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Human Molecular Genetics, 2012, Vol. 21, No. 7 **1665–1672**
doi:10.1093/hmg/ddr597
Advance Access published on December 16, 2011

A genome-wide association study identifies locus at 10q22 associated with clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients in Japanese

Kazuma Kiyotani[1], Taisei Mushiroda[1], Tatsuhiko Tsunoda[2], Takashi Morizono[2], Naoya Hosono[3], Michiaki Kubo[3], Yusuke Tanigawara[4], Chiyo K. Imamura[4], David A. Flockhart[5], Fuminori Aki[6], Koichi Hirata[7], Yuichi Takatsuka[8], Minoru Okazaki[9], Shozo Ohsumi[10], Takashi Yamakawa[11], Mitsunori Sasa[12], Yusuke Nakamura[1,13,*] and Hitoshi Zembutsu[13]

[1]Laboratory for Pharmacogenetics, [2]Laboratory for Medical Informatics and [3]Laboratory for Genotyping Development, RIKEN Center for Genomic Medicine, Yokohama 230-0045, Japan, [4]Department of Clinical Pharmacokinetics and Pharmacodynamics, School of Medicine, Keio University, Tokyo 160-8582, Japan, [5]Division of Clinical Pharmacology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA, [6]Department of Surgery, Itoh Surgery and Breast Clinic, Kochi 780-0085, Japan, [7]1st Department of Surgery, Sapporo Medical University, Sapporo 060-8543, Japan, [8]Department of Breast Surgery, Kansai Rosai Hospital, Hyogo 060-0006, Japan, [9]Department of Surgery, Sapporo Breast Surgical Clinic, Sapporo 060-0006, Japan, [10]Department of Breast Oncology, Shikoku Cancer Center, Ehime 791-0280, Japan, [11]Department of Surgery, Yamakawa Breast Clinic, Kochi 780-8081, Japan, [12]Department of Surgery, Tokushima Breast Care Clinic, Tokushima 770-0052, Japan and [13]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan

Received September 24, 2011; Revised November 26, 2011; Accepted December 12, 2011

Although many association studies of polymorphisms in candidate genes with the clinical outcomes of breast cancer patients receiving adjuvant tamoxifen therapy have been reported, genetic factors determining individual response to tamoxifen are not fully understood. To identify genetic polymorphisms associated with clinical outcomes of patients with tamoxifen treatment, we conducted a genome-wide association study (GWAS). We studied 462 Japanese patients with hormone receptor-positive, invasive breast cancer receiving adjuvant tamoxifen therapy. Of them, 240 patients were analyzed by genome-wide genotyping using the Illumina Human610-Quad BeadChips, and two independent sets of 105 and 117 cases were used for replication studies. In the GWAS, we detected significant associations with recurrence-free survival at 15 single-nucleotide polymorphisms (SNPs) on nine chromosomal loci (1p31, 1q41, 5q33, 7p11, 10q22, 12q13, 13q22, 18q12 and 19p13) that satisfied a genome-wide significant threshold (log-rank $P = 2.87 \times 10^{-9}$–9.41×10^{-8}). Among them, rs10509373 in *C10orf11* gene on 10q22 was significantly associated with recurrence-free survival in the replication study (log-rank $P = 2.02 \times 10^{-4}$) and a combined analysis indicated a strong association of this SNP with recurrence-free survival in breast cancer patients treated with tamoxifen (log-rank $P = 1.26 \times 10^{-10}$). Hazard ratio per C allele of rs10509373 was 4.51 [95% confidence interval (CI), 2.72–7.51; $P = 6.29 \times 10^{-9}$]. In a combined analysis of rs10509373 genotype with previously identified genetic makers, *CYP2D6* and *ABCC2*, the number of risk alleles of these three genes had cumulative effects on recurrence-free survival among 345 patients receiving tamoxifen monotherapy (log-rank $P = 2.28 \times 10^{-12}$).

*To whom correspondence should be addressed at: Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. Tel: +81 354495372; Fax: +81 354495433; Email: yusuke@ims.u-tokyo.ac.jp

In conclusion, we identified a novel locus associated with recurrence-free survival in Japanese breast cancer patients receiving adjuvant tamoxifen therapy.

INTRODUCTION

Tamoxifen has been the gold standard for endocrine treatment of patients with estrogen receptor (ER)-positive breast cancers. However, 30–50% of patients with adjuvant tamoxifen therapy experience relapse and subsequently die of the disease (1,2), indicating individual differences in responsiveness to tamoxifen.

Tamoxifen is metabolized to the highly active metabolites, 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyltamoxifen (endoxifen). It is reported that these metabolites are the active therapeutic moieties, having 100-fold greater affinity to ER and 30–100-fold greater potency in suppressing estrogen-dependent cell proliferation than those of tamoxifen (3–5). Inter-individual differences in the formation and elimination of these active metabolites could be one of the important factors affecting variability in the response to tamoxifen. Most previous reports focused on the genes involved in the pharmacokinetics of tamoxifen and its metabolites seek genetic variations which determine the individual response to tamoxifen. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6), which is the key enzyme responsible for the generation of endoxifen, is thought to be the most promising predictor of plasma concentration of endoxifen and clinical efficacy of tamoxifen in breast cancer patients (6–14). Schroth *et al.* (15) recently reported outstanding results in 1325 breast cancer patients, providing sufficiently powered evidence for an association between *CYP2D6* genotype and clinical outcomes in patients treated with tamoxifen in the adjuvant setting. Besides *CYP2D6*, several genes, such as *CYP2C19*, *CYP3A5*, sulfotransferase 1A1 (*SULT1A1*), UDP-glucuronosyltransferase 2B15 (*UGT2B15*) and ATP-binding cassette sub-family C member 2 (*ABCC2*), were reported as possible candidates associated with the clinical outcomes of tamoxifen therapy (7,10,14,16); however, associations of these candidate genes have not yet been sufficiently validated. Therefore, individual differences in responsiveness to tamoxifen still remain, even if the effects of genetic polymorphisms of *CYP2D6* were considered, suggesting the existence of other genetic factor(s).

In this study, to identify responsible loci for the clinical outcomes of tamoxifen therapy, we performed a genome-wide association study (GWAS) by genotyping over 610 000 single-nucleotide polymorphisms (SNPs) and identified the novel locus containing chromosome 10 open-reading frame 11 (*C10orf11*) gene associated with recurrence-free survival in the breast cancer patients treated with tamoxifen.

RESULTS

Patient characteristics

We recruited 462 Japanese patients with breast cancer receiving adjuvant tamoxifen therapy. Table 1 summarizes the characteristics of all of these patients who were pathologically diagnosed to have a hormone receptor-positive, invasive breast cancer. Their median age at the time of surgery was 51 years old (range, 27–84 years), the median follow-up period was 6.8 years (range, 0.6–23.5 years) and the median tamoxifen treatment period was 4.8 years (range, 0.6–6.3 years). Among the characteristics listed in Table 1, tumor size and nodal status showed significant associations with the recurrence-free survival [$P = 0.000215$; hazard ratio (HR), 1.71; 95% confidence interval (CI), 1.29–2.27; and $P = 0.0138$; HR, 1.83; 95% CI, 1.14–3.09, respectively] in the Cox proportional hazards analysis, whereas the other factors were not associated with recurrence-free survival (Supplementary Material, Table S1).

Genome-wide association and replication studies

We conducted a GWAS of recurrence-free survival of 240 Japanese patients with breast cancer who received tamoxifen monotherapy using Illumina Human610-Quad BeadChips. After the standard quality control, association analysis was performed for 470 796 SNPs by the trend log-rank test. We generated a quantile–quantile plot (Supplementary Material, Fig. S1) and obtained the genomic control inflation factor (λ_{GC}) of 1.023, indicating a low possibility of false-positive associations resulting from population stratification. We detected significant associations with recurrence-free survival at 15 SNPs in nine genetic regions (1p31, 1q41, 5q33, 7p11, 10q22, 12q13, 13q22, 18q12 and 19p13) that satisfied a genome-wide significant threshold of $P < 1.06 \times 10^{-7}$ (Fig. 1). To further validate the results of GWAS, we carried out a replication study using an independent 105 breast cancer patients. We genotyped 9 of the 15 SNPs because 6 of them were highly linked to another SNP ($r^2 > 0.80$; Supplementary Material, Table S2). We found that rs10509373 in *C10orf11* gene on 10q22 was significantly associated with recurrence-free survival in the replication stage (log-rank $P = 4.18 \times 10^{-4}$; Table 2 and Fig. 2). The associations of the other SNPs were not replicated (Supplementary Material, Table S2). Furthermore, a combined result of the GWAS and first replication study strongly suggested an association of this locus with recurrence-free survival in breast cancer patients treated with tamoxifen (log-rank $P = 2.19 \times 10^{-10}$). We also genotyped rs10509373 using additional 117 samples, which include the patients receiving tamoxifen after chemotherapy and observed a significant association (log-rank $P = 1.86 \times 10^{-2}$). A combined *P*-value of all samples was 2.19×10^{-10}, suggesting the significant association with recurrence-free survival in breast cancer patients treated with tamoxifen (Fig. 2 and Supplementary Material, Table S3). In Cox proportional hazards analysis, *C10orf11* genotype (rs10509373) was an independent indicator of the recurrence-free survival after adjustment for tumor size and nodal status ($P = 6.28 \times 10^{-8}$; Table 2). The adjusted HRs of rs10509373

Table 1. Characteristics of patients

Characteristic	No. of patients (%) GWAS	First replication	Second replication	Total
No.	240	105	117	462
Age at surgery (years)				
Median	51	50	48	51
Range	31–83	35–84	27–71	27–84
Follow-up (years)				
Median	7.2	5.2	6.2	6.8
Range	1.1–23.5	0.6–19.3	1.0–15.5	0.6–23.5
Tamoxifen treatment (years)				
Median	4.9	4.0	4.7	4.8
Range	1.0–6.1	0.6–6.0	0.7–6.3	0.6–6.3
Menopausal status				
Pre-menopause	101 (42.1)	40 (38.1)	75 (64.1)	216 (46.8)
Post-menopause	131 (54.6)	40 (38.1)	35 (29.9)	206 (44.6)
Unknown	8 (3.3)	25 (23.8)	7 (6.0)	40 (8.7)
Tumor size (cm)				
≤2	138 (57.5)	57 (54.3)	48 (41.0)	243 (52.6)
2.1–5	92 (38.3)	34 (32.4)	56 (47.9)	182 (39.4)
>5	1 (0.4)	2 (1.9)	12 (10.3)	15 (3.2)
Unknown	9 (3.8)	12 (11.4)	1 (0.9)	22 (4.8)
Nodal status				
Negative	193 (80.4)	88 (83.8)	74 (63.2)	355 (76.8)
Positive	44 (18.3)	13 (12.4)	41 (35.0)	98 (21.2)
Unknown	3 (1.3)	4 (3.8)	2 (1.7)	9 (1.9)
ER status				
Positive	173 (72.1)	87 (82.9)	98 (83.8)	358 (77.5)
Negative	24 (10.0)	2 (1.9)	12 (10.3)	38 (8.2)
Unknown	43 (17.9)	16 (15.2)	7 (6.0)	66 (14.3)
PR status				
Positive	167 (69.6)	77 (73.3)	87 (74.4)	331 (71.6)
Negative	28 (11.7)	11 (10.5)	22 (18.8)	61 (13.2)
Unknown	45 (18.8)	17 (16.2)	8 (6.8)	70 (15.2)
Her-2				
Positive[a]	3 (1.3)	5 (4.8)	6 (5.1)	14 (3.0)
Negative	82 (34.2)	28 (26.7)	60 (51.3)	170 (36.8)
Unknown	155 (64.6)	72 (68.6)	51 (43.6)	278 (60.2)
Treatment				
Tamoxifen alone	240 (100.0)	105 (100.0)	0 (0.0)	345 (76.7)
Tamoxifen + AC or EC	0 (0.0)	0 (0.0)	41 (35.0)	41 (8.9)
Tamoxifen + CMF	0 (0.0)	0 (0.0)	32 (27.4)	32 (6.9)
Tamoxifen + other chemotherapies	0 (0.0)	0 (0.0)	44 (37.6)	44 (9.5)
Events				
No event	210 (87.5)	89 (84.8)	98 (55.4)	397 (85.9)
Locoregional events	9 (3.8)	0 (0.0)	0 (0.0)	9 (1.9)
Distant metastasis events	12 (5.0)	15 (14.3)	17 (9.6)	44 (9.5)
Contralateral breast events	9 (3.8)	1 (1.0)	2 (1.1)	12 (2.6)

AC, adriamycin + cyclophosphamide; EC, epirubicin + cyclophosphamide; CMF, cyclophosphamide + methotrexate + 5-fluorouracil.
[a]Score of 3+ in immunohistochemistry.

C allele was 4.51 (95% CI, 2.72–7.51), suggesting that C allele was a risk allele for breast cancer recurrence.

To further identify SNPs associated with recurrence-free survival in patients receiving tamoxifen therapy, we genotyped 130 tag SNPs for fine mapping on chromosome 10q22 (Chr. 10: 77.35–78.70 Mb) where the most significant association with the recurrence-free survival was observed (Fig. 3 and Supplementary Material, Table S4). Although no SNPs showed a stronger association than the landmark SNP, rs10509373, fine mapping of this region indicated that a 172-kb linkage disequilibrium (LD) block (77.67–77.84 Mb) including *C10orf11* was likely to contain the genetic variant(s) associated with recurrence-free survival in patients receiving tamoxifen therapy.

Combination analysis with previously identified gene loci

As we previously identified significant associations of *CYP2D6* and *ABCC2* rs3740065 genotypes with recurrence-free survival in patients treated with tamoxifen among the patients receiving tamoxifen monotherapy (Supplementary Material, Table S5) (7), we investigated a combined effect of *C10orf11* genotype in addition to *CYP2D6* and *ABCC2* genotypes on the recurrence-free survival by classifying the 345

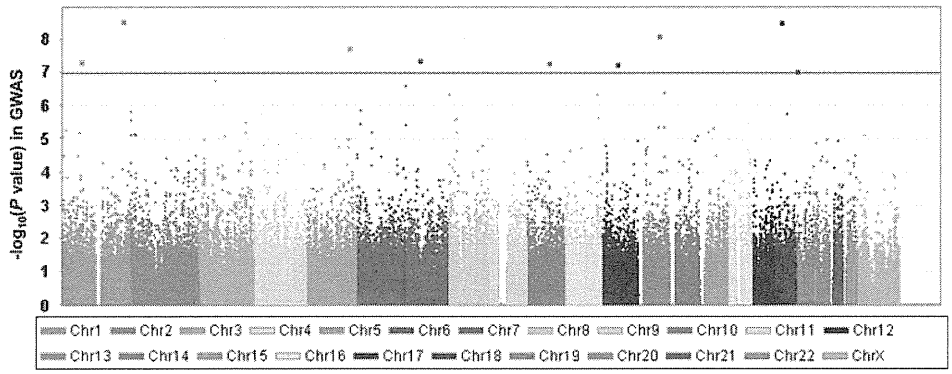

Figure 1. Results of the GWAS. Manhattan plot showing the $-\log_{10}$-transformed *P*-value of SNPs in the GWAS for 240 Japanese patients with breast cancer receiving tamoxifen monotherapy. The red line indicates the genome-wide significance level ($P = 1.06 \times 10^{-7}$).

Table 2. Association analysis of rs10509373 in C10orf11 with recurrence-free survival in breast cancer patients receiving tamoxifen therapy

SNP	Chr	Chr location[a]	Allele (risk)	Study set	Risk allele frequency		Log-rank *P*	Univariate		Multivariate[b]	
					Event	No event		HR (95% CI)[c]	*P*-value	HR (95% CI)[c]	*P* value
rs10509373	10	77827578	T/C (C)	GWAS	0.117	0.021	5.19×10^{-8}	7.70 (3.25–18.22)	3.41×10^{-6}	9.64 (3.85–24.12)	1.29×10^{-6}
				First replication	0.094	0.017	4.18×10^{-4}	7.93 (2.06–30.58)	2.65×10^{-3}	5.96 (1.49–23.86)	1.17×10^{-2}
				GWAS + first replication	0.109	0.020	2.19×10^{-10}	7.34 (3.58–14.98)	4.90×10^{-8}	9.18 (1.39–22.62)	6.07×10^{-9}
				Second replication	0.132	0.036	1.86×10^{-2}	2.72 (1.13–6.53)	2.53×10^{-2}	2.92 (1.14–7.49)	2.55×10^{-2}
				Combined replications	0.114	0.027	2.02×10^{-4}	3.21 (1.65–6.22)	5.67×10^{-4}	3.20 (1.53–6.69)	1.97×10^{-3}
				Combined all	0.115	0.024	1.26×10^{-10}	4.51 (2.72–7.51)	6.29×10^{-9}	4.53 (2.62–7.83)	6.28×10^{-8}

Chr, chromosome; CI, confidence interval; GWAS, genome-wide association study.
[a]Based on NCBI 36 genome assembly.
[b]Adjusted for tumor size and nodal status.
[c]HR per one allele.

patients into 6 groups (0, 1, 2, 3, 4 and 5 risk allele groups) according to the number of risk alleles of the three genes. Kaplan–Meier analysis revealed the number of risk alleles of these three genes to have cumulative effects on recurrence-free survival (log-rank $P = 2.24 \times 10^{-12}$; Fig. 4). In the Cox proportional hazards analysis of 345 patients, the *CYP2D6* and *ABCC2* genotypes showed similar associations with recurrence-free survival to those in previous analysis of 282 patients ($P = 1.99 \times 10^{-4}$ and 8.51×10^{-4}, respectively; Supplementary Material, Table S6) (7). In the multivariate analysis, rs10509373 in *C10orf11* still showed a significant association even after adjustment of *CYP2D6* and *ABCC2* genotypes in addition to tumor size and nodal status ($P = 4.74 \times 10^{-7}$; Supplementary Material, Table S6), indicating that rs10509373 is an independent risk factor of breast cancer recurrence. Furthermore, combined analysis of *C10orf11*, *CYP2D6* and *ABCC2* revealed that genotypes of the three genes have cumulative effects on recurrence-free survival ($P = 2.28 \times 10^{-12}$), and adjusted HR for risk of recurrence computed for patients carrying three or more risk alleles increased from 6.51-fold (three risk alleles) to 119.51-fold (five risk alleles) compared with those carrying one risk allele (Supplementary Material, Table S6). In the subgroup analysis of menopausal status, we identified the significant associations in both subgroups of pre- and postmenopausal patients, although the stronger association was observed in postmenopausal group than in the premenopausal patients (Supplementary Material, Table S7).

DISCUSSION

This study represents the first GWAS which attempts to identify genetic variants associated with clinical outcomes of tamoxifen therapy and successfully revealed that a marker SNP, rs10509373, on chromosome 10q22 was significantly associated with recurrence-free survival in 462 Japanese patients with breast cancer receiving tamoxifen monotherapy. Furthermore, combined analysis of this SNP with previously identified predictors, *CYP2D6* and *ABCC2*, revealed that the number of risk alleles of the three genes have cumulative effects on recurrence-free survival in tamoxifen-treated breast cancer patients.

The most significantly associated SNP in this study, rs10509373 (combined $P = 1.26 \times 10^{-10}$), is located in a 172-kb LD block which contains the 3′ region of *C10orf11*

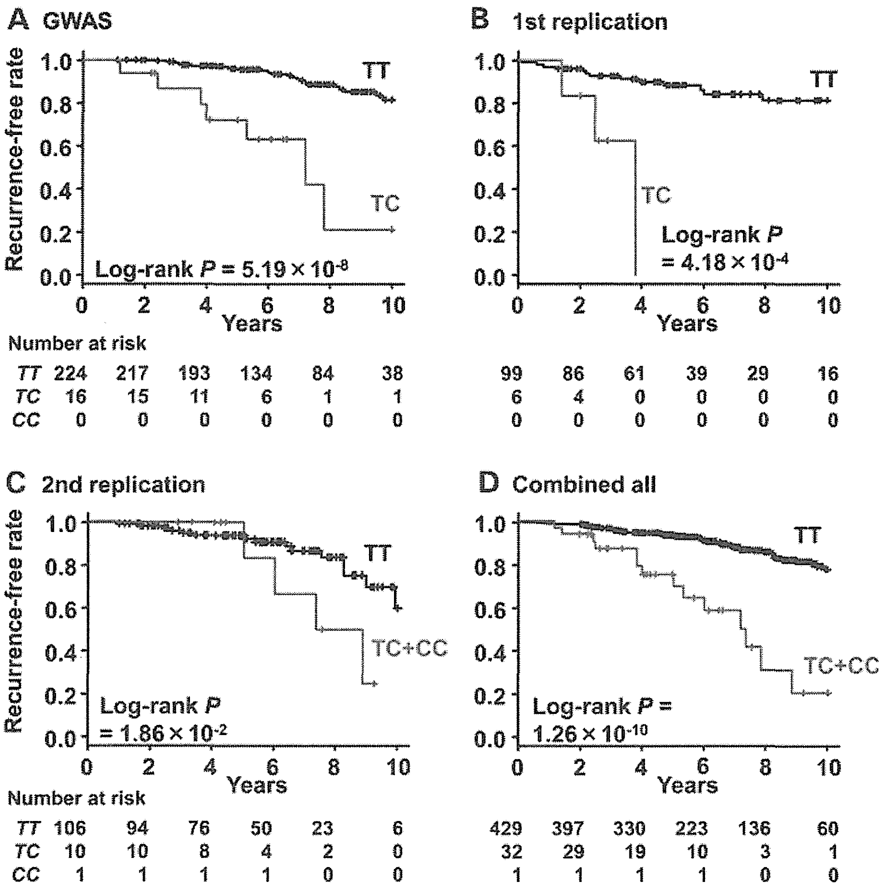

Figure 2. Kaplan–Meier estimates of recurrence-free survival for *C10orf11* rs10509373 genotype in 240 patients genotyped in the genome-wide association study (**A**), in 105 patients genotyped in the first replication study (**B**), in 117 patients genotyped in the second replication study (**C**) and in 462 patients in the combination analysis (**D**).

gene. The fine mapping of this region indicated that a peak association was located in intron 5 of *C10orf11* gene, suggesting that *C10orf11* is likely to be a causative gene to determine the clinical outcomes of breast cancer patients treated with tamoxifen. Because no associated SNPs were found in exon region by re-sequencing of *C10orf11* (Supplementary Material, Table S8), a genetic variant(s) within this LD block might alter C10orf11 transcriptional activity. C10orf11 protein, comprising 198 amino acids, is predicted to contain leucine-rich repeat domain and to have the capacity of protein binding in the SMART database (http://smart.embl-heidelberg.de/), although no report has clarified its function. It is reported that *C10orf11* region overlaps with ultraconserved elements (UCEs), perfectly constrained elements between the human, mouse and rat genomes (17–19). Their functional roles have not been completely elucidated yet; however, UCEs are thought to possess some essential functional properties. It is reported that paired box 2, encoded by *PAX2* gene on 10q24, which regulates *ERBB2* transcription and is involved in acquiring tamoxifen resistance (20), and special AT-rich sequence-binding protein-1 encoded by *SATB1* gene on 3p23, which delineates epigenetic modification and is associated with breast tumor growth and metastasis (21), are located in the UCE-rich regions (17). We further examined the association of pharmacokinetic data with *C10orf11* genotype; however, no significant difference was observed between *C10orf11* genotype and plasma levels of endoxifen and 4-hydroxytamoxifen in 98 breast cancer patients taking 20 mg/day tamoxifen (Supplementary Material, Fig. S2). According to our gene expression database (in-house), the C10orf11 transcript is expressed in breast cancer cells in clinical tissues. We examined the effects of rs10509373 on the expression levels of C10orf11 in peripheral blood mononuclear cells and brain using a public database SNPExpress (22). However, no significant associations were observed ($P = 0.63$ and 0.93, respectively) possibly because of the quite low expression of C10orf11 in these tissues. The association of *C10orf11* genotype was significant in the second replication samples, which include the patients receiving tamoxifen alone after chemotherapy (Table 2); however, neither *CYP2D6* nor *ABCC2* genotypes were significantly associated with clinical outcomes in the second replication samples as shown in our previous study (Supplementary Material, Table S5) (23). These lines of evidence might suggest that C10orf11 is involved in acquiring tamoxifen resistance or determining the characteristics of breast cancer, although further functional analysis will be needed to clarify the biological mechanisms which could have effects on the

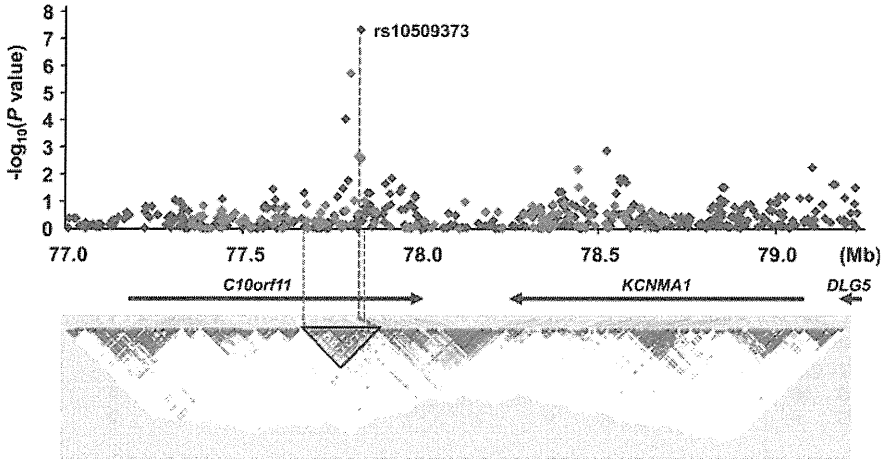

Figure 3. Association mapping and LD map of 10q22. Blue diamond dots represent $-\log_{10}$-transformed *P*-values of SNPs genotyped by Illumina Human610-Quad BeadChips in the GWAS, and red diamond dots show $-\log_{10}$-transformed *P*-values of the SNPs of fine mapping. Arrows indicate the position of known genes. The *D′*-based LD map (MAF $\geq$ 0.10) is drawn using genotype data of 240 patients with breast cancer enrolled in the GWAS.

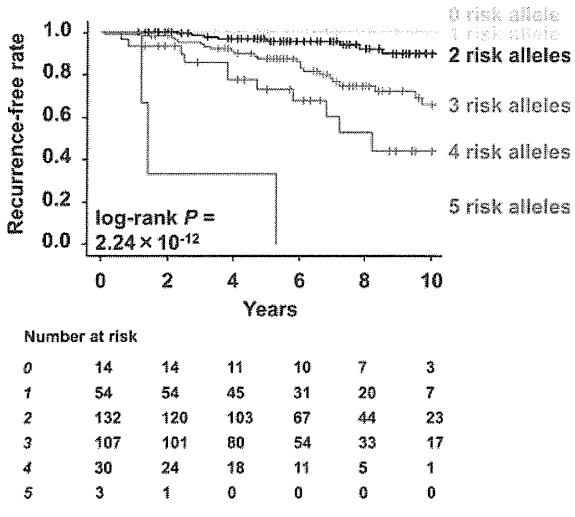

Figure 4. Combined effects of *C10orf11*, *CYP2D6* and *ABCC2* genotypes on clinical outcomes of tamoxifen monotherapy. Kaplan–Meier estimates of recurrence-free survival rate for combined effects of *C10orf11* rs10509373, *CYP2D6* and *ABCC2* rs3740065 genotypes. The 345 patients receiving tamoxifen monotherapy were classified into six groups (0, 1, 2, 3, 4 and 5 risk allele groups) based on the number of risk alleles of these three genes.

clinical outcomes of breast cancer patients receiving tamoxifen therapy.

Several research groups focused on the genes involved in the pharmacokinetics of tamoxifen or its metabolites have investigated genetic variants of *CYP2C19*, *CYP3A5*, *UGT2B15* and *SULT1A1* as candidate genes associated with clinical outcomes of tamoxifen therapy (7,10,14,16). In our GWAS, no SNP in these candidate genes showed significant association with recurrence-free survival in patients treated with tamoxifen (log-rank $P = 3.14 \times 10^{-2}$–9.90×10^{-1}). According to the previous reports, the effect sizes of the above candidate genes were not so large, indicating that the sample size used in our study might not have enough power to detect associations of the SNPs with the tamoxifen efficacy.

Another group hypothesized that non-genomic steroid signaling and cross-talk with growth factor signaling pathways may contribute to the clinical outcomes of the patients treated with tamoxifen and reported that *TC21* promoter polymorphism was significantly associated with an unfavorable tamoxifen treatment outcome; however, no significant association was observed at SNPs in *TC21* gene in our GWAS (log-rank $P = 1.19 \times 10^{-1}$–9.98×10^{-1}) (24). The *P*-values of the SNPs in the *ESR1*, *ESR2* and *PGR* genes, which encode ERα, ERβ and progesterone receptor (PR), respectively, ranged from 1.33×10^{-2} to 9.88×10^{-1}, indicating no significant association.

In conclusion, our GWAS using 462 Japanese patients with breast cancer identified a new locus, containing the *C10orf11* gene, associated with the clinical outcomes of breast cancer patients treated with tamoxifen. These findings provide new insights into personalized selection of hormonal therapy for the patients with breast cancer. However, large-scale replication study and further functional analysis are required to verify our results and to clarify their biological mechanisms which have effects on the clinical outcomes of patients receiving tamoxifen therapy.

MATERIALS AND METHODS

Patients

A total of 462 patients with primary breast cancer (including the 282 patients reported previously (6,7)) were recruited at Shikoku-*10 collaborative group (Tokushima Breast Care Clinic, Yamakawa Breast Clinic, Shikoku Cancer Center, Kochi University Hospital, and Itoh Surgery and Breast Clinic), Kansai Rosai Hospital, Sapporo Breast Surgical Clinic and Sapporo Medical University Hospital. Of them, 240 patients who were recruited from September 2007 to September 2008 were used for a GWAS analysis, and the remaining 105 patients who were recruited from October 2008 to January 2010 were analyzed in a first replication study. All patients were Japanese women pathologically diagnosed with

ER-positive and/or PR-positive, invasive breast cancer who received adjuvant tamoxifen monotherapy without any other treatments after surgical treatment between 1986 and 2008. In addition, we analyzed 117 patients who had been treated with tamoxifen monotherapy after receiving chemotherapy as the second replication set (23). Data on primary breast cancer diagnosis or recurrence were confirmed from patients' medical record. Patients without recurrence were censored at the date of the last consultation. Recurrence-free survival time was defined as the time from surgical treatment to diagnosis of the recurrence of a breast cancer (locoregional, distant metastasis and contralateral breast events) or death. Patients received tamoxifen 20 mg/day for 5 years; tamoxifen was stopped at the time a recurrence was identified. ER and PR status were evaluated by enzyme immunoassay or immunohistochemistry. The cut-off for human epidermal growth factor receptor 2 overexpression was defined as 3+ immunohistochemical staining (25). Nodal status was determined according to the International Union against Cancer tumor-node-metastasis classification. This study was approved by the Institutional Review Board in the Institute of Medical Science, The University of Tokyo (Tokyo, Japan), and written informed consent was obtained from all patients.

Genotyping and quality control

Genomic DNA was extracted from peripheral blood ($n = 424$) or frozen breast tissue ($n = 38$) using Qiagen DNA Extraction Kit (Qiagen, Valencia, CA, USA). In the GWAS, 240 patients were genotyped using the Illumina Human610-Quad BeadChip (Illumina, San Diego, CA, USA). Quality control of SNPs was achieved by excluding SNPs with low call rate (<99%) and SNPs with Hardy–Weinberg equilibrium P-value $< 1.0 \times 10^{-6}$. SNPs with a minor allele frequency (MAF) <0.01 were also excluded from further analysis. A total of 470 796 SNPs passed the filters and were further analyzed. We used multiplex polymerase chain reaction-based Invader Assay (Third Wave Technologies, Madison, WI, USA) on ABI PRISM 7900HT (Applied Biosystems, Foster City, CA, USA) for the replication study and fine mapping (26). For fine mapping, tag SNPs were selected from the HapMap phase II JPT data (http://www.hapmap.org/) (27) with the following criteria: a pairwise $r^2 > 0.80$ and an MAF ≥ 0.01 using Haploview software (28).

Genotyping of *CYP2D6* and *ABCC2* rs3740065 was performed using real-time Invader (Third Wave Technologies) and TaqMan assays (Applied Biosystems) as described previously (7,29,30). To evaluate the effects of *CYP2D6* alleles, we defined all of the decreased and null alleles (including **4*, **5*, **10*, **14*, **21* and **41*, and gene-duplication alleles, **10–*10* and **36–*36*) as allele '*V*', and alleles of **1* and duplicated **1–*1* as allele '*wt*' as described previously (7).

Statistical analysis

Recurrence-free survival curves were estimated using the Kaplan–Meier method. Statistical significance of a relationship between clinical outcomes and genetic polymorphism was assessed by the trend log-rank test. The value of λ_{GC} was calculated from the median of the trend log-rank test statistics (31). Cox proportional hazards analysis was used to identify significant prognostic clinical factors and to test for an independent contribution of genetic factors to recurrence-free survival. To examine potential confounding, age was treated as a continuous variable, tumor size was treated as an ordinal variable, and the other covariates were treated as categorical variables. Genotypes were analyzed by assigning an ordinal score to each genotype (0 for homozygous non-risk alleles, 1 for heterozygous risk alleles and 2 for homozygous risk alleles). Combination effects were investigated by adding up the number of risk alleles of *CYP2D6*, *ABCC2* and *C10orf11* genes. All polymorphisms evaluated in this study were tested for deviation from Hardy–Weinberg equilibrium with the use of a χ^2-test. Statistical tests provided two-sided P-values, and a significance level of $P < 0.05$ was used. We used a significance level of $P < 1.06 \times 10^{-7}$ (0.05 of 470 796) in the GWAS and 5.56×10^{-3} (0.05 of 9) in the replication study to adjust multiple testing by the strict Bonferroni correction. Statistical analyses were carried out using SPSS (version 17.0, SPSS, Chicago, IL, USA) and the R statistical environment version 2.9.2 (http://www.r-project.org/).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENTS

We express our gratitude to all the study participants. We thank Yuka Kikuchi, Aiko Ohno, Shoko Higuchi and Kumi Matsuda for technical assistance. We thank all other members and staff for their contribution to the sample collection and the completion of our study.

Conflict of Interest statement. None declared.

FUNDING

This work was supported mainly by a Grant-in-Aid for Leading Project of Ministry of Education, Culture, Sports, Science and Technology of Japan (to Y.N.). This work was also supported in part by Grant-in-Aids for Young Scientists (B) (22790179) of Ministry of Education, Culture, Sports, Science and Technology of Japan (to K.K.), the Kobayashi Institute for Innovative Cancer Chemotherapy (to K.K.), the Japan Research Foundation for Clinical Pharmacology (to K.K.), and the Takeda Science Foundation (to K.K.).

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胆と膵 Vol. 34 (2) p. 143～148, 2013

ゲノムワイド関連解析による ジェムシタビン副作用関連遺伝子の同定

前佛　均[1]・清谷　一馬[2]・宇野　智子[3]・木村　康利[3]
薤田　泰誠[2]・光畑　直喜[4]・伊奈志乃美[5]・鬼原　史[3]
山上　裕機[5]・平田　公一[3]・中村　祐輔[1]

要約：抗癌剤による副作用発現の有無は多くの要因が関係して規定されているものと考えられるが，遺伝的要因もその重要な因子の一つと考えられている。われわれはジェムシタビンにより引き起こされる重篤な骨髄抑制と関連する遺伝子多型（一塩基多型：SNP）を同定するため，164例のジェムザール単剤治療症例を用いてゲノムワイド関連解析および再現性確認のためのreplication studyを行った。その結果ジェムシタビンによる副作用と強い関連をもつ可能性の高い四つのSNPを含む遺伝領域を同定した（*DAPK1*上のrs11141915：$P=1.27\times10^{-6}$，2q12に存在するrs1901440：$P=3.11\times10^{-6}$，*PDE4B*上のrs12046844：$P=4.56\times10^{-5}$，3q29に存在するrs11719165：$P=5.98\times10^{-5}$）。同定された四つのSNPを用いて副作用リスクに働くと考えられるgenotypeの合計数に応じて各症例を点数化したところ，点数の高い症例では低い症例に比べて有意に副作用の発現率が高くなることが示された。今回同定された四つの遺伝子多型を用いたスコアリングシステムはジェムザールによる副作用の投与前診断に有用となる可能性が示された。

Key words：ジェムシタビン，骨髄抑制，ゲノムワイド関連解析，遺伝子多型

はじめに

現在胆膵悪性疾患をはじめ多くの悪性腫瘍に対する治療薬として適応となっているジェムシタビン（ジェムザール®）は骨髄抑制をはじめ，有害事象の発生頻度が決して少なくない薬剤であるが，その副作用の発現を規定する遺伝的要因についてはいまだ十分に解明されていないのが現状である。生命の設計図とも言われる人の遺伝情報（ゲノム配列）は同じ人間といえども個人間でわずかな違いが存在することが知られており，遺伝子多型（一塩基多型）と呼ばれる塩基配列の個人差を比較することで副作用の発現と関係する遺伝子を同定しようとする解析が進んできており，一部は日常臨床に応用されている。近年，ゲノム全体にわたり一塩基多型をgenotypingする技術が進歩し，ゲノムワイド関連解析（genome-wide association study，GWAS：「ジーワス」と呼ばれることが多い）という方法によりこれまで副作用との関連が全く知られていなかった新たな副作用関連遺伝子を発見する試みがなされるようになってきた。ジェムシタビンはSLC28A1，SLC28A3，SLC 29A1などの薬剤輸送タンパクを介して血中から細胞内に入り[1～3]，deoxycyti-

A Genome-wide Association Study Identifies Four Genetic Markers for Hematological Toxicities in Cancer Patients Receiving Gemcitabine Therapy
Hitoshi Zembutsu et al

1）東京大学医科学研究所ヒトゲノム解析センター（〒113-0033 文京区本郷7-3-1）
2）理化学研究所ゲノム医科学研究センター
3）札幌医科大学医学部外科学第一講座
4）呉共済病院泌尿器科
5）和歌山県立医科大学外科学第二講座

dine kinase (dCK), cytidine deaminase (CDA) などの酵素により代謝を受けることが知られていることから[4]，これらの既知遺伝子上の多型と副作用との関係を調べた報告はいくつか存在するが，現在のところ副作用と強い関連を示す遺伝子多型は同定されていない。本研究はゲノムワイド関連解析を通じジェムザールによる副作用と強い関係を有する遺伝子多型を同定することで，副作用予測診断へ応用することを目的として行われた[5]。

Ⅰ．ジェムシタビンによる有害事象

ジェムシタビン単剤による抗腫瘍治療を受けた174症例を対象に解析を行った（表1）。174例中grade 3以上の白血球/好中球減少症をきたした54例をcase，副作用を示さなかった120例をcontrolとしcase-control studyを行った。解析は21例のcaseおよび58例のcontrolをゲノムワイド関連解析（GWAS）に用い，33例のcaseおよび62例のcontrolをGWAS結果の再現性確認のためのreplication studyに用いた。case-control間で有意な性差を認めず（$P>0.64$），年齢分布にも有意差を認めなかった（$P>0.53$）。疾患別では，半分以上の症例が膵癌（56.9%）でその他肺癌（20.1%），胆管癌（18.4%）などであった。GWASで用いた症例と，再現性確認のためのreplication studyで用いたサンプル間で疾患分布に有意な差を認めなかった。

Ⅱ．ゲノムワイド関連解析によるジェムシタビン副作用関連候補遺伝子の同定

ジェムシタビン投与により骨髄抑制（>grade 3）が認められた21例と，投与により有害事象を認めなかった58例を用いて，ゲノム全体にわたり（約610,000 SNP）遺伝子多型をスクリーニングした。得られた各症例の610,000 SNPのgenotype情報を用いてcase-control関連解析（Fisherの正確検定）を行った。その結果，もっとも副作用と強い関連を示した遺伝子多型（SNP）は$P=0.000006690$を示した。図1にゲノム全体にわたるマーカーSNPとジェムシタビン副作用との関連の強さをグラフで表したもの（マンハッタンプロット）を示すが，ジェムシタビンの副作用と関係するSNPはゲノム全体にわたり散在している可能性を示している。

Ⅲ．ジェムシタビン副作用関連候補遺伝子のreplication study

ゲノムワイド関連解析の結果の再現性を確認するために，有意差上位100 SNPについて33例のcaseおよび62例のcontrolを用いて関連解析を行った。100 SNPに対するreplication studyの結果$P<0.05$を示す4 SNPが同定された（表2）。4 SNPとジェムシタビンによる骨髄抑制との関連はそれぞれ9番染色体上のrs11141915が$P=2.77\times10^{-3}$，2番染色体上のrs1901440は$P=1.82\times10^{-2}$，1番染色体上のrs12046844は$P=3.09\times10^{-2}$，3番染色体上のrs11719165は$P=4.61\times10^{-2}$を示した。さらにこの4 SNPについてGWASで用いたcaseおよびcontrol症例をそれぞれ加えて解析した結果，いずれもゲノムワイド有意水準である1.07×10^{-7}に達するSNPは存在しなかったものの，9番染色体上のrs11141915は$P=1.27\times10^{-6}$，オッズ比4.10（95% CI：2.21-7.62），2番染色体上のrs1901440は$P=3.11\times10^{-6}$，オッズ比34.00（95% CI：4.29-269.48），1番染色体上のrs12046844は$P=4.56\times10^{-5}$，オッズ比4.13（95% CI：2.10-8.14），3番染色体上のrs11719165は$P=5.98\times10^{-5}$，オッズ比2.60（95% CI：1.63-4.14）を示し，この4 SNPを含む遺伝的領域はジェムシタビンによる骨髄抑制と何らかの関連を示す結果となった。また，4遺伝領域の中で9番染色体上の領域については*DAPK1*，1番染色体上の領域については*PDE4B*という既知の遺伝子を含んでいた。

Ⅳ．遺伝子多型情報を用いたジェムシタビンによる骨髄抑制予測診断モデル

ジェムシタビンによる骨髄抑制と関連が示唆された4SNPはmultiple logistic regression解析の結果それぞれ独立した副作用予測因子であったため，この4SNPを用いた骨髄抑制予測診断システムについて検討を行った。四つのSNPについて骨髄抑制リスクに働くと考えられるgenotypeを持っている場合，それぞれのSNPについて1点を与え，もっていない場合には0点として各症例合計点数別に骨髄抑制発現群（case）と副作用を認めなかった群（control）で分布を調べた結果が表3および図2である。スコア0または1を示した113例中骨髄抑制群は11.5%，スコア2については60.9%，スコア3については86.7%が骨髄抑制発現群が占めており，コントロール群に比べ有意に高いスコ

表 1 Patients' characteristics

Stage	Platform	Source	No. samples	Female (%)	Age (mean ± SD)	Cancer types, *N*			
						Pancreatic	Lung	Bile duct	Others
GWAS									
ADR	Illumina HumanHap610-Quad	BioBank Japan	21	45.0	64.8 ± 10.9	12	6	1	2
non-ADR	Illumina HumanHap610-Quad	BioBank Japan	58	41.8	64.0 ± 8.7	23	19	10	1
Replication study									
ADR	Invader assay	BioBank Japan, Sapporo Medical University, Wakayama Medical University, Kure Kyosai Hospital	33	35.5	64.2 ± 9.9	28	3	4	3
non-ADR	Invader assay	BioBank Japan, Sapporo Medical University, Wakayama Medical University, Kure Kyosai Hospital	62	30.2	64.9 ± 9.0	36	7	17	2

ADR : adverse drug events

表 2 Summary of association results of GWAS and replication study

SNP	Chromosome	Chromosome location*	Gene	Allele 1/2 (risk)	Stage	ADR				non-ADR				*P* value			False discovery rate	Odds ratio (95%CI) †
						11	12	22	RAF	11	12	22	RAF	Allelic	Dominant	Recessive		
rs11141915	9	89425614	*DAPK1*	T/G (T)	GWAS	18	3	0	0.93	21	30	7	0.62	1.27×10^{-4}	1.04×10^{-4}	1.80×10^{-1}		7.94 (2.32-27.25)
					Follow up	22	11	0	0.83	23	31	8	0.62	2.77×10^{-3}	9.23×10^{-3}	4.73×10^{-2}	0.185	3.05 (1.45-6.41)
					Combined	40	14	0	0.87	44	61	15	0.62	1.27×10^{-6}	6.91×10^{-6}	6.11×10^{-3}		4.10 (2.21-7.62)
rs1901440	2	134154429	No gene	A/C (C)	GWAS	11	3	7	0.40	31	27	0	0.23	4.42×10^{-2}	1.00×10^{-0}	4.01×10^{-5}		60.52 (5.45-632.87)
					Follow up	20	8	5	0.27	42	19	1	0.17	1.30×10^{-1}	5.05×10^{-1}	1.82×10^{-2}	0.655	10.89 (1.22-97.64)
					Combined	31	11	12	0.32	73	46	1	0.20	1.44×10^{-2}	7.39×10^{-1}	3.11×10^{-6}		34.00 (4.29-269.48)
rs12046844	1	66010967	*PDE4B*	T/C (C)	GWAS	1	5	15	0.83	12	32	14	0.52	3.93×10^{-4}	1.95×10^{-4}	1.67×10^{-1}		7.86 (2.56-24.12)
					Follow up	4	10	19	0.73	7	34	21	0.61	1.50×10^{-1}	3.09×10^{-2}	1.00×10^{-0}	0.545	2.65 (1.11-6.31)
					Combined	5	15	34	0.77	19	66	35	0.57	3.05×10^{-4}	4.56×10^{-5}	3.43×10^{-1}		4.13 (2.10-8.14)
rs11719165	3	196067377	No gene	C/T (C)	GWAS	9	10	2	0.67	5	27	26	0.32	1.15×10^{-4}	3.49×10^{-3}	1.21×10^{-3}		4.27 (2.01-9.05)
					Follow up	9	16	8	0.52	7	31	24	0.36	4.61×10^{-2}	1.78×10^{-1}	8.12×10^{-2}	0.741	1.87 (1.02-3.42)
					Combined	18	26	10	0.57	12	58	50	0.34	5.98×10^{-5}	3.26×10^{-3}	3.66×10^{-4}		2.60 (1.63-4.14)

RAF, risk allele frequency : CI, confidence interval : GWAS, genome-wide association study.
*Based on NCBI 36 genome assembly.
†Odds ratios were shown for the model with minimum *P* values.

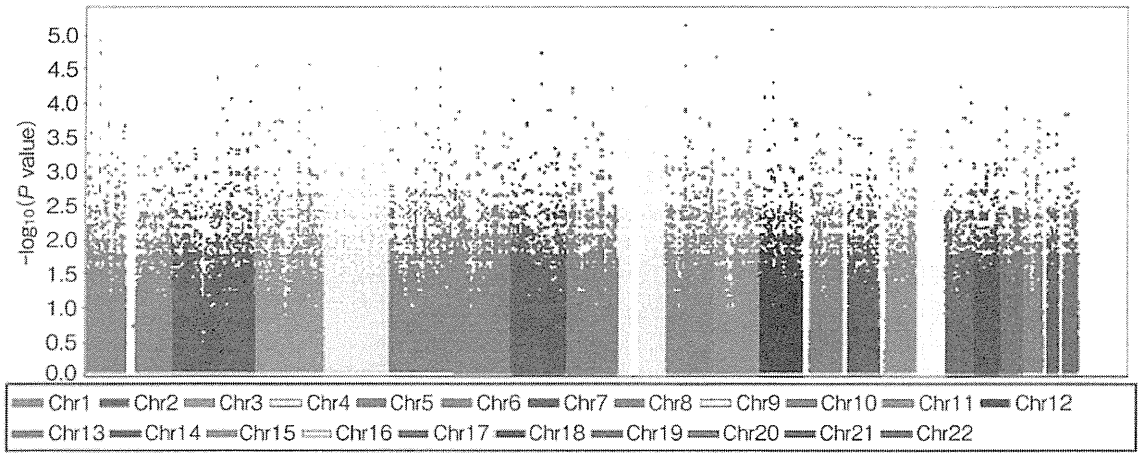

図 1 マンハッタンプロット

ゲノム全体のマーカー SNP（点）について各染色体を横軸に，ジェムシタビンによる骨髄抑制との関連の強さを縦軸に表示している。ほとんどの SNP（点）が下方に位置し関連が認められない一方で，いくつかの SNP は強い関連がある可能性が示されている。

表 3 Prediction scores of gemcitabine-induced sever leukopenia/neutropenia using rs11141915, rs1901440, rs12046844 and rs11719165

Score	ADR, *N*（%）(*N*=54)	non-ADR, *N*（%）(*N*=120)	Odds ratio（95% CI）*P* value	General control, *N*（%）(*N*=934)
0	4（7.4%）	50（41.7%）	1.00（reference）	271（29.0%）
1	9（16.7%）	50（41.7%）		423（45.3%）
2	28（51.9%）	18（15.0%）	11.97（5.23-27.37）6.25×10^{-10}	194（20.8%）
3	13（24.1%）	2（1.7%）	50.00（10.13-246.90）4.13×10^{-9}	46（4.9%）
		（trend test）	9.91（5.56-17.67）1.31×10^{-14}	

CI, confidence interval.

アを示すことが確認された（trend test $P=1.31\times10^{-14}$）。さらに日本人一般集団をこのスコアリングシステムにあてはめた場合の分布を検討した結果，0 点が 29.0%，1 点が 45.3%，2 点が 20.8%，3 点が 4.9%になることが示され，このスコアリングシステムをジェムシタビン治療開始前に応用することで骨髄抑制の危険性が少なくより安全かつ適切な治療選択に有用となる可能性が示された（図 2）。

V．考　察

われわれはゲノムワイド関連解析によりジェムシタビンによる骨髄抑制と深い関係があると考えられる遺伝領域として，9 番，2 番，1 番，3 番染色体上の遺伝子多型（SNP）rs11141915，rs1901440，rs12046844，and rs11719165 をそれぞれ同定した。さらにこの四つの遺伝子多型を組み合わせて解析することによりジェムシタビンによる骨髄抑制をより正確に予測できる可能性が示唆された。

本研究において rs11141915 は最もジェムシタビンによる骨髄抑制と強い関連（$P=0.00000127$，オッズ比 4.10），を示したが，この SNP は *DAPK1* 遺伝子の 3 番目のイントロン上に存在する。*DAPK1* 遺伝子はリン酸化酵素の一種で骨髄や末梢血細胞において発現していることが知られている。この遺伝子はジェムシタビンを含む抗癌剤に対する耐性と何らかの関係があることが指摘されており，機序は不明だがジェムシタビンによる骨髄抑制を引き起こす上で重要な役割を担っている可能性が高いものと考えられる[6]。

また，rs12046844 はジェムシタビンによる骨髄抑制との関連が $P=0.0000456$，オッズ比 4.13 であったが，このSNPを含む領域には*PDE4B*遺伝子が含まれていた。*PDE4B* 遺伝子は加水分解酵素の一種であるが，好中球や単球などで機能しており炎症細胞の活性調節を担っている。また肺癌においてジェムシタビン耐性に関係していることが指摘されていることも考慮する

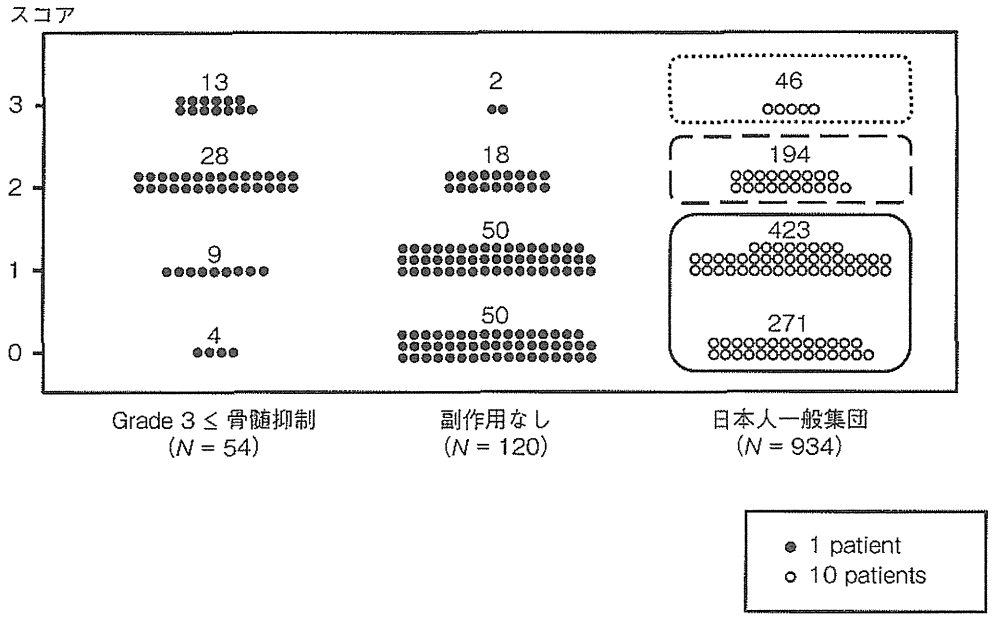

図 2　四つの遺伝情報を用いたジェムシタビン骨髄抑制予測診断システム
4 SNP について骨髄抑制リスクジェノタイプの合計数に応じて各症例をスコアリングした場合の分布図。

と，*PDE4B* はジェムシタビンによる骨髄抑制において重要な役割を担っている可能性が考えられる。

ジェムシタビンが体内に入り細胞に到達し細胞内で代謝を受ける過程において CDA，dCK，SLC28A1，SLC28A3，SLC29A1 などの遺伝子が関係していることは知られているが[7~14]，今回のゲノムワイド関連解析の結果からは有意水準を超える強い関連を見いだすことはできなかった。つまり，ジェムシタビンによる骨髄抑制はこれまで知られていないメカニズムによって引き起こされている可能性を示唆するものではないかと考えられる。

最後に今回同定された四つの遺伝子多型を含む遺伝領域はジェムシタビンによる骨髄抑制と何らかの関連があることが示唆され，さらにこの四つの遺伝子多型情報を用いた骨髄抑制予測システムによりジェムシタビン治療を行う前に骨髄抑制のリスクを回避できる可能性が考えられる。このようなゲノム情報に基づいた適切かつ安全な治療は今後ジェムシタビンに限らず，多くの薬剤についても応用されていくものと考えられる。

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CYP2D6 遺伝子多型による TAM 投与量調節治療

演題番号 ：OJ-173
笹 三徳[1]　清谷 一馬[2,5]　筵田 泰誠[2]　今村 知世[3]　谷川原 佑介[3]
細野 直哉[4]　前佛 均[2]　中村 祐輔[2]

1. とくしまブレストケアクリニック
2. 東京大学医科学研究所
3. 慶應大学医学部　臨床薬剤学
4. 理化学研究所
5. 徳島大学疾患ゲノムセンター

<はじめに>CYP2D6 は TAM 代謝においては重要な酵素である。日本人女性では*10 をはじめ酵素活性減弱・消失を示す多型(V)が高頻度に認められ、同症例では TAM の効果が減弱している可能性がある。
<対象と方法>TAM 服用患者 98 名について、CYP2D6 遺伝子型別に TAM 投与量を調節し（wt/V: 30mg/day, V/V:40mg/day）、endoxifen、TAM などの血中濃度測定、副作用調査を行った。
<結果>TAM 増量により、血液中の endoxifen は wt/*10、*10/*10 症例でそれぞれ 1.4, 1.7 倍に増加し、wt/wt 症例での 20mg/day 投与とほぼ同じ水準まで改善した。血液中の 4-OH TAM も同じ結果を示した。副作用は、TAM 増量により有意に増加したものはなかった。
<結語>CYP2D6 遺伝子多型別に TAM 投与量を調節することは治療成績向上に寄与する可能性が示唆された。

World J Surg (2013) 37:1379–1387
DOI 10.1007/s00268-013-1989-6

World Journal of Surgery

Prognosis and Predictors of Surgical Complications in Hepatocellular Carcinoma Patients With or Without Cirrhosis after Hepatectomy

Toru Mizuguchi · Masaki Kawamoto · Makoto Meguro · Yukio Nakamura · Shigenori Ota · Thomas T. Hui · Koichi Hirata

Published online: 12 March 2013

Abstract
Background Although poor liver function is associated with a high morbidity rate and poor prognosis in hepatocellular carcinoma (HCC) patients, the exact effects of liver pathology on the surgical outcomes of HCC patients are poorly understood. The purpose of this study was to assess how the liver pathology of HCC patients affects their prognosis and complications rate after liver resection.
Methods Between January 2006 and November 2010, 149 consecutive hepatocellular carcinoma patients, including 79 noncirrhosis patients and 70 cirrhosis patients, were enrolled in this study.
Results Among the noncirrhotic patients, operative time, fresh frozen plasma (FFP) transfusion requirement, tumor size, and serum retinol binding protein (RBP) levels were significantly higher in the complications group than in the complications-free groups. On the other hand, in the cirrhotic patients the prothrombin time (PT) and indocyanine green retention value at 15 min ($ICGR_{15}$) of the complications group were significantly lower and higher, respectively, than those of the complications-free group. In the noncirrhotic patients, recurrence-free survival and overall survival did not differ between the complications and complications-free groups. On the other hand, in the cirrhotic patients, the recurrence-free survival and overall survival of the complications-free group were significantly longer than those of the complications group.
Conclusions In the noncirrhotic patients, surgical complications had no prognostic effect, whereas they had a significant survival impact in the cirrhotic patients. The surgical strategy for HCC should be based on the patient's pathological background.

T. Mizuguchi (✉) · M. Kawamoto · M. Meguro · Y. Nakamura · S. Ota · K. Hirata
Department of Surgery I, Sapporo Medical University Hospital, Sapporo Medical University School of Medicine, S-1, W-16, Chuo-Ku, Sapporo, Hokkaido 060-8543, Japan
e-mail: tmizu@sapmed.ac.jp

T. T. Hui
Department of Surgery, Children's Hospital & Research Center Oakland, 747 52nd Street, Oakland, CA 94609, USA

Introduction

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer worldwide and the third most common cause of cancer-related death [1, 2]. The optimal management strategy for HCC depends on both tumor-related factors and host liver function [3, 4]. Although the frequency of nonalcoholic steatohepatitis-related HCC has recently increased [5, 6], most HCC still develops in patients with viral hepatitis-associated liver disease [1, 2]. In the era when no effective viral therapy was available, a high incidence of recurrence after treatment was inevitable in HCC patients. Therefore, surgery for HCC tended to be avoided in patients with good liver function [7]. In addition, the high mortality rate of liver resection itself encouraged patients and doctors to select interventional therapy instead of a surgical approach.

However, liver surgery techniques have improved, and the mortality rate after liver resection was nearly zero in recent cases [8, 9]. In addition, technical developments have encouraged surgeons to select a laparoscopic approach instead of conventional open surgery [10, 11]. In liver resection for HCC, the current goal is to reduce the morbidity rate as much as possible and improve patient prognosis. Liver transplantation is considered to be the best curative approach for HCC, but liver resection should be

considered in cases in which it would be expected to achieve a good prognosis [9, 12]. Furthermore, the mortality rate of liver resection is lower than that of liver transplantation in the early stages of HCC, and among patients with good liver function the long-term prognosis of patients who undergo liver resection is comparable to that of patients that undergo liver transplantation [9].

Although poor liver function is associated with a high morbidity rate and poor prognosis in HCC patients [13, 14], the exact effects of liver pathology on the surgical outcomes of HCC patients are poorly understood. Therefore, the purpose of this study was to identify how liver pathology affected the prognosis and complications rates of consecutive HCC patients who underwent liver resection.

Patients and methods

Between January 2006 and November 2010, 149 consecutive hepatocellular carcinoma patients who underwent hepatectomy were enrolled in this study after providing informed consent. Mortality was defined as any in-hospital death that occurred within 90 days of surgery. Postoperative complications were defined and classified according to the modified Clavien classification system [15]. Briefly, Grade I complications were defined as any deviation from the normal postoperative course that did not require special treatment. Grade II complications were defined as those that required pharmacological treatment with drugs. Grade III complications were defined as those that required surgical or radiological intervention with (IIIb) or without (IIIa) general anesthesia. Grade IV complications were defined as life-threatening complications involving single (IVa) or multiple (IVb) organ dysfunction. Grade V complications were defined as those that caused the death of the patient. For grade IV or worse complications, liver failure was defined as a serum bilirubin concentration of greater than 5 mg/dl that lasted for more than 2 days. Renal dysfunction/insufficiency was defined as oliguria (<400 ml/day) combined with a sustained serum creatine level elevation of more than 1.1 mg/dl. Bleeding was diagnosed by endoscopic examination. Wound seroma/infection was defined as any wound that split open regardless of whether bacteria were detected. Ascites was defined as fluid discharge of more than 300 ml/day for more than 3 days.

We divided the patients into two groups: the noncirrhosis group (79 patients) and the cirrhosis group (70 patients). The study design conformed to the ethical guidelines of the Declaration of Helsinki, and obtained informed consent was obtained from each subject before their registration.

Assessment of clinical and operative variables

Before hepatectomy, we performed laboratory tests to assess the patients' serum levels of type IV collagen (Col), hyaluronic acid (HA), prealbumin (PreALB), retinol binding protein (RBP), hepatocyte growth factor (HGF), alpha fetoprotein (AFP), and protein induced by vitamin K absence or antagonists-II (PIVKAII), as well as their indocyanine green retention value at 15 min ($ICGR_{15}$) and ^{99}m-technetium-labeled galactosyl serum albumin (GSA) scintigraphy index (HH15, LHL15) values. Their intraoperative data and any complications that occurred during hospitalization also were recorded. Tumor size and number were assessed by pathological examinations. All laboratory tests were conducted in the early morning on the day of assessment. The model for end-stage liver disease (MELD) score of each patient was calculated using the following formula: $9.57 \times \text{Ln}$ (creatinine mg/dL) $+ 3.78 \times \text{Ln}$ (bilirubin mg/dL) $+ 11.20 \times \text{Ln}$ (PT-INR) $+ 6.43$, based on laboratory tests [16].

The Child–Pugh score with Pugh's modification was calculated as the sum of the scores for five clinical parameters [ascites (none = one point, moderate = two points, severe = three points), serum bilirubin (<2 mg/dl = one point, 2–3 mg/dl = two points, >3 mg/dl = three points), serum albumin (>3.5 g/dl = one point, 2.8–3.5 g/dl = two points, <2.8 g/dl = three points), hepatic encephalopathy (absent = one point, grade 1 or 2 = two points, grade 3 or 4 = three points), and prothrombin index [>70 % = one point, 40–70 % = two points, <40 % = three points]). Then, the patients were classified into three groups with different expected survivals according to their Child–Pugh scores (Child–Pugh A = 5–6 points, Child–Pugh B = 7–9 points, Child–Pugh C = 10 or more points) [17].

Surgical procedure

All liver resections were performed with the Pringle maneuver after more than 300 ml of intraoperative bleeding. Hepatic flow was not controlled if the intraoperative bleeding was less than 300 ml. A Cavitron ultrasonic aspirator (CUSA) was used for liver parenchymal dissection, and an argon laser beam coagulator or saline-associated monopolar electrocautery was used to achieve hemostasis. Antibiotics were administered 30 min before the laparotomy and every 3 h during the operation. All of the sutures and ties, except those used for skin closure, were absorbable (Vicryl or PDS, Johnson & Johnson Gateway, Piscataway, NJ). The periwound skin was washed with 500 ml of warm saline before skin closure. A closed-type intra-abdominal drain and a subcutaneous drain were installed for 2–3 days after the liver resection.

The operation type was classified as follows: hepatic resection (Hr) 0: partial resection including tumor enucleation; Hr S: sub-segmentectomy; Hr 1: mono-segmentectomy; Hr 2: bi-segmentectomy including right hepatectomy, left hepatectomy, and central bi-segmentectomy; and Hr 3: tri-segmentectomy.

Statistical analysis

For the statistical analyses, demographic data and perioperative laboratory test results were extracted from the clinical database, and the differences among the groups were compared using the χ^2 test followed by the post-hoc 2×2 Fisher's exact test, when necessary. Continuous variables were compared using the Mann–Whitney U test. Logistic regression analysis was used to identify the most relevant risk factors for complications. The factors affecting overall survival were assessed using the Kaplan–Meier method, with comparisons performed using the log-rank test and univariate or multivariate analyses performed using the Cox proportional hazards regression model. All calculations were performed using the StatView 5.0 software package (Abacus Concepts Inc., Berkeley, CA) or SPSS 16.0 (SPSS Inc., Chicago, IL). Receiver operating characteristic (ROC) curves, which were used to calculate the area under the ROC curve (AUC), were produced using the MedCalc software package (Ver 8.0.1.0, Mariakerke, Belgium). All results are expressed as median values (minimum value-maximum value). P values <0.05 were considered to be statistically significant.

Results

Of the 149 consecutive patients in which we performed hepatectomy for HCC, postoperative pathological liver evaluations found that 79 patients had noncirrhotic livers, and 70 patients had cirrhotic livers. The two groups displayed similar morbidity rates, and there were no significant differences in the frequencies of any of the complications included in the Clavien classification (Table 1).

Table 2 shows the clinical demographics of the noncirrhotic patients. In univariate analysis of these patients, the operative time, fresh frozen plasma (FFP) transfusion requirement, tumor size, MELD score, and γ-glutamyl transpeptidase (gGT), and retinol binding protein (RBP) levels of the complications group were found to be significantly higher than those of the complications-free group. Multivariate analysis demonstrated that all of these indicators, except gGT and the MELD score, were significantly increased in the complications group. The area under the curve (AUC) values of these indicators were all greater than 0.65 and were significantly different (Fig. 1a–d). Interactive dot diagrams were used to determine the ideal cutoff values for each parameter, which were 3.1 mg/dl for RBP (Fig. 1e), 373 min for operative time (Fig. 1f), 0 U for FFP transfusion requirement (Fig. 1g), and 5.5 cm for tumor size (Fig. 1h).

Table 1 Postoperative complications suffered by noncirrhotic and cirrhotic patients after hepatectomy for hepatocellular carcinoma

Clavien classification	Noncirrhotic ($n = 79$)		Cirrhotic ($n = 70$)	
	GI–GIII	GIV–GV	GI–GIII	IV–GV
Liver failure	1	1	1	1
Bleeding	1		2	
Bile leakage	3		1	
Respiratory distress	5		1	1
Renal dysfunction/failure	2		2	
Intra-abdominal abscess	3		2	
Wound seroma/infection	5		3	
Pleural effusion	3		5	
Ascites	3		10	
	28 events/ 22 patients		29 events/ 24 patients	

Table 3 shows the clinical demographics of the cirrhotic patients. In univariate analysis of these patients, the prothrombin time (PT), choline esterase (CholE) levels, Child–Pugh score, and MELD score of the complications group were found to be significantly higher than those of the complications-free group, whereas the indocyanine green retention value at 15 min ($ICGR_{15}$) of the complications group was significantly lower than that of the complications-free group. Of these, PT and the $ICGR_{15}$ achieved significance in the multivariate analysis. The AUC values of both of these parameters were greater than 0.65 and were significantly different (Fig. 2a, b). Interactive dot diagrams demonstrated the ideal cutoff values for each of these parameters, which were 82.8 % for PT (Fig. 2c) and 9.6 % for the $ICGR_{15}$ (Fig. 2d).

We next examined the recurrence-free survival (Fig. 3a, c) and overall survival (Fig. 3b, d) rates of the noncirrhotic (Fig. 3a, b) and cirrhotic patients (Fig. 3c, d). Among the non-cirrhotic patients, the complications and complications-free groups displayed similar recurrence-free survival and overall survival rates. On the other hand, among the cirrhotic patients, the complications-free group demonstrated significantly longer recurrence-free survival and overall survival than the complications group.

Discussion

In this study, we showed that the risk factors for perioperative complications differed between noncirrhotic

Table 2 Clinical demographics of the noncirrhotic patients who underwent initial hepatectomy for hepatocellular carcinoma ($N = 79$)

	Complications	Complications-free	Univariate	Multivariate
Etiology			0.262	
B	8	25		
C	6	20		
BC	1	0		
NBNC	7	12		
Operation			0.054	
0	5	24		
S	3	13		
1	6	11		
2	4	8		
3	4	1		
Stage			0.678	
1	1	8		
2	16	32		
3	3	10		
4	2	7		
Operative time (min)	415.6 ± 143.9	332.7 ± 134.8	0.019	0.043
Bleeding (ml)	857.8 ± 609.3	552.4 ± 1261.1	0.282	
Blood transfusion (U)	1.9 ± 3.2	0.6 ± 2.4	0.053	
FFP transfusion (U)	4.0 ± 6.1	1.1 ± 3.1	0.006	0.001
Tumor size (cm)	6.62 ± 4.06	4.22 ± 3.14	0.009	0.001
Tumor number	1.6 ± 1.2	1.6 ± 1.5	0.979	
Age (year)	67.7 ± 9.2	67.9 ± 11.2	0.931	
Height (cm)	161.9 ± 7.6	160.5 ± 7.5	0.463	
Weight (kg)	61.4 ± 10.2	58.7 ± 9.8	0.289	
BMI	23.5 ± 3.1	22.7 ± 3.2	0.307	
ALB (g/dl)	3.81 ± 0.53	3.93 ± 0.35	0.252	
Bil (mg/dl)	0.61 ± 0.34	0.72 ± 0.34	0.189	
PT (%)	92.9 ± 9.4	92.8 ± 13.5	0.979	
Plt ($\times 10^4$)	18.2 ± 6.8	17.7 ± 11.5	0.844	
AT (%)	96.9 ± 16.8	94.4 ± 17.2	0.577	
AST (IU/L)	43.4 ± 25.6	38.8 ± 39.4	0.617	
ALT (IU/L)	41.4 ± 29.7	36.4 ± 30.9	0.516	
gGT (IU/L)	117.2 ± 180.6	52.7 ± 47.1	0.022	0.056
CholE (IU/L)	245.3 ± 79.1	250.4 ± 66.1	0.776	
Col (ng/ml)	5.52 ± 2.83	4.99 ± 1.46	0.322	
HA (ng/ml)	160.1 ± 143.5	141.6 ± 207.1	0.712	
BTR	6.49 ± 2.21	6.53 ± 1.79	0.941	
ICG R15 (%)	10.1 ± 8.5	10.8 ± 6.7	0.692	
RBP (mg/dl)	4.47 ± 3.68	2.68 ± 1.13	0.005	0.024
PreALB (mg/dl)	21.3 ± 8.1	18.8 ± 6.6	0.195	
HGF (ng/ml)	0.34 ± 0.11	0.29 ± 0.13	0.199	
HH15	0.602 ± 0.062	0.584 ± 0.069	0.299	
LHL15	0.930 ± 0.027	0.933 ± 0.029	0.675	
Child–Pugh score	5.318 ± 0.477	5.281 ± 0.701	0.818	
MELD score	8.901 ± 5.314	7.402 ± 1.156	0.046	0.095

patients and cirrhotic patients who had undergone liver resection for HCC. In addition, the effects of surgical complications on postoperative recurrence-free survival and overall survival also differed among these groups. These results indicate that the pathological state of the patient's liver should be taken into account when