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厚生労働科学研究費補助金

がん臨床研究事業

切除不能進行・再発胃がんに対する個別化治療に関する研究

平成 22 年度～平成 24 年度　総合研究報告書

研究代表者　山田　康秀

平成 25（2013）年 3 月

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研究代表者　　山田　康秀　　国立がん研究センター中央病院

研究要旨

HER2陰性切除不能進行・再発胃癌患者を対象に、ドセタキセル＋シスプラチン＋S-1併用療法（DCS療法）を試験治療とし、標準治療であるシスプラチン＋ S-1(CS)療法に対する優越性を検証するためのランダム化比較試験を核として　①HER2による治療の個別化、②クレアチニンクリアランスによる治療の個別化、③組織型による治療法の個別化、④予後予測分子マーカーによる治療法の個別化を目指す。

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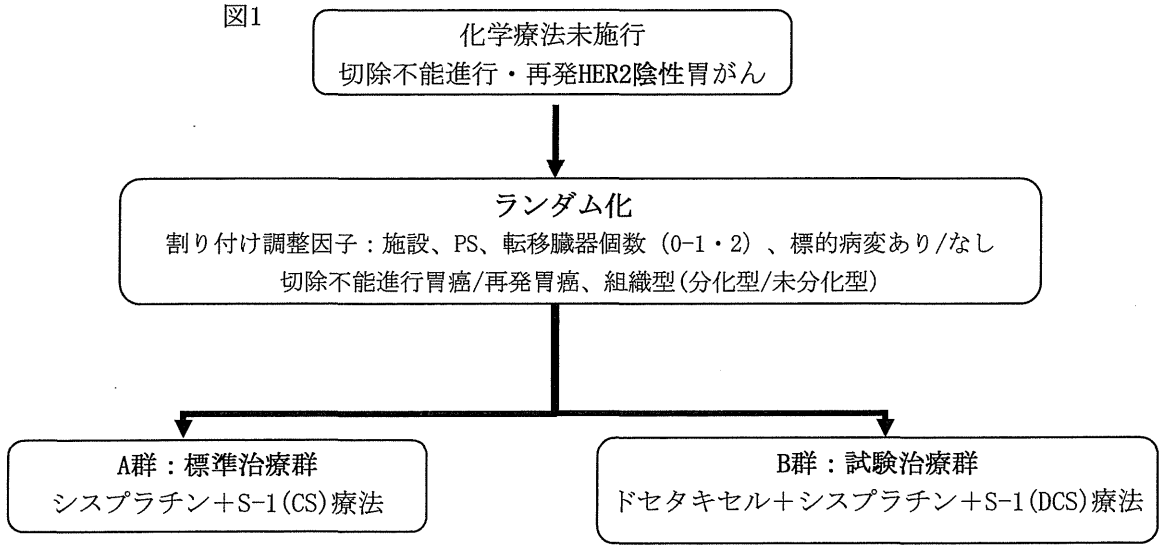
図1



A．研究目的

図1に示すように、胃癌(HER2陰性)を対象に、ドセタキセル+シスプラチン+S-1（DCS）療法を試験治療とし、標準治療であるシスプラチン+S-1(CS)療法に対する優越性を第Ⅲ相試験で検証することを目的とする。

1)クレアチニンクリアランス値に従って抗癌剤投与量を変更することにより、重篤な副作用を回避するための腎機能による治療の個別化、2)組織型による治療法の個別化の有用性を確認する。得られた抗癌剤効果予測因子候補である分子マーカー(excision repair cross-complementing group 1 (ERCC1)など)や組織型（未分化型/分化型腺癌）別に、その有用性および妥当性を検証する

JCOG9912 付随研究から予後因子、効果予測因子としての可能性が考えられた ERCC1、チミジル酸合成酵素(TS)、ジヒドロピリミジン脱水素酵素（DPD)、などと抗腫瘍効果の関係も確認することとし分子マーカーによる個別化治療の確立を目指す。また本試験内で DCS 療法および CS 療法の効果、および薬物有害反応の予測因子、予後因子を同定し検証する。

B．研究方法(図１参照)

HER2陰性切除不能進行・再発胃癌患者を対象に、ドセタキセル＋シスプラチン＋S-1併用療法（DCS療法）を試験治療とし、標準治療であるシスプラチン＋ S-1(CS)療法に対する優越性をランダム化比較にて検証する。HER2陽性である場合はトラスツズマブを抗癌剤に併用することが推奨されるため、本試験の対象とはしない。

主要評価項目は全生存期間、筆頭副次的評価項目は分化型腺癌/未分化型腺癌のサブグループ毎の全生存期間（組織型別にDCS群、CS群の治療効果が異なる場合、結果の解釈を事前に定めている）、副次的評価項目は無増悪生存期間、奏効割合、用量強度、有害事象発生割合、Grade 4の非血液毒性発生割合、早期死亡割合、治療関連死亡発生割合、分化型腺癌/未分化型腺癌のサブグループ毎の無増悪生存期間、分化型腺癌/未分化型腺癌のサブグループ毎の奏効割合とする。

試験治療(DCS)群では、第１コース開始前のクレアチニンクリアランス値に応じて、シスプラチンおよび S-1 の投与量を変更する。クレアチニンクリアランスは Cockcroft-Gault 式※により推測値を求める。

※Cockcroft-Gault 式を以下に示す：男性：Ccr＝{(140-年齢)×体重（kg）} / {72×血清 Cr 値(mg/dL)}、女性：Ccr＝0.85×{(140-年齢)×体重（kg）} / {72×血清 Cr 値(mg/dL)}。

ERCC1 など mRNA 定量のための薄切標本の作成は各々の参加施設で行う。原発巣切除標本（ホルマリン固定パラフィン包埋）から mRNA 量を測定する患者では解析用として 10 枚（10 μm 厚)、原発巣内視鏡生検標本を用いて測定する患者では遺伝子発現解析用として 15 枚を測定施設に送付する。H-E (hematoxylin and eosin) 染色用としての薄切標本は、1 検体あたり 5μm 厚 1 枚のプレパラートを用意する。mRNA 発現量の測定は、抗癌剤に対する腫瘍反応を規定する因子を解析することにより腫瘍に即した個別化化学療法が可能になるとの考えから、同因子の腫瘍内における mRNA 発現量を RT-PCR 法により北里大学消化器内科で行う。レーザー捕獲顕微鏡を用いて、マーキングした H-E 染色像を参照しながら 10 μm 厚のプレパラート上の腫瘍細胞のみを正確に切り出す。切り出した腫瘍細胞から RNA を抽出し、高精度かつ高感度微量検出可能な Real time RT-PCR 法を用いて mRNA 発現量の解析を行う。

従来、このようなmRNA発現解析は新鮮凍結標本を用いることが必要とされてきたが、北里大学は、通常病理検査標本であるホルマリン固定パラフィン包埋薄切標本から解析に十分なRNAを抽出する技術を有している。

（倫理面への配慮）

参加患者の安全性確保については、適格条件やプロトコール治療の中止変更規準を厳しく設けており、試験参加による不利益は最小化される。また、「臨床研究に関する倫理指針」およびヘルシンキ宣言などの国際的倫理原則に従い以下を遵守する。

1) 研究実施計画書のIRB承認が得られた施設のみから患者登録を行う。
2) すべての患者について登録前に充分な説明と理解に基づく自発的同意を本人より文書で得る。
3) データの取り扱い上、患者氏名等直接個人が識別できる情報を用いず、かつデータベースのセキュリティを確保し、個人情報（プライバシー）保護を厳守する。

研究の第三者的監視：JCOG（Japan Clinical Oncology Group）に所属する研究班は共同で、Peer reviewと外部委員審査を併用した第三者的監視機構としての各種委員会を組織し、科学性と倫理性の確保に努めている。本研究も、JCOGのプロトコール審査委員会、効果・安全性評価委員会、監査委員会、放射線治療委員会などによる第三者的監視を受けることを通じて、科学性と倫理性の確保に努める。

C．研究結果

本研究の結果、HER2陰性胃癌に対する新たな標準治療を確立することができる。また、実臨床における高齢癌患者の増加に際し、体表面積に加え年齢、性を考慮した腎機能の指標であるクレアチニンクリアランス値を用い、より適正化した個々の患者の初回抗癌剤投与量を設定することの有用性、重篤な有害事象を回避することによる治療継続性を検証する。

本研究による先行研究の結果、ERCC1は切除不能進行・再発胃癌の独立した予後不良因子であり、ERCC1 mRNA量は分化型腺癌に比べ未分化型腺癌で高い傾向がみられた。本第Ⅲ相試験では試験対象全体の治療成績の解析に加え、組織型別の対象集団に対する治療効果を確認する。

また本試験の付随研究として、抗癌剤の効果予測法を開発するために、癌部生検組織および血液検体を用いて網羅的遺伝子解析およびプロテオミクス解析などを国立がん研究センター研究所で行う。余剰検体はJCOGバイオバンクに保存し、検査法の進歩により新たな解析が必要な場合は再利用する。

D．考察

本年度4月より症例登録を開始する。予定登録患者数、750名、登録期間は4.5年、追跡期間は登録終了後1.5年、総研究期間として6年を予定している。

E．結論

対照(CS)群の全生存期間中央値は13.5ヶ月と予想される。試験治療(DCS)群がこれを3ヶ月上回るか否かを検出する優越性臨床試験として計画した場合、症例集積期間4.5年、追跡期間1.5年、有意水準片側5%、検出力80%と仮定すると、この差に必要な症例数は732例となる。若干の不適格、除外症例を見込んで、１群375例、2群併せて750例の症例集積を目標とする。

平成25年3月5日現在、186名（参加施設は48施設）が登録された。試験開始後の症例集積速度（16.9人/月）は既に予定（13.8人/月）を上回っている。

F．研究発表

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3. 知的財産等の出願・登録状況（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

研究成果の刊行に関する一覧表

雑誌

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ORIGINAL ARTICLE

Additive effects of drug transporter genetic polymorphisms on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients

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Abstract
Purpose Effects of genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in addition to "*UGT1A1*28* or **6*" on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients were investigated.
Methods Associations between transporter haplotypes/variations along with *UGT1A1*28* or **6* and SN-38 area under the time–concentration curve (AUC) or neutropenia were examined in irinotecan monotherapy (55 patients) and irinotecan–cisplatin-combination therapy (62 patients).
Results Higher SN-38 AUC values were observed in *ABCB1* 2677G>T (A893S) (**2* group) for both regimens. Associations of grade 3/4 neutropenia were observed with *ABCC2* −1774delG (**1A*), *ABCG2* 421C>A (Q141K) and IVS12 + 49G>T (*#IIB*) and *SLCO1B1* 521T>C (V174A) (**15* · *17*) in the irinotecan monotherapy, while they were

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evident only in homozygotes of *ABCB1*2*, *ABCG2#IIB*, *SLCO1B1*15 · 17* in the cisplatin-combination therapy. With combinations of haplotypes/variations of two or more genes, neutropenia incidence increased, but their prediction power for grade 3/4 neutropenia is still unsatisfactory.
Conclusions Certain transporter genotypes additively increased irinotecan-induced neutropenia, but their clinical importance should be further elucidated.

Keywords Irinotecan · Transporter · Genetic polymorphism · Haplotype

Introduction

Irinotecan, an anticancer prodrug, is widely used for treating a broad range of carcinomas including colorectal and lung cancers. However, unexpected severe diarrhea and neutropenia are important clinical side effects from irinotecan treatment. The active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1], and is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (UGTs), such as UGT1A1, UGT1A7, and UGT1A9, to form an inactive metabolite, SN-38 glucuronide (SN-38G) [2–4]. Irinotecan is also inactivated by CYP3A4 to produce 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin (NPC) [5]. Irinotecan and its metabolites are excreted into the bile and urine via the action of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp/ABCB1), multiple resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2) [6]. Transport of SN-38 from the plasma into the liver is mediated by the organic anion transporting polypeptide C (OATP-C/SLCO1B1) [7]. Most of the previous pharmacogenetic studies on irinotecan have focused on *UGT1A1* polymorphisms and have shown clinical relevance of *UGT1A1*28*, a repeat polymorphism in the TATA box [−54_−39A(TA)$_6$TAA>A(TA)$_7$TAA or −40_−39ins TA], to severe toxicities [8–10]. Based on these findings, in 2005, the Food and Drug Administration (FDA) of the United States approved an amendment for the label of Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028) and the clinical use of a genetic diagnostic kit for the **28* allele. In parallel with this advance in the USA, clinical relevance to severe neutropenia of *UGT1A1*6* [211G>A (G71R)], another low-activity allele detected specifically in East-Asians, as well as **28* was demonstrated in several studies on Asian patients [11–14]. Accordingly, in June 2008, the Ministry of Health, Labor and Welfare of Japan approved changes to irinotecan labels (Campto and Topotecin) by adding a caution for the risk of severe toxicities in patients either homozygous or compound heterozygous for *UGT1A1*28* and **6* (**28/*28*, **6/*6*, **28/*6*) and the clinical use of a diagnostic kit for *UGT1A1*28* and **6*. Severe toxicities, however, are found in patients without **6/*6*, **28/*28*, and **28/*6*; therefore, other factors responsible for irinotecan toxicities should be identified.

Several clinical studies have suggested polymorphisms of the drug transporter genes, such as *ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1*, might affect irinotecan pharmacokinetics (PK)/pharmacodynamics (PD) in Caucasian and Asian patients. However, the results obtained from different ethnic populations with various irinotencan regimens are still controversial, and the genetic markers examined also differ [13, 15–26]. We previously identified a number of haplotypes/variations of transporter genes, including *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in Japanese [12, 26–29], but their clinical significance, either alone or in combination, in irinotecan therapy has not yet been examined.

This study aimed to identify the genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1* which can affect irinotecan PK/PD in Japanese cancer patients. We carefully stratified the patients considering the irinotecan regimen (irinotecan monotherapy or combination therapy with cisplatin) and *UGT1A1* genotype (*UGT1A1 *6* or **28*), and examined additive effects of transporter haplotypes/variations on the area under the time–concentration curves (AUC) of the toxic metabolite SN-38 and on the risk of severe neutropenia.

Patients and methods

Patients

The patients used in this study were the same as those described in a previous paper [12], where details on the eligibility criteria for irinotecan therapy, patient profiles, and irinotecan regimens were described. In this study, 55 patients with irinotecan monotherapy (100 mg/m^2 weekly or 150 mg/m^2 biweekly) and 62 patients with combination therapy of irinotecan (60 mg/m^2 weekly or 70 mg/m^2 biweekly) and cisplatin (60 or 80 mg/m^2, respectively) were included. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants.

Analyses on genetic polymorphisms and PK/PD

Patients' data on genetic variations and haplotypes of *UGT1A1*, *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were

previously obtained [12, 26–29]. Regarding *ABCG2*, combination haplotypes were newly defined using the previously reported haplotypes from three linkage disequilibrium (LD) blocks [28]. Patients' PK data on the area under the concentration–time curve (AUC) and toxicities were previously obtained [12].

Association analyses

Associations of transporter genotypes with AUC/dose values for irinotecan, SN-38 and SN-38G, absolute neutrophil count (ANC) nadir, and incidence of grade 3 diarrhea or grade 3/4 neutropenia were investigated. For SN-38 AUC/dose and neutropenia, the patients were stratified by the presence of *UGT1A1*6* or **28* (*UGT+*). Statistical significance (two-sided, $P < 0.1$) was determined by the Mann–Whitney (MW) test or Jonckheere–Terpstra (JT) test for AUC/dose, and by Fisher's exact test and chi-square test (for trend) for incidence of grade 3 and 4 toxicities, using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, CA, USA) and StatXact version 6.0 (Cytel Inc., Cambridge, MA). Multiplicity adjustment was not applied to bivariate analysis, and contributions of the candidate genetic markers to SN-38 AUC/dose values and ANC nadir were further determined by multiple regression analysis after logarithmic transformation of the AUC/dose values and ANC nadir counts. The variables examined were age, sex, body surface area, history of smoking or drinking, performance status, serum biochemistry (GOT, ALP, creatinine) at baseline, the ANC at baseline (for neutropenia), and genetic markers including *UGT1A1*6* or **28* (*UGT+*) and the transporter haplotypes. The variables in the final models were selected by the forward and backward stepwise procedure at a significance level of 0.20 using JMP version 7.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

Definition of major transporter haplotypes and their selected markers

For screening transporter gene polymorphisms affecting irinotecan PK/PD, major haplotypes and their tagging single nucleotide polymorphisms (SNPs) from *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were selected (Table 1) according to their frequencies (more than 5%) and/or from preliminary results obtained from all patients treated with irinotecan.

For *ABCB1* block 1[26], the haplotype group *BJL*, which consists of **1B* (having −1789G>A), **1J* (having −1789G>A and −371A>G) and **1L* (having −1789G>A and −145C>G), was selected because an association of the marker SNP −1789G>A with lower expression levels of P-gp has been reported [30]. *ABCB1* block 2 **2* was originally defined as haplotypes containing three SNPs, 1236C>T, 2677G>T (A893S) and 3435C>T [31]. Since the **9* haplotype with 1236C>T, 2677G>T (A893S) without 3435C>T [16] showed the same trend for PK/PD as **2* (data not shown), the current study classified the

Table 1 List of major transporter haplotypes and their markers analyzed for Japanese cancer patients

Gene	Haplotype	Tagging SNP	Abbreviation used in this paper	Haplotype frequency	
				Monotherapy ($N = 110$)[a]	With cisplatin ($N = 124$)[a]
ABCB1	*BJL*[b] (block 1)	−1789G>A		0.182	0.210
	**2* group[c] (block 2)	2677G>T(A893S)	*B*	0.382	0.379
	**10* group[d] (block 2)	2677G>A(A893T)		0.182	0.169
	**1b* (block 3)	IVS27-182G>T		0.200	0.169
ABCC2	**1A*	−1774delG	*C*	0.373	0.371
	**1C/G*	3972C>T(I1324I)		0.218	0.266
ABCG2	#*IIB* [**1a–*2–*1b*][e]	421C>A(Q141K), IVS12 + 49G>T	*G*	0.200	0.274
	#*IIIC* [**1b–*3–*1c*][e]	34G>A(V12M), IVS9-30A>T		0.164	0.097
SLCO1B1	**1b*	388A>G(N130D)		0.373	0.573
	**15 · 17*	521T>C(V174A)	*S*	0.191	0.153

[a] Number of chromosome

[b] *BJL* consists of **1B* (having −1789G>A), **1J* (having −1789G>A and −371A>G) and **1L* (having −1789G>A and −145C>G) previously defined [26]

[c] **2* Group includes **2*, **9*, **12* and **14* haplotypes previously defined [26]

[d] **10* Group includes **10* and **13* haplotypes previously defined [26]

[e] Combination of *ABCG2* haplotypes of three blocks [block (−1)–block 1–block 2] previously defined [28]

haplotypes with 2677G>T (A893S), *2, *9, *12 and *14 [26], as the *2 group (*2 in this paper). Similarly, the *10 group was classified as haplotypes with 2677G>A (A893T), i.e., *10 and *13, since no differences in PK/PD parameters were observed between these haplotypes. The *4, *6, and *8 haplotypes in block 2 [16, 26] showed no significant effect in the current analysis (data not shown). The *ABCB1* block 3 *1b haplotype containing IVS27-182G>T was selected because our previous study showed it was associated with an increased renal clearance of SN-38 [16].

Based on reports showing possible functional alterations of −1774delG [32] and 3972C>T (I1324I) [18, 24], *ABCC2* haplotypes containing those variations were classified as *1A and "*1C and *1G (*1C/G)", respectively, according to our previous definition: *1A, −1774delG; *1C, −24C>T and 3972C>T; *1G, 3972C>T [27]. *ABCC2*2* [1246G>A (V417I)] and *1H [2934G>A (S978S)] [27] showed no statistically significant effects (data not shown).

The *ABCG2* combinatorial haplotypes were newly defined as combinations of haplotypes across the three blocks [block (−1)–block 1–block 2] previously reported [28]. Major combinations in 177 patients were the wild type #IA (frequency = 0.291), #IIB [containing 421C>A (Q141K) and IVS12 + 49G>T] (0.251) and #IIIC [containing 34G>A (V12M) and IVS9-30A>T] (0.107). Note that #IIB and #IIIC are subgroups of block 1 *2 [421C>A (Q141K)] and block 1*3 [34G>A (V12M)], respectively [28].

The *SLCO1B1* haplotypes used were the major haplotypes *1b [containing 388A>G (N130D) without 521T>C (V174A)] [33] and *15 · 17 [containing 521T>C (V174A)], the functional relevance of which has been reported [34].

Association of transporter genotypes with AUC values

Since we previously found that some PK parameters, including AUC/dose, Cmax/dose and $t_{1/2}$ for irinotecan and/or its metabolites, as well as incidence of grade 3/4 toxicities were affected by irinotecan regimen [12], the following analyses were conducted using the two groups of patients; i.e., those treated with irinotecan monotherapy (100–150 mg/m^2 for initial dosage) or by combination therapy with cisplatin (60–70 mg/m^2 for initial dose of irinotecan). Since SN-38 AUC levels were largely dependent on the *UGT1A1* genotype "*6 or *28" [12], the associations of transporter genotypes with SN-38 AUC values were analyzed within the groups stratified by the marker *UGT1A1* "*6 or *28" (*UGT+*); i.e., *UGT−/−*, *UGT+/−* and *UGT+/+*. Since the SN-38 AUC/dose level of one patient with haplotypes *ABCB1*2* [2677G>T (A893S)] and *14 [2677G>T (A893S) and 1345G>A (E448K)] showed an outlying value (indicated as "*a*" in Fig. 1), this patient was excluded from the statistical analysis. In this study, we preliminarily found that effect of each transporter genotype on irinotecan PK/PD was generally small. However, it was hypothesized that multiple transporter genotypes might act additively as described below. Accordingly, we adopted a statistical significance level of $P = 0.1$ (two-sided) to pick up candidate polymorphisms for further evaluation of their combined effects.

Figure 1 shows the association of transporter genotypes with SN-38 AUC values in the irinotecan monotherapy. In all patients (ALL), higher values of the SN-38 AUC/dose were observed in the *ABCB1*2/*2* [1.64-fold of −/−, $P = 0.095$ (MW test)] (Fig. 1b) and *ABCG2#IIB* [1.24-fold of −/−, $P = 0.078$ (MW test)] genotypes (Fig. 1e) and lower values were observed in the *ABCB1*1b* (block 3) [0.78-fold of −/−, $P = 0.008$ (MW test)] (Fig. 1c) genotype. In *UGT−/−* patients, an increase in SN-38 AUC/dose was observed in the *ABCB1 BJL* [1.22-fold of −/−, $P = 0.073$ (MW test)] (Fig. 1a) and *ABCG2#IIB* [1.21-fold of −/−, $P = 0.082$, (MW test)] genotypes (Fig. 1e). In *UGT* (+/− and +/+) patients, an increase in SN-38 AUC/dose in *SLCO1B1*15 · 17 (S)* [1.59-fold of −/−, $P = 0.036$ (MW test)] was also observed (Fig. 1f). Multiple regression analysis for the SN-38 AUC/dose (logarithm-transformed values) in the irinotecan monotherapy revealed significant associations of *ABCB1*2/*2* (coefficient = 0.212 ± 0.075, $P = 0.007$), along with *UGT+/−* (0.113 ± 0.054, $P = 0.040$) and *UGT+/+* (0.225 ± 0.088, $P = 0.014$) in the final model [$R^2 = 0.226$, Intercept = 0.281 (log 10^{-3}h m^2/L), $N = 53$].

Regarding other compounds, *ABCB1*2/*2* also showed higher irinotecan AUC/dose (1.27-fold) [66.2 (48.2–82.4) [median (25th–75th percentiles)] for *2/*2 vs. 52.2 (40.6–61.9) for −/− and *2/−; $P = 0.063$ (MW test)] and SN-38G AUC/dose (1.62-fold) [18.0 (14.6–27.7) for *2/*2 vs. 11.1 (7.7–14.2) for −/− and *2/−; $P = 0.002$ (MW test)]. Conversely, lower irinotecan AUC/dose for *ABCB1*10/*10* (0.79-fold) [54.8 (44.4–65.7) for −/− vs. 43.3 (40.6–54.1) for *10/*10; $P = 0.062$ (JT test)] was detected.

For the combination therapy with cisplatin, an increase of the SN-38 AUC/dose for *ABCB1*2/*2* (1.43-fold) in *non-UGT+/+* patients (*UGT−/−* and *UGT+/−*) ($N = 55$) [3.57 (2.72–4.19) for *2/*2 vs. 2.51 (1.99–3.28) for −/− and *2/−; $P = 0.032$ (MW test)], and a decrease for *ABCB1*1b* (0.80-fold) in *UGT−/−* patients ($N = 35$) [2.03 (1.72–2.33) for *1b/− and *1b/*1b vs. 2.55 (2.02–3.31) for −/−; $P = 0.026$ (MW test)] were observed. Multivariate analysis, however, showed no significant contributions of these transporter haplotypes to the SN-38 AUC/dose values.

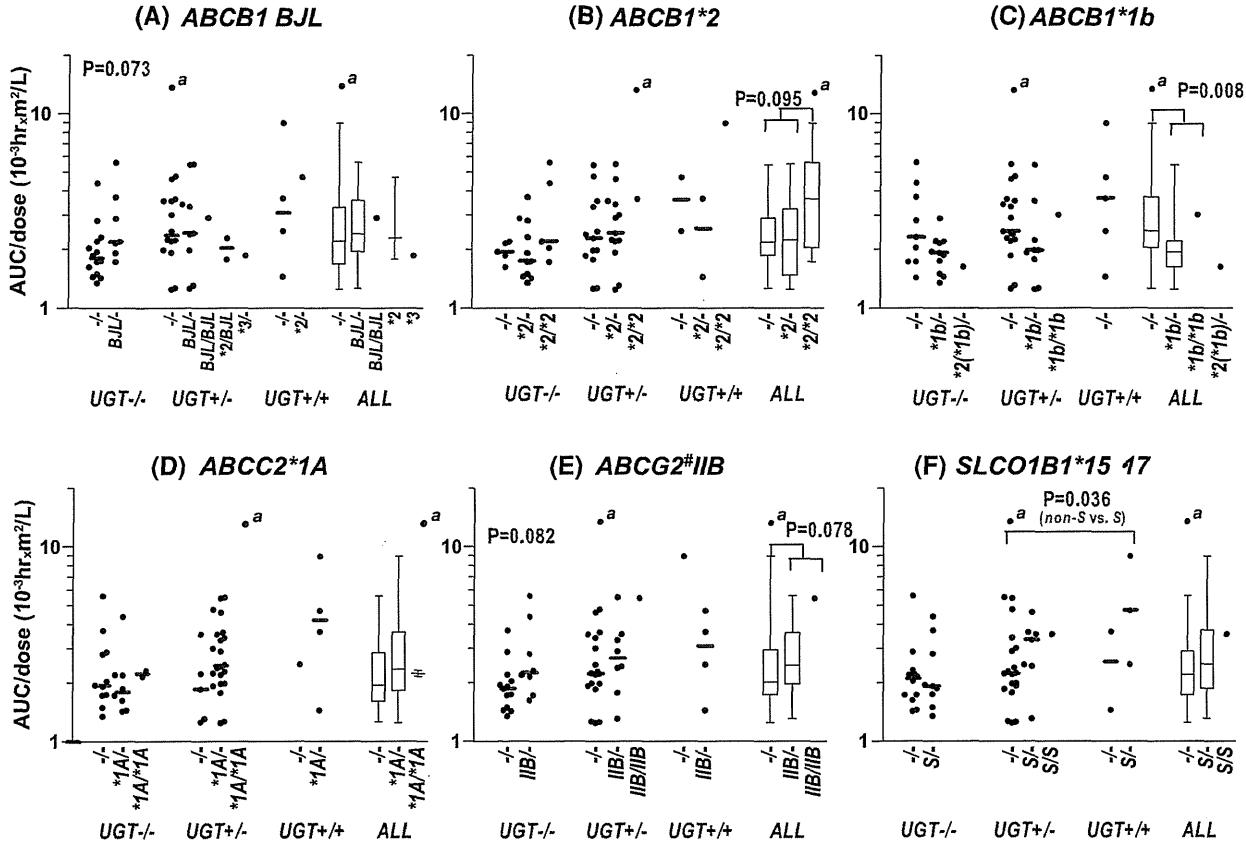

Fig. 1 Effects of transporter genotypes on SN-38 AUC/dose in irinotecan monotherapy ($N = 54$). *a* Excluded from statistical analysis. The *bars* represent the medians. *UGT+* = *UGT1A1*6* or **28*. **a** *BJL* contains −1789G>A, *2 (block 1) = 325G>A (E109K), **3* (block 1) = 304G>A (G102R); **b** *2 (block 2) contains 2677G>T (A893S); **c** **1b* (block 3) = IVS27-182G>T, *2 (block 3) = 3751G>A (V1251I); **d** **1A* contains −1774delG; **e** *IIB* contains 421C>A (Q141K) and IVS12 + 49G>T; **f** *S* = *SLCO1B1*15 · 17* containing 521T>C (V174A)

Effects of transporter genotypes on toxicities in irinotecan monotherapy

Since 80 and 100% of *UGT+/+* patients showed grade 3/4 neutropenia in the irinotecan monotherapy and combination therapy with cisplatin, respectively, neutropenia incidence was analyzed only in the *non-UGT+/+* population. Two patients were excluded from the analysis; one patient who showed an outlier SN-38 value (indicated as "*a*" in Fig. 1) and a second patient from the cisplatin-combination therapy group who discontinued irinotecan therapy.

In terms of incidence of grade 3/4 neutropenia in irinotecan monotherapy (Table 2), *ABCC2*1A*-dependent increases [0, 25.8 and 50.0% for −/−, **1A*/− and **1A*/**1A*, respectively; $P = 0.014$ (chi-square test for trend)] were observed in *UGT* (−/− and +/−) patients. Higher incidence with *ABCG2#IIB* was also found in *UGT* (−/− and +/−) patients [9.5% for −/− and 35.3% for *#IIB*/− and *#IIB*/*#IIB*, respectively; $P = 0.049$ (Fisher's exact test)], and with *SLCO1B1*15 · 17*(*S*) in the *UGT+/−* patients [15.0, 28.6 and 100% for −/−, *S*/− and *S*/*S*, respectively; $P = 0.076$ (chi-square test for trend)].

Multiple regression analysis for the ANC nadir (logarithm-transformed values) was conducted. The final model [$R^2 = 0.466$, Intercept = 1.088 (log counts/μL), $N = 52$] revealed associations of *ABCC2*1A*/**1A* (coefficient = −0.339 ± 0.088, $P = 0.0004$), *ABCG2#IIB* (−0.131 ± 0.067, $P = 0.057$) and *SLCO1B1*15 · 17* (−0.136 ± 0.066, $P = 0.046$) in addition to *UGT+/−* (−0.134 ± 0.073, $P = 0.074$) and *UGT+/+* (−0.238 ± 0.117, $P = 0.047$) and ANC at baseline (0.541 ± 0.226, $P = 0.021$), but association of *ABCB1*2*/**2* was not significant (−0.158 ± 0.095, $P = 0.104$).

Although total incidence of grade 3 diarrhea was low (11%), an *ABCB1*2*-dependent increase was observed [0, 15.4 and 28.6% for −/−, *2/− and *2/*2, respectively; $P = 0.022$ (chi-square test for trend)]. Note that all patients who experienced grade 3 diarrhea had neither the *ABCC2*1C/G* nor *ABCG2#IIIC* genotypes.

Table 2 Effects of transporter genotypes on incidences of grade 3/4 neutropenia in Japanese patients treated with irinotecan monotherapy

Gene	Genotype	*UGT*−/−				*UGT*+/−				*UGT* (−/−, +/−)			
		No./total	%	*P* value		No./total	%	*P* value		No./total	%	*P* value	
				Exact[a]	Trend[b]			Exact[a]	Trend[b]			Exact[a]	Trend[b]
ABCB1	*BJL* (block 1)[c]												
	−/−	3/14	21.4	>0.1		4/15	26.7	>0.1	>0.1	7/29	24.1	>0.1	>0.1
	+/−	0/7	0.0			2/9	22.2			2/16	12.5		
	+/+					0/1	0.0			0/1	0.0		
	*2 group (block 2)												
	−/−	1/5	20.0	>0.1[d]	>0.1	5/14	35.7	>0.1[d]	>0.1	6/19	31.6	>0.1[d]	>0.1
	+/−	1/11	9.1			0/13	0.0			1/24	4.2		
	+/+	1/5	20.0			1/1	100			2/6	33.3		
	1b (block 3)[e]												
	−/−	2/9	22.2	>0.1		4/18	22.2	>0.1	>0.1	6/27	22.2	>0.1	>0.1
	+/−	0/11	0.0			2/9	22.2			2/20	10.0		
	+/+					0/1	0.0			0/1	0.0		
ABCC2	*1A												
	−/−	0/11	0.0	>0.1	0.031	0/5	0.0	>0.1		0/16	0.0	0.022	0.014
	+/−	2/8	25.0			6/23	26.1			8/31	25.8		
	+/+	1/2	50.0							1/2	50.0		
ABCG2	#*IIB*												
	−/−	0/13	0.0	0.042		3/19	15.8	>0.1	>0.1	3/32	9.4	0.049	0.057
	+/−	3/8	37.5			3/8	37.5			6/16	37.5		
	+/+					0/1	0.0			0/1	0.0		
SLCO1B1	*15 · 17												
	−/−	2/12	16.7	>0.1		3/20	15.0	>0.1	0.076	5/32	15.6	>0.1	>0.1
	+/−	1/9	11.1			2/7	28.6			3/16	18.8		
	+/+					1/1	100			1/1	100		

[a] Fisher's exact test for (−/−) versus (+/− and +/+)

[b] Chi-square test for trend

[c] Three patients bearing *2 (block 1) or *3 (block 1) were excluded

[d] Fisher's exact test for (−/− and +/−) versus (+/+)

[e] One patient bearing *2 (block 3) was excluded

Effects on toxicities in combination therapy with cisplatin

Since only four patients (6.0%) experienced grade 3 diarrhea from the cisplatin-combination therapy, association analysis for diarrhea was not done.

Grade 3/4 neutropenia incidence was higher with *ABCB1*2* [47.1, 63.3 and 85.7% for −/−, *2/− and *2/*2, respectively; $P = 0.073$ (chi-square test for trend)] in *UGT* (−/− and +/−) patients. In *UGT*−/− patients, a higher incidence was also observed with *ABCG2*#IIB* [55.6, 83.3 and 100% for −/−, #*IIB*/− and #*IIB*/#*IIB*, respectively; $P = 0.075$ (chi-square test for trend)]. Conversely, the incidence was lower with *ABCG2*#IIIC* [71.4% for −/−, and 25% for #*IIIC*/− and #*IIIC*/#*IIIC*, respectively; $P = 0.006$ (Fisher's exact test)] in *UGT* (−/− and +/−) patients. Notably, all patients homozygous for *ABCG2*#IIB* ($N = 5$) or *SLCO1B1*15 · 17* ($N = 1$) experienced grade 3/4 neutropenia. The effect of *ABCC2*1A* on neutropenia was not consistent among the *UGT* genotypes in contrast to the results from the monotherapy. Multiple regression analysis was not applied to the neutropenia parameters in the cisplatin-combination therapy because, as described in the next section, contributions of minor variations could not be ignored.

Minor genetic variations possibly related to grade 4 neutropenia

We have detected a number of rare non-synonymous variations of the transporter genes to which statistical analysis could not be applied. Since grade 4 neutropenia