別紙３　　厚生労働科学研究費補助金（がん臨床研究事業）

分担研究報告書

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

分担研究者　　辻　晃仁　高知医療センター　腫瘍内科科長

研究要旨：個別化治療戦略の開発として、StageIV、再発胃癌患者に対し、分化型腺癌と未分化型腺癌に分けて治療開発を行うこととし、ドセタキセル+シスプラチン+Ｓ-1併用療法を試験治療とし、標準治療であるS-1/シスプラチン療法に対する優越性をランダム化比較第ＩＩＩ相試験で検証することを予定している。

A. 研究目的

切除不能・再発胃癌患者に対する初回治療の現在の標準治療はS-1/シスプラチン併用(SP)療法である。さらに延命効果を高めることを目的として、SP 療法にドセタキセルを併用する多剤併用療法であるDCS 療法が開発され、第Ⅱ相試験で奏効率 82.5% (95%CI, 70.7-94.2) 、無増悪期間中央値8.7 か月(95%CI, 6.7-10.7)、全生存期間中央値 18.0 か月(95%CI, 15.0-20.9)と有望な治療成績が報告された(Nakayama, ASCO 2009)。しかしながら、3 剤を併用するDCS 療法は、グレード4 の好中球減少が35%、発熱性好中球減少が8%にみられ、消化器症状など非血液毒性の頻度も高く患者にとっての不利益もある。一方、抗がん剤治療の効果を投与前に予測し、無効な抗がん剤投与を回避することにより、患者に無益な副作用を与えずに延命効果を高める個別化治療戦略の開発がなされている。
今回我々はStageIV、再発胃癌患者に対し、分化型腺癌(diffuse)と未分化型腺癌(intestinal)に分けて治療開発を行うこととし、未分化型腺癌、分化型腺癌ともに、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S-1/シスプラチン療法に対する優越性をランダム化比較第ＩＩＩ相試験で検証することとした。

B. 研究方法

切除不能進行・再発胃癌の組織型による個別化化学療法に関するS-1/シスプラチン併用療法とドセタキセル/シスプラチン/S-1併用療法の無作為化第III相試験（PC1013「胃がんに対するDCS vs CS」）を計画した。予後不良である未分化型腺癌でのDCS療法の優越性を検証するデザインとした。また複数の遡及的検討から、TS高発現が予後不良因子であることも示されており、高TS群でのDCS療法の優越性に関して併せて検証する。

C. 研究結果

「胃がんに対するDCS vs CS」のプロトコールコンセプトを作成、JCOG運営委員会での検討・承認を受け、現在プロトコールを作成中である。

F. 研究発表

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別紙3

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書
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

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研究要旨

はじめに

胃癌は病理学的には低分化腺癌（diffuse type）および分化腺癌（intestinal type）に大別される（両者が混在したmixed typeも存在する）。前者では癌細胞間の接着が弱く、個々の細胞が明瞭な腺腔を形成することなく浸潤し、進行すると腹膜播種を呈す傾向があるのに対し、後者では癌細胞同士が集合して腺管様構造を形成し、進行すると血行性転移をきたしやすい、という特徴がある。近年の分子生物学的研究により、胃癌についてもその分子生物学的特性が明らかになりつつある。中でも、human epidermal growth factor 2（HER2）は内在性チロシンキナーゼ活性を有する膜貫通型糖タンパク質で、胃癌においても7～34%でHER2が陽性であることが報告されている[1-3]。また、HER2は胃癌において負の予後因子である可能性が指摘されている[1, 3]。

TrastuzumabはHER2に対するモノクローナル抗体であり、本邦でHER2陽性乳癌に対し保険承認されている。胃癌に関しては、2009年の米国臨床腫瘍学会（ASCO）にて、HER2陽性胃癌に対する無作為化比較試験（ToGA trial）において、Trastuzumab の5-FUまたはCapecitabine + Cisplatin（CDDP)への上乗せ効果(Median Overall survival: 13.8ヶ月 vs 11.1ヶ月、hazard ratio 0.74; 95% CI 0.60-0.91; p= 0.0046)が報告された[4]。胃癌のHER2陽性率は高分化腺癌で高い傾向がある[5]が、今回、HER2陽性のスキルス胃癌症例に対してTrastuzumab併用化学療法で完全奏効（CR）が得られた1例を経験したため報告する。

Ⅰ．症例

【患者】62歳、男性
【主訴】心窩部痛
【既往歴】高血圧
【現病歴】高血圧にて近医通院中、2006年3月から心窩部痛あり、上部消化管内視鏡、腹部エコーが施行され、胃癌、肝転移が疑われたため、2006年4月当院紹介受診となった。
【現症】身長159cm、体重61kg、performance status（ECOG)=1
【主な検査所見】
白血球8660/μL、赤血球394万/μL、Hb 12.9g/dL、Ht 36.8%、血小板35.9万/μL、CRP 0.26mg/dL、総蛋白7.2g/dL、ALB 4.2g/dl、ALT 53IU/L、BUN 23.0mg/dL、Cre 0.97mg/dL、Na 140mEq/L、K 3.8mEq/L、CEA 101.4ng/mL、CA19-9 468.9U/mL
【臨床経過】
精査の結果、スキルス（4型）胃癌（図1, 2）、多発肝転移・リンパ節転移（StageⅣ: cT3N3M1）と診断し、化学療法の方針となった。原発巣からの生検病理組織は、低分化腺癌主体（por2 ＞ tub2：図3）であったものの、HER2を検索したところ強陽性（免疫組織化学染色（IHC）3+、FISH比11.74）と判明した。 ToGA試験［Capecitabine（または5-FU）+CDDP±Trastuzumab療法］参加を希望され、適格であったため登録となった。2006年5月からCapecitabine 1000mg/m^2 day1-14、CDDP 80mg/m2 day1、Trastuzumab 負荷用量8mg/kg day1、維持量6mg/kgを3週間1サイクルとして開始した（Capecitabine、CDDPは6サイクルまで）。有害事象については、1サイクル中に、食欲不振、嘔気嘔吐grade 2が出現し、一時Capecitabineを休薬した。2サイクル開始時には好中球1350/μLと低下していたため、規定に従い、Capecitabine、CDDPを75%へ減量した。2サイクル後のCTにて肝転移巣は著明に縮小、腹部リンパ節は不明瞭化し、部分奏効（PR）と判定し、治療を継続した。その後は特に問題となる有害事象は認めず、10サイクル後のCTにて、CRと判定、2ヶ月後のCTでCR確定となった（図4）。その後も治療を継続したが、23サイクル目直前のCTで多発腹膜播種を認め、試験治療を中止した（治療開始から15ヶ月）。Paclitaxelや5FU/Methotrexateによる後治療を施行したが、2008年9月、原病の悪化により永眠された（初回治療から2年4ヶ月）。

Ⅱ．考察

本例は、画像所見上4型を呈し、組織学的にもdiffuse typeが主体の胃癌であったが、HER2陽性であり、Trastuzumab併用療法によるclinical benefitが得られることを示唆する症例である。

ToGA試験の結果では、奏効率はTrastuzumab併用群と非併用群ではそれぞれ47%と35%であり、Trastuzumab併用群では有意に奏効率が高かった（p=0.0017）。CR率

も、組織型は明らかにされていないが、それぞれ16例（5%）と7例（2%）であり、有意差は認めないものの、Trastuzumab併用群に高い傾向があった（p=0.0599）。

また、Lauren分類[6]におけるHER2陽性率はintestinal typeがdiffuseもしくはmixed typeと比べ、高いと報告されている（32.2% vs 6.1%／20.4%; p<0.001）[5]。また、Overall survival（OS）のサブグループ解析では、diffuse typeではTrastuzumabの併用によるbenefitが見られなかったと報告されている[4]。一方、探索的解析ではあるが、IHC2+/FISH＋またはIHC3＋群では、OS中央値はTarastuzumab併用群で16.0ヶ月、非併用群では11.8ヶ月（hazard ratio 0.65, 95%CI 0.51-0.83）と更に延長が認められている[4]。

つまり、ToGA試験全体の結果からは、画像所見上、diffuse typeの可能性が高いスキルス胃癌においては「Trastuzumab併用によるbenefitは低い」と判断され、今後の日常診療においてHER2検索の意義も疑問視される恐れがある。しかし、本例のように画像所見上4型を呈し、病理学的にもdiffuse type主体の症例についてもTrastuzumab併用療法が考慮されるべきであり、今後Trastuzumabを進行胃癌に使用していくにあたり、HER2は画像や病理所見を問わず、検索する意義があると考える。

F．研究発表

1．論文発表：未

2．学会発表：胃癌学会

G．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

（別紙３）

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書
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

研究分担者　　奥野　達哉　神戸大学消化器内科　特定助教

研究要旨　抗がん剤血中濃度、患者遺伝子解析による消化器癌の化学療法に対する治療効果、並びに有害事象出現予測因子の解明

A. 研究目的

近年、消化器癌に対する化学療法（放射線化学療法）の有効性が次第に明らかになってきており、食道癌では手術と同等の成績が示されるようになり、また他の消化器癌でも延命やQOLの向上に寄与できることが明らかになってきている。しかし、化学療法（放射線化学療法）の普及とともに問題点も浮上しており、一つは有害事象の出現予測が困難であること、もう一つは治療効果の個人差が大きいことである。すなわち、同じ容量の薬剤使用、同じ病期であっても有害事象の程度、治療効果は個人差が非常に大きいことが判明している。しかしながら、副作用、治療効果の症例ごとの差異が一体何に起因するのかは未だに解明されておらず、治療前にそれを予測することは困難である。そこで我々は、遺伝子多型、癌組織における癌関連遺伝子の発現や、治療中の患者個々抗がん剤血中濃度と有害事象、治療効果との関連性について検討することとした。

B. 研究方法

説明者が説明文書にのっとって本研究内容を説明し、対象患者がこの内容を充分に理解し、かつ「研究協力への同意書」に自署することにより研究協力への同意を表明した場合、約 5ml の血液を通常の静脈採血法で採取する。また消化管内視鏡検査時に生検鉗子により癌組織を約 1mm 径を 1~2 カ所採取する。提供された試料は共同研究機関である神戸大学薬剤部に提供され、薬物血中濃度測定、血液と生検組織からの DNA、RNA 抽出を行う。消化器癌に対する（放射線）化学療法における有害事象、治療効果と関連の可能性のある遺伝子型を網羅的に解析し、治療効果や副作用と関連した遺伝子型の同定を行う。

（倫理面への配慮）

本研究は、厚生科学審議会が平成１２年に定めた「遺伝子解析研究に付随する倫理的問題等に対応するための指針」並びに平成１３年３月２９日に文部科学省、厚生労働省、経済産業省の３省庁合同で作成された「ヒトゲノム・遺伝子解析研究に関する倫理指針」に準じて、ヒトゲノム・遺伝子解析研究の特殊性を十分考慮し作成され、当院の倫理委員会も承認している。

C. 研究結果

以前、我々は、食道癌Stage II/III患者におけるFP-RT療法の治療効果において、CR（腫瘍消失）率からみればTS 遺伝子の 5`-TSERが3Rである群、3`-TSUTR に6bpの挿入がある群、GSTP1-105位がValである群においてCR率が高く、予後因子である（Favourable genotype）可能性を認めた為、3遺伝子型のうち2個以上を持つ患者群と、1個以下しか持たない群の生存曲線比較を行った(combined analysis)結果、Stage II/III食道癌患者の予後を推定でき得る可能性を見出した（P=0.0197）為、Favourable genotypeを複数伴う事が生存予後に関して重要であるとの論文報告を行った（Am J Clin Oncol. 2007;30(3):252-7)。今回我々は食道癌放射線化学療法患者において、

TNFRSF1B 遺伝子の A1466G 遺伝子型に着目し 1466 A を持つ患者群では、CR を得る可能性が高い事を見出した(p = 0.040)。しかし、追加検討した M196R/T587G または C1493T 遺伝子型では治療効果予測は行い得なかった（J Exp Clin Cancer Res. 2010 Jul 20;29:100.）。

D. 考察

TNF-α は炎症にかかわる重要なサイトカインであるが、二つの受容体を有する。このうち TNFR2 をコードする、NFRSF1B 遺伝子は第 1 番染色体短腕に位置し、以前から複数の多型が報告されている。この分野については消化器領域においては炎症性腸疾患等の分野で、その発現量分析や遺伝子型との関係が報告されてきたが、最近は癌領域においても文献的報告がなされてきた。今回我々は 3' 非翻訳領域の 1466 A/G に注目し解析を行い、A を持つ患者群が G のみの患者群に比し優位に CR を得、治療効果も高い傾向があることを見出した。

E. 結論

食道癌患者における FP-RT 療法の治療効果において TS 遺伝子型や、GSTP 遺伝子型が、また、今回の検討では TNFRSF1B3' 非翻訳領域の 1466 A/G に注目し解析を行い、1466 A を持つ患者群が優位に CR を得、治療効果も高いことを見出した。今後、この分野の研究が食道癌化学放射線療法患者だけでなく進行消化器癌患者に対する治療成績向上の一助とするため、今後も検討を重ねる所存である。

F. 健康危険情報

なし

G. 研究発表

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2．学会発表

なし

H. 知的財産権の出願・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

別紙4

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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Y. Horita, Y. Yamada, Y. Hirashima, K. Kato, T. Nakajima, Y. Shimada.	Effects of bevacizumab on plasma concentration of irinotecan and its metabolites in advanced colorectal cancer patients	Cancer Chemother Pharmacol	65	467-471	2010

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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 (**1IB*) 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.

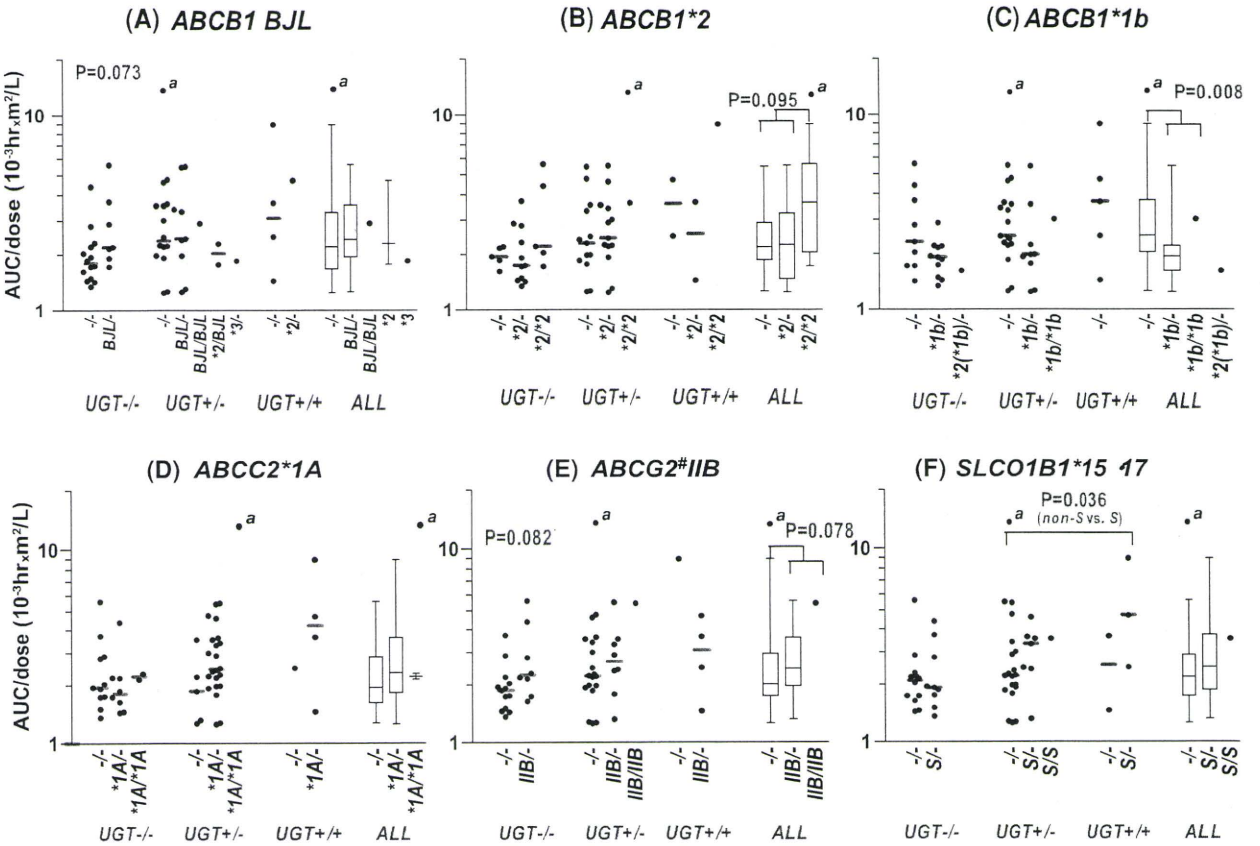

Fig. 1 Effects of transporter genotypes on SN-38 AUC/dose in irinotecan monotherapy ($N = 54$). *a* Excluded from statistical analysis. The *bars* represent the medians. *UGT+* = *UGT1A1*6* or **28*. **a** *BJL* contains −1789G>A, *2 (block 1) = 325G>A (E109K), **3* (block 1) = 304G>A (G102R); **b** *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

Table 3 Minor genetic variations detected in non-*UGT*+/+ patients who experienced grade 4 neutropenia

ID	Gene	Genetic variation	
		Nucleotide change (amino acid substitution)	Haplotype[a]
b1	*ABCB1*	304G>C (G102R)	*Block 1 *3*
b2(B)[b]		1804G>A (D602N)	*Block 2 *12*
b3(B)[b]		1342G>A (E448K)	*Block 2 *14*
b4		3043A>G (T1015A)	*Block 2 *16*
b5		3751G>A (V1251I)	*Block 3 *2*
c1	*ABCC2*	1177C>T (R393W)	*7
g1	*ABCG2*	376C>T (Q126X)	*Block 1 *4*
g2		1465T>C (F489L)	*Block 2 *2*
g3		1723C>T (R575X)	*Block 2 *5*
s1(S)[c]	*SLCO1B1*	1007C>G (P336R)	
s2		311T>A (M104K)	
u1	*UGT1A1*	−3279T>G, 1941C>G	*#60-#1B* (+/+)

[a] Defined in previous papers for *ABCB1* [26], *ABCC2* [27], *ABCG2* [28] and *UGT1A1* [35]

[b] Linked with *ABCB1*2* (*B*)

[c] Linked with *SLCO1B1*15 · 17* (*S*)

occurred in *non-UGT*+/+ patients at rates of 8.0% (4/50) in the irinotecan monotherapy and 20% (11/55) in the cisplatin-combination therapy, we investigated possible contributions of these minor transporter variations and another low-activity *UGT*-haplotype, *UGT1A1#60-#1B* [35], to severe neutropenia.

Among the rare variations detected, eleven heterozygous transporter genetic variations and one *UGT1A1#60-#1B* homozygote were found in *non-UGT*+/+ patients who experienced grade 4 neutropenia (Table 3). These variations include an amino acid substitution leading to reduced in vitro activity, *ABCG2* 1465T>C (F489L) [36], and the stop codons, *ABCG2* 376C>T (Q126X) and 1723C>T (R575X) [28].

Additive effects of transporter gene haplotypes on neutropenia

Since multiple transporters are involved in irinotecan PK/PD, severity of toxicity might depend on the number and combinations of the low-activity variants, each of which does not effectively affect PD. To examine this possibility, we surveyed relationships between ANC nadirs and combinations of haplotypes associated with grade 3/4 neutropenia ($P < 0.1$) and the minor variations associated with grade 4 neutropenia (listed in the previous section); the data for selected haplotypes/variations are depicted in Fig. 2. For the combination therapy with cisplatin (Fig. 2b), homozygous *SLCO1B1*15 · 17* was included, but *ABCC2*1A* was excluded since its effect in the cisplatin-combination therapy was not consistent among the *UGT* genotypes.

In the irinotecan monotherapy, ANC nadirs in most patients with either one or more of *ABCG2#IIB*, *SLCO1B1*15 · 17* and the minor variations were lower than the median ANC nadirs of both *UGT*−/− and *UGT*+/− patients without them (None) (Fig. 2a). In particular, the effects were more evident in patients bearing two or more of the selected haplotypes/variations (including the *UGT*+). Among the patients who experienced grade 3 or 4 neutropenia, 80% of patients had two or more candidate haplotypes/variations in the *UGT* (−/− and +/−) group (Fig. 2a).

In *UGT*+/− patients with the cisplatin-combination therapy, ANC nadirs of the patients with *ABCB1*2/*2*, *ABCG2#IIB/#IIB*, *SLCO1B1*15 · 17/*15 · 17* or any minor variations, and their combinations were lower than the median values of patients without these markers (None), except for one patient with *ABCB1*2/*2* and *SLCO1B1*15 · 17* (*B/B* + *S/*−) (Fig. 2b). Also, in *UGT*−/− and *UGT*+/− patients, the effects were more evident in the patients with two or more of the selected haplotypes/variations. Among the patients who experienced grade 4 neutropenia, 82% of patients had two or more candidate haplotypes/variations in the *UGT* (−/− and +/−) group (Fig. 2b).

It was noted that the additive effect of *g1* [*ABCG2* 376C>T (Q126X)] was not observed in the heterozygotes (*g1*/−), but was evident in the compound heterozygotes with another *ABCG2* genetic polymorphism, *#IIB*, (*G/g1*) (Fig. 2a, b).

Regarding the combined effects of the above transporter genotypes on SN-38 AUC values, higher levels were observed in patients with the candidate haplotypes/variations of two or more genes in the monotherapy, but this trend was not always evident in the cisplatin-combination therapy patients (data not shown).

Discussion

In this study, we showed possible additive effects of transporter and *UGT1A1* genotypes on irinotecan PK and PD. Since multiple transporters are involved in irinotecan PK, it is likely that a functional alteration of one of the responsible transporters can be compensated by other transporters; thus, changes in PK/PD parameters by transporter genotypes may not always be large. However, the overall elimination rate of irinotecan or its metabolites might be altered under the conditions of simultaneously reduced activities of multiple transporters, higher irinotecan doses, or reduced UGT activity.

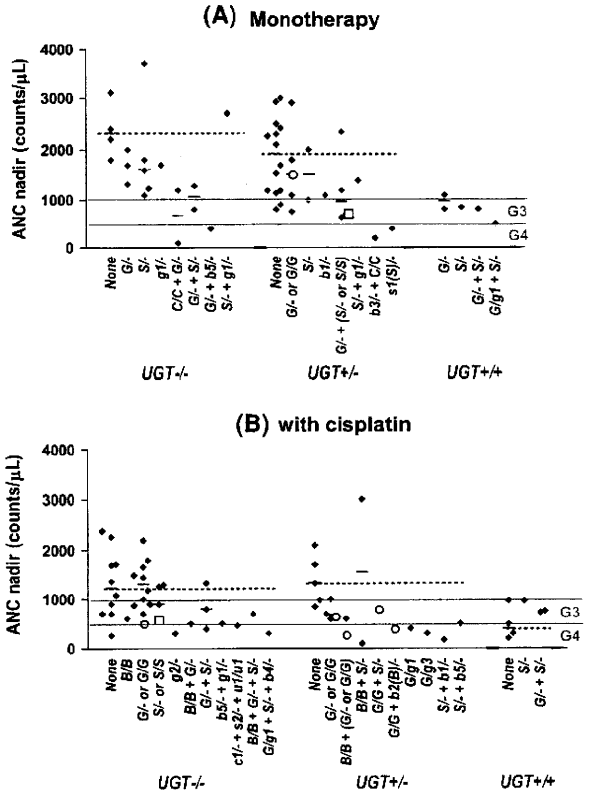

Fig. 2 Additive effects of transporter haplotypes/variations on ANC nadirs in irinotecan monotherapy (**a**) and combination therapy with cisplatin (**b**). *UGT+ = UGT1A1*6* or **28*; *B = ABCB1*2*; *C = ABCC2*1A*; *G = ABCG2#IIB* (*open circle*, *#IIB/#IIB*); *S = SLCO1B1*15 · 17* (*open square*, **15 · 17/*15 · 17*); *b1–u1* = minor variations listed in Table 3. **a** *None = non-(C, G, S* or *minors)*, **b** *None = non-(B, G, S* or *minors)*. The *bar* in each genotype represents the median. The *dotted lines* in each *UGT* genotype show the median values of patients without any selected transporter polymorphisms/variations (*None*). The *lines* (G3 and G4) represent the border of grade 3 and 4 neutropenia

In the irinotecan monotherapy, the increasing effect of *ABCB1*2/*2* (block 2) on SN-38 AUC/dose was evident while contributions of *ABCB1 BJL* (block 1), *ABCB1*1b* (block 3), *ABCG2#IIB* and *SLCO1B1*15 · 17* were not significant in the multivariate analysis. For neutropenia, additive effects were suggested for *ABCC2*1A/*1A*, *ABCG2#IIB*, *SLCO1B1*15 · 17*, and possibly some minor genetic variations in addition to *UGT1A1*6* or **28* (Fig. 2a). The association of *ABCB1*2* (block 2) with grade 3 diarrhea was also observed.

In the combination therapy with cisplatin, an increase in the SN-38 AUC/dose by *ABCB1*2* and for a decrease by *ABCB1*1b* were observed, but the multivariate analysis did not show their significant contributions. Regarding neutropenia, additive effects of *ABCB1*2/*2*, *ABCG2#IIB/#IIB*, and possibly, *SLCO1B1*15 · 17/*15 · 17* and some minor variations were suggested (Fig. 2b).

Thus, in both regimens, the associations of *ABCB1*2* (block 2) with higher SN-38 AUC/dose levels and toxicities (diarrhea or neutropenia), and additive effects of *ABCG2#IIB* and *SLCO1B1*15 · 17* with *UGT1A1*6* or **28* on neutropenia were observed. The current study also suggests that combination genotypes with two or more genes could have a greater effect on neutrophil count reduction than a single gene, indicating a quantitative property of multiple genetic factors affecting phenotype. These findings could partly explain a large interindividual variation in irinotecan toxicities within each *UGT* genotype.

In this study, influences of the transporter genotypes on SN-38 AUC/dose did not always correlate to an influence on neutropenia as observed in the combination therapy with cisplatin and in the case of *ABCB1*2* (block 2) in the monotherapy. Although weak negative correlations were observed between the SN-38 AUC level and ANC nadir, the SN-38 AUC values of patients who exhibited grade 3/4 neutropenia (ANC nadir < 1,000 counts/μL) were fairly diverse, especially in the combination therapy with cisplatin (Fig. 3). It is likely that the extent of toxicities depends not only on systemic exposure levels of the active metabolite for which hepatic UGT activity is a large contributor, but also on the elimination from the target cells (neutrophil progenitor cells or enterocytes) where transporter function might be more critical.

Our previous study showed the association of *ABCB1* block 2 **2* [1236C>T, 2677G>T (A893S) and 3435C>T] with lower renal clearance of irinotecan and its metabolites [16]. The current data obtained in the irinotecan monotherapy also suggest higher AUC/dose for irinotecan, SN-38G, and SN-38 with *ABCB1*2/*2*. Since a high affinity of P-gp for irinotecan is known, lower elimination rate of irinotecan could also result in higher plasma levels of its metabolites. Other studies have also suggested associations of the haplotype 1236T–2677T (corresponding to our **2* group in this study) with a reduced excretion rate of P-gp substrates [37] and SN-38 [25], and associations of the haplotype 2677T–3435T (corresponding to our **2* group in this study) with paclitaxel-induced neutropenia [38].

For *ABCC2*, *ABCC2* −1774delG, a tagging SNP of **1A*, was reported to be associated with low promoter activity and cholestatic or mixed-type hepatitis [32]. Patients with *ABCC2*1A/*1A* together with *ABCB1*2/*2* or *ABCG2#IIB* showed higher values of SN-38 AUC (Fig. 1) and neutropenia in the monotherapy (Fig. 2a), but these trends were not evident in the *UGT−/−* patients treated with cisplatin-combination therapy (data not shown). Thus, the effects of *ABCC2* might be dependent on combinations with other genetic and non-genetic factors. Conflicting clinical outcomes of *ABCC2* 3972C>T, a marker of **1C/G*, were reported to cause higher AUC of irinotecan and its