Targeted sequencing reveals genetic variants associated with sensitivity of 79 human cancer xenografts to anticancer drugs

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Abstract. Although there has been progress moving from a ‘one-size-fits-all’ cytotoxic approach to personalized molecular medicine, the majority of patients with cancer receive chemotherapy using cytotoxic anticancer drugs. The sequencing analysis of 409 genes associated with cancer was conducted in the present study using 59 DNA sequences extracted from human cancer xenografts implanted into nude mice, of which sensitivity to 9 cytotoxic anticancer drugs [5-fluorouracil, nimustine, adriamycin, cyclophosphamide, cisplatin, mitomycin C (MMC), methotrexate, vincristine (VCR), and vinblastine] was examined. The present study investigated the association between the sensitivities of the xenografts to the 9 anticancer drugs and the frequency of single nucleotide variants (SNV). The correlation between the expression level of the genes and sensitivities to the 9 drugs in the above xenografts was also estimated. In the screening study using 59 xenografts, 3 SNVs (rs1805321, rs62456182 in PMS1 Homolog 2, Mismatch Repair System Component and rs13382825 in LDL Receptor Related Protein 1B), were associated with sensitivity to VCR and MMC, respectively (P<0.001). A replication study of 596 SNVs was subsequently performed, which indicated P<0.05 in the screening study using independent samples of 20 xenografts. A combined result of the screening and replication studies indicated that 35 SNVs were potentially associated with sensitivities to one or more of the nine anticancer drugs (Pcombined=0.0011-0.035). Of the 35 SNVs, rs16903989 and rs201432181 in Leukemia Inhibitory Factor Receptor α and Adhesion G Protein-Coupled Receptor A2 were commonly associated with sensitivity to 2 or 4 anticancer drugs, respectively. These findings provide novel insights which may benefit the development of personalized anticancer therapy for patients with cancer in the future.

Introduction

Over the past decade, the understanding of human cancer and development of molecular targeted therapies have benefitted from genomic technologies (1). A large proportion of patients with cancer suffer adverse effects from molecular targeted or cytotoxic agents while exhibiting no effective response in terms of tumor shrinkage (2). Although molecular targeted therapy is a standard cancer treatment, anticancer therapies using cytotoxic drugs remain a gold standard approach for cancer treatment (3-5). The efficacy of cytotoxic anticancer drugs varies among individual patients (6-8). Although a number of recent studies have attempted to establish a diagnostic method for predicting chemosensitivity (9-12), to the best of our knowledge, no clinically applicable genetic markers for the prediction of sensitivity or resistance to cytotoxic anticancer drugs have been developed. In order to distinguish which patients may respond to certain drugs from those who may not, prior to initiating treatment, to offer a ‘cancer precision medicine’ program of more effective chemotherapy and also to relieve patients from severe adverse events, a larger set of genetic variants in tumors must be identified to serve as accurate predictive markers for each anticancer drug.

The development of next generation sequencing technologies has revolutionized cancer genomic research because it provides a comprehensive method of detecting genomic alterations (somatic mutations) in cancer cells (13-15). A number of studies have reported an association between clinical outcomes and variant allele frequencies (VAFs) in tumors (16-20). As the properties of cancer cells may be influenced by complicated interactions among genes associated
with cancer, such as oncogenes or tumor suppressor genes expressed in cancer cells (21-23), the present study hypothesized that the genetic variants of these genes within the tumors may serve important roles in determining the response to cytotoxic anticancer drugs.

In the current study, to identify genetic markers for sensitivity or resistance to 9 cytotoxic anticancer drugs, all exons of 409 genes associated with cancer from 79 cancer xenografts in mice that had been established from 12 different human organs were sequenced. The association between single nucleotide variants (SNVs) detected in the xenografts and sensitivities to the 9 cytotoxic anticancer drugs were then investigated using a nonparametric approach. The present study identifies the genes associated with cancer that may also be associated with sensitivity to ≥1 of the 9 anticancer drugs examined. The results of the current study may help to elucidate the mechanism that causes the different clinical responses to chemotherapy among patients and may be applicable in the development of a prediction system to optimize treatment.

Materials and methods

Xenografts, anticancer drugs and examination of xenografts for sensitivity to anticancer drugs. A total of 79 human cancer xenografts, including 12 breast cancers, 12 gastric cancers, 10 neuroblastosmas, 10 non-small-cell lung cancers, 7 gliomas, 6 pancreatic cancers, 5 colon cancers, 5 choriocarcinomas, 4 small-cell lung cancers, 4 hematopoietic cancers, 7 gliomas, 6 pancreatic cancers, 5 colon cancers, 5 neuroblastomas, 10 non-small-cell lung cancers, 7 gliomas, 6 pancreatic cancers, 1 ovarian cancer, and 1 osteosarcoma were transplanted to athymic BALB/c-nu mice (weight, 26.3±1.8 g; age, 8-10 weeks) and maintained by serial subcutaneous treatment (6 mice per xenograft). Chemosensitivity was studied using the mean values for the treatment and control groups on day 14, as described previously (25,26). All animal studies were approved by the institutional committee of Central Institute for Experimental Animals, and conducted according to previously described protocols (27). Mice were sacrificed 21 days after drug administration.

Gene expression analysis. Total RNA was extracted from xenograft untreated tissues using ISOGEN (Nippon Gene Co., Ltd., Toyama, Japan) according to the manufacturer's protocol. To eliminate genomic DNA contamination, samples were treated with Recombinant DNase (RNase-free; Takara Bio, Inc., Otsu, Japan) following the manufacturer's protocol. cDNA was prepared from 5 µg total RNA using SuperScript III reverse transcriptase (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Firstly, 5 µg total RNA, 1 µl oligo dT primers (Invitrogen; Thermo Fisher Scientific, Inc.) and diethyl pyrocarbonate (DEPC) water were mixed to a total volume of 16 µl. This mixture was incubated at 70°C for 10 min and then chilled on ice for 5 min. The following components were added: 5 µl 5X first strand buffer (Invitrogen; Thermo Fisher Scientific, Inc.), 1 µl 25 mM dNTP (Wako Pure Chemical Industries, Ltd.), 2.5 µl 100 mM DTT (Invitrogen; Thermo Fisher Scientific, Inc.) and 0.5 µl Recombinant RNase Inhibitor (Takara Bio, Inc.), followed by 1.5 µl SuperScript III Reverse Transcriptase. This reaction mixture was incubated at 42°C for 50 min and terminated by heating to 70°C for 15 min. The cDNA products were stored at -20°C until required. mRNA expression profiles were obtained from an in-house cDNA microarray consisting of 23,040 genes, as described previously (25,26). For the 69 genes (Table I) whose expression was not available in the aforementioned profile, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was completed, using the SYBR Green Real-Time PCR system (Thermo Fisher Scientific, Inc.) and the StepOnePlus and 7900HT Fast Real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.), following the manufacturer's protocols. Each PCR reaction mixture contained 5 µl Fast SYBR Green Master Mix (2x) (Applied Biosystems; Thermo Fisher Scientific, Inc.), 0.2 µl of each primer (10 pmol/µl) (Sigma-Aldrich; Merck KGaA), 1 µl cDNA and DEPC water (Ambion; Thermo Fisher Scientific, Inc.), for a total volume of 10 µl. The reaction was performed at 95°C for 20 sec, 40 cycles of 95°C for 3 sec and 60°C for 30 sec, 95°C for 15 sec, 60°C for 1 min and 95°C for 15 sec. The sequences of the primers are shown in Table I. The level of mRNA was assessed using the relative standard curve method, relative to β-actin reference gene (28).

Sample preparation and targeted next-generation sequencing. Tumor genomic DNA was extracted from 79 xenografts using the QIAamp DNA Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. In the screening study, targeted next generation sequencing was performed in 59 xenografts (12 breast cancers, 12 gastric cancers, 10 neuroblastosmas, 10 non-small-cell lung cancers, 7 gliomas, 6 pancreatic cancers, 1 ovarian cancer and 1 osteosarcoma) using the Ion AmpliSeq Comprehensive Cancer Panel (CCP;
| Gene    | Primer sequence                      |
|---------|--------------------------------------|
| MTRR    | Forward 5'-AGCCTACTCTCAAGACTGCA-3'   |
|         | Reverse 5'-CAAGTTATAGCCTGGAGATTG-3'  |
| ADAMTS20| Forward 5'-GAAATACTTGTGAGGCGC-3'     |
|         | Reverse 5'-GCACGGCCTCCTCGAACG-3'     |
| ASXL1   | Forward 5'-TTCACGGTCAAGAAGGATGC-3'   |
|         | Reverse 5'-GGCCTATTAGACCCACG-3'      |
| ADGRA2/ | Forward 5'-AGAAGGTGAGGATGTTGATG-3'   |
| GPR124  | Reverse 5'-AGACGTTGGAGGATGTTGATG-3'  |
| ADGRB3/ | Forward 5'-AACGGGCGAAGAGGATGAGA-3'   |
| BAI3    | Reverse 5'-TGTGGCATTCAGGGGACATTG-3'  |
| AKT1    | Reverse 5'-TCAACACTTCTCTGTTGCG-3'    |
|         | Reverse 5'-GAAAGTGCGCTTGGACCTG-3'    |
| AMER1/  | Forward 5'-GGGATATGCAAGAAGGACCT-3'   |
| FAM12B  | Reverse 5'-CTTGCTGAGACTCCTGAG-3'     |
| ATR     | Forward 5'-TGTAGAATGGAAGGATGAGA-3'   |
|         | Reverse 5'-AACCTCTCCCCCGTACCC-3'     |
| BCL3    | Forward 5'-AACCTCCTACACCCCTATAC-3'   |
|         | Reverse 5'-CGTTCTGCTCAGAAGCG-3'      |
| BCL6    | Forward 5'-AGCCTATGATCTCGAGAT-3'     |
|         | Reverse 5'-TCTTCCAGGGAGGGATG-3'      |
| BRIP1/  | Forward 5'-CCACCTTCGTCGCAAAGTGA-3'   |
| FANCJ   | Reverse 5'-TCTGTTCAAGGACACTCG-3'     |
| CDH1    | Forward 5'-ATTCTTCCCCAGCAGCAG-3'     |
|         | Reverse 5'-TCCACGGCGTAGACACAG-3'     |
| CRBN    | Forward 5'-TCCCTTCGCTAAGAACAAG-3'    |
|         | Reverse 5'-AAAGCCCAACATCAGCGGAA-3'   |
| CRTC1   | Forward 5'-GGTCCCCGGAATCAACATCT-3'   |
|         | Reverse 5'-GAGGTTAGAGAAGGCTTCCA-3'   |
| CDKN2A  | Forward 5'-GCCGGCTACCCCGAGGAG-3'     |
|         | Reverse 5'-GGAAAGCGGGGTGGT-3'        |
| CMPK1   | Forward 5'-ATGGAAGGGAAGGAGGATG-3'    |
|         | Reverse 5'-TCCACACTCTCTTCTGACA-3'    |
| CYP2C19 | Forward 5'-GTAATTTGCGCTGAGGAAA-3'    |
|         | Reverse 5'-CAGTGGCGAAGAGGAGGATG-3'   |
| CYP2D6  | Forward 5'-ACCAGCCTCACAGGCTGCT-3'    |
|         | Reverse 5'-TTCAGGATCGAACAGGATC-3'    |
| DDIT3   | Forward 5'-TGTAAAAGTAGGAGGGGTTG-3'   |
|         | Reverse 5'-TGGCTTTTCACTGGTTGATG-3'   |
| EP300   | Forward 5'-AAATGCCGAAAGATGTTG-3'     |
|         | Reverse 5'-TGGAAGTTCGCTTGAGGAGG-3'   |
| ERBB3   | Forward 5'-CAACCTCTAGCCAGCTGTC-3'    |
|         | Reverse 5'-ATCAGGCCCACTCTCCATCT-3'   |
| ERCC1   | Forward 5'-ACCAGAGTACATTCCATCGG-3'   |
|         | Reverse 5'-TCTTGGACCCGCTTCTGGA-3'    |
| FANC2D  | Forward 5'-GGGATATTGCTTGCTTGAG-3'    |
|         | Reverse 5'-GTCAGGTGTTGGCTTCTCT-3'    |
| FAS     | Forward 5'-GATGAAACGACTCTGTGG-3'     |
|         | Reverse 5'-TCAACAATCATCTACTTCTG-3'   |
| FLCN    | Forward 5'-GAAGCAGAGCAGCTGAG-3'      |
|         | Reverse 5'-CAGTGTGACGAGCATGTCG-3'    |
| FH      | Forward 5'-TGTGAGGGTTGAACCTGGGA-3'   |
|         | Reverse 5'-ATGTTGACCTGGTGAGGAAA-3'   |
| GNA1I   | Forward 5'-TACGAGGAGCACAAGGGCA-3'    |
|         | Reverse 5'-GTCGTTAGACATCTCTGAGTC-3'  |
| HNF1A   | Forward 5'-TGTGAGGAGGAGCCCCAGC-3'    |
|         | Reverse 5'-CTCTGGCTCCTTCCTTCTG-3'    |
| IKBKE   | Forward 5'-GGAAGATTCCTCCTGCTTTATG-3' |
|         | Reverse 5'-TGCACTGTACAGGATGCTC-3'    |
| ITGA10  | Forward 5'-ACTTAGGTGACTCAACACTGG-3'  |

Table I. Sequences of primers used for qRT-PCR.
Thermo Fisher Scientific, Inc.), which targets the exons of 409 tumor suppressor genes and frequently cited and mutated oncogenes. DNA concentrations were determined using the TaqMan Rnase P Detection Reagents kit (Termo Fisher Scientific, Inc.). Barcoded amplicon libraries for individual DNA samples were prepared using the Ion Xpress Barcode Adapters and the Ion AmpliSeq Library kit 2.0 (Thermo Fisher Scientific, Inc.) following the manufacturer’s protocol. Pooled barcoded libraries were subsequently conjugated with sequencing beads by emulsion PCR and enriched using the Ion PI Hi-Q Chef kit and Ion Chef (Thermo Fisher Scientific, Inc.) following the manufacturer’s protocol. Adapters and the Ion AmpliSeq Library kit 2.0 (Thermo Fisher Scientific, Inc.) were used to identify variations from the reference sequence where mm10 ≥ hg19 were considered as reads derived from the mouse genome and subsequently removed. The Variant Caller plugin (version 5.0.2.1; Thermo Fisher Scientific, Inc.) was used to identify variations from the reference sequence (hg19). In the replication study, targeted sequencing was performed with 8-10 samples per Ion PI Chip V3 using the Ion Proton system (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. Sequencing reads generated were aligned with the human genome build 19 (hg19) and mouse genome build 38 (mm10). Reads with an alignment score where mm10 x hg19 were considered as reads derived from the mouse genome and subsequently removed. The Variant Caller plugin (version 5.0.2.1; Thermo Fisher Scientific, Inc.) was used to identify variations from the reference sequence (hg19). In the replication study, targeted sequencing was performed in 20 xenografts, including 5 colon cancers, 5 choriocarcinomas, 4 small-cell lung cancers, 4 hematopoietic cancers and 2 ovarian cancers. PolyPhen2 (genetics.bwh.harvard.edu/pph2/) and SIFT (sift.jcvi.org/) were used for the computational prediction of the functional changes that amino acid substitutions may have on protein function. Variants were predicted to be ‘benign’, ‘possibly damaging’ or ‘probably damaging’ by Polyphen2, and ‘tolerated’ or ‘damaging’ by SIFT.

| Gene | Primer sequence |
|------|----------------|
| PTPRT | Forward 5'-CAATGGAAATGCGGACACAGC-3' | Reverse 5'-GATCACAAGCCGGTACTTGCC-3' |
| RECQL4 | Forward 5'-CCTCGTCGTCACTCATGGAAT-3' | Reverse 5'-GACAGACATCCGGTCCTTCC-3' |
| REL | Forward 5'-TCTCTCGTGTCTCCTGAAACC-3' | Reverse 5'-CCTCTCCGACTTCCACCA-3' |
| RUNX1 | Forward 5'-CATCGCCTTCTAAAGGTTGGT-3' | Reverse 5'-GGTCTCTCATGCTGGCTAG-3' |
| SMO | Forward 5'-TGAAATCGCTACAAGTCG-3' | Reverse 5'-CCAGCTCATGTCGGCACT-3' |
| SAMD9 | Forward 5'-ATGGCAAAGCAACTTAACCTTCC-3' | Reverse 5'-CCATTCCAGCTCTGGTCCG-3' |
| TAF1L | Forward 5'-TCCCTCAGTCTGTCGAGA-3' | Reverse 5'-TCTCAGACGAGCTGAGAAT-3' |
| TET1 | Forward 5'-CATTACGTCAGACTTTAAGCCCT-3' | Reverse 5'-CGGTTGATTGATGTCTTTT-3' |
| TNFAIP3 | Forward 5'-ACCCCATTTGCTCCTGCTATT-3' | Reverse 5'-CAATCCCTCCCCGCTCTTGT-3' |
| TCF12 | Forward 5'-CTTCGTACCTACCCACAGT-3' | Reverse 5'-CTTGGGGAGGAAAGTCTTTG-3' |
| β-actin | Forward 5'-GAATGAGTACGCTCCCTGGC-3' | Reverse 5'-GGTCCTCAAGTGCTGTACAGCAGG-3' |

**Statistical analysis.** Xenografts were classified into three groups according to variant allele frequencies (VAFs), low (<10%), middle (10-90%) and high (>90%), and the difference of sensitivity to each anticancer drug (T/C (%)) among the groups was examined using a nonparametric approach (Mann-Whitney U-test for two groups or Kruskal-Wallis test for three groups). To identify genes, which may distinguish patients who may respond to the anticancer drugs, from those who may not, SNVs of which the difference between the maximum and the minimum VAF was <50% were removed from further analysis. P<8.39x10^-10 was determined to indicate a statistically significant difference in the replication study for the adjustment of multiple testing by the strict Bonferroni correction. A Pearson correlation coefficient was performed to estimate the association between the gene expression and sensitivity to each anticancer drug. Combination effects were investigated by totalizing by the score of each VAF group.

**Results**

Identification of the candidate SNVs associated with chemosensitivity. To identify genetic variants significantly associated with the efficacy of one or more of nine anticancer drugs (5FU, ACNU, ADR, CPM, DDP, MMC, MTX, VCR and VLB) examined in the current nude mouse system, all exons of 409 genes associated with cancer using 59 xenografts derived from breast cancer, gastric cancer, neuroblastoma, non-small-cell lung cancer, glioma, pancreatic cancer, ovarian cancer and osteosarcoma at the screening stage were sequenced. A total of 5,494 SNVs were identified in the sequence analysis of the 59 xenografts, and the median number of SNVs called in one sample was 988. A total of 2,206 SNVs with a difference between the maximum and the minimum VAF <50% were removed from further analysis, and 2,087, 2,134, 2,134, 2,134, 2,134, 2,134, 1,944, 2,124 and 2,124 SNVs were assessed for sensitivity to 5FU, ACNU, ADR, CPM, DDP, MMC, MTX, VCR and VLB, respectively. The xenografts were classified into three groups, low (<10%), middle (10-90%) and high (>90%) VAF, and the association between the VAF group and sensitivities to cytotoxic anticancer drugs was assessed using the Kruskal-Wallis test or Mann-Whitney U-test. Chemosensitivity was calculated as T/C and the variants whose allele frequency was higher in xenografts with lower T/C as were defined as ‘chemosensitive variants’ and variants whose allele frequency was higher in xenografts with higher T/C as ‘chemoresistant variants’. As presented in Table II, when 59 xenografts were analyzed in a screening study, 43-98 SNVs exhibited a potential association with chemosensitivity. A Pearson correlation coefficient was calculated to determine the association between the VAF and chemosensitivity. The strict Bonferroni correction was used to correct for multiple testing. The top 10 variants that revealed the smallest P-values are displayed in Tables III-XI.

In the screening study using 59 xenografts, three SNVs were observed to exhibit associations (P<0.001) with the associated genes; rs1805321 (P=0.00018; Table X) and rs62456182 (P=0.00054; Table X) in PMS1 Homolog 2, Mismatch Repair System Component, and rs13382825 (P=0.00092; Table VIII) in LDL Receptor Related Protein 1B. The three SNVs were associated with sensitivity to MMC and VCR (no. 1 and 2 in Table X), respectively (Tables VIII
and X). The xenografts with higher VAFs of rs1805321 and rs62456182 demonstrated an increased response to VCR compared with those that exhibited a lower variant allele frequency of the two SNVs (Table X). By contrast, xenografts with higher VAFs of rs13382825 exhibited a decreased response to MMC compared with those that presented with lower variant allele frequencies (Table VIII), suggesting that this genetic variant is associated with resistance to MMC.

Replication study using additional xenografts. To further validate the result of the screening-stage analysis, a replication study was performed, using 596 SNVs showing P<0.05 in ≥1 anticancer drugs in the screening set using independent samples of 20 xenografts. No SNVs revealed significant levels of association in the replication study following Bonferroni correction, including rs1805321, rs62456182 and rs13382825, which demonstrated an association (P<0.001) with VCR (no. 1 and 2; Table X) and MMC (no. 1; Table VIII) in the screening study.

A combined result of the screening and replication studies suggested potential associations of 35 SNVs, which exhibited a stronger association in the combined study than those in screening study, with sensitivity to ≥1 anticancer drugs (Table XII). However, significant association was not observed in these SNVs (0.0011<Pcombined<0.035 in Table XII) following Bonferroni correction. The SNV rs1805321, which is located in exon 19 of GPR124 demonstrated a higher response to ACNU, MMC, VLB and ADR (Pcombined=0.0013, 0.0040, 0.017 and 0.029, respectively; no. 3 Table XII), however, no significant association was observed between the expression level of GPR124 and sensitivity to these 4 cytotoxic anticancer drugs in the present study (ACNU, MMC, VLB and ADR; Table XII).

Combination analysis with markedly associated SNVs with chemosensitivity. A combined effect of markedly associated SNVs with chemosensitivity was investigated (Pcombined<0.01) on sensitivities to ADR, 5FU, ACNU and CPM (Table XII). The xenografts were scored 0, 1 and 2 based on the allele frequency of the chemosensitive variants (Pcombined<0.01) as low (<10%), middle (10-90%), and high (>90%), respectively. Furthermore, the xenografts were scored 2, 1 and 0 depending on the allele frequency of the chemoresistant variants (Pcombined<0.01) as low (<10%), middle (10-90%), and high (>90%), respectively. The xenografts were then classified into 4-6 groups according to the sum of the scores. The combination analysis using rs4589708, rs113962761 and rs1050171 revealed a cumulative effect on sensitivity to ADR (P=0.000012; Fig. 2). Similarly, combination analysis using strongly associated SNVs with sensitivity to 5FU, ACNU and CPM (P<0.01), also revealed a cumulative effect on sensitivity to them (P=0.00025, P=0.000076 and P=0.00021, respectively, data not shown).

Discussion

The present study conducted two-step association studies between frequencies of SNVs in 409 genes (three VAF groups; <10%, 10-90%, >90%) and the sensitivities to 9 cytotoxic anticancer drugs using 79 human cancer xenografts, and identified 35 SNVs with potential associations to sensitivity or resistance to ≥1 cytotoxic anticancer drugs in a combined study. The SNV demonstrating the lowest P-value in the combined study was rs79555258 (no. 1 in Table XII) in Activin A Receptor Type 2A (ACVR2A; P=0.0011). As presented in Fig. 1, xenografts with more variant alleles of rs79555258 in the three studies (screening, replication and combined) exhibited a lower response to CPM than those with less variant alleles, suggesting that this variant may be associated with resistance to CPM. The correlation analysis between gene expression and drug sensitivity demonstrated a significantly positive correlation between the expression level of LIFR and sensitivity to VCR (r=0.42, P=0.00031) and CPM (r=0.36, P=0.0020) as presented in Table XII (no. 16). The xenografts with more variant alleles in rs201432181, which is located in exon 19 of GPR124, demonstrated a higher response to ACNU, MMC, VLB and ADR (Pcombined=0.0013, 0.0040, 0.017 and 0.029, respectively; no. 3 Table XII), however, no significant association was observed between the expression level of GPR124 and sensitivity to these 4 cytotoxic anticancer drugs in the present study (ACNU, MMC, VLB and ADR; Table XII).

Identification of SNVs associated with multi-drug sensitivity. Of the 35 SNVs, that demonstrated a potential association with sensitivity to ≥1 anticancer drugs examined, rs16903989 and rs201432181 (no. 16 and 3, respectively; Table XII) were commonly associated with sensitivity to 2 (VCR and CPM) and 4 (ACNU, MMC, VLB and ADR) drugs, respectively. Xenografts with more variant alleles in rs16903989, which is located in intron 9 of Leukemia Inhibitory Factor Receptor Alpha (LIFR), exhibited a higher response to VCR and CPM (Pcombined=0.0098 and 0.026, respectively; Table XII). The correlation analysis between gene expression and drug sensitivity demonstrated a significantly positive correlation between the expression level of LIFR and sensitivity to VCR (r=0.42, P=0.00031) and CPM (r=0.36, P=0.0020) as presented in Table XII (no. 16). The xenografts with more variant alleles in rs201432181, which is located in exon 19 of GPR124, demonstrated a higher response to ACNU, MMC, VLB and ADR (Pcombined=0.0013, 0.0040, 0.017 and 0.029, respectively; no. 3 Table XII), however, no significant association was observed between the expression level of GPR124 and sensitivity to these 4 cytotoxic anticancer drugs in the present study (ACNU, MMC, VLB and ADR; Table XII).
### Table III. Single nucleotide variants potentially associated with sensitivity to 5-fluorouracil.

| No. | Chr | SNP ID     | Position     | Gene    | Allele Ref./Variant | Sensitivity | Study set       | Variant allele frequency |
|-----|-----|------------|--------------|---------|---------------------|-------------|----------------|-------------------------|
|     |     |            |              |         |                     |             |                | <10% | 10-90% | >90% | P-value |
| 1   | 1   | rs11121691 | 11181327     | MTOR    | C/T                 | Sensitive   | Screening      | 52  | 3      | 1    | 0.00536 |
|     |     |            |              |         |                     |             | Replication    | 19  | 1      | 0    | NA      |
|     |     |            |              |         |                     |             | Combined       | 71  | 4      | 1    | 0.03340 |
| 2   | 14  | rs8020503  | 51239067     | NIN     | C/G                 | Sensitive   | Screening      | 25  | 0      | 31   | 0.00668 |
|     |     |            |              |         |                     |             | Replication    | 5   | 1      | 14   | 0.67994 |
|     |     |            |              |         |                     |             | Combined       | 30  | 1      | 45   | 0.01397 |
| 3   | 2   | rs1128919  | 148657117    | ACVR2A  | G/A                 | Sensitive   | Screening      | 15  | 28     | 13   | 0.01129 |
|     |     |            |              |         |                     |             | Replication    | 3   | 6      | 11   | 0.23334 |
|     |     |            |              |         |                     |             | Combined       | 18  | 34     | 24   | 0.11270 |
| 4   | 7   | rs3802064  | 92731586     | SAMD9   | A/G                 | Resistant   | Screening      | 46  | 8      | 2    | 0.01191 |
|     |     |            |              |         |                     |             | Replication    | 17  | 2      | 1    | 0.83228 |
|     |     |            |              |         |                     |             | Combined       | 63  | 10     | 3    | 0.02524 |
| 5   | 18  | -          | 22642739     | ZNF521  | A/G                 | Sensitive   | Screening      | 43  | 13     | 0    | 0.01218 |
|     |     |            |              |         |                     |             | Replication    | 19  | 1      | 0    | NA      |
|     |     |            |              |         |                     |             | Combined       | 62  | 14     | 0    | 0.00564 |
| 6   | 7   | rs78644495 | 98552958     | TRRAP   | G/A                 | Resistant   | Screening      | 46  | 10     | 0    | 0.01244 |
|     |     |            |              |         |                     |             | Replication    | 15  | 5      | 0    | 0.51253 |
|     |     |            |              |         |                     |             | Combined       | 61  | 15     | 0    | 0.10691 |
| 7   | 10  | rs2435352  | 43600689     | RET     | A/G                 | Resistant   | Screening      | 34  | 16     | 6    | 0.01268 |
|     |     |            |              |         |                     |             | Replication    | 11  | 4      | 5    | 0.78343 |
|     |     |            |              |         |                     |             | Combined       | 45  | 20     | 11   | 0.02998 |
| 8   | 10  | rs11574851 | 104160959    | NFkB2   | C/T                 | Sensitive   | Screening      | 46  | 9      | 1    | 0.01305 |
|     |     |            |              |         |                     |             | Replication    | 15  | 1      | 4    | 0.17591 |
|     |     |            |              |         |                     |             | Combined       | 61  | 10     | 5    | 0.01631 |
| 9   | 22  | rs3818120  | 41523770     | EP300   | G/A                 | Resistant   | Screening      | 47  | 9      | 0    | 0.01354 |
|     |     |            |              |         |                     |             | Replication    | 16  | 3      | 1    | 0.04714 |
|     |     |            |              |         |                     |             | Combined       | 63  | 12     | 1    | 0.41164 |
| 10  | 22  | rs20554    | 41553259     | EP300   | G/A                 | Resistant   | Screening      | 47  | 9      | 0    | 0.01354 |
|     |     |            |              |         |                     |             | Replication    | 16  | 3      | 1    | 0.04714 |
|     |     |            |              |         |                     |             | Combined       | 63  | 12     | 1    | 0.41164 |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-' this variant is not identified in dbSNP; Ref., reference; NA, not available.
Table IV. Single nucleotide variants potentially associated with sensitivity to nimustine.

| No. | Chr | SNP ID   | Position   | Gene | Allele Ref./Variant | Sensitivity | Study set          | Variant allele frequency |
|-----|-----|----------|------------|------|---------------------|-------------|--------------------|-------------------------|
|     |     |          |            |      |                     |             |                    | <10%  | 10-90% | >90% | P-value |
| 1   | 19  | rs3218066| 30,312,874 | CCNE1| C/T                 | Sensitive   | Screening          | 38    | 18     | 1   | 0.00224 |
|     |     |          |            |      |                     |             | Replication        | 16    | 3      | 1   | 0.92463 |
|     |     |          |            |      |                     |             | Combined           | 54    | 21     | 2   | 0.00379 |
| 2   | 19  | rs3218068| 30,313,344 | CCNE1| T/C                 | Sensitive   | Screening          | 38    | 18     | 1   | 0.00224 |
|     |     |          |            |      |                     |             | Replication        | 16    | 3      | 1   | 0.92463 |
|     |     |          |            |      |                     |             | Combined           | 54    | 21     | 2   | 0.00379 |
| 3   | 4   | rs7688174 | 40,244,982 | RHOH | C/G                 | Resistant   | Screening          | 53    | 1      | 3   | 0.00828 |
|     |     |          |            |      |                     |             | Replication        | 19    | 1      | 0   | NA      |
|     |     |          |            |      |                     |             | Combined           | 72    | 2      | 3   | 0.02986 |
| 4   | 5   | rs6962    | 256,509    | SDHA | G/A                 | Resistant   | Screening          | 51    | 6      | 0   | 0.01003 |
|     |     |          |            |      |                     |             | Replication        | 19    | 1      | 0   | NA      |
|     |     |          |            |      |                     |             | Combined           | 70    | 7      | 0   | 0.00849 |
| 5   | 11  | rs5030171 | 32,449,417 | WTI  | C/G                 | Resistant   | Screening          | 12    | 17     | 28  | 0.01105 |
|     |     |          |            |      |                     |             | Replication        | 2     | 6      | 12  | 0.92759 |
|     |     |          |            |      |                     |             | Combined           | 14    | 23     | 40  | 0.01873 |
| 6   | 11  | rs5030170 | 32,449,420 | WTI  | C/A                 | Resistant   | Screening          | 12    | 17     | 28  | 0.01105 |
|     |     |          |            |      |                     |             | Replication        | 2     | 6      | 12  | 0.92759 |
|     |     |          |            |      |                     |             | Combined           | 14    | 23     | 40  | 0.01873 |
| 7   | 5   | rs10039029| 251,469    | SDHA | G/A                 | Resistant   | Screening          | 49    | 7      | 1   | 0.01148 |
|     |     |          |            |      |                     |             | Replication        | 19    | 1      | 0   | NA      |
|     |     |          |            |      |                     |             | Combined           | 68    | 8      | 1   | 0.01366 |
| 8   | 1   | rs76717731| 193,107,192| CDC73| C/T                 | Resistant   | Screening          | 52    | 5      | 0   | 0.01303 |
|     |     |          |            |      |                     |             | Replication        | 16    | 3      | 1   | 0.53863 |
|     |     |          |            |      |                     |             | Combined           | 68    | 8      | 1   | 0.08246 |
| 9   | 11  | rs74662318| 4,150,239  | RRMI | T/G                 | Resistant   | Screening          | 48    | 9      | 0   | 0.01422 |
|     |     |          |            |      |                     |             | Replication        | 17    | 3      | 0   | 0.56000 |
|     |     |          |            |      |                     |             | Combined           | 65    | 12     | 0   | 0.02893 |
| 10  | 5   | rs28363396| 138,148,036| CTNNA1| A/G                | Sensitive   | Screening          | 51    | 6      | 0   | 0.01557 |
|     |     |          |            |      |                     |             | Replication        | 18    | 2      | 0   | 0.16531 |
|     |     |          |            |      |                     |             | Combined           | 69    | 8      | 0   | 0.20441 |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). ‘-’, this variant is not identified in dbSNP; Ref., reference; NA, not available.
Table V. Single nucleotide variants potentially associated with sensitivity to adriamycin.

| No. | Chr | SNP ID   | Position  | Gene   | Allele Ref./Variant | Sensitivity | Study set     | Variant allele frequency | P-value |
|-----|-----|----------|-----------|--------|---------------------|-------------|--------------|--------------------------|---------|
|     |     |          |           |        |                     |             |              | <10% 10-90% >90%        |         |
| 1   | 11  | rs77233576 | 44,130,665 | EXT2   | A/C                 | Resistant   | Screening     | 51 5 1             | 0.00115 |
|     |     |          |           |        |                     |             | Replication   | 14 5 1             | 0.43313 |
|     |     |          |           |        |                     |             | Combined      | 65 10 2            | 0.01565 |
| 2   | 9   | rs464826  | 136,913,355 | BRD3   | T/C                 | Resistant   | Screening     | 15 15 27          | 0.00131 |
|     |     |          |           |        |                     |             | Replication   | 5 7 8             | 0.88274 |
|     |     |          |           |        |                     |             | Combined      | 20 22 35          | 0.03060 |
| 3   | 2   | rs117225004 | 141,259,253 | LRP1B  | T/C                 | Resistant   | Screening     | 53 3 1             | 0.00315 |
|     |     |          |           |        |                     |             | Replication   | 20 0 0            | NA      |
|     |     |          |           |        |                     |             | Combined      | 73 3 1             | 0.00355 |
| 4   | 15  | rs2229765 | 99,478,225 | IGF1R  | G/A                 | Resistant   | Screening     | 26 25 6            | 0.00363 |
|     |     |          |           |        |                     |             | Replication   | 10 7 3            | 0.58702 |
|     |     |          |           |        |                     |             | Combined      | 36 32 9            | 0.01565 |
| 5   | 15  | rs2293117 | 99,478,713 | IGF1R  | T/C                 | Resistant   | Screening     | 26 25 6            | 0.00363 |
|     |     |          |           |        |                     |             | Replication   | 10 7 3            | 0.58702 |
|     |     |          |           |        |                     |             | Combined      | 36 32 9            | 0.01565 |
| 6   | 7   | rs113962761 | 50,450,446 | IKZF1  | C/T                 | Resistant   | Screening     | 47 10 0            | 0.00365 |
|     |     |          |           |        |                     |             | Replication   | 19 1 0            | NA      |
|     |     |          |           |        |                     |             | Combined      | 66 11 0            | 0.00147 |
| 7   | 5   | rs16903989 | 38,504,303 | LIFR   | A/T                 | Sensitive   | Screening     | 28 23 6            | 0.00509 |
|     |     |          |           |        |                     |             | Replication   | 14 5 1            | 0.59174 |
|     |     |          |           |        |                     |             | Combined      | 42 28 7            | 0.03189 |
| 8   | 1   | rs138622243 | 47,691,061 | TAL1   | G/T                 | Sensitive   | Screening     | 54 2 1             | 0.00591 |
|     |     |          |           |        |                     |             | Replication   | 19 0 1            | NA      |
|     |     |          |           |        |                     |             | Combined      | 73 2 2             | 0.00764 |
| 9   | 22  | rs180812  | 23,657,735 | BCR    | G/A                 | Resistant   | Screening     | 30 3 24            | 0.00662 |
|     |     |          |           |        |                     |             | Replication   | 8 1 11            | 0.33467 |
|     |     |          |           |        |                     |             | Combined      | 38 4 35            | 0.01663 |
| 10  | 6   | rs12196767 | 51,776,535 | PKHD1  | T/C                 | Resistant   | Screening     | 41 15 1            | 0.00950 |
|     |     |          |           |        |                     |             | Replication   | 14 6 0            | 0.59174 |
|     |     |          |           |        |                     |             | Combined      | 55 21 1            | 0.01484 |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphisms; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). ‘-’ this variant is not identified in dbSNP; Ref., reference; NA, not available.
### Table VI. Single nucleotide variants potentially associated with sensitivity to cyclophosphamide.

| No. | Chr | SNP ID | Position  | Gene  | Allele Ref./Variant | Sensitivity | Study set   | <10% | 10-90% | >90% | P-value |
|-----|-----|--------|-----------|-------|---------------------|-------------|-------------|------|--------|------|---------|
| 1   | 6   | rs4331993 | 152,793,572 | SYNE1 | T/A                | Resistant   | Screening   | 48   | 4      | 7    | 0.00119 |
|     |     |         |           |       |                     |             | Replication | 16   | 4      | 0    | 0.60273 |
|     |     |         |           |       |                     |             | Combined    | 64   | 8      | 7    | 0.00139 |
| 2   | 6   | rs1024195 | 56,507,135  | DST   | T/C                | Sensitive   | Screening   | 24   | 23     | 12   | 0.00188 |
|     |     |         |           |       |                     |             | Replication | 10   | 6      | 4    | 0.27755 |
|     |     |         |           |       |                     |             | Combined    | 34   | 29     | 16   | 0.14291 |
| 3   | 2   | rs79555258 | 148,680,526 | ACVR2A | T/C                | Resistant   | Screening   | 55   | 3      | 1    | 0.00312 |
|     |     |         |           |       |                     |             | Replication | 18   | 0      | 2    | 0.02313 |
|     |     |         |           |       |                     |             | Combined    | 73   | 3      | 3    | 0.00109 |
| 4   | 12  | rs3217786 | 4,383,158  | CCND2  | T/C                | Resistant   | Screening   | 24   | 3      | 32   | 0.00378 |
|     |     |         |           |       |                     |             | Replication | 5    | 0      | 15   | 0.12606 |
|     |     |         |           |       |                     |             | Combined    | 29   | 3      | 47   | 0.00247 |
| 5   | 14  | rs8020503 | 51,239,067 | NIN   | C/G                | Resistant   | Screening   | 27   | 0      | 32   | 0.00602 |
|     |     |         |           |       |                     |             | Replication | 5    | 1      | 14   | 0.09672 |
|     |     |         |           |       |                     |             | Combined    | 32   | 1      | 46   | 0.07844 |
| 6   | 5   | rs28363396 | 138,148,036 | CTNNA1 | A/G                | Sensitive   | Screening   | 53   | 6      | 0    | 0.00675 |
|     |     |         |           |       |                     |             | Replication | 18   | 2      | 0    | 0.84988 |
|     |     |         |           |       |                     |             | Combined    | 71   | 8      | 0    | 0.02011 |
| 7   | 18  | -       | 22,642,750 | ZNF521 | G/C                | Resistant   | Screening   | 55   | 4      | 0    | 0.00796 |
|     |     |         |           |       |                     |             | Replication | 19   | 1      | 0    | NA     |
|     |     |         |           |       |                     |             | Combined    | 74   | 5      | 0    | 0.10716 |
| 8   | 7   | rs2360885 | 151,971,043 | MLL3  | T/C                | Resistant   | Screening   | 22   | 37     | 0    | 0.00844 |
|     |     |         |           |       |                     |             | Replication | 0    | 20     | 0    | NA     |
|     |     |         |           |       |                     |             | Combined    | 22   | 57     | 0    | 0.03116 |
| 9   | 3   | -       | 128,202,753 | GATA2 | G/A                | Resistant   | Screening   | 10   | 49     | 0    | 0.00862 |
|     |     |         |           |       |                     |             | Replication | 0    | 20     | 0    | NA     |
|     |     |         |           |       |                     |             | Combined    | 10   | 69     | 0    | 0.01830 |
| 10  | 14  | rs1152783 | 99,642,360 | BCL11B | C/G                | Resistant   | Screening   | 52   | 6      | 1    | 0.00895 |
|     |     |         |           |       |                     |             | Replication | 16   | 3      | 1    | 0.92461 |
|     |     |         |           |       |                     |             | Combined    | 68   | 9      | 2    | 0.12611 |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphisms; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). ‘-‘, this variant is not identified in dbSNP; Ref., reference; NA, not available.
Table VII. Single nucleotide variants potentially associated with sensitivity to cisplatin.

| No. | Chr | SNP ID | Position | Gene  | Allele Ref./Variant | Sensitivity | Study set    | Variant allele frequency |
|-----|-----|--------|----------|-------|--------------------|-------------|--------------|--------------------------|
|     |     |        |          |       |                    |             |              | <10% 10-90% >90% P-value |
| 1   | 18  | -      | 22,642,741 | ZNF521 | A/G               | Resistant   | Screening       | 34 23 0 0.00331          |
|     |     |        |          |       |                    |             | Replication     | 11 9 0 0.51842          |
|     |     |        |          |       |                    |             | Combined        | 45 32 0 0.02421          |
| 2   | 6   | rs2228480 | 152,420,095 | ESR1 | G/A               | Resistant   | Screening       | 44 8 5 0.00403          |
|     |     |        |          |       |                    |             | Replication     | 15 3 2 0.58609          |
|     |     |        |          |       |                    |             | Combined        | 59 11 7 0.00419          |
| 3   | 17  | rs11653832 | 5,424,906  | NLRP1 | C/G               | Sensitive   | Screening       | 54 1 2 0.00774          |
|     |     |        |          |       |                    |             | Replication     | 19 0 1 NA              |
|     |     |        |          |       |                    |             | Combined        | 73 1 3 0.11849          |
| 4   | 17  | rs11653580 | 5,424,991  | NLRP1 | G/A               | Sensitive   | Screening       | 54 1 2 0.00774          |
|     |     |        |          |       |                    |             | Replication     | 19 0 1 NA              |
|     |     |        |          |       |                    |             | Combined        | 73 1 3 0.11849          |
| 5   | 17  | rs56872041 | 5,433,841  | NLRP1 | A/G               | Sensitive   | Screening       | 54 1 2 0.00774          |
|     |     |        |          |       |                    |             | Replication     | 19 0 1 NA              |
|     |     |        |          |       |                    |             | Combined        | 73 1 3 0.11849          |
| 6   | 17  | rs35596958 | 5,433,966  | NLRP1 | T/C               | Sensitive   | Screening       | 54 1 2 0.00774          |
|     |     |        |          |       |                    |             | Replication     | 19 0 1 NA              |
|     |     |        |          |       |                    |             | Combined        | 73 1 3 0.11849          |
| 7   | 17  | rs34733791 | 5,437,285  | NLRP1 | G/A               | Sensitive   | Screening       | 54 1 2 0.00774          |
|     |     |        |          |       |                    |             | Replication     | 19 0 1 NA              |
|     |     |        |          |       |                    |             | Combined        | 73 1 3 0.11849          |
| 8   | 18  | rs79073678 | 56,414,592 | MALT1 | T/C               | Sensitive   | Screening       | 43 6 8 0.00953          |
|     |     |        |          |       |                    |             | Replication     | 15 3 2 0.81174          |
|     |     |        |          |       |                    |             | Combined        | 58 9 10 0.02702         |
| 9   | 1   | rs1318056  | 179,112,145 | ABL2  | C/G               | Sensitive   | Screening       | 54 1 2 0.01006          |
|     |     |        |          |       |                    |             | Replication     | 18 2 0 0.61429          |
|     |     |        |          |       |                    |             | Combined        | 72 3 2 0.05767          |
| 10  | 10  | rs755793  | 123,310,871 | FGFR2 | A/G               | Sensitive   | Screening       | 52 3 2 0.01078          |
|     |     |        |          |       |                    |             | Replication     | 18 2 0 0.89974          |
|     |     |        |          |       |                    |             | Combined        | 70 5 2 0.03628          |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). ‘-‘, this variant is not identified in dbSNP; Ref., reference; NA, not available.
Table VIII. Single nucleotide variants potentially associated with sensitivity to mitomycin C.

| No. | Chr | SNP ID     | Position      | Gene  | Allele | Ref./Variant | Sensitivity | Study set      | <10% | 10-90% | >90% | P-value |
|-----|-----|------------|---------------|-------|--------|-------------|-------------|----------------|------|--------|------|---------|
| 1   | 2   | rs13382825 | 141,528,435   | LRP1B | T/C    | Resistant   | Screening   | 49 1 1         | 0.00092 | 0.25630 | 0.00793 |
|     |     |            |               |       |        |             | Replication  | 17 1 2         |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 66 10 3        |       |         |      |         |
| 2   | 7   | rs2230585  | 100,410,597   | EPHB4 | G/A    | Resistant   | Screening   | 34 14 11       | 0.00266 | 0.01083 | 0.01284 |
|     |     |            |               |       |        |             | Replication  | 8 8 4          |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 42 22 15       |       |         |      |         |
| 3   | 5   | rs216123   | 149,460,553   | CSF1R | A/G    | Sensitive   | Screening   | 42 13 4        | 0.00310 | 0.24816 | 0.00728 |
|     |     |            |               |       |        |             | Replication  | 13 3 4         |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 55 16 8        |       |         |      |         |
| 4   | 11  | rs2295081  | 32,439,038    | WT1   | T/C    | Resistant   | Screening   | 15 20 24       | 0.00431 | 0.98644 | 0.04518 |
|     |     |            |               |       |        |             | Replication  | 3 4 13         |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 18 24 37       |       |         |      |         |
| 5   | 9   | rs686346   | 135,978,378   | RALGDS| T/C    | Resistant   | Screening   | 33 16 10       | 0.00591 | 0.05342 | 0.00397 |
|     |     |            |               |       |        |             | Replication  | 7 8 5          |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 40 24 15       |       |         |      |         |
| 6   | 11  | rs16754    | 32,417,945    | WT1   | T/C    | Resistant   | Screening   | 16 20 23       | 0.00723 | NA      |      |         |
|     |     |            |               |       |        |             | Replication  | 20 0 0         |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 36 20 23       |       |         |      | 0.01174 |
| 7   | 14  | rs17111401 | 81,528,412    | TSHR  | T/A    | Sensitive   | Screening   | 42 9 8         | 0.00758 | 0.63346 | 0.04605 |
|     |     |            |               |       |        |             | Replication  | 17 1 2         |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 59 10 10       |       |         |      |         |
| 8   | 18  | -          | 22,642,750    | ZNF52I| G/C    | Resistant   | Screening   | 55 4 0         | 0.00794 | NA      |      |         |
|     |     |            |               |       |        |             | Replication  | 19 1 0         |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 74 5 0         |       |         |      | 0.09450 |
| 9   | 7   | rs56173078 | 100,420,155   | EPHB4 | A/G    | Sensitive   | Screening   | 55 3 1         | 0.00907 | NA      |      |         |
|     |     |            |               |       |        |             | Replication  | 20 0 0         |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 75 3 1         |       |         |      | 0.01301 |
| 10  | 5   | rs2229992  | 112,162,854   | APC   | T/C    | Resistant   | Screening   | 6 19 34        | 0.00954 | 0.48368 | 0.06409 |
|     |     |            |               |       |        |             | Replication  | 2 6 12         |       |         |      |         |
|     |     |            |               |       |        |             | Combined     | 8 25 46        |       |         |      |         |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNPs, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '‑' this variant is not identified in dbSNP; Ref., reference; NA, not available.
Table IX. Single nucleotide variants possibly associated with sensitivity to methotrexate.

| No. | Chr | SNP ID   | Position   | Gene   | Allele Ref/Variant | Sensitivity | Study set       | Variant allele frequency |
|-----|-----|----------|------------|--------|--------------------|-------------|------------------|-------------------------|
|     |     |          |            |        |                    |             |                  | <10% | 10-90% | >90% | P-value |
| 1   | 2   | rs62154469 | 100,209,627 | AFF3   | C/T                | Sensitive   | Screening       | 38 | 10 | 2 | 0.00146 |
|     |     |          |            |        |                    |             | Replication     | 14 | 3 | 1 | 0.07029 |
|     |     |          |            |        |                    |             | Combined        | 52 | 13 | 3 | 0.15022 |
| 2   | 18  | -        | 22,642,744  | ZNF521 | A/G                | Resistant   | Screening       | 28 | 22 | 0 | 0.00317 |
|     |     |          |            |        |                    |             | Replication     | 11 | 7 | 0 | 0.68283 |
|     |     |          |            |        |                    |             | Combined        | 39 | 29 | 0 | 0.01315 |
| 3   | 9   | rs4489420 | 139,418,260 | NOTCH1 | A/G                | Sensitive   | Screening       | 2  | 9  | 39 | 0.00457 |
|     |     |          |            |        |                    |             | Replication     | 2  | 0  | 16 | 0.20492 |
|     |     |          |            |        |                    |             | Combined        | 4  | 9  | 55 | 0.02555 |
| 4   | 19  | rs1048290 | 10,600,442  | KEAP1  | G/C                | Sensitive   | Screening       | 22 | 11 | 17 | 0.00778 |
|     |     |          |            |        |                    |             | Replication     | 5  | 4  | 9  | 0.01099 |
|     |     |          |            |        |                    |             | Combined        | 27 | 15 | 26 | 0.03367 |
| 5   | 1   | rs4870   | 2,488,153   | TNFRSF14 | A/G             | Sensitive   | Screening       | 35 | 11 | 4 | 0.00822 |
|     |     |          |            |        |                    |             | Replication     | 9  | 5  | 4  | 0.06420 |
|     |     |          |            |        |                    |             | Combined        | 44 | 16 | 8 | 0.04414 |
| 6   | 6   | rs7747060 | 56,476,262  | DST    | T/C                | Resistant   | Screening       | 28 | 17 | 5  | 0.01127 |
|     |     |          |            |        |                    |             | Replication     | 12 | 3  | 3  | 0.80779 |
|     |     |          |            |        |                    |             | Combined        | 40 | 20 | 8  | 0.04457 |
| 7   | 6   | rs17215781| 152,570,274 | SYNE1  | A/G                | Sensitive   | Screening       | 47 | 3  | 0  | 0.01305 |
|     |     |          |            |        |                    |             | Replication     | 18 | 0  | 0  | NA         |
|     |     |          |            |        |                    |             | Combined        | 65 | 3  | 0  | 0.02790 |
| 8   | 19  | rs273269  | 18,279,638  | PIK3R2 | T/C                | Sensitive   | Screening       | 1  | 2  | 47 | 0.01342 |
|     |     |          |            |        |                    |             | Replication     | 0  | 0  | 18 | NA         |
|     |     |          |            |        |                    |             | Combined        | 1  | 2  | 65 | 0.01020 |
| 9   | 5   | rs75732095| 149,495,537 | PDGFRB | G/A                | Sensitive   | Screening       | 28 | 15 | 7  | 0.01376 |
|     |     |          |            |        |                    |             | Replication     | 12 | 3  | 3  | 0.94673 |
|     |     |          |            |        |                    |             | Combined        | 40 | 18 | 10 | 0.07319 |
| 10  | 15  | rs316618  | 41,796,498  | LTK    | T/A                | Resistant   | Screening       | 47 | 3  | 0  | 0.01383 |
|     |     |          |            |        |                    |             | Replication     | 17 | 0  | 1  | NA         |
|     |     |          |            |        |                    |             | Combined        | 64 | 3  | 1  | 0.00644 |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). ‘-‘, this variant is not identified in dbSNP; Ref., reference; NA, not available.
Table X. Single nucleotide variants potentially associated with sensitivity to vincristine.

| No. | Chr | SNP ID       | Position   | Gene      | Allele Ref/Variant | Sensitivity | Study set       | <10% | 10-90% | >90% | P-value |
|-----|-----|--------------|------------|-----------|--------------------|-------------|-----------------|------|--------|------|---------|
| 1   | 7   | rs1805321    | 6,026,988  | PMS2      | G/A                | Sensitive   | Screening       | 24   | 15     | 17   | 0.00018 |
|     |     |              |            |           |                    |             | Replication     | 10   | 6      | 4    | 0.93002 |
|     |     |              |            |           |                    |             | Combined        | 34   | 21     | 21   | 0.00172 |
| 2   | 7   | rs62456182   | 6,038,722  | PMS2      | T/C                | Sensitive   | Screening       | 22   | 18     | 16   | 0.00054 |
|     |     |              |            |           |                    |             | Replication     | 10   | 6      | 4    | 0.93002 |
|     |     |              |            |           |                    |             | Combined        | 32   | 24     | 20   | 0.00372 |
| 3   | 1   | rs2453056    | 120,477,998| NOTCH2    | C/A                | Resistant   | Screening       | 52   | 3      | 1    | 0.00293 |
|     |     |              |            |           |                    |             | Replication     | 20   | 0      | 0    | NA      |
|     |     |              |            |           |                    |             | Combined        | 72   | 3      | 1    | 0.00437 |
| 4   | 17  | rs1136201    | 37,879,588 | ERBB2     | A/G                | Resistant   | Screening       | 47   | 8      | 1    | 0.00386 |
|     |     |              |            |           |                    |             | Replication     | 14   | 4      | 2    | 0.21126 |
|     |     |              |            |           |                    |             | Combined        | 61   | 12     | 3    | 0.01309 |
| 5   | 7   | rs228006     | 6,026,775  | PMS2      | T/C                | Sensitive   | Screening       | 1    | 4      | 51   | 0.00508 |
|     |     |              |            |           |                    |             | Replication     | 1    | 3      | 16   | 0.29807 |
|     |     |              |            |           |                    |             | Combined        | 2    | 7      | 67   | 0.57955 |
| 6   | 3   | rs3732565    | 134,968,232| EPHB1     | C/T                | Sensitive   | Screening       | 49   | 7      | 0    | 0.00927 |
|     |     |              |            |           |                    |             | Replication     | 18   | 1      | 1    | 0.84994 |
|     |     |              |            |           |                    |             | Combined        | 67   | 8      | 1    | 0.06335 |
| 7   | 1   | rs5277       | 186,648,197| PTGS2     | C/G                | Sensitive   | Screening       | 50   | 5      | 1    | 0.01139 |
|     |     |              |            |           |                    |             | Replication     | 18   | 2      | 0    | 0.70514 |
|     |     |              |            |           |                    |             | Combined        | 68   | 7      | 1    | 0.01819 |
| 8   | 9   | rs2290889    | 93,639,849 | SYK       | G/A                | Sensitive   | Screening       | 50   | 5      | 1    | 0.01183 |
|     |     |              |            |           |                    |             | Replication     | 19   | 1      | 0    | NA      |
|     |     |              |            |           |                    |             | Combined        | 69   | 6      | 1    | 0.02838 |
| 9   | 3   | rs762803844  | 71,247,577 | FOXP1     | G/T                | Sensitive   | Screening       | 45   | 11     | 0    | 0.01185 |
|     |     |              |            |           |                    |             | Replication     | 20   | 0      | 0    | NA      |
|     |     |              |            |           |                    |             | Combined        | 65   | 11     | 0    | 0.04620 |
| 10  | 5   | rs16903989   | 38,504,303 | LIFR      | A/T                | Sensitive   | Screening       | 27   | 23     | 6    | 0.01225 |
|     |     |              |            |           |                    |             | Replication     | 14   | 5      | 1    | 0.09051 |
|     |     |              |            |           |                    |             | Combined        | 41   | 28     | 7    | 0.00983 |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNPs, single nucleotide polymorphisms; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). ‘-‘, this variant is not identified in dbSNP; Ref., reference; NA, not available.
Table XI. Single nucleotide variants potentially associated with sensitivity to vinblastine.

| No. | Chr | SNP ID   | Position     | Gene   | Allele  | Sensitivity | Study set       | Variant allele frequency |
|-----|-----|----------|--------------|--------|---------|-------------|-------------------|--------------------------|
|     |     |          |              |        | Ref./Variant |             |                  | <10% | 10-90% | >90% | P-value |
| 1   | 5   | rs351855 | 176,520,243  | FGFR4  | G/A     | Resistant   | Screening        | 22   | 22     | 12   | 0.00337 |
|     |     |          |              |        |         |             | Replication      | 8    | 6      | 6    | 0.08904 |
|     |     |          |              |        |         |             | Combined        | 30   | 28     | 18   | 0.00225 |
| 2   | 18  | -        | 22,642,741   | ZNF521 | A/G     | Resistant   | Screening        | 33   | 23     | 0    | 0.00613 |
|     |     |          |              |        |         |             | Replication      | 11   | 9      | 0    | 0.42416 |
|     |     |          |              |        |         |             | Combined        | 44   | 32     | 0    | 0.08059 |
| 3   | 18  | rs79073678| 56,414,592   | MALT1  | T/C     | Sensitive   | Screening        | 42   | 6      | 8    | 0.00767 |
|     |     |          |              |        |         |             | Replication      | 15   | 3      | 2    | 0.89077 |
|     |     |          |              |        |         |             | Combined        | 57   | 9      | 10   | 0.05146 |
| 4   | 3   | -        | 37,067,095   | MLHI   | A/T     | Sensitive   | Screening        | 49   | 7      | 0    | 0.00960 |
|     |     |          |              |        |         |             | Replication      | 9    | 11     | 0    | 0.34137 |
|     |     |          |              |        |         |             | Combined        | 58   | 18     | 0    | 0.15800 |
| 5   | 1   | rs117505788| 6,535,149   | PLEKHG5| A/G     | Resistant   | Screening        | 52   | 3      | 1    | 0.01140 |
|     |     |          |              |        |         |             | Replication      | 18   | 2      | 0    | 0.84983 |
|     |     |          |              |        |         |             | Combined        | 70   | 5      | 1    | 0.20339 |
| 6   | 9   | rs16909898| 98,231,008   | PTCH1  | A/G     | Resistant   | Screening        | 46   | 8      | 2    | 0.01377 |
|     |     |          |              |        |         |             | Replication      | 18   | 1      | 1    | 0.34380 |
|     |     |          |              |        |         |             | Combined        | 64   | 9      | 3    | 0.01214 |
| 7   | 9   | rs1805155 | 98,238,379   | PTCH1  | A/G     | Resistant   | Screening        | 46   | 8      | 2    | 0.01377 |
|     |     |          |              |        |         |             | Replication      | 18   | 1      | 1    | 0.34380 |
|     |     |          |              |        |         |             | Combined        | 64   | 9      | 3    | 0.01214 |
| 8   | 9   | rs28448271| 98,239,730   | PTCH1  | G/A     | Resistant   | Screening        | 46   | 8      | 2    | 0.01377 |
|     |     |          |              |        |         |             | Replication      | 18   | 1      | 1    | 0.34380 |
|     |     |          |              |        |         |             | Combined        | 64   | 9      | 3    | 0.01214 |
| 9   | 11  | rs77233576| 44,130,665   | EXT2   | A/C     | Resistant   | Screening        | 50   | 5      | 1    | 0.01647 |
|     |     |          |              |        |         |             | Replication      | 14   | 5      | 1    | 0.62003 |
|     |     |          |              |        |         |             | Combined        | 64   | 10     | 2    | 0.59593 |
| 10  | 3   | rs59684491| 37,067,097   | MLHI   | A/T     | Sensitive   | Screening        | 49   | 6      | 1    | 0.01677 |
|     |     |          |              |        |         |             | Replication      | 13   | 6      | 1    | 0.96834 |
|     |     |          |              |        |         |             | Combined        | 62   | 12     | 2    | 0.08250 |

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNPs, single nucleotide polymorphisms; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). ‘-’ , this variant is not identified in dbSNP; Ref., reference; NA, not available.
Table XII. Summary of results for screening and replication study of 35 single nucleotide variants associated with sensitivity to cytotoxic anticancer drugs.

| No. | Drug | Chr | SNP ID | Position | Gene | Allele Ref./variant | Feature | Prediction of functional effect | Sensitivity | Study set | Number of samples in VAF group | P-value | Expression |
|-----|------|-----|--------|----------|------|---------------------|---------|-------------------------------|-------------|----------|-------------------------------|---------|------------|
| 1   | CPM  | 2   | rs79555258 | 148,680,526 | ACVR2A | T/C | Intron 9 | Benign | Resistant | Screening | 55 | <10% 3 1 0.00312 -0.02 0.85 |
|     |      |     |         |           |       |         |         | Tolerated (0.43) | Sensitive | Replication | 18 | 10-90% 0 2 0.02313 |
|     |      |     |         |           |       |         |         | Combined | 73 | >90% 3 3 0.00109 |
| 2   | ACNU | 1   | rs3218625  | 186,643,541 | PTGS2 | C/T | Exon 10 (G587R) | Benign | Resistant | Screening | 55 | <10% 2 0 0.04147 -0.30 0.15 |
|     |      |     |         |           |       |         |         | Tolerated (0.43) | Sensitive | Replication | 17 | 10-90% 3 0 0.00807 |
|     |      |     |         |           |       |         |         | Combined | 72 | >90% 5 0 0.00117 |
| 3a  | ACNU | 8   | rs201432181 | 37,699,794  | GPR124 | A/T | Exon 19 (D1313V) | Possibly damaging (0.664) | Resistant | Screening | 55 | <10% 2 0 0.02030 0.14 0.47 |
|      |      |     |         |           |       |         |         | Tolerated (0.12) | Sensitive | Replication | 18 | 10-90% 2 0 0.02319 |
|      |      |     |         |           |       |         |         | Combined | 73 | >90% 4 0 0.00126 |
| 3a  | ACNU | 8   | rs201432181 | 37,699,794  | GPR124 | A/T | Exon 19 (D1313V) | Possibly damaging (0.664) | Resistant | Screening | 57 | <10% 2 0 0.02117 -0.28 0.15 |
|      |      |     |         |           |       |         |         | Tolerated (0.12) | Sensitive | Replication | 18 | 10-90% 2 0 0.18538 |
|      |      |     |         |           |       |         |         | Combined | 75 | >90% 4 0 0.00404 |
| 3a  | MMC  | 8   | rs201432181 | 37,699,794  | GPR124 | A/T | Exon 19 (D1313V) | Possibly damaging (0.664) | Resistant | Screening | 54 | <10% 2 0 0.03044 -0.16 0.42 |
|      |      |     |         |           |       |         |         | Tolerated (0.12) | Sensitive | Replication | 18 | 10-90% 2 0 0.84983 |
|      |      |     |         |           |       |         |         | Combined | 72 | >90% 4 0 0.01706 |
| 3a  | VLB  | 8   | rs201432181 | 37,699,794  | GPR124 | A/T | Exon 19 (D1313V) | Possibly damaging (0.664) | Resistant | Screening | 55 | <10% 2 0 0.03933 0.31 0.11 |
|      |      |     |         |           |       |         |         | Tolerated (0.12) | Sensitive | Replication | 18 | 10-90% 2 0 0.61416 |
|      |      |     |         |           |       |         |         | Combined | 73 | >90% 4 0 0.02917 |
| 3a  | ADR  | 8   | rs201432181 | 37,699,794  | GPR124 | A/T | Exon 19 (D1313V) | Possibly damaging (0.664) | Resistant | Screening | 47 | <10% 10 0 0.00365 -0.07 0.47 |
|      |      |     |         |           |       |         |         | Tolerated (0.12) | Sensitive | Replication | 19 | 10-90% 1 0 NA |
|      |      |     |         |           |       |         |         | Combined | 66 | >90% 11 0 0.00157 |
| 3a  | ADR  | 7   | rs113962761 | 50,450,446  | IKZF1  | C/T | Intron 5 | Benign | Resistant | Screening | 39 | <10% 18 2 0.04828 0.20 0.09 |
|      |      |     |         |           |       |         |         | Tolerated (0.43) | Sensitive | Replication | 12 | 10-90% 6 2 0.03822 |
|      |      |     |         |           |       |         |         | Combined | 51 | >90% 24 4 0.00243 |
| 5   | CPM  | 2   | rs2020910  | 48,030,692  | MSH6  | T/A | Exon 5 (T1102T) | Benign | Resistant | Screening | 24 | <10% 3 32 0.00378 0.02 0.84 |
|      |      |     |         |           |       |         |         | Tolerated (0.43) | Sensitive | Replication | 5  | 10-90% 0 15 0.12606 |
|      |      |     |         |           |       |         |         | Combined | 29 | >90% 3 47 0.00247 |
| 6   | CPM  | 12  | rs3217786  | 4,383,158   | CCND2 | T/C | Exon 1 (3'UTR) | Benign | Resistant | Screening | 37 | <10% 16 4 0.04670 0.22 0.06 |
|      |      |     |         |           |       |         |         | Tolerated (0.43) | Sensitive | Replication | 16 | 10-90% 4 0 0.01812 |
|      |      |     |         |           |       |         |         | Combined | 53 | >90% 20 4 0.00288 |
| 7   | ADR  | 7   | rs1050171  | 55,249,063  | EGFR  | G/A | Exon 20 (Q787Q) | Benign | Resistant | Screening | 43 | <10% 13 0 0.01218 -0.39 0.002 |
|      |      |     |         |           |       |         |         | Tolerated (0.43) | Sensitive | Replication | 19 | 10-90% 1 0 NA |
|      |      |     |         |           |       |         |         | Combined | 62 | >90% 14 0 0.00564 |
| No. | Drug | Chr | SNP ID     | Position     | Gene | Allele | Ref./variant | Feature | Prediction of functional effect | Number of samples in VAF group | Sensitivity | Study set | r<sup>c</sup> | P-value | r<sup>d</sup> | P-value |
|-----|------|-----|------------|--------------|------|--------|-------------|---------|-------------------------------|-------------------------------|-------------|-----------|---------|----------|----------|----------|
| 9   | ADR  | 2   | rs4589708  | 29,496,210   | ALK  | A/G    | Intron 10   |         | Sensitive                      | 4 8 45                         | 0.04147     | Screening | 0.37    | 0.47     |
| 10  | MTX  | 14  | rs3730344  | 105,241,576  | AKT1 | G/A    | Intron 5    |         | Sensitive                      | 47 3 0                         | 0.01466     | Screening | -0.03   | 0.86     |
| 11  | 5FU  | 2   | rs1863703  | 219,544,388  | STK36| A/G    | Exon 8 (K295R) | Benign (0.056) Tolerated (0.35) | Sensitive                      | 48 8 0                         | 0.02328     | Screening | 0.01    | 0.91     |
| 12  | 5FU  | 2   | rs16859180 | 219,553,468  | STK36| C/T    | Exon 12 (R477W) | Probably damaging (1.000) Damaging (0.00) | Sensitive                      | 48 8 0                         | 0.02328     | Screening | 0.01    | 0.91     |
| 13  | 5FU  | 2   | rs12993599 | 219,563,602  | STK36| G/A    | Exon 26 (R1112Q) | Benign (0.071) Tolerated (1.00) | Sensitive                      | 48 8 0                         | 0.02328     | Screening | 0.01    | 0.91     |
| 14  | ACNU | 5   | rs6962     | 256,509      | SDHA | G/A    | Exon 15 (V657I) | Benign (0.021) Tolerated (0.62) | Resistant                      | 51 6 0                         | 0.01003     | Screening | 0.08    | 0.47     |
| 15  | 5FU  | 1   | rs1699760  | 144,852,545  | PDE4DIP| C/T   | Intron 43   |         | Resistant                      | 45 11 0                        | 0.01420     | Screening | -0.16   | 0.17     |
| 16b | VCR  | 5   | rs16903989 | 38,504,303   | LIFR  | A/T    | Intron 9    |         | Sensitive                      | 27 23 6                        | 0.01225     | Screening | 0.42    | 0.0003   |
|     | CPM  | 5   | rs16903989 | 38,504,303   | LIFR  | A/T    | Intron 9    |         | Sensitive                      | 29 24 6                        | 0.04242     | Screening | 0.36    | 0.002    |
| 17  | 5FU  | 1   | rs71664012 | 144,881,666  | PDE4DIP| C/A   | Intron 24   |         | Resistant                      | 16 40 0                        | 0.02674     | Screening | -0.16   | 0.17     |
| 18  | MMC  | 8   | rs17847568 | 30,973,938   | WRN   | C/T    | Exon 20 (T781I) | Possibly damaging (0.807) Damaging (0.02) | Resistant                      | 57 0 2                         | 0.04210     | Screening | 0.05    | 0.82     |
Table XII. Continued.

| No. | Drug | Chr | SNP ID | Position | Gene | Allele Ref./variant | Feature | Prediction of functional effect | Number of samples in VAF group | Study set | Sensitivity | <10% | 10-90% | >90% | P-value | r² | P-value⁴ |
|-----|------|-----|--------|----------|------|---------------------|---------|-----------------------------|-------------------------------|-----------|-------------|------|--------|------|---------|----|---------|
| 19  | MMC  | 7   | rs78004519 | 151,860,023 | MLL3 | A/G | Exon 43 (S3547P) | Benign (0.033) Tolerated (0.30) | Resistant | Screening | 57 | 2 | 0 | 0.04887 | 0.13 | 0.34 |
|     |      |     |         |          |      |        |        |                               | Replication | 19 | 1 | 0 | NA |
|     |      |     |         |          |      |        |        |                               | Combined | 76 | 3 | 0 | 0.01530 |
| 20  | ACNU | 8   | rs75858201 | 103,308,010 | UBR5 | T/C | Exon 29 (K1222K) | Resistant | Screening | 54 | 3 | 0 | 0.04531 | -0.11 | 0.37 |
|     |      |     |         |          |      |        |        |                               | Replication | 19 | 1 | 0 | NA |
|     |      |     |         |          |      |        |        |                               | Combined | 73 | 4 | 0 | 0.01592 |
| 21  | MMC  | 8   | rs138106214 | 90,947,858 | NBN  | G/A | Intron 15 | Resistant | Screening | 56 | 3 | 0 | 0.04167 | 0.13 | 0.43 |
|     |      |     |         |          |      |        |        |                               | Replication | 19 | 1 | 0 | NA |
|     |      |     |         |          |      |        |        |                               | Combined | 75 | 4 | 0 | 0.01617 |
| 22  | VCR  | 10  | rs14877922 | 114,901,092 | TCF7L2 | G/A | Intron 5 | Resistant | Screening | 48 | 7 | 1 | 0.03119 | -0.10 | 0.40 |
|     |      |     |         |          |      |        |        |                               | Replication | 18 | 2 | 0 | 0.20720 |
|     |      |     |         |          |      |        |        |                               | Combined | 66 | 9 | 1 | 0.01649 |
| 23  | CPM  | 8   | rs17652171 | 113,662,583 | CSMD3 | A/C | Intron 18 | Resistant | Screening | 52 | 7 | 0 | 0.04256 | -0.15 | 0.21 |
|     |      |     |         |          |      |        |        |                               | Replication | 18 | 2 | 0 | 0.37710 |
|     |      |     |         |          |      |        |        |                               | Combined | 70 | 9 | 0 | 0.01711 |
| 24  | MMC  | 18  | -     | 22,642,748 | ZNF521 | A/C | Intron 7 | Resistant | Screening | 51 | 8 | 0 | 0.04618 | 0.07 | 0.58 |
|     |      |     |         |          |      |        |        |                               | Replication | 19 | 1 | 0 | NA |
|     |      |     |         |          |      |        |        |                               | Combined | 70 | 9 | 0 | 0.01743 |
| 25  | ADR  | 11  | rs10895289 | 102,199,611 | BIRC3 | A/T | Intron 1 | Sensitive | Screening | 51 | 6 | 0 | 0.01991 | -0.05 | 0.86 |
|     |      |     |         |          |      |        |        |                               | Replication | 19 | 1 | 0 | NA |
|     |      |     |         |          |      |        |        |                               | Combined | 70 | 7 | 0 | 0.01977 |
| 26  | MMC  | 2   | rs61749494 | 60,689,441 | BCL11A | T/C | Exon 4 (E202E) | Sensitive | Screening | 46 | 13 | 0 | 0.03616 | 0.07 | 0.84 |
|     |      |     |         |          |      |        |        |                               | Replication | 16 | 3 | 1 | 0.21878 |
|     |      |     |         |          |      |        |        |                               | Combined | 62 | 16 | 1 | 0.01994 |
| 27  | VCR  | 13  | rs2491231 | 28,610,183 | FLT3  | A/G | Intron 10 | Resistant | Screening | 14 | 13 | 29 | 0.04949 | -0.25 | 0.12 |
|     |      |     |         |          |      |        |        |                               | Replication | 3 | 4 | 13 | 0.19167 |
|     |      |     |         |          |      |        |        |                               | Combined | 17 | 17 | 42 | 0.02057 |
| 28  | 5FU  | 14  | rs67737119 | 95,591,070 | DICER1 | G/A | Intron 8 | Resistant | Screening | 22 | 14 | 20 | 0.02660 | -0.02 | 0.85 |
|     |      |     |         |          |      |        |        |                               | Replication | 9 | 5 | 6 | 0.61376 |
|     |      |     |         |          |      |        |        |                               | Combined | 31 | 19 | 26 | 0.02387 |
| 29  | VCR  | 6   | rs8192585  | 32,188,823 | NOTCH4 | G/A | Exon 4 (S244L) | Sensitive | Screening | 51 | 5 | 0 | 0.04130 | 0.51 | 0.38 |
|     |      |     |         |          |      |        |        |                               | Replication | 18 | 2 | 0 | 0.34416 |
|     |      |     |         |          |      |        |        |                               | Combined | 69 | 7 | 0 | 0.02414 |
Table XII. Continued.

| No. | Drug | Chr | SNP ID   | Position  | Gene  | Allele Ref./variant | Feature | Sensitivity       | Study set | Number of samples in VAF group | P-value | Expression |
|-----|------|-----|----------|-----------|-------|---------------------|---------|-------------------|-----------|-------------------------------|---------|------------|
|     |      |     |          |           |       |                     |         |                   |           | <10% | 10-90% | >90% |                  |<10% | 10-90% | >90% |    |
| 30  | 5FU  | 1   | rs1539243| 206,647,787| IKBKE | T/C                | Exon 4 (I67I) | Resistant | Screening         | 2      | 3   | 51    |       | 0.03643 | 0.05 | 0.79 |
|     |      |     |          |           |       |                     |         |                   |           | Replication                 | 0      | 1   | 19    | NA    |             |      |        |
|     |      |     |          |           |       |                     |         |                   |           | Combined                     | 2      | 4   | 70    | 0.02733 |          |      |        |
| 31  | ADR  | 17  | rs2735611| 8,048,283 | PER1  | G/A                | Exon 18 (G749G) | Resistant | Screening         | 38     | 17  | 2     | 0.03587 | -0.13 | 0.53 |
|     |      |     |          |           |       |                     |         |                   |           | Replication                 | 12     | 7   | 1     | 0.84700 |          |      |        |
|     |      |     |          |           |       |                     |         |                   |           | Combined                     | 50     | 24  | 3     | 0.02888 |          |      |        |
| 32  | DDP  | 1   | rs12037217| 85,742,023| BCL10 | C/A               | Exon 1 (A5S) | Resistant | Screening         | 53     | 4   | 0     | 0.03770 | -0.27 | 0.73 |
|     |      |     |          |           |       |                     |         |                   |           | Replication                 | 18     | 2   | 0     | 0.44969 |          |      |        |
|     |      |     |          |           |       |                     |         |                   |           | Combined                     | 71     | 6   | 0     | 0.02953 |          |      |        |
| 33  | 5FU  | 18  | -        | 22,642,744| ZNF521| A/G                | Intron 7 | Resistant | Screening         | 32     | 24  | 0     | 0.03429 | -0.39 | 0.002 |
|     |      |     |          |           |       |                     |         |                   |           | Replication                 | 13     | 7   | 0     | 0.52596 |          |      |        |
|     |      |     |          |           |       |                     |         |                   |           | Combined                     | 45     | 31  | 0     | 0.03358 |          |      |        |
| 34  | CPM  | 1   | rs139822181| 144,863,320| PDE4DIP| T/C               | Exon 37 (K208R) | Probable damaging (-0.998) | Damaging (0.02) | Screening | 50     | 9   | 0     | 0.04988 | 0.11  | 0.37 |
|     |      |     |          |           |       |                     |         |                   |           | Replication                 | 19     | 1   | 0     | NA     |          |      |        |
|     |      |     |          |           |       |                     |         |                   |           | Combined                     | 69     | 10  | 0     | 0.03433 |          |      |        |
| 35  | ADR  | 20  | rs62206933| 31,023,500| ASXL1 | C/T                | Exon 13 (H995H) | Resistant | Screening         | 51     | 6   | 0     | 0.04955 | -0.04 | 0.84 |
|     |      |     |          |           |       |                     |         |                   |           | Replication                 | 18     | 2   | 0     | 0.48819 |          |      |        |
|     |      |     |          |           |       |                     |         |                   |           | Combined                     | 69     | 8   | 0     | 0.03538 |          |      |        |

5FU, 5-fluorouracil; ACNU, nimustine; ADR, adriamycin; CPM, cyclophosphamide; DDP, cisplatin; MMC, mitomycin C; MTX, methotrexate; VCR, vincristine; VLB, vinblastine; Chr, chromosome; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). ‘-’, this variant is not identified in dbSNP; Ref., reference; NA, not available; ‘variant allele was suggested to cause multidrug sensitive (ACNU, MMC, VLB and ADR); ‘variant allele was suggested to cause multidrug sensitive (VCR and CPM); ‘expression r: Pearson correlation coefficient (r) had been calculated to estimate positive (sensitive) or negative (resistant) correlation between the gene expression level and sensitivity to each anticancer drug; ‘expression P-value, P-value of Pearson correlation coefficient.
rs16903989, which was located in intron 9 of the LIFR gene was commonly associated with sensitivity to CPM and VCR. LIFR forms a heterodimer with a signal transducer, gp130 and leads to activation of the Janus kinase/signal transducer and activator of transcription and mitogen activated protein kinase cascades (34). LIFR has been demonstrated to be downregulated in breast cancer and was identified as a metastasis suppressor (35, 36). A single nucleotide polymorphism in LIFR (rs3729740) was reported to be a potential predictive marker for sensitivity to a molecular-targeted drug, cetuximab (37). Furthermore, the expression level of LIFR was revealed to be associated with sensitivity to VCR in glioblastoma cells (38), and the data of the current study also indicated a positive correlation between the expression level of LIFR and sensitivity to VCR. Although the role of LIFR in response to anticancer therapy has not yet been clarified, this gene may be associated with a common mechanism of drug response. The current study also demonstrated that rs201432181 in GPR124 was typically associated with sensitivity to 4 anticancer drugs (ACNU, ADR, MMC and VLB). rs201432181 is a nonsynonymous substitution (p.D1313V), and the effect of the substitution on protein function was predicted to be ‘possibly damaging’ by Polyphen2. GPR124 is known to regulate vascular endothelial growth factor-induced tumor angiogenesis in vitro (39). Therefore, the promotion of tumor angiogenesis by activation of pathway involved with GPR124 may enhance the delivery of anticancer drugs.

To investigate the tissue specificity of the chemosensitivity-related SNVs identified in the current study, subgroup analysis for breast and gastric cancer xenografts was performed as they included the largest number of tissues (n=12 each) used in the present study. SNVs that were commonly associated with chemosensitivity in the xenografts derived from breast and gastric cancer were identified (rs79555258 for CPM, P=0.031 and 0.086, respectively). By contrast, the study also observed the SNVs associated with chemosensitivity in the xenografts derived from breast cancer, but not in those from gastric cancer.

Of the 409 genes sequenced using CCP in the current study, Excision Repair Cross-Complementation Group 1, Excision Repair Cross-Complementation Group 2, AKT1 and Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit α have previously been reported to be candidates or promising predictors for sensitivity to cisplatin (40-42). However, no SNVs associated with these genes demonstrated a significant association with cisplatin in the current study, this is potentially because the sample size was too small. Further studies using a large number of xenografts and clinical samples are required to confirm whether they may be a predictive marker for sensitivity to cisplatin clinically.

In conclusion, the present study used 79 human cancer xenografts implanted into nude mice to identify 35 possible genetic variants associated with the sensitivity or resistance to ≥1 anticancer drugs from a total of 9. These findings provide novel insights into personalized selection of chemotherapy for patients with cancer, however; further functional analysis is required to verify the results of the current study and to clarify their biological mechanisms, which have effects on drug response. The current study also demonstrated that rs79555258 and sensitivity to CPM. The xenografts with higher variant allele frequency in rs79555258 exhibited a lower response to CPM compared with those that presented with a lower variant allele frequency. The (A) screening study, (B) replication study and (C) combined study are presented where the sensitivity to CPM is represented by relative tumor volume of T with respect to C. ‘x’ represents a single xenograft. Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside represents the median. The whiskers outside the box extend to the highest and lowest value within 1.5 times the IQR. CPM, cyclophosphamide; T, treated mice; C, control.

Figure 1. Association between rs79555258 and sensitivity to CPM. The xenografts with higher variant allele frequency in rs79555258 exhibited a lower response to CPM compared with those that presented with a lower variant allele frequency. The (A) screening study, (B) replication study and (C) combined study are presented where the sensitivity to CPM is represented by relative tumor volume of T with respect to C. ‘x’ represents a single xenograft. Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside represents the median. The whiskers outside the box extend to the highest and lowest value within 1.5 times the IQR. CPM, cyclophosphamide; T, treated mice; C, control.

Figure 2. Combined effects of rs4589708, rs13962761 and rs1050171 on sensitivity to ADR. The distribution of ADR sensitivity is presented in the four score groups. The xenografts were classified into four groups based on the sum of the score given to each variant allele frequency group for the three single nucleotide variants. ‘x’ represents a single xenograft. Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside represents the median. The whiskers outside the box extend to the highest and lowest value within 1.5 times the IQR. ADR, Adriamycin; T, treated mice; C, control.

Figure 2. Combined effects of rs4589708, rs13962761 and rs1050171 on sensitivity to ADR. The distribution of ADR sensitivity is presented in the four score groups. The xenografts were classified into four groups based on the sum of the score given to each variant allele frequency group for the three single nucleotide variants. ‘x’ represents a single xenograft. Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside represents the median. The whiskers outside the box extend to the highest and lowest value within 1.5 times the IQR. ADR, Adriamycin; T, treated mice; C, control.
the clinical outcomes of patients receiving the chemotherapy. Accumulation of data is expected to lead to ‘cancer precision medicine’ using more effective and less harmful anticancer drugs.

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