25. Purcell S, Neale B, Todd Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, *et al*: **PLINK: a tool set for whole genome association and population based linkage analyses.** *Am J Hum Genet* 2007, **81**:559 575.
26. De Jager PL, Chibnik LB, Cui J, Reischl J, Lehr S, Simon KC, Aubin C, Bauer D, Heubach JF, Sandbrink R, *et al*: **Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: a weighted genetic risk score.** *Lancet Neurol* 2009, **8**:1111 1119.
27. Clapham DE: **Calcium signaling.** *Cell* 2007, **131**:1047 1058.
28. Price VH: **Treatment of hair loss.** *N Engl J Med* 1999, **341**:964 973.
29. Li GR, Deng XL: **Functional ion channels in stem cells.** *World J Stem Cells* 2011, **3**:19 24.
30. Endo K, Takeshita T, Kasai H, Sasaki Y, Tanaka N, Asao H, Kikuchi K, Yamada M, Chenb M, O'Shea JJ, *et al*: **STAM2, a new member of the STAM family, binding to the Janus kinases.** *FEBS Lett* 2000, **477**:55 61.
31. Yamada M, Ishii N, Asao H, Murata K, Kanazawa C, Sasaki H, Sugamura K: **Signal transducing adaptor molecules STAM1 and STAM2 are required for T cell development and survival.** *Mol Cell Biol* 2002, **22**:8648 8658.
32. Liu J, Sun K, Bai Y, Zhang W, Wang X, Wang Y, Wang H, Chen J, Song X, Xin Y, *et al*: **Association of three gene interaction among MTHFR, ALOX5AP and NOTCH3 with thrombotic stroke: a multicenter case control study.** *Hum Genet* 2009, **125**:649 656.
33. Manev H, Manev R: **5 Lipoxygenase (ALOX5) and FLAP (ALOX5AP) gene polymorphisms as factors in vascular pathology and Alzheimer's disease.** *Med Hypotheses* 2006, **66**:501 503.
34. Karnik P, Tekeste Z, McCormick TS, Gilliam AC, Price VH, Cooper KD, Mirmirani P: **Hair follicle stem cell specific PPARgamma deletion causes scarring alopecia.** *J Invest Dermatol* 2009, **129**:1243 1257.
35. Petukhova L, Duvic M, Hordinsky M, Norris D, Price V, Shimomura Y, Kim H, Singh P, Lee A, Chen WV, *et al*: **Genome wide association study in alopecia areata implicates both innate and adaptive immunity.** *Nature* 2010, **466**:113 117.
36. Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W: **Beta Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin.** *Cell* 2001, **105**:533 545.
37. Zhang Y, Andl T, Yang SH, Teta M, Liu F, Seykora JT, Tobias JW, Piccolo S, Schmidt Ullrich R, Nagy A, *et al*: **Activation of beta catenin signaling programs embryonic epidermis to hair follicle fate.** *Development* 2008, **135**:2161 2172.
38. Young P, Boussadia O, Halfter H, Grose R, Berger P, Leone DP, Robenek H, Charnay P, Kemler R, Suter U: **E cadherin controls adherens junctions in the epidermis and the renewal of hair follicles.** *EMBO J* 2003, **22**:5723 5733.
39. Samuelov L, Sprecher E, Tsuruta D, Biro T, Kloepper JE, Paus R: **P cadherin regulates human hair growth and cycling via canonical Wnt signaling and transforming growth factor beta2.** *J Invest Dermatol* 2012, **132**:2332 2341.

doi:10.1186/bcr3475
Cite this article as: Chung *et al.*: A genome wide association study of chemotherapy induced alopecia in breast cancer patients. *Breast Cancer Research* 2013 **15**:R81.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

Journal of Human Genetics (2013) 58, 327 333
© 2013 The Japan Society of Human Genetics All rights reserved 1434 5161/13
www.nature.com/jhg

REVIEW

Important and critical scientific aspects in pharmacogenomics analysis: lessons from controversial results of tamoxifen and *CYP2D6* studies

Kazuma Kiyotani[1,2], Taisei Mushiroda[2], Hitoshi Zembutsu[3] and Yusuke Nakamura[3,4]

Tamoxifen contributes to decreased recurrence and mortality of patients with hormone receptor-positive breast cancer. As this drug is metabolized by phase I and phase II enzymes, the interindividual variations of their enzymatic activity are thought to be associated with individual responses to tamoxifen. Among these enzymes, CYP2D6 is considered to be a rate-limiting enzyme in the generation of endoxifen, a principal active metabolite of tamoxifen, and the genetic polymorphisms of *CYP2D6* have been extensively investigated in association with the plasma endoxifen concentrations and clinical outcome of tamoxifen therapy. In addition to *CYP2D6*, other genetic factors including polymorphisms in various drug-metabolizing enzymes and drug transporters have been implicated to their relations to clinical outcome of tamoxifen therapy, but their effects would be small. Although the results of association studies are controversial, accumulation of the evidence has revealed us the important and critical issues in the tamoxifen pharmacogenomics study, namely the quality of genotyping, the coverage of genetic variations, the criteria for sample collection and the source of DNAs, which are considered to be common problematic issues in pharmacogenomics studies. This review points out common critical issues in pharmacogenomics studies through the lessons we have learned from tamoxifen pharmacogenomics, as well as summarizes the results of pharmacogenomics studies for tamoxifen treatment.
Journal of Human Genetics (2013) **58,** 327–333; doi:10.1038/jhg.2013.39; published online 9 May 2013

Keywords: ABCC2; C10orf11; endoxifen; estrogen receptor; genome-wide association study; P450 2D6; pharmacogenomics study design; single-nucleotide polymorphism

INTRODUCTION

Tamoxifen, a selective estrogen receptor (ER) modulator, has been widely used for the treatment and prevention of recurrence for patients with hormone receptor (ER or progesterone receptor) positive breast cancers. As >70% of breast cancers are hormone receptor positive, thousands of breast cancer patients worldwide initiate to take endocrine treatment including tamoxifen each year. In pre and postmenopausal patients with primary breast cancer, 5 years of adjuvant tamoxifen significantly reduced recurrence rate as well as cancer specific mortality for 15 years after their primary diagnosis.[1] However, approximately one third of patients treated with adjuvant tamoxifen experience a recurrent disease,[1,2] implicating possible individual differences in responsiveness to tamoxifen.

Tamoxifen is metabolized to more active metabolites or inactive forms by phase I and phrase II enzymes, including cytochrome P450s (CYPs), sulfotransferases (SULTs) and UDP glucuronosyltransferases (UGTs). The polymorphisms in these drug metabolizing enzymes are considered to affect individual differences in plasma concentrations of active tamoxifen metabolites and clinical outcome in breast cancer patients treated with tamoxifen. Among these enzymes, CYP2D6 has been most extensively investigated owing to its significant role in production of active metabolites, endoxifen and 4 hydroxytamoxifen.

This review summarizes current reports on the relationships of genetic polymorphisms and other biomarkers to individual differences in clinical outcome of breast cancer patients with tamoxifen treatment. In addition, we investigate reasons or causes of discordant results for the association between *CYP2D6* genetic variations and clinical outcome, and would like to highlight various problematic issues in pharmacogenomics studies.

TAMOXIFEN METABOLISM

Tamoxifen is extensively metabolized by phase I and phase II enzymes in the human liver (Figure 1).[3,4] Tamoxifen itself has low affinity to the ER as only 1.8% of the affinity of 17β estradiol.[3]

[1]Division of Genome Medicine, Institute for Genome Research, The University of Tokushima, Tokushima, Japan; [2]Research Group for Pharmacogenomics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan; [3]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan and [4]Department of Medicine and Center for Personalized Therapeutics, The University of Chicago, Chicago, IL, USA
Correspondence: Dr Y Nakamura, Department of Medicine and Surgery, The University of Chicago, 900E 57th Street, KCBD 6130, Chicago 60637, IL, USA.
E mail: ynakamura@bsd.uchicago.edu
Received 19 March 2013; revised 11 April 2013; accepted 12 April 2013; published online 9 May 2013

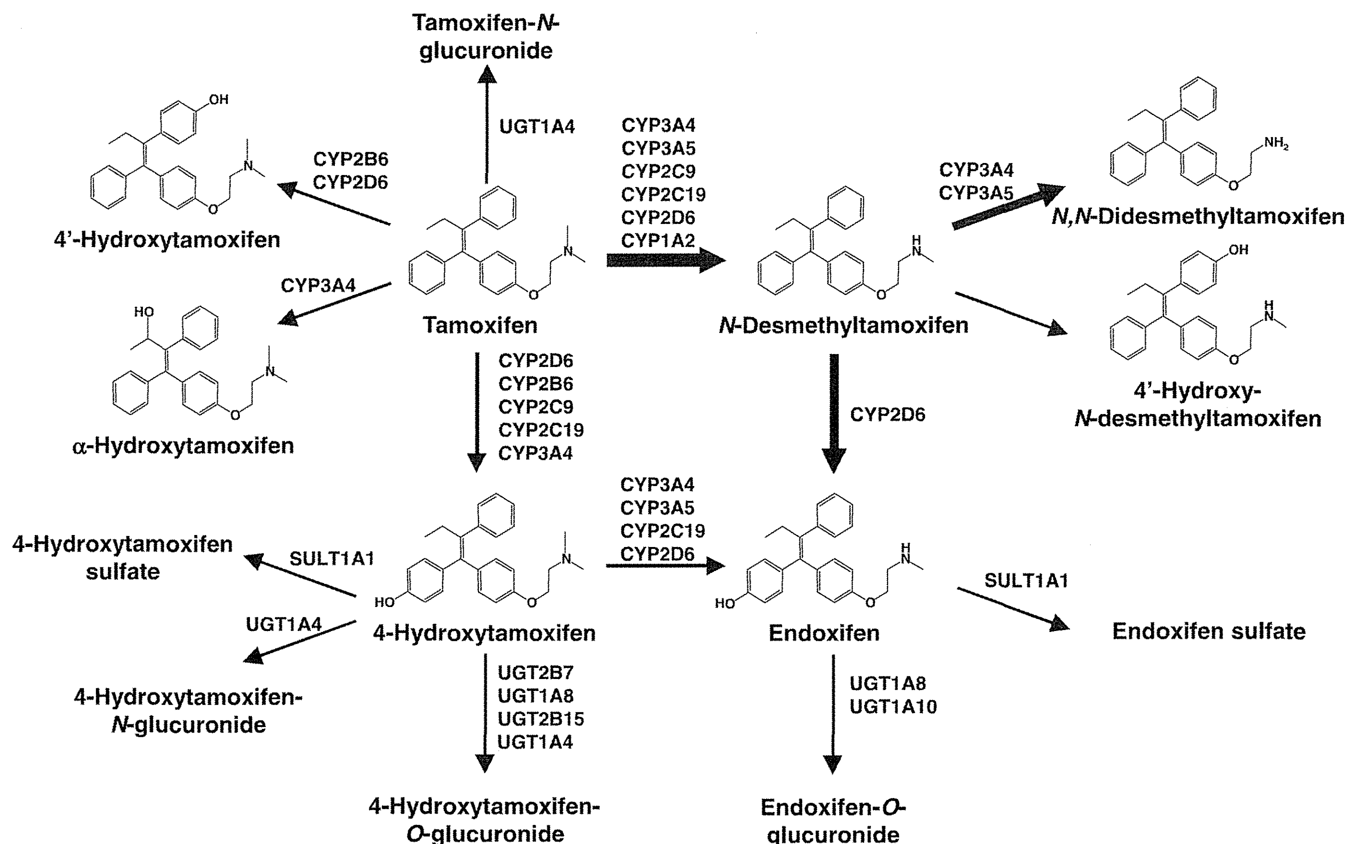

Figure 1 Metabolic pathways of tamoxifen in human. Major metabolic pathways are highlighted with bold arrows.

The major metabolite *N* desmethyltamoxifen is formed by *N* demethylation, which is catalyzed mainly by CYP3A4 and CYP3A5, with small contribution by CYP2D6, CYP1A2, CYP2C9 and CYP2C19.[5–9] *N* desmethyltamoxifen shows weak affinity to the ER similar to tamoxifen.[3,4] However, 4 hydroxytamoxifen, which is formed by 4 hydroxylation of tamoxifen, has 100 fold higher affinity to the ER and 30 to 100 fold greater potency in suppressing estrogen dependent breast cancer cell proliferation than tamoxifen.[3,10–12] This conversion is catalyzed by CYP2D6, CYP2B6, CYP2C9, CYP2C19 and CYP3A4.[5,13–15] Endoxifen (4 hydroxy *N* desmethyltamoxifen) has a potency equivalent to 4 hydroxytamoxifen,[10,16,17] and its plasma concentration level exceed that of 4 hydroxytamoxifen by several folds, suggesting endoxifen to be a principal active metabolite.[9–11] Endoxifen formation from *N* desmethyltamoxifen is predominantly catalyzed by CYP2D6.[18] Several additional metabolites, such as *N,N* didesmethyltamoxifen, 4′ hydroxy *N* desmethyltamoxifen and α hydroxytamoxifen were reported, but no other highly active metabolite has been described so far.[4]

Tamoxifen and these metabolites are further metabolized by phase II enzymes, such as SULTs and UGTs. SULT1A1 is considered to be the primary SULT responsible for the sulfation of 4 hydro xytamoxifen and endoxifen.[19,20] UGT1A8, UGT1A10, UGT2B7, UGT2B15 and UGT1A4 are involved in the *O* glucuronidation of 4 hydroxytamoxifen and endoxifen.[21–23] Tamoxifen and 4 hydroxytamoxifen are glucuronidated by UGT1A4 to the corresponding N^+ glucuronides.[24,25] The genetic variations of these drug metabolizing enzymes are possible to affect tamoxifen metabolism.

GENETIC POLYMORPHISMS OF *CYP2D6*

CYP2D6 is one of the most important CYP isoforms owing to its central role in the metabolism of a number of clinically important drugs.[26] The *CYP2D6* gene is located on chromosome 22q13.1, containing two neighboring pseudogenes, *CYP2D7* and *CYP2D8*. This locus is extremely polymorphic with over 80 allelic variants, a subset of which should affect the gene product and result in wide interindividual and ethnic differences in CYP2D6 activity.[27] Commonly, four CYP2D6 phenotypes are defined on the basis of their *in vivo* metabolic capacities: poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM) and ultra rapid metabolizer (UM).[28,29] It has been reported that the PM phenotype, which is caused by carrying two null alleles, is present in 5 10% of Caucasians.[30] The *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5* and *CYP2D6*6* are major null alleles that are related to the PM phenotype and account for nearly 95% of the PMs in Caucasians.[31] Among them, *CYP2D6*4* shows the highest frequency as 17.5 23.0%.[27] *CYP2D6*5*, which is found at a frequency of ~5%, lacks an entire *CYP2D6* gene. In contrast, <1% of Asians show the PM phenotype,[32] and most Asians are categorized as IMs because of the high frequency of a *CYP2D6*10* allele.[33,34] The *CYP2D6*14*, *CYP2D6*18*, *CYP2D6*21*, *CYP2D6*36* and *CYP2D6*44* were null alleles found in Asian populations, although their frequencies are very low.[35–38] The frequencies of UMs, who carry a duplicated/multiplied

wild type *CYP2D6* gene(s), are 10 15% in Caucasian, whereas UMs are uncommon in Asians. As described here, because the *CYP2D6* gene locus is complex, genotyping of *CYP2D6* variants, especially *CYP2D6*5*, is technically not so easy. Although the accuracy of genotyping partly depends on the quality of DNAs and the platforms of genotyping, wrong genotyping results sometimes cause incorrect interpretation of the research outcome, and result in both false positive conclusions and false negative conclusions.

CYP2D6 GENOTYPE AND CLINICAL OUTCOME OF TAMOXIFEN THERAPY

In recent years, we have seen an explosion of interest in the clinical relevance of *CYP2D6* genotype on outcome of breast cancer patients who are treated with tamoxifen. Prospective cohort studies of adjuvant tamoxifen treatment have revealed a wide interindividual variation in the steady state plasma concentrations of active metabo lites, endoxifen and 4 hydroxytamoxifen during tamoxifen treatment in patients carrying *CYP2D6* genetic variants.[8,9,11] The patients homozygous for null alleles (categorized as PM) showed nearly one fourth of endoxifen concentration in plasma, compared with those carrying two normal alleles (categorized as EM).[8,9] The patients carrying two alleles that encode a low function enzyme, including *CYP2D6*10* and *CYP2D6*41* (categorized as inter mediate metabolizer), had nearly 50% of plasma endoxifen concentration compared with the controls.[4,39–41] These patients with low endoxifen concentration were suspected to have a poorer clinical outcome.

As shown in Table 1, a number of studies have reported the association between the *CYP2D6* genotype and clinical outcome of breast cancer patients receiving the tamoxifen therapy. One of the first studies reported by Goetz *et al.*[42,43] demonstrated that homozygous carriers of *CYP2D6*4* allele had a shorter relapse free survival (RFS) and disease free survival than the patients for heterozygous or homozygous for the wild type allele (hazard ratio (HR), 1.85; $P=0.18$ for RFS: HR, 1.86; $P=0.089$ for disease free survival). Following these reports, Schroth *et al.*[44] published retrospective analysis of 1,325 breast cancer patients with adjuvant tamoxifen monotherapy, and observed that PMs revealed a significantly higher risk of recurrence than EMs with HR of 2.12 for a time to recurrence ($P=0.003$). These associations were supported by several research groups.[45–50] In Asians, we reported the significant effects of *CYP2D6* genotype (especially *CYP2D6*10*) on RFS in Japanese patients receiving adjuvant tamoxifen monotherapy (HR, 9.52; $P=0.000036$).[40,51] The worse clinical outcome of tamoxifen therapy in the patients carrying *CYP2D6*10* was confirmed in Chinese, Korean, Thai and Malaysian populations.[52–55] However, several discordant results have been also reported.[56–61] More recently, two retrospective analyses of large prospective trails, the ATAC (Alimidex, Tamoxifen, Alone or in Combination) trial and the Breast International Group (BIG) 1 98 trial, were reported.[62,63] In the ATAC analysis, there was no significant association between any of CYP2D6 phenotypic groups and recurrence rates in 588 patients treated with tamoxifen (HR, 1.22; $P=0.44$; PM relative to EM).[62] Similarly, in the BIG 1 98 analysis, no significant difference was found among different CYP2D6 metabolizer groups and cancer free survival in 973 breast cancer patients (HR, 0.58; $P=0.35$; EM vs PM).[63] As discussed in previous reports, there may be several confounding factors or critical errors in the experimental designs to explain these discrepancies.

One of the most important issues in the pharmacogenomics study is the quality of genotype data. This should be influenced by (i) the accuracy of genotyping methods, (ii) coverage of genotyped alleles and (iii) DNA source. In both of the ATAC and BIG 1 98 studies,[62,63] the authors mentioned the high reproducibility of genotyping methods because of the concordance of genotyping results in duplicate determinations. However, this does not fully guarantee the accuracy of their genotype results. Their genotype results were highly deviated from Hardy Weinberg equilibrium (χ^2 $P=10$ ^{92} for *CYP2D6*4*) probably because they used the low quality genomic DNA extracted from formalin fixed paraffin embedded tumor tis sues.[64–67] Therefore, they excluded *CYP2D6*5* from the analyses, and performed 60 cycle PCR to detect 1846 G>A (*CYP2D6*4*), which is likely to lead to the misgenotyping results. The importance of wide coverage of *CYP2D6* alleles was clearly demonstrated by Schroth *et al.*[68] In the report, the increase of genotyping coverage was shown to increase HR for RFS as well as enhance the statistical power. In our samples, we also detected a lower HR of 5.83 without *CYP2D6*5* genotyping data than that of 9.52 (*wt/wt* vs *V/V*, $N=282$; unpublished data). In addition, nearly 30% frequency of loss of heterozygosity at the chromosome 22q, where the *CYP2D6* gene is located, in breast cancer cells definitely causes misclassification of patients and leads to misinterpretation of the results if one uses DNAs isolated from tumor tissues (particularly caner cell rich samples).[69]

The second critical issue is selection of study participants. To evaluate the effects of *CYP2D6* genotype on tamoxifen efficacy, it is scientifically certain that the patients treated only with tamoxifen should be selected. As shown in Table 1, most of studies showing the 'null' association included the patients who were treated with a combination of tamoxifen and chemotherapy. We reported significant effects of *CYP2D6* genotypes on shorter RFS when we analyzed patients treated with the tamoxifen monotherapy (HR, 9.52; $P=0.0032$; $N=282$), but not when we analyzed those with the combination chemotherapy (HR, 0.64; $P=0.44$; $N=167$).[70,71] In a combined population (total 449 patents, including 37.2% of those with the combination therapy), HR dropped to 2.45 (95% confidence interval, 1.30 4.54) for *wt/wt* vs *V/V* (unpublished data).

These lines of evidence clearly tell us the importance of complete *CYP2D6* genotyping using germline DNAs isolated from very carefully selected samples with tamoxifen monotherapy. All of 'null' association studies lacked one or multiple elements of these essential factors, as shown in Table 1. Therefore, large prospective studies satisfying these conditions are needed to make a definite conclusion for the value of *CYP2D6* genotyping in tamoxifen therapy.

The patients carrying decreased or impaired function *CYP2D6* alleles consistently showed lower plasma endoxifen concentrations than those having the homozygous normal genotype.[4,8,9,11,39–41] Plasma endoxifen levels were suggested to associate with clinical outcome of tamoxifen treated patients.[72] Therefore, several research groups recently conducted *CYP2D6* genotype based dose adjustment studies.[73–75] Irvin *et al.*[74] demonstrated that endoxifen levels were significantly increased when the dose was increased from 20 40 mg in intermediate metabolizer and PM patients; however, endoxifen levels in PM patients were still significantly lower than the normal individuals. We also investigated the effects of the increase of tamoxifen dose from 20 to 30 mg or 40 mg in the patients heterozygous or homozygous for variant alleles, respectively, and demonstrated that endoxifen concentrations were significantly increased to a similar level of the CYP2D6 normal patients who took 20 mg of tamoxifen (Figure 2).[75] In these studies, the incidence of adverse events was not affected by the dose adjustment. Although further verification is required

Table 1 Summary of studies evaluating association of *CYP2D6* genotype with response to adjuvant tamoxifen therapy

							Association results			
Studies	*Number of patients*	*DNA source*	*Tamoxifen therapy*	*% of monotherapy*	*Tamoxifen dose*	*Outcome*	*Hazard ratio (95% CI)*	*P-value*	CYP2D6*5 *genotyping*	CYP2D6 *groups*[a]
Positive association										
Goetz *et al* [42]	190	FFPE tumors	Monotherapy	100%	20 mg per day for 5 years	DFS	2 44 (1 22–4 90)	0 012	No	*wt/wt+ wt/*4 vs *4/*4*
Goetz *et al* [43]	180	FFPE tumors	Monotherapy	100%	20 mg per day for 5 years	RFS	3 20 (1 37–7 55)	0 007	No	*wt/wt vs* PM
Schroth *et al* [45]	206	FFPE tumors	Monotherapy	100%	–[b]	RFS	2 24 (1 16–4 33)	0 02	Yes	EM vs decreased
Newman *et al* [46]	115	PBMC	+ Chemotherapy and/or rad at on	63 5%	20 mg per day med an >4 years	RFS	1 9 (0 8–4 8)	0 19	Yes	*wt/wt+ wt/V vs V/V*
K yotan *et al* [51]	58	PBMC	Monotherapy	100%	20 mg per day for 5 years	RFS	10 04 (1 17–86 27)	0 036	Yes	*wt/wt vs *10/*10*
Xu *et al* [52]	152	PBMC	Monotherapy	100%	–	DFS	4 7 (1 1–20 0)	0 04	No	*100C/C+ C/T vs T/T*
Schroth *et al* [44]	1 325	PBMC 44 5%[67] Tumor sect ons 55 5%[67]	Monotherapy	100%	For 5 years	RFS	1 49 (1 12–2 00) 2 12 (1 28–3 50)	0 006 0 003	Yes	*wt/wt vs* hetEM/ M *wt/wt vs* PM
B j *et al* [47]	85	PBMC	–	–	–	Breast cancer morta ty	4 1 (1 1–15 9)	0 04	No	*wt/wt vs *4/*4*
K yotan *et al* [40]	282	PBMC	Monotherapy	100%	20 mg per day for 5 years	RFS	4 44 (1 31–15 00) 9 52 (2 79–32 45)	0 017 0 0032	Yes	*wt/wt vs wt/V wt/wt vs V/V*
Ramon *et al* [48]	91	PBMC	+ Chemotherapy	39 8%	–	DFS	–	0 016[c]	Yes	Others vs PM
Park *et al* [53]	110	PBMC	+ Chemotherapy	21 8%	20 mg per day med an 3 9 years	RFS	5 59 (0 93–33 5)	0 05	Yes	EM vs PM
Teh *et al* [54]	95	PBMC	–		20 mg per day	Recurrence event	13 14 (1 54–109 94)[d]	0 004	Yes	EM vs M
Sukasem *et al* [55]	48	PBMC	+ Chemotherapy	6 3%	–	DFS	6 85 (1 48–31 69)	0 01	Yes	EM vs M
Damodaran *et al* [49]	132	PBMC	+ Chemotherapy	6 8%	For 5 years	RFS	7 15 (1 77–28 89)	0 006	Yes	Score ⩽0 5 vs score ⩾1
Goetz *et al* [50]	453	FFPE tumors	Monotherapy	100%	20 mg per day for 5 years	D sease event	2 45 (1 05–5 73)[d]	0 04	No	EM vs PM
Null association										
Nowe *et al* [56]	160	FFPE tumors	+ Chemotherapy and/or rad at on	14 2%	–	DFS	0 67 (0 33–1 35)	0 19	No	*wt/wt vs wt/*4+ *4/*4*
Wegman *et al* [57]	76	Fresh frozen tumors	+ Chemotherapy and/or rad at on	–	40 mg per day for 2 years	RFS	<1 0[e]	-	No	*wt/wt vs wt/*4+ *4/*4*
Wegman *et al* [58]	103	Fresh frozen tumors	–	–	40 mg per day for 2 years	RFS	0 87 (0 38–1 97)	0 74	No	*wt/wt vs wt/*4+ *4/*4*
	111	Fresh frozen tumors	–	–	40 mg per day for 5 years	RFS	0 33 (0 08–1 43)	0 14	No	*wt/wt vs wt/*4+ *4/*4*
Ok sh ro *et al* [59]	173	PBMC	+ Chemotherapy and/or gosere n	42 2%	20 mg per day med an 52 months	RFS	0 60 (0 18–1 92)	0 39	No	*100C/C+ C/T vs T/T*
K yotan *et al* [70]	167	PBMC	+ Chemotherapy	0%	20 mg per day for 5 years	RFS	1 05 (0 48–2 27) 0 64 (0 20–1 99)	0 91 0 44	Yes	*wt/wt vs wt/V wt/wt vs V/V*
Abraham *et al* [60]	3 155	PBMC	+ Chemotherapy	48 4%	20 mg per day	RFS	1 57 (0 64–3 84)	0 32	Yes	*Others vs* PM
Park *et al* [61]	130	PBMC	+ Chemotherapy and/or aromatase nh b tors	18 2%	–	RFS	1 34 (0 42–4 28)	0 63	Yes	*wt/wt+ wt/V vs V/V*
Rae *et al* [62]	588	FFPE tumors	+ Chemotherapy	95 7%	20 mg per day for 5 years	RFS	1 22 (0 76–1 96)	0 44	No	EM vs PM
Regan *et al* [63]	973	FFPE tumors	Monotherapy	100%	20 mg per day for 5 years	RFS	0 58 (0 28–1 21)	0 35	No	EM vs PM

Abbrev at ons C confidence nterva DFS d sease-free surv va EM extens ve metabo zer FFPE forma n-fixed paraffin-embedded M ntermed ate metabo zer PBMC per phera b ood mononuc ear ce PM poor metabo zer RFS recurrence-free surv va

Defin t on of a e es *wt *1, *1-*1* or **2 im *9,*10 *10-*10, *17* or **41 pm *3 *4 *5 *6 *14 *21* or **36-*36 V, im* or *pm*

Defin t on of genotype groups *wt/wt* 2 *wt* a e es EM *wt/wt* or *wt/im* M *im/im* or *im/pm* hetEM/ M *wt/im wt/pm im/im* or *im/pm* PM 2 *pm* a e es decreased *wt/pm im/im im/pm* or *pm/pm;* score⩽0 5, *im/pm* or *pm/pm;* score⩽1, *wt/wt, wt/im, wt/pm* or *im/im*

[a]Genotype group was reass gned us ng reported data

[b]Not reported

[c]Log- ank test *P*-va ue

[d]Odds rat o

[e]Not ca cu ated hazard rat o accord ng to *CYP2D6* genotypes

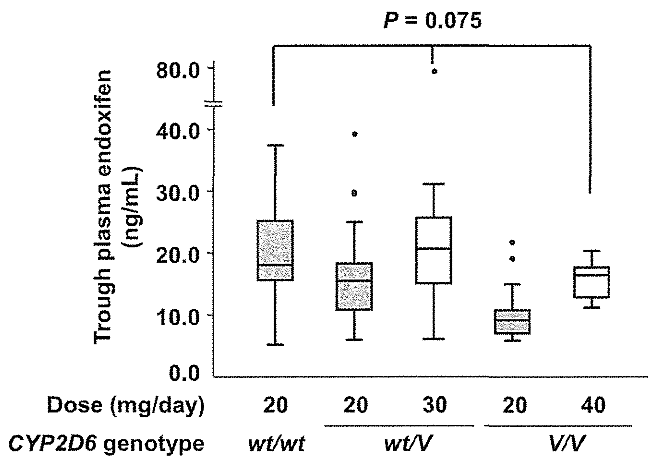

Figure 2 Steady state plasma concentration of endoxifen before and after dose escalation of tamoxifen in breast cancer patients. The horizontal line indicates the median concentration, the box covers the 25th 75th percentiles, and the maximum length of each whisker is 1.5× the interquartile range, dots outside the whiskers are outliers. Data from Kiyotani *et al.*[75]

especially for PM patients, these results suggest that increased tamoxifen dose is an effective way to maintain the effective endoxifen concentration for the patients carrying decreased function or null alleles of *CYP2D6*.

POLYMORPHISMS IN OTHER GENES AND CLINICAL OUTCOME OF TAMOXIFEN THERAPY

Other CYPs, including CYP2C9, CYP2C19, CYP3A4 and CYP3A5, UGTs and SULTs are also involved in the metabolism of tamoxifen. Among them, *CYP3A5*3* is well investigated in association with tamoxifen metabolism or clinical outcome of tamoxifen therapy; however, no significant association was observed.[4,9,42,45,76,77] For *CYP2C19*, a significant association with clinical outcome of tamoxifen treatment was found in carriers of *CYP2C19*17*,[45] but not in the carriers of *CYP2C19*2* or *CYP2C19*3*.[45,59] However, the results have also been contradictive and not conclusive.[78,79] Several investigations on genetic variations in the *SULT1A1* gene, including single nucleotide polymorphisms (SNPs) and copy number variations, found no clear association with tamoxifen efficacy[56,58,79] and tamoxifen metabolism.[9,57] Further analysis would be required by consideration of 'allele copy number' of *SULT1A1*, as demonstrated in the case of *CYP2D6*.[80–82]

There are several reports investigating the involvement of drug transporters in disposition of tamoxifen and its active metabolites, endoxifen and 4 hydroxytamoxifen. ABCB1 (P glycoprotein, multi drug resistance protein 1) is an ATP dependent, efflux transporter with broad substrate specificity widely appreciated for its role in mediating cellular resistance to many anticancer agents.[83] ABCB1 is reported to be involved in the transport of active tamoxifen metabolites.[84,85] Several *ABCB1* polymorphisms have been reported, including 2667 G>A/T and 3435C>T; however, no SNPs were significantly associated with clinical outcome of tamoxifen therapy.[40,54] ABCC2 (multidrug resistance associated protein 2) has an important role in the biliary excretion of glucuronides or sulfates of drugs, including tamoxifen and its metabolites.[17] We found an intronic SNP of *ABCC2* (rs3740065), which is in strong linkage disequilibrium ($r^2 = 0.89$) with 1774G/delG, to be significantly associated with clinical outcome of patients with tamoxifen therapy through the screening using haplotype tagging SNPs.[40,86] An *in vitro* study reporting that ABCC2 was expressed at higher levels in tamoxifen resistant breast cancer cells suggests the possibility that active metabolites of tamoxifen are transported by ABCC2 from breast cancer cells.[87]

We also identified a novel locus, containing *C10orf11*, associated with RFS in the breast cancer patients treated with tamoxifen alone by the genome wide association study encompassing a total of 462 Japanese patients (HR, 4.53; $P = 6.28 \times 10^{-8}$).[88] At present, however, no report is available regarding the function of the C10orf11 protein. Large scale replication study and further functional analysis are required to verify these associations, and to clarify their biological significance and mechanisms that have effects on the clinical outcome of patients receiving tamoxifen therapy.

OTHER FACTORS AFFECTING CLINICAL OUTCOME OF TAMOXIFEN THERAPY

As well as the genetic polymorphisms modifying the tamoxifen pharmacokinetics, characteristic of cancers, including gene expression profiles or genomic alterations, are also one of important determinants of individual response to tamoxifen. Many molecules have been identified to be involved in the tamoxifen resistance.[89,90] Several microarray analyses revealed the gene signatures to predict the outcome of adjuvant tamoxifen therapy, such as breast cancer intrinsic subtype,[91,92] 21 gene signature (used as OncotypeDX)[93] and *HOXB13/IL17BR* expression ratio.[94,95] Goetz *et al.*[96] reported that combination of *CYP2D6* genotype and *HOXB13/IL17BR* was significantly associated with disease free survival (log rank $P = 0.004$) and overall survival (log rank $P = 0.009$). More recently, Ellis *et al.*[97] clarified the elevated frequency of somatic mutations and genome structure changes in aromatase inhibitor resistant tumors by whole genome sequencing. Therefore, prediction of individual response to tamoxifen using cancer characteristics seems to be effective, and may affect the association results of genetic markers.

CONCLUSION

Although a large number of investigations on tamoxifen pharmaco genomics have been performed, the association results between *CYP2D6* genotype and clinical outcome are still controversial. However, accumulation of the evidence clarifies some of the causes of these controversial results, particularly some scientific issues in the false negative results, and implies the importance of the quality of genotyping as well as sample selections in the tamoxifen pharmaco genomics study. The important issues learned from the tamoxifen and breast cancer studies are commonly applicable in pharmacogenomics studies. As we are aiming to establish the personalized medicine system in which we select a right patient and provide an appropriate dose of a right drug, the pharmacogenomics study also requires the accurate genotyping using a sufficient number of appropriate patients in order to obtain truly positive results and avoid false positive and false negative results. Finally, genotype guided dose adjustment based on the *CYP2D6* genotypes will be a good example for the personalized medicine. To reduce the medical care cost without losing the quality of medical care, it is very important to use the drugs, which are available at lower cost, on the basis of individual genetic information. As several novel associated SNPs/loci have been identified, integration of genotypes of *CYP2D6* and other genes as well as tumor characteristics should be the future approach to predict clinical efficacy of tamoxifen and provide better quality of lives to breast cancer patients.

1 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* **365,** 1687 1717 (2005).
2 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* **351,** 1451 1467 (1998).
3 Wakeling, A. E. & Slater, S. R. Estrogen-receptor binding and biologic activity of tamoxifen and its metabolites. *Cancer Treat. Rep.* **64,** 741 744 (1980).
4 Murdter, T. E., Schroth, W., Bacchus-Gerybadze, L., Winter, S., Heinkele, G., Simon, W. *et al.* Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. *Clin. Pharmacol. Ther.* **89,** 708 717 (2011).
5 Boocock, D. J., Brown, K., Gibbs, A. H., Sanchez, E., Turteltaub, K. W. & White, I. N. Identification of human CYP forms involved in the activation of tamoxifen and irreversible binding to DNA. *Carcinogenesis* **23,** 1897 1901 (2002).
6 Crewe, H. K., Notley, L. M., Wunsch, R. M., Lennard, M. S. & Gillam, E. M. Metabolism of tamoxifen by recombinant human cytochrome P450 enzymes: formation of the 4-hydroxy, 4'-hydroxy and *N*-desmethyl metabolites and isomerization of *trans*-4-hydroxytamoxifen. *Drug Metab. Dispos.* **30,** 869 874 (2002).
7 Coller, J. K., Krebsfaenger, N., Klein, K., Wolbold, R., Nussler, A., Neuhaus, P. *et al.* Large interindividual variability in the *in vitro* formation of tamoxifen metabolites related to the development of genotoxicity. *Br. J. Clin. Pharmacol.* **57,** 105 111 (2004).
8 Borges, S., Desta, Z., Li, L., Skaar, T. C., Ward, B. A., Nguyen, A. *et al.* Quantitative effect of *CYP2D6* genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin. Pharmacol. Ther.* **80,** 61 74 (2006).
9 Jin, Y., Desta, Z., Stearns, V., Ward, B., Ho, H., Lee, K. H. *et al.* CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. *J. Natl. Cancer Inst.* **97,** 30 39 (2005).
10 Johnson, M. D., Zuo, H., Lee, K. H., Trebley, J. P., Rae, J. M., Weatherman, R. V. *et al.* Pharmacological characterization of 4-hydroxy-*N*-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res. Treat.* **85,** 151 159 (2004).
11 Stearns, V., Johnson, M. D., Rae, J. M., Morocho, A., Novielli, A., Bhargava, P. *et al.* Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J. Natl. Cancer Inst.* **95,** 1758 1764 (2003).
12 Clarke, R., Liu, M. C., Bouker, K. B., Gu, Z., Lee, R. Y., Zhu, Y. *et al.* Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling. *Oncogene* **22,** 7316 7339 (2003).
13 Crewe, H. K., Ellis, S. W., Lennard, M. S. & Tucker, G. T. Variable contribution of cytochromes P450 2D6, 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. *Biochem. Pharmacol.* **53,** 171 178 (1997).
14 Dehal, S. S. & Kupfer, D. CYP2D6 catalyzes tamoxifen 4-hydroxylation in human liver. *Cancer Res.* **57,** 3402 3406 (1997).
15 Coller, J. K., Krebsfaenger, N., Klein, K., Endrizzi, K., Wolbold, R., Lang, T. *et al.* The influence of *CYP2B6*, *CYP2C9* and *CYP2D6* genotypes on the formation of the potent antioestrogen Z-4-hydroxy-tamoxifen in human liver. *Br. J. Clin. Pharmacol.* **54,** 157 167 (2002).
16 Borgna, J. L. & Rochefort, H. Hydroxylated metabolites of tamoxifen are formed *in vivo* and bound to estrogen receptor in target tissues. *J. Biol. Chem.* **256,** 859 868 (1981).
17 Lien, E. A., Solheim, E., Lea, O. A., Lundgren, S., Kvinnsland, S. & Ueland, P. M. Distribution of 4-hydroxy-*N*-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. *Cancer Res.* **49,** 2175 2183 (1989).
18 Desta, Z., Ward, B. A., Soukhova, N. V. & Flockhart, D. A. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system *in vitro*: prominent roles for CYP3A and CYP2D6. *J. Pharmacol. Exp. Ther.* **310,** 1062 1075 (2004).
19 Falany, J. L., Pilloff, D. E., Leyh, T. S. & Falany, C. N. Sulfation of raloxifene and 4-hydroxytamoxifen by human cytosolic sulfotransferases. *Drug Metab. Dispos.* **34,** 361 368 (2006).
20 Gjerde, J., Hauglid, M., Breilid, H., Lundgren, S., Varhaug, J. E., Kisanga, E. R. *et al.* Effects of CYP2D6 and SULT1A1 genotypes including SULT1A1 gene copy number on tamoxifen metabolism. *Ann. Oncol.* **19,** 56 61 (2008).
21 Nishiyama, T., Ogura, K., Nakano, H., Ohnuma, T., Kaku, T., Hiratsuka, A. *et al.* Reverse geometrical selectivity in glucuronidation and sulfation of *cis*- and *trans*-4-hydroxytamoxifens by human liver UDP-glucuronosyltransferases and sulfotransferases. *Biochem. Pharmacol.* **63,** 1817 1830 (2002).
22 Ogura, K., Ishikawa, Y., Kaku, T., Nishiyama, T., Ohnuma, T., Muro, K. *et al.* Quaternary ammonium-linked glucuronidation of *trans*-4-hydroxytamoxifen, an active metabolite of tamoxifen, by human liver microsomes and UDP-glucuronosyltransferase 1A4. *Biochem. Pharmacol.* **71,** 1358 1369 (2006).
23 Sun, D., Sharma, A. K., Dellinger, R. W., Blevins-Primeau, A. S., Balliet, R. M., Chen, G. *et al.* Glucuronidation of active tamoxifen metabolites by the human UDP glucuronosyltransferases. *Drug Metab. Dispos.* **35,** 2006 2014 (2007).
24 Kaku, T., Ogura, K., Nishiyama, T., Ohnuma, T., Muro, K. & Hiratsuka, A. Quaternary ammonium-linked glucuronidation of tamoxifen by human liver microsomes and UDP-glucuronosyltransferase 1A4. *Biochem. Pharmacol.* **67,** 2093 2102 (2004).
25 Sun, D., Chen, G., Dellinger, R. W., Duncan, K., Fang, J. L. & Lazarus, P. Characterization of tamoxifen and 4-hydroxytamoxifen glucuronidation by human UGT1A4 variants. *Breast Cancer Res.* **8,** R50 (2006).
26 Ingelman-Sundberg, M. Genetic polymorphisms of cytochrome *P*450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J.* **5,** 6 13 (2005).
27 Bradford, L. D. *CYP2D6* allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* **3,** 229 243 (2002).
28 Sachse, C., Brockmoller, J., Bauer, S. & Roots, I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am. J. Hum. Genet.* **60,** 284 295 (1997).
29 Griese, E. U., Zanger, U. M., Brudermanns, U., Gaedigk, A., Mikus, G., Morike, K. *et al.* Assessment of the predictive power of genotypes for the *in-vivo* catalytic function of CYP2D6 in a German population. *Pharmacogenetics* **8,** 15 26 (1998).
30 Broly, F., Gaedigk, A., Heim, M., Eichelbaum, M., Morike, K. & Meyer, U. A. Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of CYP2D6 in a European population. *DNA Cell Biol.* **10,** 545 558 (1991).
31 Sachse, C., Brockmoller, J., Hildebrand, M., Muller, K. & Roots, I. Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolizers of debrisoquine. *Pharmacogenetics* **8,** 181 185 (1998).
32 Nakamura, K., Goto, F., Ray, W. A., McAllister, C. B., Jacqz, E., Wilkinson, G. R. *et al.* Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin. Pharmacol. Ther.* **38,** 402 408 (1985).
33 Yokota, H., Tamura, S., Furuya, H., Kimura, S., Watanabe, M., Kanazawa, I. *et al.* Evidence for a new variant *CYP2D6* allele *CYP2D6J* in a Japanese population associated with lower *in vivo* rates of sparteine metabolism. *Pharmacogenetics* **3,** 256 263 (1993).
34 Tateishi, T., Chida, M., Ariyoshi, N., Mizorogi, Y., Kamataki, T. & Kobayashi, S. Analysis of the *CYP2D6* gene in relation to dextromethorphan *O*-demethylation capacity in a Japanese population. *Clin. Pharmacol. Ther.* **65,** 570 575 (1999).
35 Wang, S. L., Huang, J. D., Lai, M. D., Liu, B. H. & Lai, M. L. Molecular basis of genetic variation in debrisoquin hydroxylation in Chinese subjects: polymorphism in RFLP and DNA sequence of *CYP2D6*. *Clin. Pharmacol. Ther.* **53,** 410 418 (1993).
36 Yokoi, T., Kosaka, Y., Chida, M., Chiba, K., Nakamura, H., Ishizaki, T. *et al.* A new *CYP2D6* allele with a nine base insertion in exon 9 in a Japanese population associated with poor metabolizer phenotype. *Pharmacogenetics* **6,** 395 401 (1996).
37 Chida, M., Yokoi, T., Nemoto, N., Inaba, M., Kinoshita, M. & Kamataki, T. A new variant *CYP2D6* allele (*CYP2D6*21*) with a single base insertion in exon 5 in a Japanese population associated with a poor metabolizer phenotype. *Pharmacogenetics* **9,** 287 293 (1999).
38 Yamazaki, H., Kiyotani, K., Tsubuko, S., Matsunaga, M., Fujieda, M., Saito, T. *et al.* Two novel haplotype of *CYP2D6* gene in a Japanese population. *Drug Metab. Pharmacokinet.* **18,** 269 271 (2003).
39 Lim, H. S., Lee, J. H., Lee, S. K., Lee, S. E., Jang, I. J. & Ro, J. Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. *J. Clin. Oncol.* **25,** 3837 3845 (2007).
40 Kiyotani, K., Mushiroda, T., Imamura, C. K., Hosono, N., Tsunoda, T., Kubo, M. *et al.* Significant effect of polymorphisms in *CYP2D6* and *ABCC2* on clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients. *J. Clin. Oncol.* **28,** 1287 1293 (2010).
41 Lim, J. S., Chen, X. A., Singh, O., Yap, Y. S., Ng, R. C., Wong, N. S. *et al.* Impact of *CYP2D6*, *CYP3A5*, *CYP2C9* and *CYP2C19* polymorphisms on tamoxifen pharmacokinetics in Asian breast cancer patients. *Br. J. Clin. Pharmacol.* **71,** 737 750 (2011).
42 Goetz, M. P., Rae, J. M., Suman, V. J., Safgren, S. L., Ames, M. M., Visscher, D. W. *et al.* Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J. Clin. Oncol.* **23,** 9312 9318 (2005).
43 Goetz, M. P., Knox, S. K., Suman, V. J., Rae, J. M., Safgren, S. L., Ames, M. M. *et al.* The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. *Breast Cancer Res. Treat.* **101,** 113 121 (2007).
44 Schroth, W., Goetz, M. P., Hamann, U., Fasching, P. A., Schmidt, M., Winter, S. *et al.* Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. *JAMA* **302,** 1429 1436 (2009).
45 Schroth, W., Antoniadou, L., Fritz, P., Schwab, M., Muerdter, T., Zanger, U. M. *et al.* Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. *J. Clin. Oncol.* **25,** 5187 5193 (2007).
46 Newman, W. G., Hadfield, K. D., Latif, A., Roberts, S. A., Shenton, A., McHague, C. *et al.* Impaired tamoxifen metabolism reduces survival in familial breast cancer patients. *Clin. Cancer Res.* **14,** 5913 5918 (2008).
47 Bijl, M. J., van Schaik, R. H., Lammers, L. A., Hofman, A., Vulto, A. G., van Gelder, T. *et al.* The CYP2D6*4 polymorphism affects breast cancer survival in tamoxifen users. *Breast Cancer Res. Treat.* **118,** 125 130 (2009).
48 Ramón, Y., Altés, A., Paré, L., del Rio, E., Alonso, C., Barnadas, A. *et al.* Impact of CYP2D6 polymorphisms in tamoxifen adjuvant breast cancer treatment. *Breast Cancer Res. Treat.* **119,** 33 38 (2010).
49 Damodaran, S. E., Pradhan, S. C., Umamaheswaran, G., Kadambari, D., Reddy, K. S. & Adithan, C. Genetic polymorphisms of *CYP2D6* increase the risk for recurrence of breast cancer in patients receiving tamoxifen as an adjuvant therapy. *Cancer Chemother. Pharmacol.* **70,** 75 81 (2012).
50 Goetz, M. P., Suman, V. J., Hoskin, T. L., Gnant, M., Filipits, M., Safgren, S. L. *et al.* CYP2D6 metabolism and patient outcome in the Austrian Breast and Colorectal Cancer Study Group trial (ABCSG) 8. *Clin. Cancer Res.* **19,** 500 507 (2013).
51 Kiyotani, K., Mushiroda, T., Sasa, M., Bando, Y., Sumitomo, I., Hosono, N. *et al.* Impact of *CYP2D6*10* on recurrence-free survival in breast cancer patients receiving adjuvant tamoxifen therapy. *Cancer Sci.* **99,** 995 999 (2008).

52 Xu, Y., Sun, Y., Yao, L., Shi, L., Wu, Y., Ouyang, T. *et al.* Association between CYP2D6*10 genotype and survival of breast cancer patients receiving tamoxifen treatment. *Ann. Oncol.* **19,** 1423 1429 (2008).
53 Park, H. S., Choi, J. Y., Lee, M. J., Park, S., Yeo, C. W., Lee, S. S. *et al.* Association between genetic polymorphisms of *CYP2D6* and outcomes in breast cancer patients with tamoxifen treatment. *J. Korean Med. Sci.* **26,** 1007 1013 (2011).
54 Teh, L. K., Mohamed, N. I., Salleh, M. Z., Rohaizak, M., Shahrun, N. S., Saladina, J. J. *et al.* The risk of recurrence in breast cancer patients treated with tamoxifen: polymorphisms of *CYP2D6* and *ABCB1*. *AAPS J.* **14,** 52 59 (2012).
55 Sukasem, C., Sirachainan, E., Chamnanphon, M., Pechatanan, K., Sirisinha, T., Ativitavas, T. *et al.* Impact of *CYP2D6* polymorphisms on tamoxifen responses of women with breast cancer: a microarray-based study in Thailand. *Asian Pac. J. Cancer Prev.* **13,** 4549 4553 (2012).
56 Nowell, S. A., Ahn, J., Rae, J. M., Scheys, J. O., Trovato, A., Sweeney, C. *et al.* Association of genetic variation in tamoxifen-metabolizing enzymes with overall survival and recurrence of disease in breast cancer patients. *Breast Cancer Res. Treat.* **91,** 249 258 (2005).
57 Wegman, P., Vainikka, L., Stal, O., Nordenskjold, B., Skoog, L., Rutqvist, L. E. *et al.* Genotype of metabolic enzymes and the benefit of tamoxifen in postmenopausal breast cancer patients. *Breast Cancer Res.* **7,** R284 R290 (2005).
58 Wegman, P., Elingarami, S., Carstensen, J., Stal, O., Nordenskjold, B. & Wingren, S. Genetic variants of *CYP3A5*, *CYP2D6*, *SULT1A1*, *UGT2B15* and tamoxifen response in postmenopausal patients with breast cancer. *Breast Cancer Res.* **9,** R7 (2007).
59 Okishiro, M., Taguchi, T., Jin Kim, S., Shimazu, K., Tamaki, Y. & Noguchi, S. Genetic polymorphisms of CYP2D6*10 and CYP2C19*2, *3 are not associated with prognosis, endometrial thickness, or bone mineral density in Japanese breast cancer patients treated with adjuvant tamoxifen. *Cancer* **115,** 952 961 (2009).
60 Abraham, J. E., Maranian, M. J., Driver, K. E., Platte, R., Kalmyrzaev, B., Baynes, C. *et al.* CYP2D6 gene variants: association with breast cancer specific survival in a cohort of breast cancer patients from the United Kingdom treated with adjuvant tamoxifen. *Breast Cancer Res.* **12,** R64 (2010).
61 Park, I. H., Ro, J., Park, S., Lim, H. S., Lee, K. S., Kang, H. S. *et al.* Lack of any association between functionally significant *CYP2D6* polymorphisms and clinical outcomes in early breast cancer patients receiving adjuvant tamoxifen treatment. *Breast Cancer Res. Treat.* **131,** 455 461 (2012).
62 Rae, J. M., Drury, S., Hayes, D. F., Stearns, V., Thibert, J. N., Haynes, B. P. *et al.* *CYP2D6* and *UGT2B7* genotype and risk of recurrence in tamoxifen-treated breast cancer patients. *J. Natl Cancer Inst.* **104,** 452 460 (2012).
63 Regan, M. M., Leyland-Jones, B., Bouzyk, M., Pagani, O., Tang, W., Kammler, R. *et al.* *CYP2D6* genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. *J. Natl Cancer Inst.* **104,** 441 451 (2012).
64 Pharoah, P. D., Abraham, J. & Caldas, C. Re: *CYP2D6* genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial and Re: *CYP2D6* and *UGT2B7* genotype and risk of recurrence in tamoxifen-treated breast cancer patients. *J. Natl Cancer Inst.* **104,** 1263 1264 (2012).
65 Nakamura, Y., Ratain, M. J., Cox, N. J., McLeod, H. L., Kroetz, D. L. & Flockhart, D. A. Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. *J. Natl Cancer Inst.* **104,** 1264 (2012).
66 Stanton Jr V. Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. *J. Natl Cancer Inst* **104,** 1265 1266 (2012).
67 Brauch, H., Schroth, W., Goetz, M. P., Murdter, T. E., Winter, S., Ingle, J. N. *et al.* Tamoxifen use in postmenopausal breast cancer: CYP2D6 matters. *J. Clin. Oncol.* **31,** 176 180 (2013).
68 Schroth, W., Hamann, U., Fasching, P. A., Dauser, S., Winter, S., Eichelbaum, M. *et al.* *CYP2D6* polymorphisms as predictors of outcome in breast cancer patients treated with tamoxifen: expanded polymorphism coverage improves risk stratification. *Clin. Cancer Res.* **16,** 4468 4477 (2010).
69 Hirano, A., Emi, M., Tsuneizumi, M., Utada, Y., Yoshimoto, M., Kasumi, F. *et al.* Allelic losses of loci at 3p25.1, 8p22, 13q12, 17p13.3, and 22q13 correlate with postoperative recurrence in breast cancer. *Clin. Cancer Res.* **7,** 876 882 (2001).
70 Kiyotani, K., Mushiroda, T., Hosono, N., Tsunoda, T., Kubo, M., Aki, F. *et al.* Lessons for pharmacogenomics studies: association study between *CYP2D6* genotype and tamoxifen response. *Pharmacogenet. Genomics* **20,** 565 568 (2010).
71 Kiyotani, K., Mushiroda, T., Nakamura, Y. & Zembutsu, H. Pharmacogenomics of tamoxifen: roles of drug metabolizing enzymes and transporters. *Drug Metab. Pharmacokinet.* **27,** 122 131 (2012).
72 Madlensky, L., Natarajan, L., Tchu, S., Pu, M., Mortimer, J., Flatt, S. W. *et al.* Tamoxifen metabolite concentrations, *CYP2D6* genotype, and breast cancer outcomes. *Clin. Pharmacol. Ther.* **89,** 718 725 (2011).
73 Barginear, M. F., Jaremko, M., Peter, I., Yu, C., Kasai, Y., Kemeny, M. *et al.* Increasing tamoxifen dose in breast cancer patients based on CYP2D6 genotypes and endoxifen levels: effect on active metabolite isomers and the antiestrogenic activity score. *Clin. Pharmacol. Ther.* **90,** 605 611 (2011).
74 Irvin, W. J. Jr., Walko, C. M., Weck, K. E., Ibrahim, J. G., Chiu, W. K., Dees, E. C. *et al.* Genotype-guided tamoxifen dosing increases active metabolite exposure in women with reduced CYP2D6 metabolism: a multicenter study. *J. Clin. Oncol.* **29,** 3232 3239 (2011).
75 Kiyotani, K., Mushiroda, T., Imamura, C. K., Tanigawara, Y., Hosono, N., Kubo, M. *et al.* Dose-adjustment study of tamoxifen based on *CYP2D6* genotypes in Japanese breast cancer patients. *Breast Cancer Res. Treat.* **131,** 137 145 (2012).
76 Tucker, A. N., Tkaczuk, K. A., Lewis, L. M., Tomic, D., Lim, C. K. & Flaws, J. A. Polymorphisms in cytochrome P4503A5 (CYP3A5) may be associated with race and tumor characteristics, but not metabolism and side effects of tamoxifen in breast cancer patients. *Cancer Lett.* **217,** 61 72 (2005).
77 Gjerde, J., Geisler, J., Lundgren, S., Ekse, D., Varhaug, J. E., Mellgren, G. *et al.* Associations between tamoxifen, estrogens, and FSH serum levels during steady state tamoxifen treatment of postmenopausal women with breast cancer. *BMC Cancer* **10,** 313 (2010).
78 Ruiter, R., Bijl, M. J., van Schaik, R. H., Berns, E. M., Hofman, A., Coebergh, J. W. *et al.* *CYP2C19*2* polymorphism is associated with increased survival in breast cancer patients using tamoxifen. *Pharmacogenomics* **11,** 1367 1375 (2010).
79 Moyer, A. M., Suman, V. J., Weinshilboum, R. M., Avula, R., Black, J. L., Safgren, S. L. *et al.* *SULT1A1*, *CYP2C19* and disease-free survival in early breast cancer patients receiving tamoxifen. *Pharmacogenomics* **12,** 1535 1543 (2011).
80 Hosono, N., Kato, M., Kiyotani, K., Mushiroda, T., Takata, S., Sato, H. *et al.* *CYP2D6* genotyping for functional-gene dosage analysis by allele copy number detection. *Clin. Chem.* **55,** 1546 1554 (2009).
81 Hosono, N., Kubo, M., Tsuchiya, Y., Sato, H., Kitamoto, T., Saito, S. *et al.* Multiplex PCR-based real-time invader assay (mPCR-RETINA): a novel SNP-based method for detecting allelic asymmetries within copy number variation regions. *Hum. Mutat.* **29,** 182 189 (2008).
82 Kiyotani, K., Shimizu, M., Kumai, T., Kamataki, T., Kobayashi, S. & Yamazaki, H. Limited effects of frequent *CYP2D6*36-*10* tandem duplication allele on *in vivo* dextromethorphan metabolism in a Japanese population. *Eur. J. Clin. Pharmacol.* **66,** 1065 1068 (2010).
83 Goda, K., Bacso, Z. & Szabo, G. Multidrug resistance through the spectacle of P-glycoprotein. *Curr. Cancer Drug Targets* **9,** 281 297 (2009).
84 Teft, W. A., Mansell, S. E. & Kim, R. B. Endoxifen, the active metabolite of tamoxifen, is a substrate of the efflux transporter P-glycoprotein (multidrug resistance 1). *Drug Metab. Dispos.* **39,** 558 562 (2011).
85 Iusuf, D., Teunissen, S. F., Wagenaar, E., Rosing, H., Beijnen, J. H. & Schinkel, A. H. P-glycoprotein (ABCB1) transports the primary active tamoxifen metabolites endoxifen and 4-hydroxytamoxifen and restricts their brain penetration. *J. Pharmacol. Exp. Ther.* **337,** 710 717 (2011).
86 Kiyotani, K., Mushiroda, T., Nakamura, Y. & Zembutsu, H. *ABCC2* and clinical outcome of tamoxifen therapy: reply to T. Lang et al. *J. Clin. Oncol.* **28,** e449 (2010).
87 Choi, H. K., Yang, J. W., Roh, S. H., Han, C. Y. & Kang, K. W. Induction of multidrug resistance associated protein 2 in tamoxifen-resistant breast cancer cells. *Endocr. Relat. Cancer* **14,** 293 303 (2007).
88 Kiyotani, K., Mushiroda, T., Tsunoda, T., Morizono, T., Hosono, N., Kubo, M. *et al.* A genome-wide association study identifies locus at 10q22 associated with clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients in Japanese. *Hum. Mol. Genet.* **21,** 1665 1672 (2012).
89 Musgrove, E. A. & Sutherland, R. L. Biological determinants of endocrine resistance in breast cancer. *Nat. Rev. Cancer* **9,** 631 643 (2009).
90 Giuliano, M., Schifp, R., Osborne, C. K. & Trivedi, M. V. Biological mechanisms and clinical implications of endocrine resistance in breast cancer. *Breast* **20** (Suppl 3), S42 S49 (2011).
91 Sørlie, T., Perou, C. M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H. *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl Acad. Sci. USA* **98,** 10869 10874 (2001).
92 Sørlie, T., Tibshirani, R., Parker, J., Hastie, T., Marron, J. S., Nobel, A. *et al.* Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc. Natl Acad. Sci. USA* **100,** 8418 8423 (2003).
93 Paik, S., Shak, S., Tang, G., Kim, C., Baker, J., Cronin, M. *et al.* A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N. Engl. J. Med.* **351,** 2817 2826 (2004).
94 Ma, X. J., Wang, Z., Ryan, P. D., Isakoff, S. J., Barmettler, A., Fuller, A. *et al.* A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. *Cancer Cell* **5,** 607 616 (2004).
95 Jansen, M. P., Sieuwerts, A. M., Look, M. P., Ritstier, K., Meijer-van Gelder, M. E., van Staveren, I. L. *et al.* *HOXB13*-to-*IL17BR* expression ratio is related with tumor aggressiveness and response to tamoxifen of recurrent breast cancer: a retrospective study. *J. Clin. Oncol.* **25,** 662 668 (2007).
96 Goetz, M. P., Suman, V. J., Couch, F. J., Ames, M. M., Rae, J. M., Erlander, M. G. *et al.* Cytochrome P450 2D6 and homeobox 13/interleukin-17B receptor: combining inherited and tumor gene markers for prediction of tamoxifen resistance. *Clin. Cancer Res.* **14,** 5864 5868 (2008).
97 Ellis, M. J., Ding, L., Shen, D., Luo, J., Suman, V. J., Wallis, J. W. *et al.* Whole-genome analysis informs breast cancer response to aromatase inhibition. *Nature* **486,** 353 360 (2012).

Cancer Science
The official journal of the Japanese Cancer Association
JCA

Genome-wide association study of chemotherapeutic agent-induced severe neutropenia/leucopenia for patients in Biobank Japan

Siew-Kee Low,[1,4] Suyoun Chung,[4,5] Atsushi Takahashi,[1] Hitoshi Zembutsu,[4] Taisei Mushiroda,[2] Michiaki Kubo[3] and Yusuke Nakamura[4,5,6]

Laboratories for [1]Statistical Analysis; [2]Pharmacogenetics; [3]Genotyping Development, Center for Genomic Medicine, RIKEN, Yokohama; [4]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [5]Department of Medicine, The University of Chicago, Chicago, Illinois, USA

(Received March 21, 2013/Revised April 22, 2013/Accepted April 22, 2013/Accepted manuscript online May 4, 2013/Article first published online June 10, 2013)

Chemotherapeutic agents are notoriously known to have a narrow therapeutic range that often results in life-threatening toxicity. Hence, it is clinically important to identify the patients who are at high risk for severe toxicity to certain chemotherapy through a pharmacogenomics approach. In this study, we carried out multiple genome-wide association studies (GWAS) of 13 122 cancer patients who received different chemotherapy regimens, including cyclophosphamide- and platinum-based (cisplatin and carboplatin), anthracycline-based (doxorubicin and epirubicin), and antimetabolite-based (5-fluorouracil and gemcitabine) treatment, antimicrotubule agents (paclitaxel and docetaxel), and topoisomerase inhibitors (camptothecin and etoposide), as well as combination therapy with paclitaxel and carboplatin, to identify genetic variants that are associated with the risk of severe neutropenia/leucopenia in the Japanese population. In addition, we used a weighted genetic risk scoring system to evaluate the cumulative effects of the suggestive genetic variants identified from GWAS in order to predict the risk levels of individuals who carry multiple risk alleles. Although we failed to identify genetic variants that surpassed the genome-wide significance level ($P < 5.0 \times 10^{-8}$) through GWAS, probably due to insufficient statistical power and complex clinical features, we were able to shortlist some of the suggestive associated loci. The current study is at the relatively preliminary stage, but does highlight the complexity and problematic issues associated with retrospective pharmacogenomics studies. However, we hope that verification of these genetic variants through local and international collaborations could improve the clinical outcome for cancer patients. (*Cancer Sci* 2013; 104: 1074–1082)

It is now widely and well recognized that medication can cause distinct heterogeneity in terms of its efficacy and toxicity among individuals. These inter individual differences could be explained in part by the common and/or rare genetic variants in the human genome. Pharmacogenomics aims to discover how genetic variations in the human genome can affect a drug's efficacy or toxicity, and thus brings great promise for personalized medicine in which genetic information can be used to predict the safety, toxicity, and/or efficacy of drugs.[1] Pharmacogenomics study for chemotherapeutic therapies is particularly important because these drugs are known to have a narrow therapeutic window; in general, a higher concentration causes toxicity and a lower concentration reduces the efficacy of the drug. Two of the well described examples are the association of genetic variants in *TPMT* with 6 mercaptopurine induced myelosuppression in treatment of pediatric acute lymphoblastic leukemia and that of *UGT1A1* variants with camptothecin related neutropenia and diarrhea in treatment of colorectal and lung cancers. The US Food and Drug Administration have recommended that variants on these two genes should be helpful for the prediction of severe adverse reactions prior to use of the drugs.[2–7]

With advances in various technologies in the life sciences, it is now possible to accurately genotype more than a million common genetic variations by genome wide high density SNP array or to characterize all genetic variants in our genome by the next generation DNA sequencing methods. Although one of the greatest drawbacks of GWAS is the requirement of the large number of samples to achieve high statistical power,[8] this issue could be overcome by the establishment of Biobank Japan in 2003 (http://biobankjp.org/).[9] Biobank Japan collected approximately 330 000 disease cases (200 000 individuals) that had either one or multiples of 47 different diseases including cancers from a collaborative network of 66 hospitals throughout Japan, with the major aim to identify genetic variants associated with susceptibility to complex diseases or those related to drug toxicity. By using the samples from Biobank Japan, a significant number of insightful findings have been published in recent years for identification of common genetic variants associated with complex diseases including cancer.[10–19] With a reasonable number of samples, it is also feasible to carry out pharmacogenomics studies on chemotherapy induced toxicity.

Neutropenia and/or leucopenia are two of the most common drug adverse events after treatment with chemotherapeutic agents, which often cause life threatening infections and the delay of treatment schedule that subsequently affect the treatment outcome. Although prophylactic granulocyte colony stimulating factor has been given to the patients as a preventive measure,[20] the underlying mechanism and susceptible risk factors that cause neutropenia have not been fully elucidated. In this study, we carried out a total of 17 sets of GWAS using 13 122 cancer patients, who received various drug regimens, to identify genetic variants associated with the risk of chemotherapeutic agent induced severe neutropenia/leucopenia in the Japanese population.

Subjects and Method

Study subjects. A total of 13 122 DNA samples from cancer patients, who received various chemotherapeutic agents, stored in Biobank Japan (University of Tokyo, Tokyo, Japan), were used in this study. Among them, 805 patients developed severe neutropenia and/or leucopenia (≥grade 3), and 4804 patients

[6]To whom correspondence should be addressed.
E-mails: yusuke@ims.u-tokyo.ac.jp; ynakamura@bsd.uchicago.edu

doi: 10.1111/cas.12186
© 2013 Japanese Cancer Association

were not reported to develop any adverse reactions after being given chemotherapeutic agents. The samples could be classified into subgroups according to the drugs used: an alkylating agent (cyclophosphamide); platinum based (cisplatin and carboplatin), anthracycline based (doxorubicin and epirubicin); antimetabolite based (5 fluorouracil and gemcitabine), antimicrotubule based (paclitaxel and docetaxel); and topoisomerase inhibitor based (camptothecin and etoposide). The grade of toxicity was classified in accordance with the US National Cancer Institute's Common Toxicity Criteria version 2.0. The adverse event description is based on the medical records collected by the medical coordinator. The patients' demographic details are summarized in Table 1. Participants of this study provided written inform consent and this project was approved by the ethical committee from the Institute of Medical Sciences, University of Tokyo and the RIKEN Center for Genomic Medicine (Yokohama, Japan).

Genotyping and quality controls. DNAs obtained from the patients' blood were genotyped using Illumina OmniExpress BeadChip (San Diego, CA, USA) that contained 733 202 SNPs. Sample quality control was carried out by methods including identity by state to evaluate cryptic relatedness for each sample and population stratification by the use of principal component analysis to exclude genetically heterogeneous samples from further analysis.[21,22] Then, our standard SNP quality control was carried out by excluding SNPs deviating from the Hardy Weinberg equilibrium ($P \leq 1.0 \times 10^{-6}$), non polymorphic SNPs, SNPs with a call rate of <0.99, and those on the X chromosome.[21,22] Q Q plot and lambda values, which were calculated between observed P values from Fisher's test allelic model against expected P values, were used to further evaluate population substructure.

Statistical analysis. Genome wide case control association analyses were evaluated using Fisher's exact method considering allelic, dominant, and recessive genetic models. Manhattan plots of the study were generated using the minimum P value among the three genetic models for each SNP.

Scoring system using wGRS. The scoring analysis was carried out using SNPs with P_{min} of $<1.0 \times 10^{-5}$ after exclusion of SNPs that are in strong linkage disequilibrium ($r^2 > 0.8$) in each GWAS. The wGRS were calculated according to De Jager *et al.*[23] Briefly, we first calculated the weight of each SNP that is the natural log of the odds ratio for each allele/genotype, considering the associated genetic model. For an additive model, we assigned a score of 2 to an individual with two risk alleles, 1 to that with one risk allele, and 0 to that with no risk allele. For a dominant model, we assigned a score of 1 to an individual with one or two risk alleles, and 0 to that with no risk allele. For a recessive model, we assigned a score of 1 to an individual with two risk alleles, and 0 to that with no or one risk allele. Then the cumulative genetic risk scores were determined by multiplying the number of risk alleles/genotype of each SNP by its corresponding weight, and subsequently took the sum across the total number of SNPs that were taken into consideration of each GWAS set. We classified the genetics risk score into four different groups created from the mean and SD: group 1, <mean − 1SD; group 2, mean − 1SD to mean; group 3, mean to mean + 1SD; and group 4, >mean + 1SD. Odds ratio, 95% confidence interval, P value, sensitivity, and specificity were calculated using group 1 as a reference. To calculate the OR in which one of the cells in the contingency table is zero, we applied the Haldane correction, used to avoid error in the calculation by adding 0.5 to all of the cells of a contingency table.

Results

After subdividing the patients by administered drugs/major drug subgroups, as previously mentioned, a total of 17 GWAS analyses were carried out by comparing the allele/genotype frequency between the patients who had developed severe neutropenia/leucopenia (grade 3/4) to those who had not developed any adverse drug reactions. The Q Q plots of each GWAS and the calculated lambda value of below 1.00 indicated no significant population stratification in each of these GWAS analyses (Fig. S1). From this study, although we could not identify any SNPs that surpassed the genome wide significant threshold (P value $< 5 \times 10^{-8}$) for showing association with the risk of neutropenia/leucopenia induced by the certain type of drug or regimen, several possible candidate loci were identified. The results of the GWAS are summarized in Table 2, Table S1, and Figure S2; the results of wGRS are summarized in Table S2.

Table 1. Demographic details of cancer patients treated with chemotherapeutic agents, whose DNA samples are stored in Biobank Japan (The University of Tokyo, Tokyo, Japan)

Category	Controls†	Grade 1/2	Grade 3/4	Category	Controls†	Grade 1/2	Grade 3/4
All	4804	1253	805	Drug subtype			
Age, years (mean)	62.9	58.7	59.6	Alkylating agent	346	266	176
Gender				Cyclophosphamide	335	255	168
Male	2604	424	318	Platinum based	743	429	428
Female	2200	829	487	Cisplatin	471	191	176
Cancer subtype				Carboplatin	262	207	261
Lung cancer	587	259	266	Anthracycline	459	240	184
Breast cancer	876	388	204	Doxorubicin	66	85	83
Ovarian cancer	140	124	74	Epirubicin	370	132	83
Gastric cancer	827	100	56	Antimetabolite	2249	512	294
Esophageal cancer	208	65	53	5 Fluorouracil	952	331	177
Colorectal cancer	1573	161	50	Gemcitabine	226	111	80
Endometrial cancer	78	72	45	Antimicrotubule agent	825	468	371
Cervical cancer	129	57	35	Paclitaxel	364	321	218
Prostate cancer	91	13	21	Docetaxel	233	143	147
Pancreatic cancer	83	36	20	Topoisomerase inhibitor	187	123	106
Liver cancer	366	16	9	Camptothecin	155	106	59
Gallbladder cancer	56	9	1	Etoposide	39	19	54
				Paclitaxel + carboplatin	166	161	150

†Individuals who did not develop any adverse drug reactions after chemotherapy.

© 2013 Japanese Cancer Association

Table 2. Association analysis of single nucleotide polymorphisms (SNPs) with different chemotherapeutic drugs/drug subgroups known to induce severe neutropenia/leucopenia

CHR	SNP	BP	RA	NRA	RAF_Case	RAF_Ctr	P_a e c	P_dom	P_rec	Pm n	OR	L95	U95	Gene	re oc
Cyc ophospham de															
16	rs2519974*	22889186	T	C	0 503	0 381	2 52E-04	4 35E-06	2 77E-01	4 35E-06	1 647	1 264	2 146	*HS3ST2*	0
1	rs10922438*	198469162	T	C	0 214	0 106	6 01E-06	1 71E-05	7 10E-02	6 01E-06	2 301	1 608	3 293	*ATP6V1G3*	23190
19	rs3745571*	6475613	T	C	0 778	0 670	4 05E-04	7 72E-06	1 00E+00	7 72E-06	1 730	1 276	2 345	*DENND1C*	0
A p at num-based drugs															
15	rs4886670*	75449674	A	C	0 320	0 227	9 86E-07	1 43E-05	8 14E-04	9 86E-07	1 605	1 330	1 937	*RPL36AP45*	29318
19	rs33428*	30937843	G	A	0 481	0 403	2 71E-04	2 78E-06	3 11E-01	2 78E-06	1 375	1 160	1 629	*ZNF536*	0
14	rs12589282*	22937656	G	T	0 535	0 437	6 30E-06	5 42E-03	4 11E-06	4 11E-06	1 480	1 250	1 752	*TRA@*	0
3	rs3845905*	66525963	G	A	0 915	0 850	4 12E-06	7 45E-05	3 65E-04	4 12E-06	1 894	1 433	2 503	*LRIG1*	0
5	rs1895302*	169542600	C	T	0 551	0 478	6 95E-04	7 41E-06	4 11E-01	7 41E-06	1 340	1 132	1 587	*FOXI1*	5871
1	rs16825455*	21837755	T	C	0 686	0 605	8 85E-05	8 62E-06	1 90E-01	8 62E-06	1 425	1 193	1 702	*ALPL*	0
C sp at n															
7	rs10253216*	16861849	T	C	0 565	0 468	2 18E-03	1 68E-07	1 00E+00	1 68E-07	1 478	1 155	1 891	*AGR2*	−17111
4	rs11944965*	63424089	T	C	0 807	0 678	3 45E-06	1 68E-06	6 65E-02	1 68E-06	1 986	1 475	2 676	*LOC644534*	47600
7	rs7797977*	16862235	C	A	0 668	0 580	4 06E-03	5 23E-01	2 17E-06	2 17E-06	1 457	1 127	1 883	*AGR2*	−17497
18	rs2406342*	74488280	T	G	0 605	0 475	3 59E-05	2 48E-06	6 71E-02	2 48E-06	1 697	1 323	2 177	*ZNF236*	−47836
20	rs6077251*	7752366	T	C	0 271	0 153	2 50E-06	3 64E-06	3 06E-02	2 50E-06	2 065	1 537	2 773	*SFRS13AP2*	59982
8	rs11774576*	27740417	A	G	0 702	0 581	6 78E-05	2 82E-06	2 62E-01	2 82E-06	1 699	1 307	2 208	*SCARA5*	0
11	rs4627050*	18822037	G	A	0 781	0 649	4 43E-06	9 01E-06	2 17E-02	4 43E-06	1 932	1 452	2 572	*PTPN5*	−8648
1	rs12142335*	108302922	A	G	0 040	0 004	9 91E-06	8 45E-06	1 00E+00	8 45E-06	9 713	3 175	29 710	*VAV3*	0
Carbop at n															
15	rs11071200*	55950082	T	G	0 060	0 008	1 25E-06	8 51E-07	1 00E+00	8 51E-07	8 241	2 888	23 520	*PRTG*	0
5	rs3822735*	35799994	G	A	0 862	0 752	7 24E-06	1 68E-06	3 50E-01	1 68E-06	2 062	1 500	2 834	*SPEF2*	0
3	rs1623879*	58027197	G	A	0 441	0 321	7 69E-05	1 89E-02	3 75E-06	3 75E-06	1 669	1 297	2 148	*FLNB*	0
15	rs936229*	75132319	G	A	0 713	0 595	7 27E-05	4 41E-06	3 01E-01	4 41E-06	1 685	1 302	2 180	*ULK3*	0
13	rs7989332*	21050575	G	T	0 853	0 738	5 47E-06	1 16E-04	1 74E-03	5 47E-06	2 056	1 507	2 806	*CRYL1*	0
3	rs3845905*	66525963	G	A	0 921	0 828	5 99E-06	2 20E-05	1 50E-02	5 99E-06	2 433	1 645	3 598	*LRIG1*	0
8	rs1714746*	4105147	G	A	0 554	0 435	1 59E-04	6 63E-02	7 44E-06	7 44E-06	1 610	1 261	2 056	*CSMD1*	0
16	rs12446319*	81774798	A	G	0 253	0 143	8 97E-06	1 27E-04	3 16E-04	8 97E-06	2 026	1 480	2 774	*CMIP*	29431
1	rs1277203*	109392837	A	G	0 730	0 626	3 50E-04	3 51E-02	9 38E-06	9 38E-06	1 615	1 243	2 098	*AKNAD1*	0
A anthracyc ne-based drugs															
5	rs10040979*	158424391	G	A	0 701	0 618	4 68E-03	5 35E-01	4 60E-07	4 60E-07	1 452	1 120	1 883	*EBF1*	0
2	rs12615435*	200638509	T	G	0 883	0 773	3 95E-06	4 09E-06	9 17E-02	3 95E-06	2 214	1 555	3 154	*LOC348751*	0
5	rs7720283*	158459721	C	T	0 775	0 706	1 29E-02	3 37E-01	4 15E-06	4 15E-06	1 431	1 078	1 898	*EBF1*	0
1	rs1367448*	68633924	C	T	0 633	0 526	5 02E-04	5 32E-06	5 12E-01	5 32E-06	1 554	1 212	1 993	*LOC100289178*	0
6	rs2505059*	98495952	G	A	0 538	0 398	5 41E-06	2 29E-04	2 04E-04	5 41E-06	1 765	1 383	2 252	*MIR2113*	23455
12	rs4149639*	6442001	C	T	0 120	0 047	7 39E-06	1 52E-05	8 16E-02	7 39E-06	2 763	1 781	4 287	*TNFRSF1A*	0
19	rs1654260*	20329111	A	G	0 625	0 488	8 49E-06	3 03E-03	2 18E-05	8 49E-06	1 749	1 365	2 240	*LOC100421704*	−3576
Doxorub c n															
15	rs11857176*	78164706	A	G	0 657	0 515	1 74E-02	1 00E+00	8 08E-07	8 08E-07	1 800	1 127	2 874	*LOC100302666*	−6274
2	rs4380275*	773278	G	A	0 392	0 152	4 99E-06	1 54E-05	2 05E-02	4 99E-06	3 604	2 041	6 365	*LOC339822*	6559
11	rs2512987*	86414282	T	C	0 681	0 417	7 02E-06	6 04E-03	3 42E-05	7 02E-06	2 985	1 855	4 803	*ME3*	−30604
Ep rub c n															
12	rs4149639*	6442001	C	T	0 163	0 042	2 89E-07	8 31E-07	3 32E-02	2 89E-07	4 443	2 571	7 677	*TNFRSF1A*	0
5	rs2964475*	5407814	C	A	0 615	0 415	4 13E-06	3 95E-04	1 10E-04	4 13E-06	2 248	1 592	3 174	*KIAA0947*	−14993
13	rs1923834*	28360487	G	A	0 916	0 770	9 40E-06	4 61E-06	3 33E-01	4 61E-06	3 236	1 823	5 744	*GSX1*	−6293
10	rs908366*	126144839	A	G	0 518	0 328	7 04E-06	1 08E-04	8 69E-04	7 04E-06	2 199	1 564	3 092	*LHPP*	−5502

doi: 10.1111/cas.12186

© 2013 Japanese Cancer Association

Table 2. (continued)

CHR	SNP	BP	RA	NRA	RAF_Case	RAF_Ctr	P_a e c	P_dom	P_rec	Pm n	OR	L95	U95	Gene	re oc
3	rs1553091*	187716886	G	A	0 452	0 358	2 65E-02	8 04E-01	7 46E-06	7 46E-06	1 480	1 053	2 080	*LOC100505844*	−22691
A ant metabo te drugs															
18	rs7228133*	4539085	C	A	0 733	0 686	2 26E-02	6 64E-01	1 70E-06	1 70E-06	1 255	1 034	1 522	*LOC284215*	243085
21	rs8127977*	26826514	A	G	0 804	0 722	1 30E-05	2 11E-06	2 67E-01	2 11E-06	1 587	1 282	1 966	*NCRNA00158*	−22501
12	rs894734*	54319727	G	A	0 849	0 776	3 84E-05	3 97E-06	6 63E-01	3 97E-06	1 619	1 279	2 050	*HOXC13*	−12849
13	rs9580312*	22754093	G	A	0 480	0 409	1 35E-03	8 09E-06	6 25E-01	8 09E-06	1 330	1 120	1 581	*LOC100506622*	−30331
21	rs2055011*	19481354	C	T	0 184	0 143	1 12E-02	2 12E-01	8 82E-06	8 82E-06	1 347	1 075	1 686	*CHODL*	−135796
12	rs12582168*	124894184	C	T	0 333	0 256	8 50E-05	9 31E-06	2 84E-01	9 31E-06	1 454	1 210	1 748	*NCOR2*	0
5-F uorourac															
7	rs10488226*	12713070	A	C	0 195	0 107	1 09E-05	3 54E-06	2 98E-01	3 54E-06	2 026	1 500	2 737	*LOC100505995*	−12175
2	rs6740660*	224943685	G	A	0 966	0 894	4 10E-06	8 83E-06	2 40E-01	4 10E-06	3 386	1 870	6 131	*SERPINE2*	−39649
4	rs1567482*	36026747	G	A	0 952	0 875	6 26E-06	1 44E-05	9 14E-02	6 26E-06	2 846	1 716	4 719	*LOC651644*	39948
2	rs6706693*	192465598	A	G	0 328	0 219	1 62E-05	2 12E-03	9 45E-06	9 45E-06	1 743	1 362	2 232	*OBFC2A*	−77200
Gemc tab ne															
18	rs9961113*	75605399	C	T	0 625	0 403	1 43E-06	3 83E-04	3 73E-05	1 43E-06	2 473	1 706	3 584	*LOC100421527*	−260017
5	rs2547917*	58713680	A	G	0 350	0 212	9 06E-04	8 79E-02	3 33E-06	3 33E-06	1 997	1 345	2 965	*PDE4D*	0
15	rs12900463*	85415386	C	T	0 219	0 115	2 24E-03	1 02E-01	4 03E-06	4 03E-06	2 154	1 342	3 457	*ALPK3*	0
22	rs9609078*	31153276	T	C	0 089	0 009	4 32E-06	9 97E-06	2 59E-01	4 32E-06	10 890	3 528	33 610	*OSBP2*	0
5	rs6863418*	173625154	A	G	0 175	0 055	1 37E-05	6 98E-06	4 55E-01	6 98E-06	3 623	2 042	6 429	*HMP19*	88972
20	rs6037430*	344079	G	A	0 894	0 730	9 74E-06	1 75E-05	7 92E-02	9 74E-06	3 109	1 805	5 359	*NRSN2*	8567
A ant m crotubu e drugs															
17	rs11651483*	12777402	C	T	0 729	0 665	1 69E-03	2 60E-01	3 37E-07	3 37E-07	1 357	1 120	1 643	*RICH2*	0
6	rs4235898*	77266188	A	G	0 830	0 740	1 05E-06	3 34E-06	6 50E-03	1 05E-06	1 718	1 377	2 142	*LOC100131680*	−103976
13	rs4771859*	93088651	G	A	0 764	0 709	5 49E-03	2 60E-01	1 47E-06	1 47E-06	1 328	1 088	1 623	*GPC5*	0
1	rs12145418*	216716320	T	G	0 334	0 274	3 04E-03	3 17E-01	2 35E-06	2 35E-06	1 331	1 104	1 604	*ESRRG*	0
6	rs9386485*	106329055	T	C	0 596	0 492	2 65E-06	2 85E-05	8 59E-04	2 65E-06	1 524	1 279	1 817	*PRDM1*	−205140
16	rs12935229*	77328895	A	G	0 249	0 182	1 83E-04	2 23E-02	4 40E-06	4 40E-06	1 495	1 214	1 840	*ADAMTS18*	0
7	rs6961860*	17085321	G	A	0 557	0 495	5 33E-03	8 87E-01	4 67E-06	4 67E-06	1 283	1 078	1 527	*LOC100131425*	156806
14	rs12882718*	86902054	T	C	0 737	0 643	5 91E-06	5 55E-06	2 03E-02	5 55E-06	1 555	1 283	1 884	*LOC100421119*	−42891
12	rs1043763*	122630909	T	C	0 668	0 574	1 36E-05	6 51E-06	3 10E-02	6 51E-06	1 496	1 248	1 795	*MLXIP*	1920
2	rs4591358*	196365890	C	T	0 302	0 215	6 60E-06	6 89E-04	1 89E-04	6 60E-06	1 578	1 297	1 920	*LOC391470*	81627
14	rs8022296*	97987857	G	A	0 663	0 608	1 06E-02	6 54E-01	7 29E-06	7 29E-06	1 269	1 058	1 521	*LOC100129345*	111127
4	rs6817170*	154374984	G	A	0 377	0 286	9 29E-06	4 61E-05	3 27E-03	9 29E-06	1 517	1 264	1 822	*KIAA0922*	−12514
Pac taxe															
1	rs922106*	90025519	T	G	0 298	0 202	2 17E-04	1 95E-02	9 28E-07	9 28E-07	1 679	1 277	2 207	*LRRC8B*	0
6	rs9386485*	106329055	T	C	0 624	0 477	1 17E-06	1 43E-05	5 24E-04	1 17E-06	1 821	1 429	2 320	*PRDM1*	−205140
8	rs2444896*	99022009	T	G	0 727	0 603	1 58E-05	2 43E-06	7 41E-02	2 43E-06	1 754	1 355	2 269	*MATN2*	0
2	rs4666360*	20335709	C	T	0 216	0 114	4 62E-06	3 24E-06	2 27E-01	3 24E-06	2 136	1 546	2 950	*RPS16P2*	19625
9	rs3138083*	35648950	A	G	0 220	0 117	3 78E-06	1 24E-05	1 09E-02	3 78E-06	2 136	1 551	2 942	*SIT1*	345
17	rs3786094*	9875205	C	T	0 528	0 422	5 30E-04	1 80E-01	5 83E-06	5 83E-06	1 531	1 206	1 944	*GAS7*	0
15	rs4886670*	75449674	A	C	0 353	0 229	7 26E-06	5 38E-05	4 26E-03	7 26E-06	1 835	1 412	2 383	*RPL36AP45*	29318
5	rs792975*	172271007	T	C	0 654	0 519	7 66E-06	1 19E-04	6 08E-04	7 66E-06	1 746	1 366	2 232	*ERGIC1*	0
Docetaxe															
9	rs3747851*	124521260	T	C	0 337	0 176	5 61E-07	1 12E-05	5 63E-04	5 61E-07	2 377	1 693	3 339	*DAB2IP*	0
7	rs4727963*	122759980	C	T	0 772	0 618	7 99E-06	1 04E-06	1 69E-01	1 04E-06	2 094	1 505	2 914	*SLC13A1*	0
14	rs1756650*	87741025	G	A	0 211	0 162	9 95E-02	9 10E-01	1 74E-06	1 74E-06	1 386	0 954	2 014	*GALC*	658333

© 2013 Japanese Cancer Association

Table 2. (continued)

CHR	SNP	BP	RA	NRA	RAF_Case	RAF_Ctr	P_a e c	P_dom	P_rec	Pm n	OR	L95	U95	Gene	re oc
13	rs488248*	106596719	T	C	0 918	0 795	3 29E-06	3 23E-05	1 17E-02	3 29E-06	2 896	1 802	4 655	*LOC728192*	−432192
6	rs12660691*	130008445	A	C	0 935	0 819	3 62E-06	4 99E-06	1 62E-01	3 62E-06	3 199	1 899	5 391	*ARHGAP18*	0
18	rs4553720*	62170726	T	C	0 377	0 281	7 77E-03	4 56E-01	6 77E-06	6 77E-06	1 547	1 131	2 116	*LOC284294*	79890
6	rs2157460*	130021128	T	C	0 932	0 820	7 07E-06	1 07E-05	1 62E-01	7 07E-06	3 013	1 806	5 025	*ARHGAP18*	0
2	rs837841*	130034012	T	G	0 714	0 662	1 49E-01	5 26E-01	8 34E-06	8 34E-06	1 279	0 930	1 759	*LOC151121*	−33653
A topo somerase nh b tors															
5	rs10074959*	104208013	T	C	0 321	0 237	3 23E-02	8 08E-01	1 13E-06	1 13E-06	1 524	1 048	2 217	*RAB9P1*	−227162
7	rs1035147*	12094966	T	G	0 981	0 877	4 01E-06	4 75E-06	5 56E-01	4 01E-06	7 294	2 587	20 559	*TMEM106B*	−155882
1	rs303386*	99589379	A	G	0 585	0 444	1 10E-03	4 30E-06	3 88E-01	4 30E-06	1 766	1 256	2 483	*LOC100129620*	0
14	rs7494275*	56231800	C	A	0 543	0 406	1 86E-03	4 26E-01	8 50E-06	8 50E-06	1 732	1 232	2 433	*RPL13AP3*	−1163
3	rs480409*	7010081	G	A	0 495	0 348	6 08E-04	1 63E-01	9 28E-06	9 28E-06	1 842	1 307	2 596	*GRM7*	0
Camptothec n															
6	rs17318866*	3837198	G	A	0 966	0 790	1 47E-06	1 78E-06	1 91E-01	1 47E-06	7 559	2 689	21 263	*FAM50B*	−12434
2	rs17027130*	41273631	C	T	0 644	0 387	2 54E-06	8 03E-04	3 91E-05	2 54E-06	2 865	1 844	4 452	*LOC729984*	−110074
1	rs303386*	99589379	A	G	0 627	0 429	3 34E-04	3 61E-06	1 06E-01	3 61E-06	2 238	1 448	3 460	*LOC100129620*	0
Etopos de															
20	rs6039763*	10183517	A	G	0 370	0 090	1 27E-05	1 54E-06	4 61E-01	1 54E-06	5 966	2 502	14 230	*LOC100131208*	0
1	rs2506991*	48098406	G	A	0 593	0 269	1 39E-05	1 11E-02	2 28E-06	2 28E-06	3 948	2 101	7 418	*LOC388630*	127794
2	rs12987465*	49715021	A	G	0 593	0 359	1 87E-03	6 26E-01	6 61E-06	6 61E-06	2 597	1 424	4 737	*FSHR*	−333355
7	rs3095008*	20255705	T	C	1 000	0 846	1 74E-05	9 39E-06	1 00E+00	9 39E-06	nf	N/A	N/A	*MACC1*	0
Pac taxe + carbop at n															
12	rs12310399*	95490248	A	G	0 708	0 567	2 60E-04	1 08E-01	2 46E-07	2 46E-07	1 852	1 329	2 580	*FGD6*	0
9	rs10785877*	137125501	T	C	0 833	0 660	7 38E-07	1 54E-05	1 98E-04	7 38E-07	2 580	1 766	3 769	*RXRA*	-92815
19	rs995834*	28866596	C	T	0 580	0 425	1 28E-04	1 20E-06	1 51E-01	1 20E-06	1 871	1 364	2 566	*LOC100420587*	307385
1	rs922107*	90022796	G	A	0 323	0 211	1 55E-03	9 07E-02	2 73E-06	2 73E-06	1 788	1 250	2 558	*LRRC8B*	0
7	rs1425132*	37562368	T	C	0 740	0 666	4 55E-02	7 35E-01	4 68E-06	4 68E-06	1 430	1 013	2 017	*LOC442668*	62349
1	rs6429703*	15339960	T	C	0 200	0 078	8 40E-06	2 59E-05	5 61E-02	8 40E-06	2 942	1 802	4 804	*RP1-21O18.1*	0

*SNPs used for we ghted genet c r sk score ana yses BP, SNP genom c ocat on; CHR, chromosome; nf, nfin ty; L95, ower 95% confidence nterva ; N/A, not app cab e; NRA, non-r sk a e e; OR, odds rat o; P_a e c, *P*-va ue from a e c mode ; P_dom, *P*-va ue from dom nant mode ; P_m n, m n mum *P*-va ue among the three mode s; P_rec, *P*-va ue from recess ve mode ; RA, r sk a e e; RAF, r sk a e e frequency; re oc , d stance of the SNP from the gene; U95, upper 95% confidence nterva

doi: 10.1111/cas.12186
© 2013 Japanese Cancer Association

Among these datasets, GWAS carried out using samples who were given: (i) any kind of platinum based chemotherapy (428 cases *vs* 743 controls); (ii) cisplatin based chemotherapy (176 cases *vs* 471 controls); or (iii) carboplatin based chemo therapy (261 cases *vs* 262 controls) identified SNPs showing the most significant association with chemotherapy induced severe neutropenia/leucopenia are: rs4886670 (P_{min} = 9.86 × 10^{-7}, OR = 1.61, 95% CI = 1.33 1.94) near *RPL36AP45* for (i); rs10253216 (P_{min} = 1.68 × 10^{-7}, OR = 1.48, 95% CI = 1.16 1.89) near *AGR2* for (ii); and rs11071200 (P_{min} = 8.51 × 10^{-7}, OR = 8.24, 95% CI = 2.89 23.5) on *PRTG* for (iii) (Table 2, Table S1, Fig. S2b). For the anthracycline based reg imen, we carried out GWAS with individuals given all anthra cycline based (184 cases *vs* 459 controls), doxorubicin based (83 cases *vs* 66 controls), and epirubicin based (83 cases *vs* 370 controls) chemotherapy, and identified three SNPs, rs10040979 (P_{min} = 4.60 × 10^{-7}, OR = 1.45, 95% CI = 1.12 1.88) in *EBF1*, rs11857176 (P_{min} = 8.08 × 10^{-7}, OR = 1.80, 95% CI = 1.13 2.87) near a hypothetical gene *LOC10030 2666*, and rs4149639 (P_{min} = 2.89 × 10^{-7}, OR = 4.44, 95% CI = 2.57 7.68) in *TNFRSF1A*, to be most significantly associ ated with the risk of high grade neutropenia/leucopenia, respectively (Table 2, Table S1, Fig. S2c). In the case of an timicrotubule agents, we carried out three different GWAS with individuals who were treated with antimicrotubule (371 cases *vs* 825 controls), paclitaxel based (218 cases *vs* 364 con trols), or docetaxel based (147 cases *vs* 233 controls) regimens. We identified three SNPs, rs11651483 (P_{min} = 3.37 × 10^{-7}, OR = 1.36, 95% CI = 1.12 1.64) in *RICH2*, rs922106 (P_{min} = 9.28 × 10^{-7}, OR = 1.68, 95% CI = 1.28 2.21) in *LRRC8B* and rs3747851 (P_{min} = 5.61 × 10^{-7}, OR = 2.38, 95% CI = 1.69 3.34) in *DAB2IP*, to be those most signifi cantly associated with the increased risk of severe neutropenia /leucopenia, respectively (Table 2, Table S1, Fig. S2e). Our previous report by Kiyotani *et al.*[24] identified four SNPs to be associated with gemcitabine induced hematological toxici ties. Three of the four SNPs were included in the current study with suggestive association, rs12046844 (P_{min} = 5.84 × 10^{-4}, OR = 2.53, 95% CI = 1.45 4.43), rs6430443 (P_{min} = 8.61 × 10^{-4}, OR = 6.33, 95% CI = 1.90 22.2; r^2 = 0.895 with rs1901440) and rs11719165 (P_{min} = 1.16 × 10^{-2}, OR = 2.36, 95% CI = 1.18 4.70) (Table S4). However, it is noted that some of the samples used in this study overlapped with those in the study reported by Kiyotani *et al.*, as both sourced sam ples from Biobank Japan.

Lastly, we also attempted to identify genetic variants associ ated with combined treatment of paclitaxel and carboplatin induced severe neutropenia/leucopenia (150 cases *vs* 166 controls), as this combined treatment is commonly used as the standard therapy for both ovarian and lung cancers. We found the most significant association with the SNP rs12310399 (P_{min} = 2.46 × 10^{-7}, OR = 1.85, 95% CI = 1.33 2.58) near the *FGD6* gene (Table 2, Table S1, Fig. S2a), which is suggested to activate CDC42, a member of the Ras like family of Rho and Rac proteins, and has a critical role in regulating the actin cytoskeleton. The second strongest association was observed at the locus encoding RXRA (P_{min} = 7.38 × 10^{-7}, OR = 2.58, 95% CI = 1.77 3.77), an important transcriptional factor. We also calculated the cumulative genetic scores using SNPs on six loci and identified that individuals in group 4 could have 188 times (95% CI = 36.1 979) higher risk of developing severe neutropenia/leucopenia than those belonging to group 1 with the sensitivity of 95.9% and the specificity of 88.9% (Table S2). Because this drug combination is of clinical importance, we further investigated the association of these six selected loci using 161 individuals who developed grade 1/2 neutropenia/leucopenia, using cases registered in the Biobank Japan. Interestingly, the association results for the six loci were moderate for grade 1/2 neutropenia/leucopenia, with intermediate allele frequency and OR between individuals without any adverse reactions and those with neutropenia/leu copenia of ≥grade 3 (Table S3). In addition, as shown in Table 3 and Figure 1, the higher the calculated score becomes, the higher the proportion and grade of neutropenia/leucopenia. The intermediate scores for patients with grade 1/2 neutrope nia/leucopenia could imply the possible usefulness of this scoring system for the prediction.

Furthermore, we used simulation to estimate how many samples are required to validate this scoring result. We started off by estimating the incidence of neutropenia/leucopenia by the combined treatment of paclitaxel and carboplatin. In Bio bank Japan, a total of 477 individuals received this combined treatment; among them, 166 individuals (35%) did not develop any adverse drug reactions, 161 (35%) developed mild neutro penia/leucopenia (grade 1 or 2) and 150 (30%) developed severe neutropenia/leucopenia (grade 3 or higher). The frequency of developing severe neutropenia/leucopenia is in agreement with a multicenter study reported by Guastalla *et al.*[25] When we assume that 100 patients who receive this combination therapy are prospectively registered, the inci dences of the adverse drug reactions are estimated as shown in Table 4. If we categorize the patients by wGRS according to the proportions indicated in Table 3 (and our hypothesis is right), the statistical power should be enough to validate by this small subset of patients. Even if two individuals in both group 1 and group 4 are incorrectly predicted, the calculated *P* value is still 0.03 by Fisher's exact test.

Discussion

In this study, we carried out GWAS analyses for a total of 17 subsets of chemotherapies to identify genetic variants that might be associated with chemotherapeutic induced neutrope nia/leucopenia with grades 3 and 4, however, we could not identify any SNPs that surpassed the genome wide significant threshold (*P* value < 5 × 10^{-8}). Through this study, we

Table 3. Weighted genetic risk score (wGRS) analysis of cancer patients who received combination treatment with paclitaxel and carboplatin

wGRS group	Score	G3G4	G1G2	G0	% G3G4	% G1G2	% G0	G3/4 versus G0			G1/2 versus G0		
								OR	95% CI	*P* value	OR	95% CI	*P* value
1	<5.802	2	21	48	0.03	0.29	0.68		REF			REF	
2	5.802 7.665	36	58	77	0.21	0.34	0.45	11.2	2.58 48.70	8.69E 05	1.72	0.93 3.19	9.55E 02
3	7.665 9.528	64	62	33	0.40	0.39	0.21	46.5	10.60 204.00	2.36E 13	4.29	2.21 8.35	1.61E 05
4	>9.528	47	20	6	0.64	0.28	0.08	188.0	36.10 979.00	4.78E 20	7.62	2.68 21.70	6.08E 05
Total		149	161	164									

95% CI, 95% confidence interval; G0, individuals who did not develop any adverse drug reaction; G1G2, grade 1 and grade 2 neutropenia (mild); G3G4, grade 3 and grade 4 neutropenia (severe); OR, odds ratio; REF, reference.

© 2013 Japanese Cancer Association

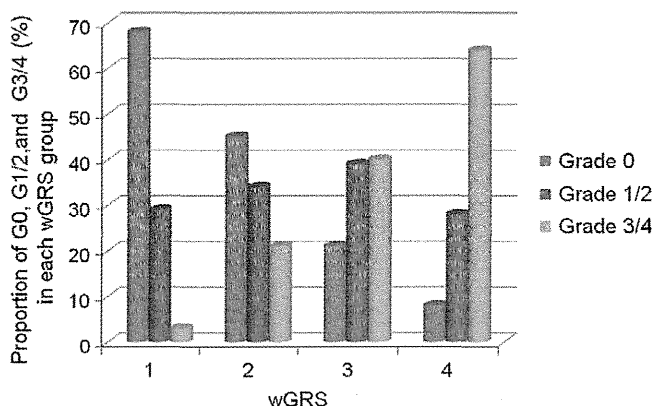

Fig. 1. Proportions of cancer patients who developed no adverse reaction (G0), mild neutropenia/leucopenia (G1/2), or severe neutro penia/leucopenia (G3/4) in each of the weighted genetic risk score (wGRS) score groups. All patients received combined treatment with paclitaxel and carboplatin and were registered with Biobank Japan. The total numbers of patients in scores 1, 2, 3, and 4 are 71, 171, 159, and 73, respectively.

Table 4. Simulation of weighted genetic risk score (wGRS) analysis for a prospective study of 100 patients who received combination treatment with paclitaxel and carboplatin

Estimated verification samples (*n* 100; 35 expected to have grade 1/2 neutropenia)					
wGRS group	G3G4	G0	OR	95% CI	*P* value
1	0	10			
2	7	17	9.0	0.47 174.00	7.82E 02
3	13	7	37.8	1.93 740.00	1.06E 03
4	10	1	147.0	5.35 4040.00	3.40E 05
Total	30	35			

95% CI, 95% confidence interval; G0, individuals without any adverse drug reaction; G3G4, grade 3 and 4 neutropenia (severe); OR, odds ratio.

encountered several important issues, which are now common problems in pharmacogenomics studies using retrospective clinical data, including confounding factors and heterogeneous treatments for individual patients (often given different combi nations of drugs, different dosage of drugs, and different time periods of treatment), that increase the complexity of studies and generate various noises in the analyses, and diminished the statistical power in the case control association studies. We understand that our current approach was not an ideal study design, but it is not easy to perfectly standardize therapy in the daily clinical practice of cancer treatment. There are several factors contributing to the variability in treatments: (i) there is some preference by doctors or by hospitals to select a particular regimen among the various recommended standard treatments; (ii) the modifications (adjustments) of the dosage or schedule according to the patient's conditions (performance status, results of laboratory tests, etc.); and (iii) although we have been collecting the clinical information, it is not perfect to collect complete clinical information in some hospitals, particularly those that do not use electronic medical records. One can say that this kind of study should be performed as a prospective design, however, due to the very rapid advances in the development of novel molecular targeted drugs and new regimens in the oncology area, the protocols have been and will be modified or improved. Hence, spending many years and a huge budget on a prospective study may result in a clini cally useless outcome, because the results are unable to be applied due to the replacement of the study protocol with a new protocol, when the results of association studies are avail able. Nevertheless, retrospective pharmacogenomic studies could be improved by implementing electronic medical record systems that could include detailed descriptions of patients' conditions and their responses to various drugs.

Although we understand the pitfalls in study designs like our present study, we need to seek possible ways to identify candi date genetic variants that might contribute to improvement in the clinical management of cancer patients, including chemo therapy induced severe neutropenia/leucopenia. Nevertheless, some of the candidate genes that we identified are of interest, considering their known functions as well as their relations with drug actions. For example, the proto oncogene *AGR2*, whose genetic variants were suggested to associate with cis platin induced neutropenia/leucopenia, encodes an anterior gradient 2 homolog (*Xenopus laevis*) that is known to play a critical role in cell migration, cell differentiation, and cell growth.[26] Cells stably expressing *AGR2* confer resistance to cisplatin *in vivo*, compared with control cells (empty vector) in a xenograft animal model.[27] The second example is *TNFRSF1A*, suggested to be associated with anthracycline based and epirubicin induced neutropenia/leucopenia. This gene encodes TNFRSF1A, which is a major receptor for TNF α. The soluble TNFRSF1A level was found to be elevated after 1 month of anthracycline based chemotherapy.[28] Addi tionally, both TNF α and TNFRSF1A are known to play a crit ical role in doxorubicin induced cardiotoxicity, in which doxorubicin stimulates an increase in circulating TNF and upregulates TNFRSF1A.[29,30] Furthermore, genetic variants on *PDE4D*, which encodes for phosphodiesterase 4D, cAMP specific, showed suggestive association with gemcitabine induced severe neutropenia/leucopenia. Ablation of *PDE4D* has been reported to impair the neutrophil function with altered chemotaxis ability and adhesion capability as well as to reduce neutrophil recruitment to the site of inflammation.[31] Besides, genetic variants on *RXRA* identified to be associated with combined treatment of paclitaxel and carboplatin induced severe neutropenia/leucopenia, encodes retinoid X receptor alpha. Disruption of this gene in mouse models moderately alters lymphocyte proliferation and survival, and affects the T helper type1/type 2 balances.[32] All of these genes might pro vide some important insights into the mechanism of various chemotherapy induced severe neutropenia/leucopenia, how ever, further validations are definitely essential.

As already described, the GWAS approach could provide a list of genetic variants that might be associated with complex phenotypes (drug responsiveness or drug induced adverse reactions) in pharmacogenomics studies. One of the clinically important aims for identification of the associated genetic variants is to establish a prediction model to identify individ uals who are at risk of adverse reactions with certain drugs or protocols. In this study, we have applied the wGRS sys tem, by which we could distinguish high risk patients from low risk individuals by counting the number of risk alleles of the suggestively associated SNPs in combination with esti mating the effect size of each SNP. One of the best examples from this study was indicated by a scoring system using six candidate SNP loci that were identified through the GWAS of severe neutropenia/leucopenia caused by combination treatment of paclitaxel and carboplatin; among 53 individuals in the high risk group (group 4) by this scoring method, 47 (89%) revealed high grade neutropenia/leucopenia. In con trast, among 50 individuals in the low risk group (group 1), only 2 (4%) revealed high grade neutropenia/leucopenia, and

doi: 10.1111/cas.12186
© 2013 Japanese Cancer Association

the odds ratio to have the severe adverse reaction in individuals belonging to group 4 was calculated to be 188 times higher than those categorized to group 1 (Table 3). Interestingly, individuals who developed grade 1/2 (mild neutropenia/leucopenia) were found to show intermediate risk scores between patients with severe neutropenia/leucopenia and those without any adverse reactions. Hence, we suggest that wGRS is an applicable method to evaluate the clinical utility of possible variants with specific phenotypes. However, the data are preliminary and require verification by an independent test sample(s) before any definitive conclusions can be drawn. But, considering that the OR of the high risk group is very high, the number of samples required for the verification (if our hypothesis is right) is not so large. In fact, we have tried to simulate a prospective study design using a model of 100 patients according to the assumption that 35% individuals will not develop any adverse drug reactions, 35% individuals will develop mild neutropenia/leucopenia (grade 1/2), and 30% will develop severe neutropenia/leucopenia (grade 3/4). As shown in Table 4, the study of 100 patients should have very strong statistical power to verify. If this is verified, as we expect, it should improve the quality of lives of cancer patients and also contribute to reducing medical care costs by avoiding unnecessary adverse events. However, to achieve success in pharmacogenomics and personalized medicine, both local and international collaborative efforts are essential.

Acknowledgments

We would like to express our heartfelt gratitude to all the patients who participated in this study. We convey our sincere appreciation to Dr Teruhiko Yoshida and Dr Hiromi Sakamoto from the National Cancer Center Research Institute for their kind support. Our thanks also goes to the members of the laboratory for statistical analysis and the laboratory for the genotyping development from the Center for Genomic Medicine for their kind support and fruitful discussions. We would like to extend our gratitude to the staff of Biobank Japan for their outstanding assistance. This work was carried out as part of the Biobank Japan Project, supported by the Ministry of Education, Culture, Sports, Sciences and Technology, Japan. In addition, this project was supported by the JSPS postdoctoral fellowship.

Disclosure Statement

The authors have no conflict of interest.

Abbreviations

GWAS	genome wide association study
OR	odds ratio
SD	standard deviation
SNP	single nucleotide polymorphism
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A
TNF α	tumor necrosis factor α
wGRS	weighted genetic risk score

References

1 Wheeler HE, Maitland ML, Dolan ME, Cox NJ, Ratain MJ. Cancer pharmacogenomics: strategies and challenges. *Nat Rev Genet* 2013; **14**: 23 34.
2 Relling MV, Hancock ML, Rivera GK *et al.* Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *J Natl Cancer Inst* 1999; **91**: 2001 8.
3 Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; **32**: 651 62.
4 Relling MV, Klein TE. CPIC: clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clin Pharmacol Ther* 2011; **89**: 464 7.
5 Innocenti F, Undevia SD, Iyer L *et al.* Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 2004; **22**: 1382 8.
6 Iyer L, Das S, Janisch L *et al.* UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J* 2002; **2**: 43 7.
7 Hoskins JM, Goldberg RM, Qu P, Ibrahim JG, McLeod HL. UGT1A1*28 genotype and irinotecan-induced neutropenia: dose matters. *J Natl Cancer Inst* 2007; **99**: 1290 5.
8 McCarthy MI, Abecasis GR, Cardon LR *et al.* Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet* 2008; **9**: 356 69.
9 Triendl R. Japan launches controversial Biobank project. *Nat Med* 2003; **9**: 982.
10 Takata R, Akamatsu S, Kubo M *et al.* Genome-wide association study identifies five new susceptibility loci for prostate cancer in the Japanese population. *Nat Genet* 2010; **42**: 751 4.
11 Akamatsu S, Takata R, Haiman CA *et al.* Common variants at 11q12, 10q26 and 3p11.2 are associated with prostate cancer susceptibility in Japanese. *Nat Genet* 2012; **44**: 426 9, S1.
12 Cui R, Kamatani Y, Takahashi A *et al.* Functional variants in ADH1B and ALDH2 coupled with alcohol and smoking synergistically enhance esophageal cancer risk. *Gastroenterology* 2009; **137**: 1768 75.
13 Cui R, Okada Y, Jang SG *et al.* Common variant in 6q26-q27 is associated with distal colon cancer in an Asian population. *Gut* 2011; **60**: 799 805.
14 Kumar V, Kato N, Urabe Y *et al.* Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. *Nat Genet* 2011; **43**: 455 8.
15 Low SK, Kuchiba A, Zembutsu H *et al.* Genome-wide association study of pancreatic cancer in Japanese population. *PLoS ONE* 2010; **5**: e11824.
16 Elgazzar S, Zembutsu H, Takahashi A *et al.* A genome-wide association study identifies a genetic variant in the SIAH2 locus associated with hormonal receptor-positive breast cancer in Japanese. *J Hum Genet* 2012; **57**: 766 71.
17 Miki D, Ochi H, Hayes CN *et al.* Variation in the DEPDC5 locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers. *Nat Genet* 2011; **43**: 797 800.
18 Miki D, Kubo M, Takahashi A *et al.* Variation in TP63 is associated with lung adenocarcinoma susceptibility in Japanese and Korean populations. *Nat Genet* 2010; **42**: 893 6.
19 Cha PC, Zembutsu H, Takahashi A, Kubo M, Kamatani N, Nakamura Y. A genome-wide association study identifies SNP in DCC is associated with gallbladder cancer in the Japanese population. *J Hum Genet* 2012; **57**: 235 7.
20 Silvestris N, Del Re M, Azzariti A *et al.* Optimized granulocyte colony-stimulating factor prophylaxis in adult cancer patients: from biological principles to clinical guidelines. *Expert Opin Ther Targets* 2012; **16**(Suppl 2): S111 7.
21 Yamaguchi-Kabata Y, Nakazono K, Takahashi A *et al.* Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. *Am J Hum Genet* 2008; **83**: 445 56.
22 Low SK, Takahashi A, Cha PC *et al.* Genome-wide association study for intracranial aneurysm in the Japanese population identifies three candidate susceptible loci and a functional genetic variant at EDNRA. *Hum Mol Genet* 2012; **21**: 2102 10.
23 De Jager PL, Chibnik LB, Cui J *et al.* Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: a weighted genetic risk score. *Lancet Neurol* 2009; **8**: 1111 9.
24 Kiyotani K, Uno S, Mushiroda T *et al.* A genome-wide association study identifies four genetic markers for hematological toxicities in cancer patients receiving gemcitabine therapy. *Pharmacogenet Genomics* 2012; **22**: 229 35.
25 Guastalla JP, Pujade-Lauraine E, Weber B *et al.* Efficacy and safety of the paclitaxel and carboplatin combination in patients with previously treated advanced ovarian carcinoma. A multicenter GINECO (Group d'Investigateurs Nationaux pour l'Etude des Cancers Ovariens) phase II study. *Ann Oncol* 1998; **9**: 37 43.
26 Wang Z, Hao Y, Lowe AW. The adenocarcinoma-associated antigen, AGR2, promotes tumor growth, cell migration, and cellular transformation. *Cancer Res* 2008; **68**: 492 7.
27 Hengel SM, Murray E, Langdon S *et al.* Data-independent proteomic screen identifies novel tamoxifen agonist that mediates drug resistance. *J Proteome Res* 2011; **10**: 4567 78.
28 Perik PJ, De Vries EG, Boomsma F *et al.* The relation between soluble apoptotic proteins and subclinical cardiotoxicity in adjuvant-treated breast cancer patients. *Anticancer Res* 2006; **26**: 3803 11.

© 2013 Japanese Cancer Association

29 Mukherjee S, Banerjee SK, Maulik M, Dinda AK, Talwar KK, Maulik SK. Protection against acute adriamycin-induced cardiotoxicity by garlic: role of endogenous antioxidants and inhibition of TNF-alpha expression. *BMC Pharmacol* 2003; **3**: 16.
30 Gilliam LA, St Clair DK. Chemotherapy-induced weakness and fatigue in skeletal muscle: the role of oxidative stress. *Antioxid Redox Signal* 2011; **15**: 2543 63.
31 Ariga M, Neitzert B, Nakae S *et al.* Nonredundant function of phosphodiesterases 4D and 4B in neutrophil recruitment to the site of inflammation. *J Immunol* 2004; **173**: 7531 8.
32 Stephensen CB, Borowsky AD, Lloyd KC. Disruption of Rxra gene in thymocytes and T lymphocytes modestly alters lymphocyte frequencies, proliferation, survival and T helper type 1/type 2 balance. *Immunology* 2007; **121**: 484 98.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Quantile quantile plots of 17 genome wide association studies of drug induced or drug subgroup induced severe neutropenia and their corresponding lambda (λ) value.

Fig. S2. (a) Manhattan plot for genome wide association study of cyclophosphamide and paclitaxel + carboplatin induced severe neutropenia/leuco penia. (b) Manhattan plot for genome wide association study of severe neutropenia/leucopenia induced by all types of platinum based agents, cisplatin, or carboplatin. (c) Manhattan plot for genome wide association study of severe neutropenia/leucopenia induced by all types of anthracy cline based agents, doxorubicin, or epirubicin. (d) Manhattan plot for genome wide association study of severe neutropenia/leucopenia induced by all types of antimetabolite agents, 5 fluorouracil, or gemcitabine. (e) Manhattan plot for genome wide association study of severe neutropenia induced by all types of antimicrotubule agents, paclitaxel, or docetaxel. (f) Manhattan plot for genome wide association study severe neutropenia/leucopenia induced by all types of topoisomerase inhibitors, camptothecin, or etoposide.

Table S1. Genome wide association study of each chemotherapy regimen with $P < 1 \times 10^{-4}$.

Table S2. Weighted genetic risk score of each genome wide association study of specific chemotherapeutic based induced severe neutropenia/leu copenia.

Table S3. Association study of cancer patients who do not develop any adverse drug reaction and those who developed neutropenia/leucopenia after being given combination treatment with paclitaxel and carboplatin.

Table S4. Association of previously reported SNPs that associated with gemcitabine induced hematological toxicity.

doi: 10.1111/cas.12186
© 2013 Japanese Cancer Association

第77回　ゲノム医学の新情報⑤
Ⅱ.遺伝子解析による薬物反応性

(2) 有害事象 ―ゲノムワイド関連解析による ゲムシタビン副作用関連遺伝子の同定

1. 東京大学医科学研究所ヒトゲノム解析センター
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

前佛　均[1]・清谷　一馬[2]・宇野　智子[3]・木村　康利[3]
Hitoshi Zembutsu　Kazuma Kiyotani　Satoko Uno　Yasutoshi Kimura（講師）

莚田　泰誠[2]・光畑　直喜[4]・伊奈志乃美[5]・鬼原　史[3]
Taisei Mushiroda　Naoki Mitsuhata（診療部長）　Shinomi Ina　Chikashi Kihara

山上　裕機[5]・平田　公一[3]・中村　祐輔[1]
Hiroki Yamaue（教授）　Koichi Hirata（教授）　Yusuke Nakamura（センター長）

はじめに

現在，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ゲムシタビン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。生命の設計図ともいわれるヒトの遺伝情報（ゲノム配列）は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[1)-4)]。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[5)]。

Surgery Frontier 20(2)：86-89, 2013

ゲノムワイド関連解析によるゲムシタビン骨髄抑制関連候補遺伝子の同定

ゲ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610,000個の遺伝子多型（single nucleotide polymorphism：SNP）をgenotypingした。得られた各症例の610,000 SNPのgenotype情報を用いてcase-control関連解析を行った結果，最も副作用と強い関連を示した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とゲムシタビン骨髄抑制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，ゲ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。

ゲムシタビンによる骨髄抑制関連候補遺伝子の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1）。4 SNPとゲ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$P = 2.77 \times 10^{-3}$，2番染色体上の

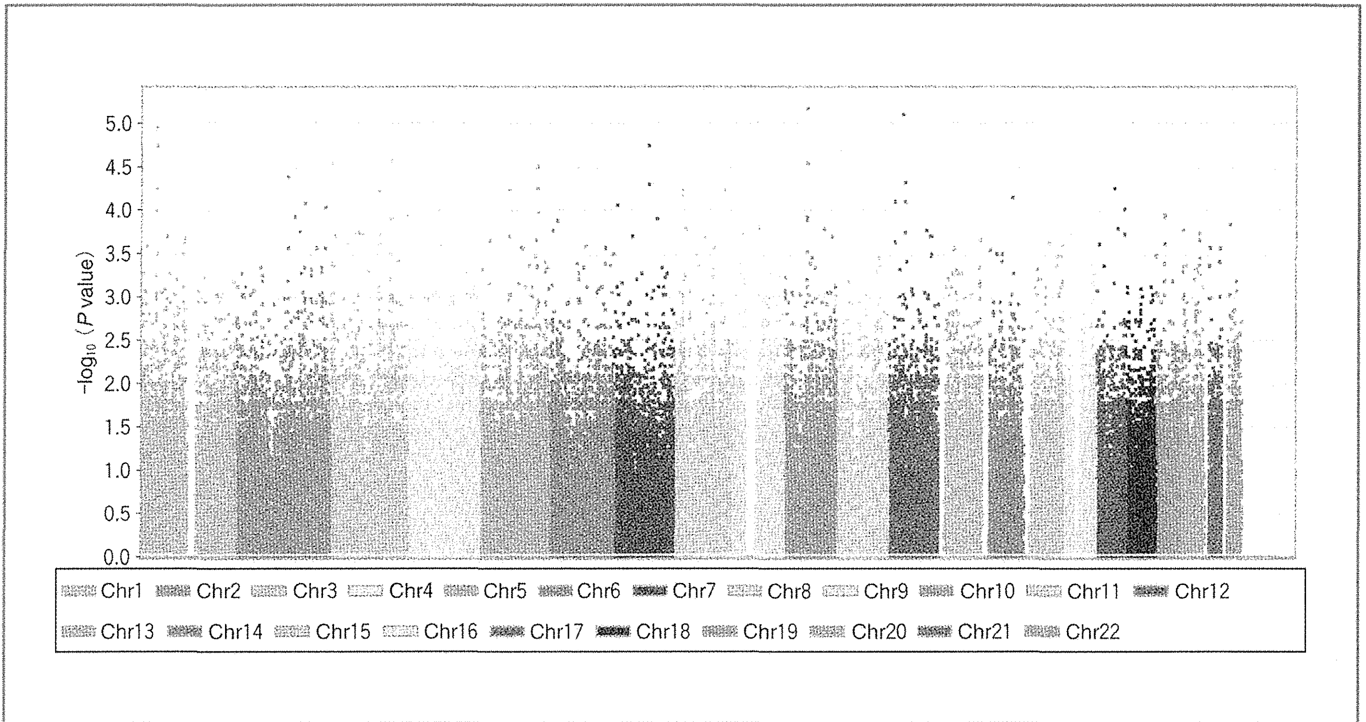

図1　マンハッタンプロット

ゲノム全体のマーカー SNP（点）について各染色体を横軸に，ゲ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。

（カラーグラビア p7　写真6参照）

表1　ゲムシタビン副作用遺伝子の関連解析の結果

SNP	染色体	遺伝子	アレルの定義 1/2（リスク）	ステージ	骨髄抑制群 11	骨髄抑制群 12	骨髄抑制群 22	骨髄抑制群 リスクアレル頻度	コントロール群 11	コントロール群 12	コントロール群 22	コントロール群 リスクアレル頻度	P値 アレルモデル	P値 優性モデル	P値 劣性モデル	オッズ比（95%CI）
rs11141915	9	*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）
				replication	22	11	0	0.83	23	31	8	0.62	2.77×10^{-3}	9.23×10^{-3}	4.73×10^{-2}	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	*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）
				replication	20	8	5	0.27	42	19	1	0.17	1.30×10^{-1}	5.05×10^{-1}	1.82×10^{-2}	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	*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）
				replication	4	10	19	0.73	7	34	21	0.61	1.50×10^{-1}	3.09×10^{-2}	1.00×10^{-0}	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	*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）
				replication	9	16	8	0.52	7	31	24	0.36	4.61×10^{-2}	1.78×10^{-1}	8.12×10^{-2}	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）

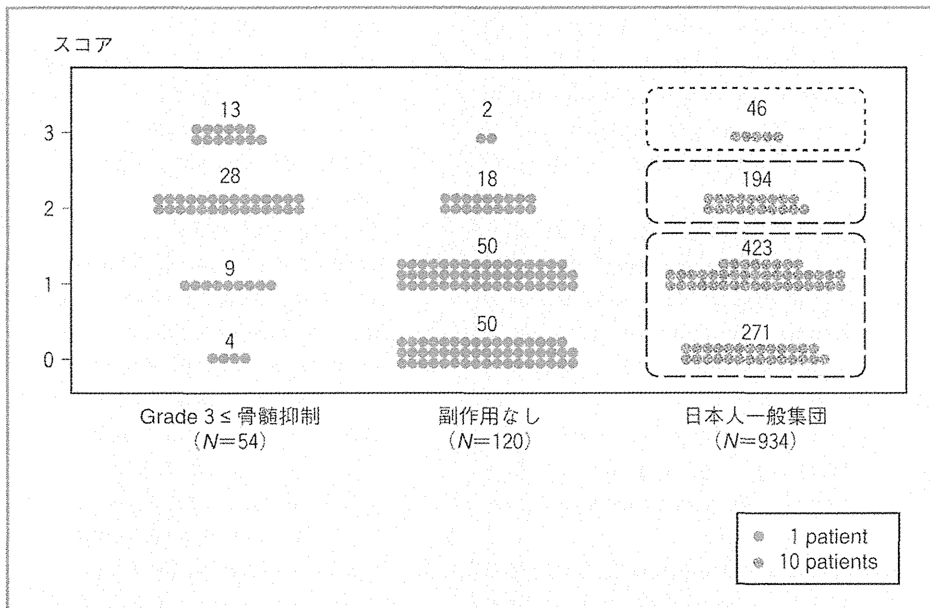

図2 4つの遺伝情報を用いたゲムシタビン骨髄抑制予測診断システム

4つの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。

rs1901440は$P = 1.82 \times 10^{-2}$，1番染色体上のrs12046844は$P = 3.09 \times 10^{-2}$，3番染色体上のrs11719165は$P = 4.61 \times 10^{-2}$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，9番染色体上のrs11141915は$P = 1.27 \times 10^{-6}$，オッズ比4.10（95% CI：2.21-7.62），2番染色体上のrs1901440は$P = 3.11 \times 10^{-6}$，オッズ比34.00（95% CI：4.29-269.48），1番染色体上のrs12046844は$P = 4.56 \times 10^{-5}$，オッズ比4.13（95% CI：2.10-8.14），3番染色体上のrs11719165は$P = 5.98 \times 10^{-5}$，オッズ比2.60（95% CI：1.63-4.14）を示し，この4 SNPを含む遺伝領域はゲムシタビンによる骨髄抑制となんらかの関連を示す結果となった。また，4遺伝領域のなかで9番染色体についてはdeath-associated protein kinase 1（*DAPK1*），1番染色体上の領域についてはphosphodiesterase 4B（*PDE4B*）という既知の遺伝子を含んでいた。

遺伝子多型情報を用いたゲムシタビンによる骨髄抑制予測診断モデル

ゲムシタビンによる骨髄抑制と関連が示唆された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。4つのSNPについて骨髄抑制リスクに働くと考えられるgenotypeを有するSNPに各1点を与え，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%が骨髄抑制発現群であり，コントロール群に比べ有意に高いスコアを示すことが確認された（trend test $P = 1.31 \times 10^{-11}$）。さらに日本人一般集団をこのスコアリングシステムに当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ゲ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）。

おわりに

最後に，今回同定された4つの遺伝子多型を含む遺伝領域はゲムシタビンによる骨髄抑制となんらかの関連があ