Table 2. Summary of studies evaluating association of *CYP2D6* genotype with response to adjuvant tamoxifen therapy

Studies	Number of patients	Tamoxifen therapy	% of monotherapy	Tamoxifen dose	Outcome[a]	Univariate		Multivariate		Comparison of *CYP2D6* genotype groups[c]
						Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value	
Goetz *et al.*,2005[41]	190	Monotherapy	100%	20 mg/day for 5 years	DFS	2.44 (1.22–4.90)	0.012	1.86 (0.91–3.82)	0.089	
Wegman *et al.*, 2005[48]	76	+Chemotherapy or radiation	not reported	40 mg/day for 2 years	RFS	not reported		<1.0[b]		wt/wt vs wt/*4+*4/*4
Nowell *et al.*, 2005[47]	160	+Chemotherapy or radiation	14.2%	not reported	DFS	not reported		0.67 (0.33–1.35)	0.19	*wt/wt vs wt/*4+*4/*4*
Goetz *et al.*, 2007[42]	180	Monotherapy	100%	20 mg/day for 5 years	RFS	3.20 (1.37–7.55)	0.007	not reported		*wt/wt vs* PM[c]
Wegman *et al.*, 2007[49]	103	not reported	not reported	40 mg/day for 2 years	RFS	not reported		0.87 (0.38–1.97)	0.74	*wt/wt vs wt/*4+*4/*4*
	111	not reported	not reported	40 mg/day for 5 years	RFS	not reported		0.33 (0.08–1.43)	0.14	*wt/wt vs wt/*4+*4/*4*
Schroth *et al.*, 2007[43]	206	Monotherapy	100%	not reported	RFS	not reported		2.24 (1.16–4.33)	0.02	EM *vs* decreased
Newman *et al.*, 2008[55]	115	Monotherapy or +chemotherapy and/or radiation	63.5%	20 mg/day, median duration >4 years	RFS	not reported		1.9 (0.8–4.8)	0.19	*wt/wt+wt/V vs V/V*
Kiyotani *et al.*, 2008[45]	58	Monotherapy	100%	20 mg/day for 5 years	RFS	8.67 (1.06–71.09)	0.044	10.04 (1.17–86.27)	0.036	*wt/wt vs *10/*10*
Xu *et al.*, 2008[46]	152	Monotherapy	100%		DFS	not reported		4.7 (1.1–20.0)	0.04	*100C/C+C/T vs T/T*
Okishiro *et al.*, 2009[53]	173	Monotherapy or +chemotherapy and/or goserelin	42.2%	20 mg/day, median 52 months	RFS	0.94 (0.34–2.60)	0.95	0.60 (0.18–1.92)	0.39	*100C/C+C/T vs T/T*
Schroth *et al.*, 2009[44]	1,325[d]	Monotherapy	100%	for 5 years	RFS	1.49 (1.12–2.00)	0.006	1.40 (1.04–1.90)	0.03	*wt/wt vs hetEM/IM*
						2.12 (1.28–3.50)	0.003	1.90 (1.10–3.28)	0.02	*wt/wt vs PM*
Bijl *et al.*, 2009[54]	85	not reported	not reported	not reported	Breast cancer mortality	not reported		4.1 (1.1–15.9)	0.04	*wt/wt vs *4/*4*
Kiyotani *et al.*, 2010[38]	282[d]	Monotherapy	100%	20 mg/day for 5 years	RFS	not reported		4.44 (1.31–15.00)	0.0170	*wt/wt vs wt/V*
						not reported		9.52 (2.79–32.45)	0.0032	*wt/wt vs V/V*
Ramón *et al.*, 2010[56]	91	Monotherapy or +chemotherapy	39.8%	not reported	DFS	not reported	0.016[e]	not reported	not reported	PM vs others

CI, confidence interval; RFS, recurrence-free survival; DFS, disease-free survival; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.

[a]Genotype group was reassigned using reported data.

Definition of alleles: *wt*, **1* or **1-*1*; *im*, **10*, **10-*10* or **41*; *pm*, **3*, **4*, **5*, **6*, **14*, **21* or **36-*36*; *V*, *im* or *pm*.

Definition of genotype groups: *wt/wt*, 2 *wt* alleles; EM; *wt/wt* or *wt/im*; hetEM/IM, *wt/im*, *wt/pm*, *im/im* or *im/pm*; PM, 2 *pm* alleles; decreased, *wt/pm*, *im/im*, *im/pm* or *pm/pm*.

[b]Not calculated hazard ratio according to *CYP2D6* genotypes.

[c]Genotype group defined as combination of *CYP2D6*4* and CYP2D6 inhibitors by Goetz *et al.*[42]

[d]These studies included patients reported previously.[41,42,45]

[e]log-lank test p value.

of 486 postmenopausal patients (206 of them received adjuvant tamoxifen).[43] In 2009, Schroth *et al.* subsequently published a retrospective analysis of 1,325 German and North American breast cancer patients who were at an early stage and treated with adjuvant tamoxifen, and observed that PMs revealed a higher risk of recurrence than EMs with HR of 1.90 for a time to recurrence ($p = 0.02$); however, no significant difference in overall survival was observed.[44] In Asians, Kiyotani *et al.* reported that *CYP2D6*10* was significantly associated with shorter RFS in Japanese patients receiving adjuvant tamoxifen monotherapy in 2008 (HR, 10.04; $p = 0.036$), and also confirmed significant association in a follow-up study of 282 Japanese patients receiving adjuvant tamoxifen monotherapy (HR, 9.52; $p = 0.000036$ for RFS).[38,45] The worse clinical outcome of tamoxifen therapy in the patients carrying *CYP2D6*10* was also confirmed in a Chinese population.[46] Although still based on retrospective analyses of tumor samples, the majority of these trials suggest that the presence of one or two variant *CYP2D6* alleles is associated with shorter RFS. However, several studies have reported discordant results. Two large retrospective studies reported an inverse association between *CYP2D6* genotype and breast cancer outcomes.[47,48] Nowell *et al.* reported a trend toward better overall survival with HR of 0.77 in a cohort of adjuvant tamoxifen-treated breast cancer patients with the *CYP2D6*4* genotype.[47] A Swedish trial reported the better outcome for patients with at least one *CYP2D6*4* allele who were treated with 40 mg of adjuvant tamoxifen for 2 years.[48] An independent and larger cohort study by the same group also suggested that women with ER-positive tumors who were homozygous for *CYP2D6*4* revealed no significant difference in DFS compared with those with *CYP2D6*1*.[49]

There may be several reasons for these discrepancies among the studies showing the positive and negative associations. As several reviews have pointed out,[50] considerable heterogeneity in sample collection or analysis among the studies described as follows makes it hard to compare them simply: 1) differences in dosage and duration of tamoxifen treatment, 2) incompleteness of allele determination, especially for *CYP2D6*5* allele, and most importantly 3) selection of study participants. Several reports assessed partly these confounding factors. We reported significant effects of *CYP2D6* genotypes on shorter recurrence-free survival only in patients with the tamoxifen monotherapy ($p = 0.000036$) but not in those with the combination chemotherapy ($p = 0.53$) as previous publications support this notion.[51] In addition, the importance of wide coverage of *CYP2D6* alleles was clearly demonstrated by Schroth *et al.*[52] They reported that by increasing genotyping coverage, HR for RFS and the associated power were increased.

Overall, many reports investigated the association of *CYP2D6* genotype and plasma concentration of endoxifen, and consistently clarified that patients carrying the *CYP2D6* genotype which decreased or impaired CYP2D6 function showed lower plasma levels of endoxifen than those having the homozygous wild-type genotype.[7,8,10,18,38,39] For association with clinical outcome, some, but not all,[47,48,53] of the studies showed worse clinical outcome in breast cancer patients with *CYP2D6* variant alleles who were treated with tamoxifen.[38,39,41–46,54–56] However, two large studies showed no association between the *CYP2D6* genotype and clinical outcome, which has raised concern about the *CYP2D6* genotype as a biomarker to predict tamoxifen efficacy.[57,58]

Genetic Polymorphisms in Other Drug-metabolizing Enzymes and Clinical Outcome of Tamoxifen Therapy

Other CYPs, UGTs and SULTs are involved in the metabolism of tamoxifen. Hence, there is a possibility that genetic variations in these genes may affect the efficacy or toxicity of tamoxifen therapy. The most important CYP isoforms are CYP3A4 and CYP3A5, which are involved in the metabolism of more than 40% of drugs. Several polymorphisms in the *CYP3A4* gene have been reported (http://www.cypalleles.ki.se/cyp3a4.htm), but their contribution may be small due to their low allelic frequencies. In contrast, genetic polymorphisms, particularly a *CYP3A5*3* allele, define much of the variation of CYP3A5 expression.[59] The frequency of the *CYP3A5*3* allele is higher in Caucasians (85–95%) than in Asians (74–77%). Although several studies investigated the association of *CYP3A5*3* with tamoxifen metabolism or clinical outcome of tamoxifen therapy, no significant association was observed.[8,18,41,43,60,61]

In *CYP2C9*, which catalyzes 4-hydroxylation of tamoxifen, more than 30 alleles have been reported (http://www.cypalleles.ki.se/cyp2c9.htm). Among them, *CYP2C9*2* and *CYP2C9*3* have been well investigated. These two alleles are present in approximately 35% of Caucasian individuals, but are much less common in Asian populations.[62] The carriers of *CYP2C9*2* or *CYP2C9*3* showed significantly lower concentrations of endoxifen and 4-hydroxytamoxifen,[18] but no significant association with clinical outcome of tamoxifen therapy was observed.[43]

For the *CYP2C19* gene, *CYP2C19*2* and *CYP2C19*3* are null alleles. *CYP2C19*2* is observed in 10–20% of Caucasians and in more than 20% of Asians. In contrast, *CYP2C19*3* is very rare in Caucasians, but is relatively high at 5–10% in Asian populations. As a result, in Caucasians, the frequency of PMs related to CYP2C19 is 3%, whereas the PM frequency in Asian populations is as high as 23%.[31,63] Recently, a new genetic variant in the promoter region of the *CYP2C19* gene, *CYP2C19*17*, which was associated with increased CYP2C19 activity *in vivo* (UM phenotype), was identified.[64,65] The frequencies of *CYP2C19*17* were reported to be 18–27% in Caucasian populations and 1–4% in Asians.[64,66] Schroth *et al.* found significant association with clinical outcome of tamoxifen treatment in carriers of

*CYP2C19*17*,[43] but not in the carriers of *CYP2C19*2* or *CYP2C19*3*.[43,53] As to tamoxifen metabolism, no significant association was reported in *CYP2C19* polymorphisms.[18]

Several investigations on *SULT1A1*2*, which causes reduced SULT1A1 activity, found no clear association with tamoxifen efficacy[47,49] nor with tamoxifen metabolism.[8,48] Recent reports by Gjerde *et al.* addressed the association of the *SULT1A1* genotype, including copy number variation, with tamoxifen metabolism.[19,61] They clarified that neither *SULT1A1* genotypes nor copy numbers influence the plasma concentration of tamoxifen and its metabolites. However, further analysis which takes into consideration the "allele copy number" of *SULT1A1* is required, as demonstrated in the case of *CYP2D6*.[67–69]

Genetic Polymorphisms in Drug Transporters and Clinical Efficacy of Tamoxifen Therapy

Although the biotransformation of tamoxifen to endoxifen has been well studied and documented as described above, there have been few reports investigating the involvement of drug transporters in the disposition of tamoxifen and its active metabolites, 4-hydroxytamoxifen and endoxifen.

ABCB1 (P-glycoprotein, MDR1) is an ATP-dependent, efflux transporter with broad substrate specificity widely appreciated for its role in mediating cellular resistance to many anticancer agents.[70] A number of investigators have performed clinical studies to reveal the relationship between drug pharmacokinetics and *ABCB1* polymorphisms. A synonymous single nucleotide polymorphism (SNP) 3435C>T was reported to be associated with higher digoxin levels after oral administration.[71] Several groups performed screenings for *ABCB1* polymorphisms.[72–75] These three SNPs, 1236C>T, 2667G>T and 3435C>T, and their haplotypes are considered to be important in the ABCB1 function.

Callaghan and Higging reported that tamoxifen directly bound to ABCB1 and inhibited ABCB1-mediated vinblastine transport, but cellar accumulation of tamoxifen itself was not influenced by ABCB1.[76] As supporting this, it was reported that *N*-desmethyltamoxifen and 4-hydroxytamoxifen as well as tamoxifen were not substrates of this transporter in the transport assay, although 4-hydroxytamoxifen showed some tendency.[77] Recently, two studies found that ABCB1 is involved in the transport of active tamoxifen metabolites, endoxifen and 4-hydroxytamoxifen.[78,79] In both reports, P-glycoprotein knockout mice showed a tendency toward higher serum concentration of endoxifen than wild-type mice although the difference was not statistically significant, suggesting that ABCB1 does not play a major role in regulating the absorption, distribution or excretion of endoxifen. With respect to the association with the clinical outcome of tamoxifen, no single nucleotide

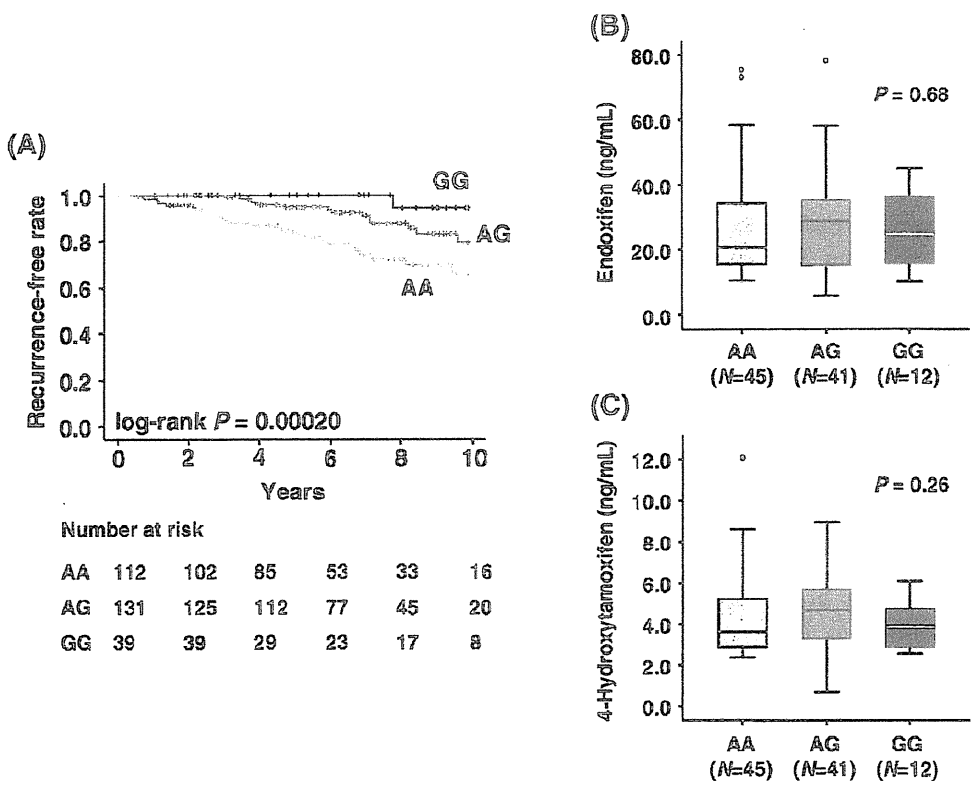

Fig. 2. Kaplan-Meier estimates of recurrent-free survival and steady-state plasma concentrations of endoxifen and 4-hydroxytamoxifen according to *ABCC2* genotype

(A) In 282 patients treated with adjuvant tamoxifen monotherapy, rs3740065 G allele was significantly associated with shorter recurrence-free survival. (B, C) Steady-state plasma concentrations of endoxifen (B) and 4-hydroxytamoxifen (C) were not significantly different among rs3740065 genotype groups.

polymorphism (SNP), which includes the SNPs described above, showed significant association in our recent report.[38]

ABCC2 (MRP2) plays an important role in the biliary excretion of conjugated drugs and xenobiotics, and also in that of some non-conjugated drugs including pravastatin and methotrexate. Tamoxifen and its metabolites are excreted into the biliary tract as glucuronides or sulfates.[16] However, there has been no report investigating the involvement of ABCC2 in the transport of tamoxifen and its active metabolites. Recent SNP screening for the *ABCC2* gene identified several common SNPs such as −1774delG (**1A*), −24C>T (**1C*) and 1249G>A (**2*).[80,81] No functional significance of 1249G>A causing Val417Ile has been shown *in vitro*,[82,83] but its *in vivo* association was reported.[84] −1774delG and −24C>T are associated with reduction of its promoter activity.[82,85] In our recent study, an intronic SNP of *ABCC2* (rs3740065) was found to be significantly associated with the clinical outcome of patients with tamoxifen therapy, whereas this SNP was not associated with plasma concentration of endoxifen or 4-hydroxytamoxifen, suggesting that the contribution of ABCC2 to biliary excretion of tamoxifen and its metabolites might be limited (Fig. 2).[38] 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.[86] As described previously,[87,88] rs3740065A/G is in strong linkage disequilibrium ($r^2 = 0.89$) with −1774G/delG, which was reported to be associated with decreased ABCC2 promoter activity. Although we believe that rs3740065 has potential to predict efficacy of tamoxifen treatment, further analyses, including replication study and functional analysis to identify the causative SNP, will be required.

Conclusion

There have been several reports on the association of *CYP2D6* genotype/phenotype and clinical outcome of breast cancer patients receiving tamoxifen therapy in large numbers of subjects. The association results with tamoxifen metabolism are consistent, but still controversial in association with clinical outcome. Investigation of the combination of the *CYP2D6* genotype and other genes, which did not affect tamoxifen pharmacokinetics, may be one of the important approaches to identify the prediction marker(s) for the clinical efficacy of tamoxifen.

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網羅的遺伝子多型解析による乳がんホルモン療法の治療効果の予測

Identification of genetic polymorphisms associated with clinical efficacy of adjuvant tamoxifen therapy in breast cancer patients

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Key words：CYP2D6、ABCC2、タモキシフェン、個別化医療、SNP

はじめに

乳がんは女性において、罹患率が最も高いがんである。タモキシフェンはエストロゲン受容体(estrogen receptor：ER) 陽性乳がんに対し、再発の予防を目的とした術後補助療法として広く用いられてきた。5年間のタモキシフェン療法により、5年および10年再発率がそれぞれ11.4%および11.8%減少することが報告されている[1]。しかしながら、タモキシフェン投与患者での5年および10年再発率はそれぞれ13.9%および22.7%であり[1]、これらの患者はタモキシフェンを服用してもその効果が十分に得られていないと考えられる。タモキシフェンは通常5年と長期間投与するため、医療経済学的観点からも、投与前に治療効果および副作用の発現を予測し、適切な患者に適切な治療を提供することが不可欠な薬物である。

タモキシフェンはプロドラッグであり、活性本体は代謝物であるエンドキシフェン（4-水酸化-*N*-脱メチルタモキシフェン）および4-水酸化タモキシフェンである。これらの活性代謝物がエストロゲンのERへの結合を阻害することにより、抗エストロゲン作用を示す（図1）。したがって、活性代謝物であるエンドキシフェンおよび4-水酸化タモキシフェンの生成や排泄に関与する薬物代謝酵素または薬物トランスポーターの活性の個人差はタモキシフェンの治療効果を左右すると考えられる。

タモキシフェンからエンドキシフェンおよび4-水酸化タモキシフェンの生成においては、CYP2D6が最も重要な酵素である[2]。CYP2D6の機能を消失する遺伝子多型である*4アレルをホモ接合体で有する患者ではタモキシフェンの再発予防効果が低いことが報告されている[3]。我々は67名の日本人乳がん患者による検討で、日本人で頻度の高い遺伝子多型である*CYP2D6*10*を有する患者ではタモキシフェン再発予防効果が低くなることを初めて明らかにした[4]。このように、*CYP2D6*遺伝子多型はタモキシフェンの再発予防効果を決定する重要な因子の一つであると考えられる。しか

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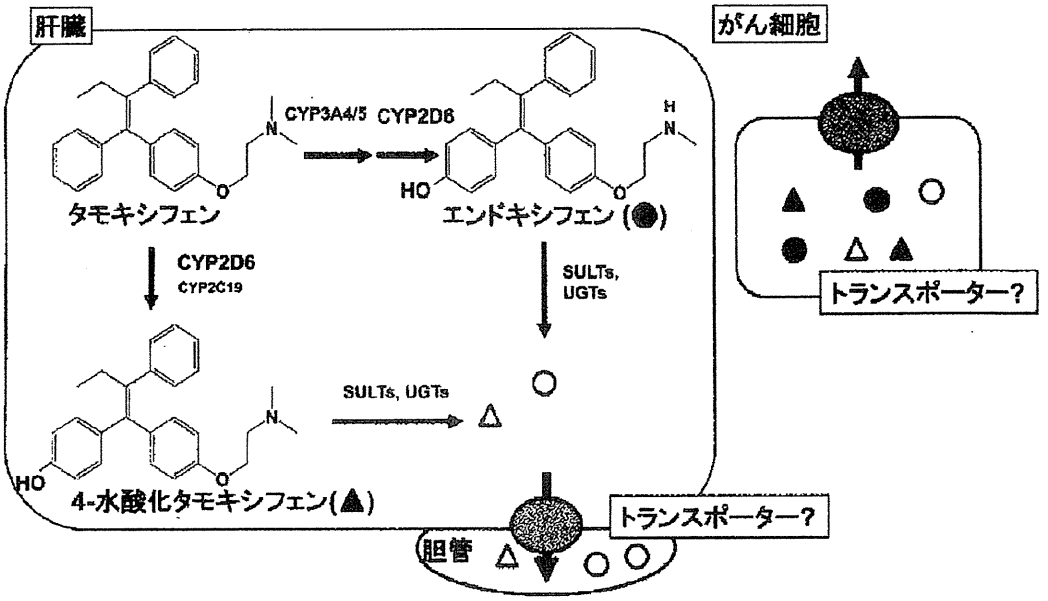

図1　タモキシフェンの代謝・排泄経路

タモキシフェンは肝臓で活性代謝物であるエンドキシフェンおよび4-水酸化タモキシフェンに変換される。エンドキシフェンおよび4-水酸化タモキシフェンはさらに硫酸抱合またはグルクロン酸抱合を受け、胆汁中に排泄される。この胆汁排泄過程にトランスポーターの関与が推測される。がん細胞では、タモキシフェンおよび活性代謝物の細胞外への排出にトランスポーターが関与することが示唆されている。
SULTs, sulfotransferases；UGTs, UDP-glucuronosyltransferases。

しながら、いずれの検討においても*CYP2D6*遺伝子多型のみではタモキシフェン再発予防効果の個人差をすべて説明することはできないことから、*CYP2D6*遺伝子多型以外にタモキシフェンの再発予防効果に影響する遺伝子多型が存在することが考えられた。

タモキシフェンから生成したエンドキシフェンおよび4-水酸化タモキシフェンはさらに硫酸抱合またはグルクロン酸抱合を受け、大部分が胆汁中に排泄される（**図1**）。これまでに報告はないが、この排泄過程にトランスポーターの関与が推測される。また、トランスポーターの発現はがん細胞において、薬剤耐性の獲得に関与している。したがって、薬物トランスポーターの遺伝子多型がタモキシフェンの再発予防効果に影響するのではないかという仮説をたてた。

本研究では、*CYP2D6*遺伝子多型とともに薬物トランスポーターの遺伝子多型を網羅的に解析することにより、タモキシフェンの再発予防効果に影響を及ぼす遺伝子多型を同定することを目的とした。

患者と方法

患者

対象患者は全て日本人女性で、1986年から2007年の間にホルモン受容体陽性の浸潤性乳がんと診断され、根治手術を受けた患者である。対象患者の全てから文書による同意を得た。本研究は理化学研究所・横浜研究所、東京大学医科学研究所および各施設の倫理審査委員会の承認を得た上で行った。

遺伝子多型と再発予防効果との関連解析では、とくしまブレストケアクリニック、やまかわ乳腺クリニック、四国がんセンター、関西ろうさい病院、札幌乳腺外科クリニック、札幌医科大学附属病院で乳がん根治手術後に5年間のタモキシフェン単剤による術後補助療法を受けており、2007年9月から2009年4月に来院した282名を対象とした。タモキシフェンの効果を適切に判断するため、併

用薬治療を受けている患者は対象外とした。

遺伝子多型と血漿中濃度との関連解析では、とくしまブレストケアクリニックに来院しており、20mg/日でタモキシフェンを服用中の98名を対象とした。タモキシフェン服用24時間後に血液を採取した。血漿中エンドキシフェンおよび4-水酸化タモキシフェンをHPLC-TOFMSにより測定した。

遺伝子型の判定

*CYP2D6*の遺伝子型（**1*-**1*、**4*、**5*、**6*、**10*、**10*-**10*、**14*、**18*、**21*、**36*-**36*および**41*）はTaqMan法（Applied Biosystems, Foster City, CA）およびreal-time Invader法（Third Wave technologies, Madison, WI）により判定した[5]。本研究では、**1*および**1*-**1*アレルを*wt*アレル、酵素活性を低下または消失させる**4*、**5*、**10*、**10*-**10*、**14*、**21*、**36*-**36*および**41*を*V*アレルとして解析を行った。

ABCB1（*MDR1*）、*ABCC2*（*MRP2*）および*ABCG2*（*BCRP*）については、haplotype-tagging SNPs（tag SNPs）を用いたスクリーニングを行った。Tag SNPsの選択は、international HapMap databaseの日本人集団（JPT）の情報を用い、以下の条件で行った：$r^2 \geq 0.8$、マイナーアレル頻度 ≥ 0.1[6]。*ABCB1*、*ABCC2*および*ABCG2*遺伝子でそれぞれ28 SNPs、11 SNPsおよび12 SNPsを選択し、合計51 SNPsをInvader法により判定した。

統計解析

タモキシフェンの再発予防効果と遺伝子多型との関連はKaplan-Meier法（trend log-rank検定）およびCox比例ハザード法により検討した。血漿中エンドキシフェンおよび4-水酸化タモキシフェンの比較はKruskal-Wallis検定により評価した。有意水準は$P<0.05$とした。トランスポーターの遺伝子多型との関連解析では、Bonferroni's correctionにより多重検定を補正し、$P<0.00098$（0.05/51）を有意水準とした。

結　果

遺伝子多型とタモキシフェン再発予防効果との関連解析[7]

表1に282名の患者背景を示す。follow-up期間の中央値は7.1年（0.8 - 23.5年）であった。**表1**に示す因子のうち、tumor sizeおよびnodal statusは無再発生存期間と有意な関連を示した（P=0.037および0.049）。

この282名について*CYP2D6*遺伝子型を判定したところ、*CYP2D6*遺伝子型の頻度は過去の知見と概ね一致した[5]。Kaplan-Meier解析では、*CYP2D6*遺伝子多型は無再発生存期間と有意な関連を示した（**図2**；log-rank P=0.00020）。Cox比例ハザード解析では、*CYP2D6*遺伝子型はその他の因子とは独立した再発予測因子であり、*wt/V*および*V/V*遺伝子型を有する患者でのハザード比は*wt/wt*と比較してそれぞれ4.44（95%信頼区間、1.31-15.00）および9.52（95%信頼区間、2.79-32.45）であった（**表2**）。

次に*ABCB1*、*ABCC2*および*ABCG2*遺伝子についてtag SNPsを選択し、タモキシフェンの再発予防効果との関連を網羅的に検討した。検討した51 tag SNPsのうち、*ABCC2*遺伝子の2 SNPs（rs3740065およびrs11190303）は、無再発生存期間と有意な関連を示した（**表3**および**図2**：log-rank P=0.00020および0.00048）。これらの2 SNPsは強い連鎖不平衡にあった（D'=0.97、r^2=0.79）。rs3740065 G/G患者と比較したとき、rs3740065 A/GおよびA/A患者でのハザード比は3.52（95%信頼区間、0.46-26.79）および10.64（95%信頼区間、1.44-78.88；P=0.00017）であった（**表2**）。*ABCB1*および*ABCG2*遺伝子のtag SNPsはいずれも有意な関連を示さなかった（log-rank P> 0.083：data not shown）。

さらに*ABCC2*と*CYP2D6*遺伝子型との組み合わせの検討を行ったところ、二つの遺伝子のリスクアレルの数の合計が増加するのに伴い、無再発生存期間が有意に短くなった（**図2**；log-rank P=

表1　患者背景

Characteristic	Total (*N* = 282) Number of patients (%)
Age at surgery, years	
Median	51
Range	31 to 83
Menopausal status	
Premenopause	123 (43.6)
Postmenopause	149 (52.8)
Unknown	10 (3.6)
Tumor size, cm	
≤2	159 (56.4)
2.1-5	106 (37.6)
>5	2 (0.7)
Unknown	15 (5.3)
Nodal status	
Negative	230 (81.6)
Positive	48 (17.0)
Unknown	4 (1.4)
ER status	
Positive	208 (73.8)
Negative	25 (8.9)
Unknown	49 (17.3)
PR status	
Positive	195 (69.1)
Negative	36 (12.8)
Unknown	51 (18.1)
Her-2	
Positive*	5 (1.8)
Negative	97 (34.4)
Unknown	180 (63.8)
Events	
No event	241 (85.5)
Locoregional events	9 (3.2)
Distant metastasis events	22 (7.8)
Contralateral breast events	10 (3.5)

ER, estrogen receptor; PR, progesterone receptor;
Her-2, human epidermal growth factor receptor 2。
* ImmunohistochemistryでScore 3+ をPositiveとした。

表2　*CYP2D6*および*ABCC2*遺伝子多型と再発予防効果との関連（Cox比例ハザード解析）

Variables	Number of patients	Number of recurrences	Hazard ratio* (95% CI)	*P* value
CYP2D6				0.000036
wt/wt	84	3	1.00 (reference)	
wt/V	135	20	4.44 (1.31-15.00)	
V/V	63	18	9.52 (2.79-32.45)	
ABCC2 rs3740065				0.00017
GG	39	1	1.00 (reference)	
AG	131	14	3.52 (0.46-26.79)	
AA	112	26	10.64 (1.44-78.88)	
CYP2D6 + *ABCC2*†				0.000000055
0	13	0	1.00 (reference)	
1	52	1		
2	109	8	4.93 (0.61-39.63)	
3	86	23	19.98 (2.69-148.65)	
4	22	9	45.25 (5.58-366.81)	

*Tumor sizeおよびnodal statusで調整した。
†*CYP2D6*および*ABCC2*のリスクアレルの合計本数。
CI, confidence interval。

表3　*ABCC2*遺伝子のtag SNPsとタモキシフェン再発予防効果との関連

SNP ID	Chromosomal location*	Position in gene	Allele†		Event			No event			Minor allele frequency		Hardy-Weinberg equilibrium *P*	Log-rank *P*
			1	2	11	12	22	11	12	22	Event	No event		
rs12268782	101523996	5' upstream region	G	A	24	15	2	174	61	6	0.23	0.15	0.83	0.082
rs2804398	101548624	Intron 7	T	A	30	8	3	175	58	8	0.17	0.15	0.062	0.79
rs2756109	101548736	Intron 7	G	T	10	22	9	110	102	28	0.49	0.33	0.58	0.0031
rs2273697	101553805	Exon 10 (Ile417Val)	G	A	32	7	1	196	43	2	0.11	0.10	0.89	0.66
rs11190291	101556000	Intron 11	C	T	32	7	1	196	43	2	0.11	0.10	0.89	0.66
rs2002042	101577921	Intron 19	C	T	19	18	4	114	101	26	0.32	0.32	0.66	0.83
rs3740065	101595683	Intron 29	A	G	26	14	1	86	117	38	0.20	0.40	0.94	0.00020‡
rs12762549	101610761	3' downstream region	G	C	12	20	8	100	110	30	0.45	0.35	0.98	0.074
rs2862691	101612513	3' downstream region	C	T	17	19	5	138	86	17	0.35	0.25	0.48	0.042
rs11598781	101623010	3' downstream region	C	T	17	19	5	136	90	15	0.35	0.25	0.92	0.036
rs11190303	101625199	3' downstream region	C	T	27	13	0	103	103	34	0.16	0.36	0.31	0.00048‡

*NCBI 36 genome assemblyに基づいた10番染色体での位置を示す。
†イベントなしの患者でのメジャーアレルをアレル1と定義した。
‡有意差あり：Bonferroni's correctionにより多重検定を補正し、$P < 0.00098$ (0.05/51) を有意水準とした。

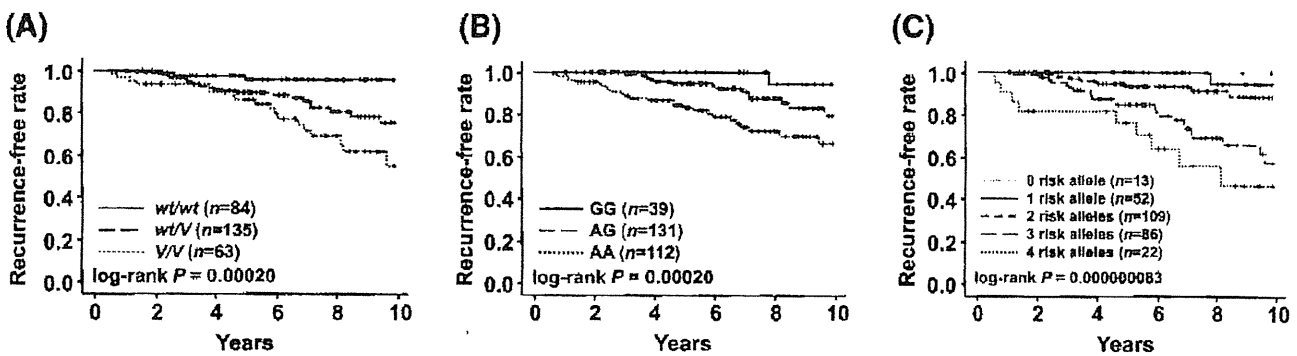

図2　タモキシフェンの再発予防効果と遺伝子多型の関連

タモキシフェン単剤による補助療法を受けている282名の乳がん患者における無再発生存率と*CYP2D6*遺伝子型（A）、*ABCC2* rs3740065遺伝子型（B）および*CYP2D6*+*ABCC2*（C）との関連をKaplan-Meier法で解析した。trend log-rank検定で有意差を検定した。

*CYP2D6*遺伝子型：*wt*、**1*、**1*-**1*；*V*、**4*、**5*、**10*、**10*-**10*、**14*、**21*、**36*-**36*、**41*。

0.000000083）。リスクアレルの数が2、3および4の患者では、1以下の患者と比較して、ハザード比は4.93（95%信頼区間、0.61-39.63）、19.98（95%信頼区間、2.69-148.65）および45.25（95%信頼区間、5.58-366.81）であった（**表2**）。

遺伝子多型とタモキシフェン代謝物の血漿中濃度との関連解析[7]

*CYP2D6*および*ABCC2*遺伝子型と血漿中エンドキシフェンおよび4-水酸化タモキシフェン濃度との関連を検討した（**図3**）。

血漿中エンドキシフェンおよび4-水酸化タモキシフェン濃度は各*CYP2D6*遺伝子型群間で有意に差があった（Kruskal-Wallis P=0.0000043および0.00052）。*CYP2D6 V/V*および*wt/V*患者での血漿中エンドキシフェン濃度の中央値はそれぞれ15.5および27.2ng/mLであり、*wt/wt*患者（35.4ng/mL）のそれぞれ43.8%および76.8%であった。4-水酸化タモキシフェンについても同様の結果であった。一方、*ABCC2* rs3740065遺伝子型では、血漿中エンドキシフェンおよび4-水酸化タモキシフェン濃度いずれとも有意な関連は認められなかった（Kruskal-Wallis P=0.68および0.26）。また、*ABCC2* rs3740065遺伝子型は血漿中タモキシフェンおよび*N*-脱メチルタモキシフェン濃度とも関連を示さなかった（data not shown）。

考　察

本検討では、乳がん術後補助療法として用いられるタモキシフェンの再発予防効果に関連する遺

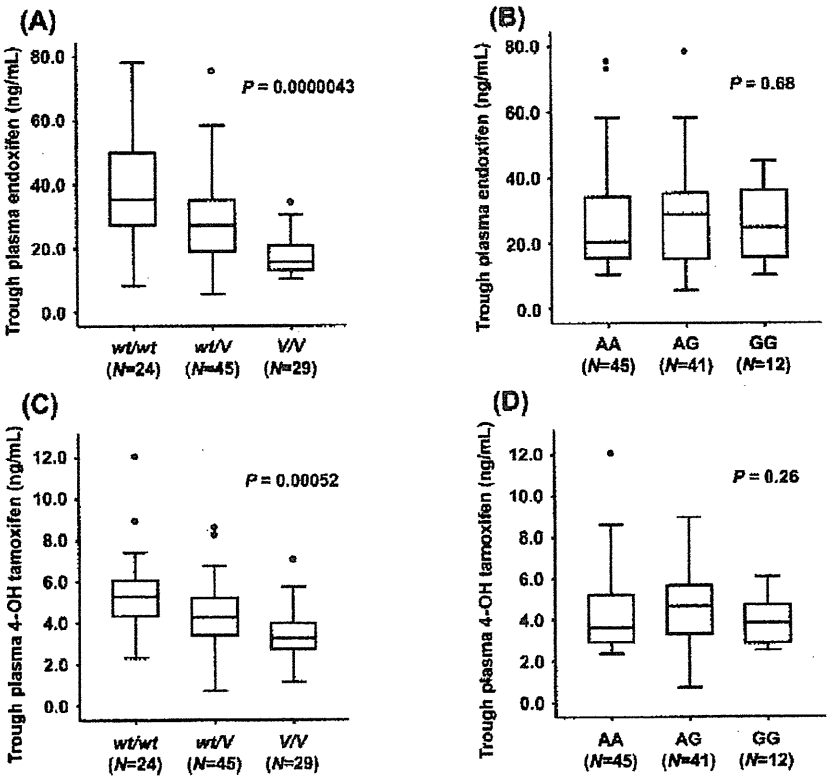

図3　血漿中エンドキシフェンおよび4-水酸化タモキシフェン濃度と遺伝子多型の関連

タモキシフェン（20mg/日）を服用中の98名の乳がん患者の血漿中エンドキシフェンおよび4-水酸化タモキシフェン濃度と*CYP2D6*遺伝子型（A、C）および*ABCC2* rs3740065遺伝子型（B、D）との関連を検討した。タモキシフェン服用24時間後に血液を採取し、血漿中エンドキシフェンおよび4-水酸化タモキシフェンをHPLC-TOFMSにより測定した。Kruskal-Wallis検定で有意差を検定した。

*CYP2D6*遺伝子型：*wt*、**1*、**1-*1*；*V*、**4*、**5*、**10*、**10-*10*、**14*、**21*、**36-*36*、**41*。

伝子多型について検討した。タモキシフェン単剤による術後補助療法を受けている282名の乳がん患者による検討で、*CYP2D6*遺伝子多型がタモキシフェンの治療効果に関連することを明らかにした。さらに、いくつかの薬物トランスポーターの遺伝子多型を網羅的に解析することにより、*ABCC2*遺伝子のrs3740065がタモキシフェンの再発予防効果と有意に関連することを明らかにした。

*CYP2D6*遺伝子多型については、これまでにいくつかのグループでタモキシフェンの治療効果との有意な関連が報告されてきた[3, 8, 9]。2009年のSchroth *et al.*の報告では、1325名の欧米人の乳がん患者による解析を行い、CYP2D6の酵素活性を低下または消失させる遺伝子多型を有する患者では無再発期間、無再発生存期間、無病生存期間が有意に短いことを明らかにしている[9]。本検討はこれに次いで多くの検体を用いた検討であり、*CYP2D6*遺伝子多型がタモキシフェンの再発予防効果に影響することを強くサポートしている。しかしながら、相反する結果も報告されている[10, 11]。これに関しては、併用治療の影響、判定した*CYP2D6*遺伝子多型の種類が重要な要因であることを明らかにしている[12, 13]。本検討と並行して収集している167名のタモキシフェン併用治療患者を用い検討したところ、*CYP2D6*遺伝子型と再発予防効果との間に有意差は認められなかった[12]。有意な関連が認められないこれまでの報告のほとんどはタモキシフェンと化学療法併用による補助

療法を行っていることからも、本検討のようにタモキシフェン単剤による補助療法を受けている患者を用いるべきであることが示唆される[12]。さらに、Schroth *et al.*は判定する*CYP2D6*アレルの種類を増やすことで、*CYP2D6*遺伝子多型とタモキシフェンの再発予防効果との関連がより明確になることを報告している[13]。更なるデータの蓄積が必要であるが、乳がん術後療法の決定に*CYP2D6*遺伝子多型の判定が用いられるようになる日はそう遠くないかもしれない。

*CYP2D6*遺伝子多型は有望なタモキシフェンの再発予防効果の予測因子であるが、*CYP2D6*遺伝子多型のみではすべてを説明することができない。*CYP2D6*以外では、*CYP2C19*、*CYP3A5*、*UGT2B15*、*SULT1A1*遺伝子多型とタモキシフェン治療効果との関連が検討されているが[8, 11]、本検討に用いた282名では、これらの遺伝子多型と無再発生存期間との間に有意な関連は認められなかった（data not shown）。そこで本検討では、トランスポーターの遺伝子多型に注目し、tag SNPsを用いたスクリーニングを行い、*ABCC2*遺伝子のイントロン29に存在するrs3740065がタモキシフェンの治療効果と有意に関連することを明らかにした。*ABCC2*遺伝子領域（約80kb）をre-sequenceした結果、rs3740065は-1774G/delG（$D'=1$、$r^2=0.89$）およびその他5 SNPsと完全連鎖不平衡であった（$D'=1$、$r^2>0.80$）。-1774G/delGは*in vitro*の検討で、*ABCC2*の発現量を低下させることが報告されている[14]。282名の乳がん患者において、-1774G/delGとタモキシフェンの再発予防効果との関連を検討したところ、rs3740065よりやや弱いながらも関連が認められた（log-rank $P=0.0023$）[15]。以上の結果より、真のcausative SNPは同定できていないが、ABCC2の発現量の上昇がタモキシフェンの再発予防効果の低下に関連していることが示唆された。これまでにABCC2がタモキシフェンまたはその代謝物を輸送することを証明した報告はないが、タモキシフェン耐性を獲得した乳がん細胞でABCC2が過剰発現しているという報告がある[16]。本検討結果では、*ABCC2*遺伝子多型はタモキシフェンおよび代謝物の血漿中濃度には影響しないことから、乳がん細胞での局所的な暴露に関与しているものと考えられる。この成果を実際の現場に生かすには、まず、この関連を別の集団で確認することが重要である。さらに、詳細な分子メカニズムの解明など多くの検討が必要であるが、タモキシフェンの再発予防効果の予測において新たな標的となりうるだろう。

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