 3 (6.2%) | 1 (4%) | |
| TYMS-6 | DD | 29 (60.4%) | 12 (48%) | 0.363 |
| | ID | 18 (37.5%) | 11 (44%) | |
| | II | 1 (2.1%) | 2 (8%) | |
| GSTP1 I105V | Ile/Ile | 36 (75%) | 16 (64%) | 0.148 |
| | Ile/Val | 9 (18.8%) | 9 (36%) | |
| | Val/Val | 3 (6.2%) | 0 | |
| XRCCI Arg399Gln | Arg/Arg | 28 (58.3%) | 15 (60%) | 0.584 |
| | Arg/Gln | 18 (37.5%) | 10 (40%) | |
| | Gln/Gln | 2 (4.2%) | 0 | |
| MTHFR C677T | CC | 30 (62.5%) | 10 (40) | 0.077 |
| | CT | 17 (35.4%) | 12 (48) | |
| | TT | 1 (2%) | 3 (12%) | |
| MDR1 C3435A/T | CC | 21 (43.8%) | 9 (36%) | 0.71 |
| | CT | 22 (45.8%) | 14 (56%) | |
| | TT | 5 (10.4%) | 2 (8%) | |
| MDR1G2677A/T | GG | 14 (29.2%) | 7 (28%) | 0.499 |
| | GT | 16 (33.3%) | 7 (28%) | |
| | AG | 3 (6.25%) | 5 (20%) | |
| | AT | 9 (18.8%) | 4 (16%) | |
| | TT | 6 (12.5%) | 2 (8%) | |
| ERCC1 C118T | CC | 20 (41.7%) | 17 (68%) | 0.027 |
| | CT | 19 (39.6%) | 8 (32%) | |
| | TT | 9 (18.75%) | 0 (0%) | |

hybridization chamber. Hybridization was carried out at 52°C for 2 hour. After hybridization, the slides were washed at 42°C with 2× SSC and 0.1% SDS for 5 min, and subsequently washed at 42°C with 1× SSC and 0.1% SDS for 5 min and with 0.5× SSC at room temperature for 5 min. The hybridization slides were scanned using LuxScan-10K/B (CapitalBio, Beijing, China). The images were analyzed using the LuxScan 3.0 software (CapitalBio, Beijing, China). The average signals after subtraction of local background were calculated from spots within each triplicate. Allele fraction (AF) scores were calculated to determine genotype [24].

*Sequencing*

Forty eight samples were carried out bidirectional sequencing and thereby used as control for validation of AF limit values. Furthermore, to confirm the accuracy of the microarray results, all heterozygous genotypes of 73 patients with CRC were validated by sequencing. Briefly, each SNP was amplified in a total volume of 15 μL containing 0.3 mmol/L of each deoxynucleoside triphosphate, 10 mmol/L Tris-HCl, 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 20% Q solution (Qiagen, Hilden, Germany), 0.16 μmol/L of each primer, 10 ng DNA, and 1.2 U Taq (TaKaRa, Dalian, China). Cycling conditions were as follows: 95°C for 3 min; 30 cycles of 95°C for 30 s, 61°C for 30 s, 72°C for 30 s; finally at 72°C for 10 min. Sequencing was carried out using Big Dye terminator chemistry on the 3730 analyzer (Applied Biosystems, CA, USA).

*Statistical analysis*

All quantitative variables were given as mean values ± SD. Hardy-Weinberg equilibrium was assessed by the chi-square test with 1 degree of freedom. The association between the SNP genotypes and drug response was tested by two -tailed Fisher exact test. Logistic regression

analysis was used to estimate the odds ratio (*ORs*) and corresponding 95% confidence intervals (*CIs*). A *P* value less than 0.05 was considered statistically significant in all analyses. All analyses were carried out with the SPSS software package Version 17.0 (SPSS, Chicago, USA).

## Results

### *Patients' characteristics*

We enrolled 73 patients with CRC. Baseline characteristics are summarized in **Table 1**. The mean age was 59 ± 14 years (range 24-87 years). 38.4% of the patients were female. There were 15.1% of stage I, 19.2% of stage II, 45.2% of stage III and 20.6% of stage IV. All patients were first diagnosed with CRC and never received anti-cancer therapy before enrollment. Treatment of 5-FU and platinum combination chemotherapy made at the time of the first diagnosis of CRC.

### *Microarray performance*

The microarray is composed of 28 oligonucleotide probes to detect a total of 13 variations, (DPYD*2, DPYD*5, DPYD*9, TYMS 6 bp Ins/Del, UGT1A1*6, UGT1A1*27, UGT1A1*28, GSTP1 Ile105Val, XRCC1 Arg399Gln, MTHFR C677T, MDR1 C3435T/A, MDR1 G2677A/T and ERCC1 C118T. To block cross-hybridization and weak signal, we set cutoff value. After normalization of signal intensities of probes, the genotype for a given polymorphic locus is determined by the ratio between the signal intensity contributed from the allele B and signals from both alleles using AF limit values as cutoffs. The AF limit values were chosen on the basis of concordance between microarray results and genotypes obtained by sequencing. Genomic DNA samples extracted from 73 CRC patients were further analyzed with the microarray to demonstrate microarray quality. All polymorphisms were successfully genotyped on the basis of AF and visual inspection. To further validate our microarray results, in the case of a heterozygote at the polymorphic site, the genotype was also determined by bidirectional sequencing. Except TYMS 6 bp Ins/Del, all results were found to be consistent with the sequencing results. For TYMS 6 bp Ins/Del, 2 microarray results were not consistent with sequencing results.

Thirty samples were randomly selected and

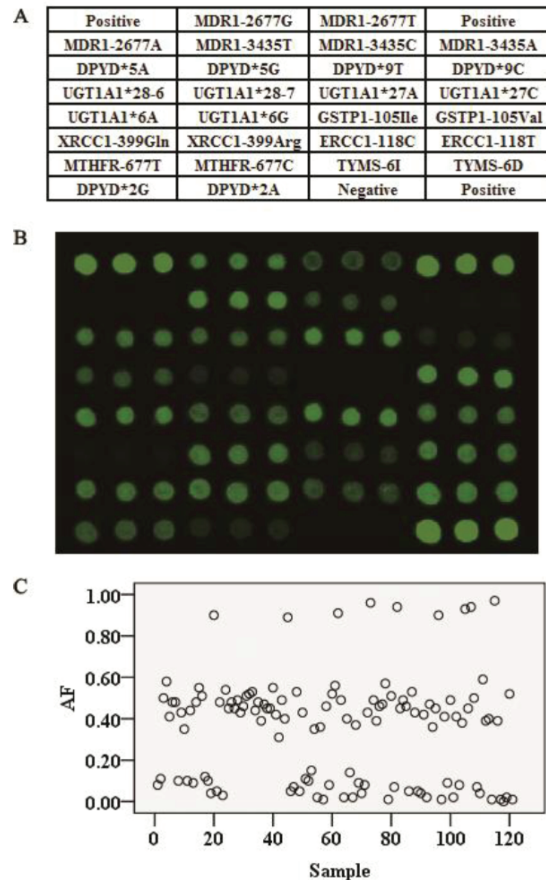


**Figure 1.** A. Microarray probe layout. All probes were spotted in triplicate. Positive: positive hybridization probe. Negative: negative hybridization probe. B. A microarray image from one individual assayed. C. Scatter plots showing the results from MDR1 C3435T genotyped in 121 individuals.

genotyped three times using the microarray. The repeatability of the microarray achieved 99%. These results showed that the microarray have a high call rate, accuracy and reproducibility. A typical microarray image from one individual is shown in **Figure 1**.

### *Association between SNPs and drug response*

UGT1A1*6 and DPYD*2 were not found in population, and UGT1A1*28, DPYD*9 and UGT1A1*27 were rare in the study population (< 5%). Since study population is small, rare variations were excluded from further analysis. The genotype distributions of other 8 SNPs were in Hardy-Weinberg equilibrium (*P* > 0.05). **Table 3** describes the genotype distributions for TYMS 6 bp Ins/Del, GSTP1 Ile105Val, XRCC1

Arg399Gln, MTHFR C677T, DPYD*5, MDR1 C3435T/A, MDR1 G2677A/T and ERCC1 C118T of 73 CRC patients treated with FOLFOX4. We found ERCC1 C118T significantly associated with response to chemotherapy (Table 4). Frequency of CC genotype of ERCC1 C118T was significantly lower in responsers than those in non-responsers (P = 0.036, OR = 0.336, 95% CI, 0.122-0.930). The association between CC genotype of ERCC1 C118T and treatment response remained significant even after adjusting for patient gender, age at diagnosis, stage, and smoking status (P = 0.017, OR = 0.241, 95% CI, 0.075-0.777). There was a weak but not significant association between MTHFR C677T TT genotype and drug response (P = 0.070). No other SNP was found to be association with treatment response.

We further examined the association between these SNPs and adverse response in all individuals. No SNP was found to be association with haematological, gastrointestinal or neurological toxicity (P > 0.05). Although 5 patients stopped treatment due to serious adverse drug reactions, no difference was also observed between these SNPs and resistance to toxicity of treatment (P > 0.05).

## Discussion

Although factors such as pathological stage, age and performance status provide a crude discrimination of prognosis in many cancers, these prognostic indicators do not accurately predict individual patient outcomes. Interindividual variability in treatment response is likely to reflect underlying molecular heterogeneity of cancer. A personalized approach of treatment selection based on molecular markers is therefore desperately needed. Currently, the majority of molecular biomarkers for predicting therapy response and prognosis in cancer patients are somatic alteration and gene or protein expression. However, obtaining of tumor tissue material has been a challenge in the setting of advance disease, which is always not resected. Therefore, it may be difficult to adopt in the routine clinical setting to add further prognostic information or prediction response using tumor material. Conversely, germline genetic variants from peripheral venous blood for the prediction of treatment response and prognosis of individual patients have been becoming much more appealing, especially in the advanced cancer setting. Detection of these genetic polymorphisms related to treatment outcomes conduces to identify the right drug and dose for individual and may ultimately realize personal medicine.

In the present study, we described a rapid microarray-based genotyping method for detecting variations of 8 genes associated with drug response. The microarray offers the unique ability to discriminate simultaneously a total of 13 variations, including 3 variations of the DPYD gene, 1 polymorphisms of the TYMS gene, 2 polymorphisms of the MDR1 gene, 1 polymorphism of the ERCC1 gene, 3 variations of the UGT1A1, 1 polymorphism of the GSTP1 gene, 1 polymorphism of the XRCC1 gene and 1 polymorphism of the MTHFR gene. Among these variations, in addition to variations associated with the response to 5-FU [7, 12, 21], irinotecan [21] and platinum [13, 16, 19, 20, 25], other polymorphisms were reported to be associated with treatment outcomes of other anti-cancer drugs, including capecitabine [26], paclitaxel [27, 28] and vinorelbine [25]. One of advantages of the method is that all DNA targets were coamplified in a single multiplex reaction, no singleplex PCR amplification required before genotype determination. To assess microarray quality, we measure concordance of genotypes determined by independent direct sequencing. Microarray results showed a high call rate and accuracy as generated by sequencing, which makes it a more attractive option for genetic testing in clinical laboratory.

ERCC1 participates in the repair of platinum-induced DNA damage. Functional variations of ERCC1 may be predictive markers for response to platinum. It has been reported that ERCC1 C118T affects the level of ERCC1 expression [18, 29]. In this study, we have investigated the relationship between ERCC1 C118T and drug response in 73 patients with CRC treated with FOLFOX4. CT or TT genotype of ERCC1 C118T exhibited a higher probability of response to treatment. This finding was in agreement with that of previous studies in which patients with CT or TT genotype of ERCC1 C118T appeared to have significantly better response to platinum-based chemotherapy compared to those with CC genotype [29, 30]. Furthermore, a meta-analysis study showed that patients with non-small cell lung cancer with CT or TT genotype of ERCC1 C118T have a good response to plati-

num-based chemotherapy [19]. Yu et al. reported that ovarian cancer cells with T allele showed a greatly reduced DNA repair capacity compared to those with C allele [18]. Since higher DNA repair capacity can lead to platinum resistance, higher probability of response to treatment in T-allele carriers in the current study may support these findings. These results indicate that ERCC1 C118T is potential biomarker of response to platinum-based chemotherapy. There was no significant association between TYMS 6 bp Ins/Del, GSTP1 Ile105Val, XRCC1 Arg399Gln, MTHFR C677T, MDR1 C3435A/T, MDR1 G2677A/T and the treatment response. Since our sample size was small, further studies in larger patient populations are needed to validate results.

In conclusion, we have developed and validated an oligonucleotide microarray for genotyping 13 variations associated with treatment outcome of chemotherapy. CC genotype of ERCC1 C118T was associated with worse response to 5-FU and platinum chemotherapy. In addition to conventional SNP genotyping, we anticipate a future in which the microarray is used in routine clinical practice, which may help doctor make more safer and effective therapy for individual cancer patients.

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