Genome-wide Identification of Chemosensitive Single Nucleotide Polymorphism Markers in Gastric Cancer

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Abstract. *A chemosensitive single nucleotide polymorphism (SNP) discovery schema is presented that utilizes (i) genome-wide SNP screening, with a human SNP array and an in vitro chemosensitivity assay, in 93 patients with gastric cancer (GC), and (ii) biological utility assessment using cell viability assays of transfected GC cells. Cytotoxicity analysis showed that most of the MKN1 and SNU638 clones transfected with the G allele of Deoxyribonuclease II beta (DNASE2B) rs3738573 were more sensitive to docetaxel than those with the C allele (p≤0.001-0.029) and most of the AGS and SNU638 clones transfected with the T allele of 5-hydroxytryptamine receptor IE (HTRIE) rs3828741 were more sensitive to paclitaxel than those with the C allele (p≤0.001-0.019). Our findings show that the two novel markers, DNASE2B rs3738573 and HTR1E rs3828741, have potential for improving the prediction of chemosensitivity of GC patients.*

Despite a declining incidence in Western countries, gastric carcinoma (GC) remains the second most common cause of cancer-related mortality worldwide (1). Surgery is the only potentially curative treatment for localized GC. Radical gastrectomy with extended lymphadenectomy is a reasonably safe procedure in experienced centers (2). However, systemic chemotherapy is necessary for advanced cases of GC. While 5-fluorouracil (5-FU) has been a key therapeutic agent for GC, oxaliplatin, paclitaxel, and docetaxel have also recently emerged as new therapeutic agents. Oxaliplatin, a third-generation diaminocyclohexane platinum compound that has a wide range of antitumor activities, appears to have a better safety profile than cisplatin in terms of adverse events (3). The response rate of patients with advanced GC to 5-FU with leucovorin and oxaliplatin (FOLFOX) is 38-43%, and the FOLFOX regimen shows a manageable toxicity profile as a first-line treatment modality for advanced GC (4). Docetaxel is a clinically well-established anti-mitotic chemotherapy agent used mainly for the treatment of breast, ovarian, and non-small cell lung cancer. It has also been found to lead to the phosphorylation of oncoprotein BCL2, which blocks apoptosis in its oncoprotein form. Docetaxel has demonstrated promising activity in GC, both as monotherapy and in combination with other agents (5). Paclitaxel induces cellular processes that lead to apoptosis or programmed cell death, even at doses that do not induce tubulin polymerization. Although the precise mechanism of this effect of paclitaxel has not yet been determined; cells exiting from mitosis without continuing to divide, then substantial DNA fragmentation, indicative of apoptosis, leads to cell death. Regulation of apoptosis-related genes may also be involved in the regulation of paclitaxel-induced cytotoxicity and resistance (6, 7).

The prognosis for patients with locally advanced GC remains poor even after potentially curative resection, with a high risk of locoregional or distant recurrence (8). The two most important problems associated with the use of chemotherapeutic agents are side-effects and drug resistance. Considering the narrow therapeutic index for

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many anticancer regimens, improving the ability to predict the response to a particular treatment is highly important to the clinician. Several commercial *in vitro* chemosensitivity tests are available (9). However, all *in vitro* assays have limitations, including reproducibility, tumor cell heterogeneity, and low rates of correlation with clinical outcomes. To identify patients most likely to benefit from a given treatment, it is important to detect molecular or genetic determinants of treatment outcomes, or predictive markers (10). As we previously reported for colorectal cancer, the identification of polymorphic, chemosensitive single nucleotide polymorphism (SNP) markers can aid in the prediction of responses to chemotherapy, and this could, in turn, enable physicians to more efficiently select patients for various regimens (11, 12).

In the current study, we used a two-step process of genome-wide chemosensitive SNP screening by array and *in vitro* chemosensitivity assay (first step) and biological utility assay (second step) to select chemosensitive SNP markers applicable to GC. Our primary aim was to discover surrogate SNP markers, capable of predicting chemotherapy response, to further aid the development of personalized chemotherapy regimens for GC patients.

Materials and Methods

Study design and patients. For the initial screening, 93 patients with sporadic GC were recruited from March 2007 to August 2008 at the Asan Medical Center (Seoul, Republic of Korea) for genome-wide SNP screening according to an *in vitro* drug response assay. Candidate SNP markers from the initial screening were further selected according to the following criteria: SNPs must be located in the linkage disequilibrium block of Japanese populations on the HapMap dataset (http://www.hapmap.org) and WGAViewer (13); minor allele frequencies must be greater than 10% in Japanese and expand Han Chinese populations; the SNPs must not depart from Hardy-Weinberg equilibrium (DHW; $p>0.01$); and, preferably, they should be non-synonymous or haplotype-tagged SNPs. For DHW assessment and clinical association analysis, 145 additional evaluable patients who had received chemotherapy were also included for the genotyping of selected SNPs, using their genomic DNA. The demographic and biological features of patients with GC evaluated in the initial screening and clinical association analysis are shown in Table I. All patients provided written informed consent, and the study protocol was approved by the Institutional Review Board for Human Genetic and Genomic Research (registration no.: 2009-0091), in accordance with the Declaration of Helsinki.

In vitro chemosensitivity assay. The chemosensitivity of tumor tissue from the initial cohort of 93 patients was assessed using oxaliplatin (Merck, Darmstadt, Germany), docetaxel (Boryong, Gyeonggi, Republic of Korea), and paclitaxel (Boryong, Gyeonggi, Republic of Korea). The cut-off concentrations of the established drugs used to distinguish *in vitro* sensitivity from resistance were set at 40 μg/ml for oxaliplatin, 75 μg/ml for docetaxel, and 10 μg/ml for paclitaxel. Histoculture drug response assay (HDRA) was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA) as a quantitative end-point to assess total tumor cell viability (14). The inhibition rate (IR) cut-off value for a positive response was previously determined as 30%, similar to that of the Response Evaluation Criteria in Solid Tumors (RECIST) (15).

Affymetrix genome-wide human SNP array. SNP mapping for the initial screening was carried out using genomic DNA from individuals who had been assessed by HDRA, according to the manufacturer's protocol (http://www.affymetrix.com), using the Affymetrix Genome-Wide Human SNP Nsp/Sty Assay Kit and SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA). The SNP Nsp/Sty Array 6.0 was scanned by Affymetrix GeneChip Operating Software (version 1.4) using an Affymetrix GeneChip Scanner 3000. Signal intensity data were initially analyzed using the dynamic model algorithm. Genotypes were then determined using Affymetrix Genotyping Console software (version 2.1) based on the Bayesian Robust Linear Model with the Mahalanobis distance classifier (BRLMM)-P algorithm. An ANOVA test was used to identify SNPs associated with quantitative HDRA drug sensitivity. A liberal p-value (<0.001) was used for the initial screening of candidate regions due to the relatively small sample size. The raw data have been deposited in the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/ projects/geo/) under accession number GSE26853.

Genotyping assay. The five chemosensitive SNPs obtained from the initial screening of 145 patients were genotyped by pyrosequencing. Briefly, DNA was extracted from white blood cells recovered from whole blood using the Wizard Purification Kit (Promega, Madison, WI, USA). The PCR protocol and sequencing primers were designed using Assay Design Software (version 1.0.6; Biotage, Uppsala, Sweden). PCR optimization was carried out using an ABI 2700 thermocycler (Applied Biosystems, Foster City, CA, USA), and then the samples were prepared on a Vacuum Prep Workstation (Biotage) according to standard protocols. Amplified PCR products were purified using streptavidin-Sepharose HP beads (Amersham Biosciences, Uppsala, Sweden), denatured, and then sequenced according to the manufacturer's recommendations.

Biological utility assessment. Human 5-hydroxytryptamine receptor 1E (*HTRIE*), excision repair cross-complementing rodent repair deficiency, complementation group 6 (*ERCC6*), and NACHT, leucine rich repeat and pyrin domain containing 2 (*NLRP2*) genes inserted in the pReceiver-M10 vector were purchased from GeneCopoeia (Rockville, MD, USA) and Deoxyribonuclease II beta (*DNASE2B*) inserted in the pCMV6 Entry vector was purchased from OriGene (Rockville, MD, USA). They were all tagged with the *c-myc* epitope. The substitution allele of each clone was generated using a site-directed mutagenesis kit (iNtRON Biotechnology, Seongnam, Republic of Korea). Mutation of each gene was confirmed by DNA sequencing analysis. Three GC cell lines (AGS, SNU638, MKN1) (KRIBB, Daejoen, Republic of Korea) were used for gene overexpression and stable cell establishment. Transfection was performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Stably expressing cells were generated by G418 selection for 10 days. Expression of the above four genes was confirmed by western blotting using anti-myc antibody (Santa Cruz

Table I. *Demographic and biological features of patients with gastric cancer in the initial screening and clinical association analysis for chemosensitive single nucleotide polymorphism markers.*

Characteristic	No. of patients (missing)		*p*-value*
	Initial screening	Clinical association	
Gender, male/female	68/25	100/45	0.493
Age, years	54.8±11.0	54.3±11.2	0.755
AJCC stage, I/II/III/IV	15/35/29/14	13/37/51/44	0.012
Extent of resection, DG/TG	42/51	60/83 (2)	0.465
Preoperative CEA level (ng/ml), >6/≤6	6/86 (1)	13/130 (2)	0.481
Preoperative CA19-9 level (U/ml), >37/≤37	6/86 (1)	23/120 (2)	0.030
Preoperative CA72-4 level (U/ml), >4/≤4	22/68 (3)	52/89 (4)	0.048
Tumor characteristics			
Lauren classification, intestinal/diffuse+mixed	40/53	49/90 (6)	0.234
Differentiation, WD/MD/PD	1/28/55 (9)	1/32/84 (28)	0.630
WHO classification, tubular/other	75/18	106/32 (7)	0.488
Lymphatic invasion, absent/present	41/52	42/101 (2)	0.021
Vascular invasion, absent/present	61/32	70/73 (2)	0.012
Perineural invasion, absent/present	39/54	56/87 (2)	0.671
EGFR expression (negative, 1 positive/2,3 positive)	66/23 (4)	105/17 (23)	0.029
p53 expression (negative, 1 positive/2,3 positive)	61/28 (4)	74/48 (21)	0.239
c-erbB2 (negative, 1 positive/2,3 positive)	79/10 (4)	107/16 (20)	0.698

*Comparison of initial screening and clinical association by Pearson's v2-test or Student's *t*-test. AJCC, American Joint Committee on Cancer (6th Ed., 2001). Other: Including papillary, signet-ring cell, mucinous, adenosquamous cell, and lymphoepithelioma-like types according to WHO classification. Significantly different between the two groups but no association with recurrence and survival in the adjuvant chemotherapy group, with the exception of stage. DG: Distal gastrectomy; TG: total gastrectomy; WD: well-differentiated; MD: moderately differentiated; PD: poorly differentiated; EGFR: epidermal growth factor receptor.

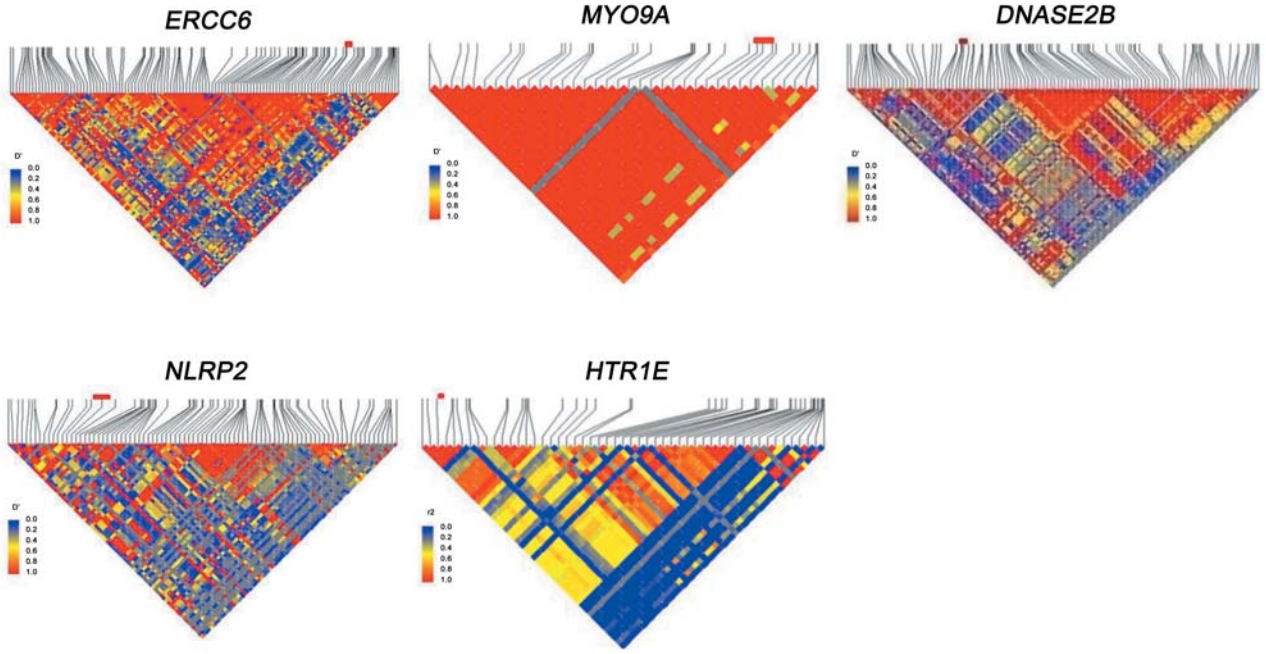

Figure 1. *Linkage disequilibrium (LD) indicating five candidate single nucleotide polymorphisms (SNPs) chemosensitive to the three drugs (marked as horizontal red bars). The red square represents regions of the highest degree of LD (D'=1). The LD blocks in the Japanese populations were obtained from the HapMap dataset (http://www.hapmap.org) and visualized with the WGAViewer (13). These SNPs were further selected on the basis of the following criteria, in order: minor allelic frequencies of greater than 5%, no departure from Hardy-Weinberg equilibrium (p>0.01), and nonsynonymous and haplotype-tagged SNPs.*

Table II. *Five candidate single nucleotide polymorphisms (SNPs) related to chemosensitivity to three drugs drawn from the initial screening and identifying steps.*

Drug	Gene	SNP ID	Alleles		Consequence
			Reference	Substitution	
Oxaliplatin	*ERCC6*	*rs2228529*	*A*	*G*	Q1413R
Oxaliplatin	*MYO9A*	*rs2415129*	*T*	*C*	G1193E
Docetaxel	*DNASE2B*	*rs3738573*	*C*	*G*	Q3H
Docetaxel	*NLRP2*	*rs4306647*	*G*	*A*	R364K
Paclitaxel	*HTR1E*	*rs3828741*	*C*	*T*	A208T

Biotechnology, Santa Cruz, CA). Four GC cell lines (AGS, SNU638: endogenous reference alleles of Myosin IX A (*MYO9A*) rs2415129-expressing cells; MKN1, SNU719: endogenous substitution alleles of *MYO9A rs2415129*-expressing cells) (KRIBB) were used for MYO9A depletion. The human MYO9A ON-TARGET plus SMART pool (Dharmacon, Lafayette, CO, USA) was used to silence the endogenous expression of MYO9A in several GC cell lines. Negative control siRNA was obtained from Bioneer (Daejoen, Republic of Korea). Transfection with siRNA was performed using Lipofectamine reagent. MYO9A expression was confirmed by Western blot with anti-MYO9A antibody 72 h after transfection (Abnova, Taipei, Taiwan, ROC). At the end of each drug treatment, MTT was added to each well in a 96-well plate and the plate was incubated at 37°C for 1 h. After treating each of the samples with 100 μl dimethyl sulfoxide (DMSO), the absorbance of the solution was read spectrophotometrically at 540 nm with a reference at 650 nm using a microtiter plate reader (SUNRISE-BASIC TECAN, Grodig, Austria).

Chemotherapy and evaluation. Eligibility criteria included histologically verified gastric adenocarcinoma, an Eastern Cooperative Oncology Group performance status of 0 or 1, and an age of 75 years or less. A total of 132 patients underwent postoperative adjuvant chemotherapy. Tumor response was assessed for measurable disease; specifically, for metastatic and recurrent GC using RECIST criteria with objective response and disease control rates calculated according to intent-to-treat analysis (15). Operable GC patients with adjuvant chemotherapy did not fulfill the primary endpoint of objective response which reflected the chemosensitivity to the respective regimen. Accordingly, recurrence and survival rates were analyzed in terms of chemosensitive SNPs by initial screening as a supplementary endpoint. Adverse events were evaluated based on the National Cancer Institute (Bethesda, MD, USA) Common Toxicity Criteria (version 3.0).

Statistical analysis. An Altman's nomogram, assuming putative chemosensitivity ranges between 25% and 75%, was used to determine the sample size to obtain 80% power to detect chemosensitive SNPs for the initial screening. Similarly, the number of patients required for the validation step was determined on the basis of reported chemosensitivity ranges between 13% and 87% for chemotherapy of metastatic cancer. Allelic frequency and Hardy-Weinberg probability were determined using GENEPOP software (http://genepop.curtin.edu.au). Genotypic and allelic frequencies were compared in terms of recurrence, disease control response, biological features and case control associations by cross-table analysis using Fisher's exact test. Potential variables were verified by multivariate analysis using binary logistic regression. Survival rates were compared using the Kaplan-Meier method with a log-rank test, and survival factors were verified using Cox's regression model. Statistical significance was defined at $p<0.05$, and all calculations were carried out using SPSS software (version 14; SPSS, Chicago, IL, USA).

Results

Initial SNP screening according to in vitro chemosensitivity. In tumor samples from 93 GC patients, the IR values in response to three different chemotherapy drugs ranged from 24.8% to 35.3%, and the percentage of chemosensitive tumors (IR, ≥30%) ranged from 37.6% to 68.8%. The average genotyping call rate among the individuals was 99.37% on a microarray. Of 2,414,251 SNPs, 168,891 SNPs were filtered out on the basis of a missing rate of >0.05 and minor allelic frequency of <0.01, leaving 2,245,360 SNPs for subsequent screening. Initial dichotomous screening identified 1,365 SNPs that were highly associated with *in vitro* chemosensitivity to the three drugs ($p<0.001$). Finally, from this initial screening, we chose five non-synonymous SNPs (nsSNPs) based on the criteria described in the study design section (Figure 1 and Table II).

In vitro cytotoxicity of transfected GC cells. SNU638, AGS, SNU719, and MKN1 human GC cell lines were used to determine the chemosensitivity of the SNPs *HTRIE rs3828741, ERCC6 rs2228529, NLRP2 rs4306647, DNASE2B rs3738573*, and *MYO9A rs2415129*. These cells were chosen for their short doubling time, easily identifiable morphological change, and selection efficiency in G418-resistant clones. Cells were cloned into stable cell lines that overexpressed reference and substitution alleles of the gene of interest. MKN1 and SNU638 cells expressing substitution allele (*C*) of *DNASE2B* 3738573 exhibited enhanced cell death ($p<0.001$-0.025 in MKN1 and $p<0.001$-0.093 in SNU638) (Figure 2A and B) in response to docetaxel treatment as compared with those

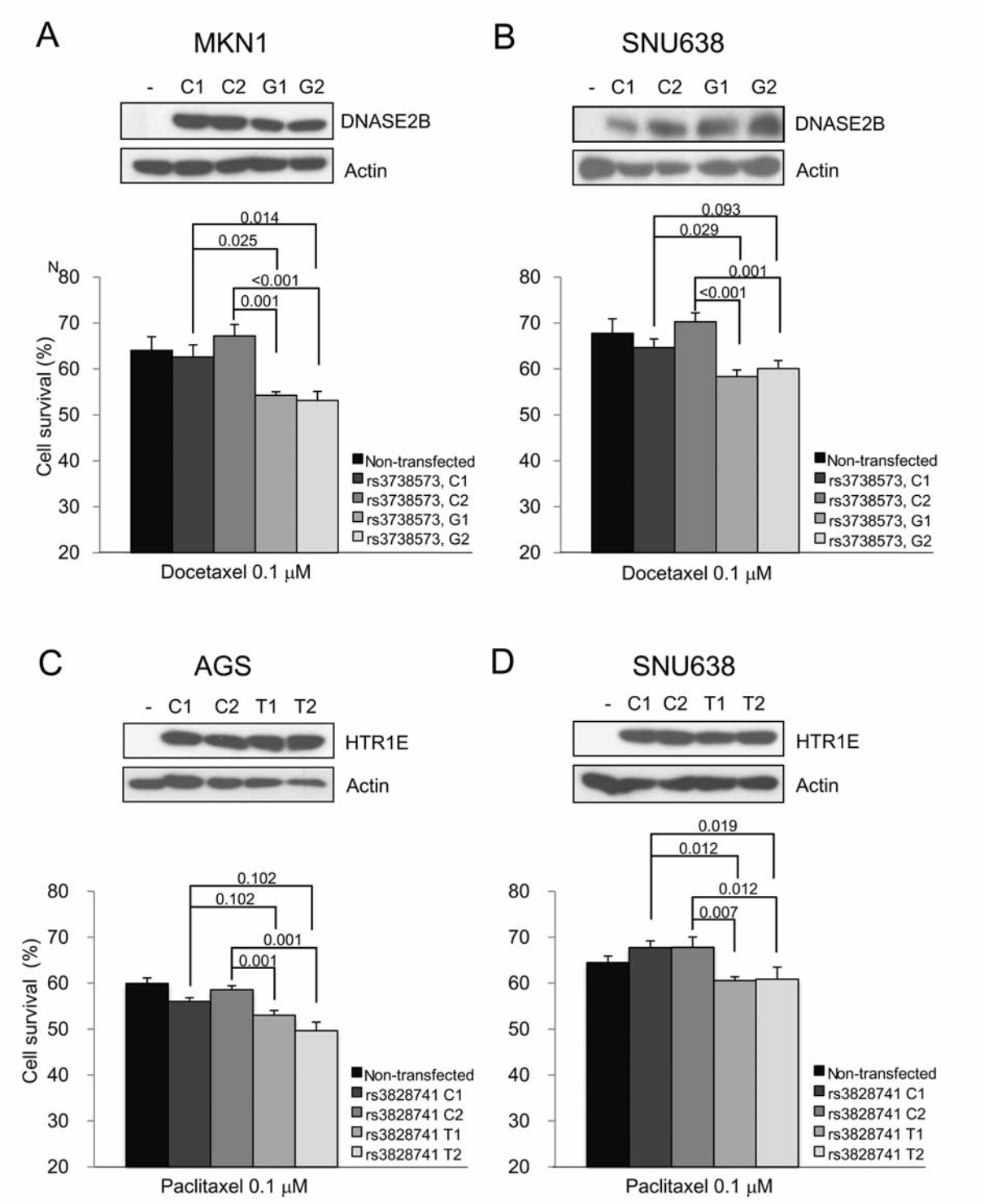

Figure 2. *Cells carrying the substitution allele of the SNP DNASE2B rs3738573 (G allele; G1 & G2 clones) are more sensitive to docetaxel-induced cell death than those with the reference allele (C allele; C1 & C2 clones) (A, B). MKN1 and SNU638 cell clones stably expressing the reference or substitution allele were incubated with docetaxel (0.1 μM) for 24 h. Cells carrying the substitution allele (T allele; T1 & T2 clones) of the SNP HTR1E rs3828741 are more sensitive to paclitaxel-induced cell death than those with the reference allele (C allele; C1 & C2 clones) (C, D). AGS and SNU638 cell clones stably expressing the reference or substitution allele were incubated with paclitaxel 0.1 μM for 24 h. Stable expression of respective alleles DNASE2B and HTR1E was confirmed by western blot using anti-myc antibody. Actin was used as a loading control. Drug response activity was determined by MTT assay. Values are means±S.E. of quadruplicates. p-values (Student's t-test) are displayed over the bars.*

Table III. *Demography, stage, and outcome of regimens in patients with gastric cancer receiving adjuvant chemotherapy and metastatic chemotherapy.*

Drugs	Mean age, years (range)	No. of patients			DFS/PFS	OS
		Gender M/F	Stage* I/II/III/IV	Response† CR/PR/SD/PD	Mean±SEM, months	
Adjuvant chemotherapy	54.3 (21-75)	92/40	13/37/51/31	NA	32.0±2.04	40.3±1.79
Oxaliplatin	55.6 (29-75)	18/11	4/6/12/7	1/12/6/10	5.5±0.9	13.3±1.9
Docetaxel	54.8 (21-75)	35/18	9/13/19/12	2/30/8/13	9.5±2.6	16.9±2.5
Paclitaxel	49.3 (36-64)	1/2	0/2/0/1	1/0/2/0	3.6±0.2	3.5±0.7

*According to the American Joint Committee on Cancer; AJCC Cancer Staging Manual, 6th ed. New York, NY, USA: Springer, 2001. †Tumor response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) criteria. DFS:Disease-free survival for adjuvant chemotherapy, PFS:progression-free survival for metastatic chemotherapy using oxaliplatin, docetaxel, and paclitaxel regimens. Objective responses (complete response [CR] + partial response [PR]) and disease control responses (CR + PR + stable disease [SD]) were 44.8% and 65.5%, respectively, in patients undergoing the oxaliplatin regimen, 60.4% and 75.5%, respectively, in patients undergoing the docetaxel regimen, and 33.3% and 100%, respectively, in patients undergoing the paclitaxel regimen. NA:Not available; OS: overall survival; PD: progressive disease.

carrying the reference allele (*G*). AGS and SNU638 cells expressing the substitution allele (*C*) of *HTRIE rs3828741* exhibited enhanced cell death in response to paclitaxel treatment as compared with those carrying the reference allele (T) (p=0.001–0.102 in AGS and p=0.007–0.019 in SNU638) (Figure 2C and D). Otherwise, no remarkable or consistent chemosensitivity to oxaliplatin for *ERCC6 rs2228529* and to docetaxel for *NLRP2 rs4306647* were observed (Figure 3). MYO9A-overexpressing cells could not be efficiently constructed, possibly due to molecular composition-based unstable transfection (295,791 bases). Instead, *MYO9A* siRNA was used to knock-down MYO9A expression in SNU638, AGS, SNU719, and MKN1 cells. MYO9A was efficiently down-regulated by specific siRNA, but exhibited no significant chemosensitivity change to oxaliplatin (Figure 4).

Identification of chemosensitive SNPs related to survival outcome in patients receiving adjuvant chemotherapy. The mean number of chemotherapy cycles administered to patients with inoperable GC was 4.8 (range, 2-11 cycles) for 29 patients treated with oxaliplatin regimens, 4.1 (range, 2-8 cycles) for 53 patients treated with docetaxel regimens, and 2.7 (range, 2-3 cycles) for three patients treated with paclitaxel regimens (Table II). Among the five SNPs found to be chemosensitive to any of these regimens, no specific genotype was associated concurrently with patients' responses, including adverse events, to these regimens (data not shown). In a multivariate analysis, disease-free survival periods (mean±SE) were significantly shorter in patients with genotypes with homozygous (*TT*) and heterozygous (*TC*) reference alleles than in patients with homozygous (*CC*) substitution alleles of *MYO9A rs2415129* (30±2 months *vs.* 43±4 months, p=0.024) (Figure 5).

Discussion

Most of the studies investigating SNPs have focused on susceptibility to specific diseases. Few studies, on the other hand, have explored the possibility of linking between SNPs and chemosensitivity. The aim of this study was to discover candidate SNP markers chemosensitive to targeted regimens. In the first step, genome-wide chemosensitive SNPs were filtered in association with individual tumor cell apoptosis to a specific regimen, using an *in vitro* chemosensitivity assay. We chose five nsSNPs: *ERCC6 rs2228529* and *MYO9A rs2415129*, which were chemosensitive to oxaliplatin, *DNASE2B rs3738573* and *NLRP2 rs4306647*, which were chemosensitive to docetaxel, and *HTRIE rs3828741*, which was chemosensitive to paclitaxel. In the next step, these candidate SNPs were assessed using a biological utility assay with cloned cells expressing specific alleles. In the cell viability assays, GC cells carrying the substitution allele (*C*) of *DNASE2B rs3738573* exhibited enhanced cell death in response to docetaxel as compared to those with the reference allele (*G*), and GC cells carrying the substitution (*C*) allele of *HTRIE rs3828741* were consistently chemosensitive to paclitaxel as compared to those with the reference allele (*T*). Clinical validation was not included in the current study as the number of patients with metastatic or recurrent GC was not sufficient to evaluate chemosensitivity.

The nsSNP *rs3738573* of *DNASE2B* leads to an amino acid change (Q3H; uncharged polar glutamine to positively charged histidine) in the signal sequence domain, which is destined to be either secreted or a part of membrane component. The signal sequence interacts with the signal recognition particle and directs the ribosome to the endoplasmic reticulum where co-translational insertion takes place. DNASE2B shares considerable sequence similarity,

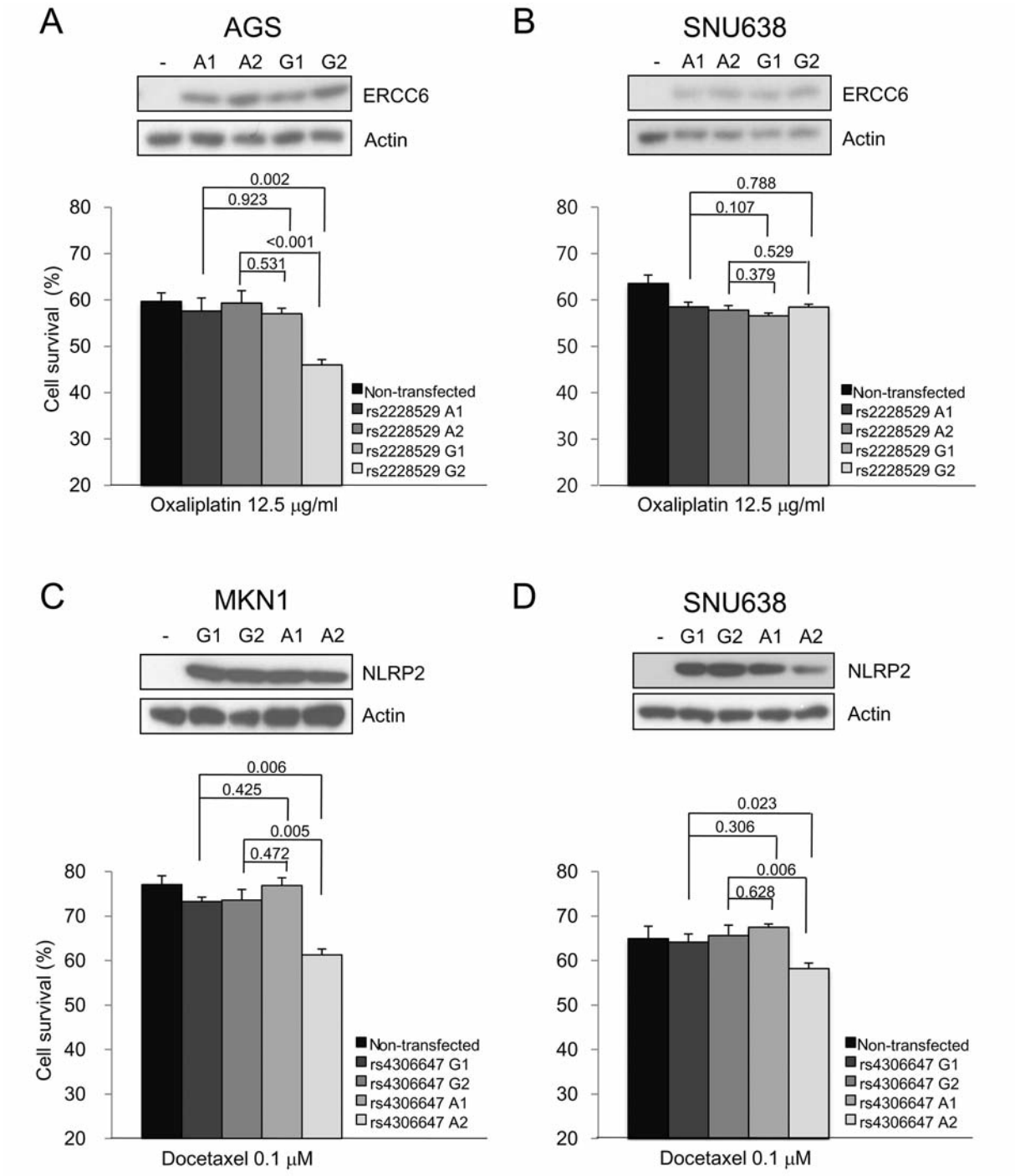

Figure 3. *Cell viability as measured by MTT assay in ERCC6 rs2228529-transfected AGS and SNU638 clones (A, B) and in NLRP2 rs4306647-transfected MKN1 and SNU638 clones (C, D) treated with oxaliplatin. Overexpression of each gene was detected by Western blot. Values are means±S.E. of quadruplicates. P-values (Student's t-test) are displayed over the bars.*

and is structurally related, to DNase II. DNase II is a well-characterized endonuclease that degrades DNA during apoptosis associated with intracellular acidification (17). Some reports have shown that DNASE2B participates in the degradation of nuclear DNA during lens cell differentiation (18). In contrast to the biological utility outcome, the median progression-free survival period was significantly shorter in those patients carrying homozygous substitution

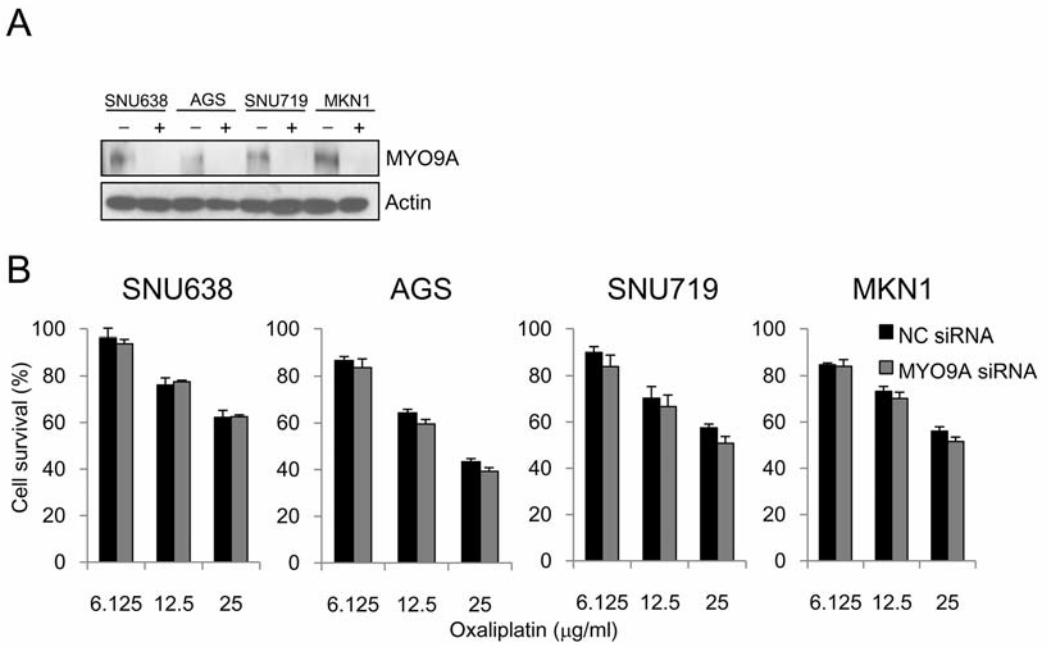

Figure 4. *Depletion of MYO9A in AGS, SNU638, MKN1, and SNU719 cells does not alter cell death induced by oxaliplatin. All four cell lines were transfected with negative control (NC; –) and MYO9A-targeting siRNAs (+). After 72 h, the cells were lysed, and expression of MYO9A was analyzed by immunoblotting total cell extracts (A). Actin was used as a loading control. At the end of the transfection, cells were treated with several doses of oxaliplatin for 24 h. Cell death was then determined by MTT assay (B). Values are means±S.E. of quadruplicates.*

alleles (*GG*) of *DNASE2B rs3738573* than in those patients carrying homozygous (*CC*) and heterozygous reference alleles (*CG*) treated with docetaxel regimens. These results suggest chemoresistance to docetaxel in patients with the *GG* allele, and a combination regimen might be required for these patients.

The nsSNP *rs3828741* of *HTRIE* leads to an amino acid change from the non-polar alanine to the uncharged polar threonine. The HTR1E is a biogenic hormone which functions as a neurotransmitter and mitogen. Several SNPs of HTR1E were reported in the central nervous system among males who attempted suicide, as well as for patients with chronic fatigue syndrome (19, 20). The activity of this receptor is mediated by G Proteins that inhibit adenylate cyclase activity. G Proteins of the Rho family are known to affect members of the BCL2 family in a different manner, however. Rho/ROCK mainly suppresses the pro-apoptotic protein BAX and enhances expression of the antiapoptotic proteins BCL_{XL} and BCL2 (21, 22). Although the current study did not assess functional association of HTR1E in terms of the respective apoptotic cascade, *HTRIE rs3828741* might be associated with proapoptotic activation with paclitaxel chemosensitivity.

In the current study, *MYO9A* knock-down was used to test the chemosensitivity of endogenous reference and substitution allele *MYO9A rs2415129*-expressing cells. MYO9A deficiency did not cause differences in chemosensitivity to oxaliplatin. The recurrence rate in patients with *MYO9A rs2415129* was high and the disease-free survival period was short in patients with homozygous (TT) and heterozygous reference alleles (TC). It has been reported that MYO9A is an actin-dependent motor molecule with a Rho GTPase-activating (GAP) domain, that is expressed in maturing ependymal epithelial cells, and that its absence leads to impaired maturation of ependymal cells (23, 24). Discrepancies between biological utility assay results and clinical outcomes may be due to the molecular complexity of the MYO9A superfamily, which comprises more than 30 classes of actin-based molecular motors. Unfortunately, we were unable to find any remarkable or consistent chemosensitivity on the part of *ERCC6 rs2228529* to oxaliplatin, nor was *NLRP2 rs4306647* chemosensitive to docetaxel. These are therefore not likely to be useful as potent chemosensitivity markers for the indicated regimens.

The two novel markers of the current study, *DNASE2B rs3738573* and *HTR1E rs3828741*, may improve the prediction of GC patient sensitivity to docetaxel and paclitaxel, respectively. These candidate SNPs still require further validation in larger clinical cohorts to ascertain whether they truly hold predicting capabilities over patient's responsiveness to these regimens.

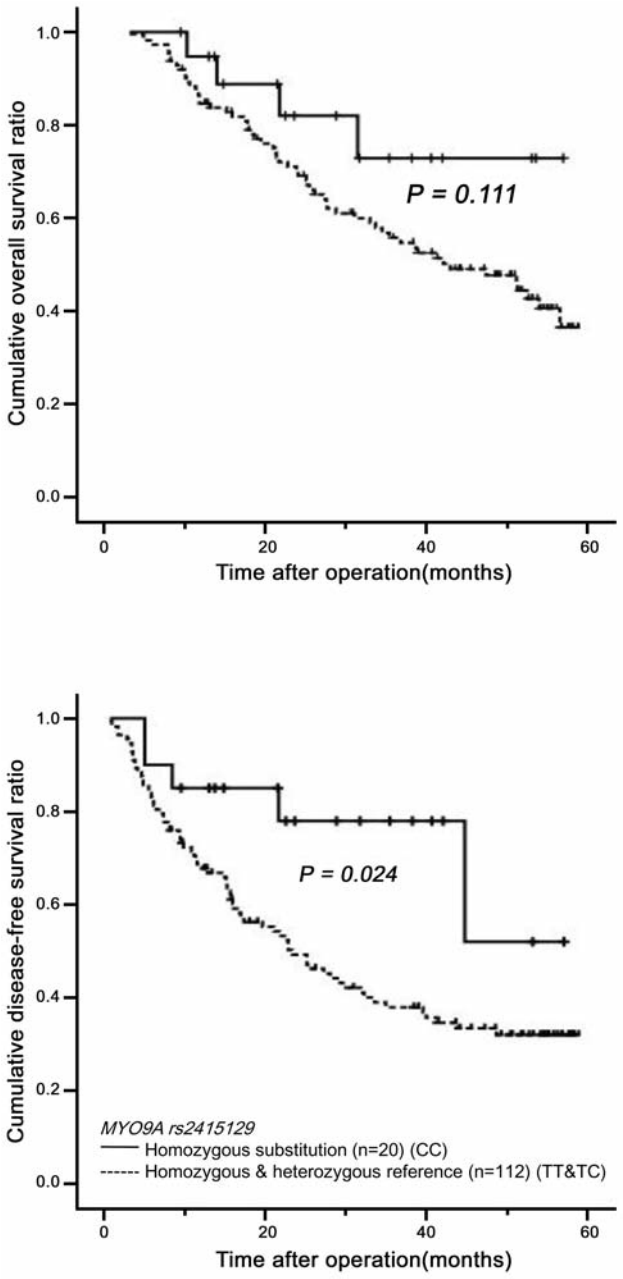

Figure 5. *Overall survival and disease-free survival periods (mean±SEM) according to MYO9A rs2415129 genotype in gastric cancer patients treated with adjuvant chemotherapy.*

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