厚生労働科学研究費補助金

がん臨床研究事業

切除不能胆道がんに対する治療法の確立に関する研究

平成１９年度　総括研究報告書

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目次

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主任研究者　奥坂　拓志　国立がんセンター中央病院　医長

研究要旨：本研究では切除不能胆道がんの予後の改善を目指し、新規抗がん剤であるS-1を用いた化学療法の有用性を、ゲムシタビンを用いた化学療法とのランダム化比較試験にておいて検証することを目的とする。最初にS-1単独療法とS-1とゲムシタビンの併用療法とのランダム化第Ⅱ相試験を実施し、より有用性が期待できるレジメンを慎重に選択することとし、本年度はこのランダム化第Ⅱ相試験をJCOG試験として実施するための作業が進められた。平成19年7月にJCOGプロトコールレビュー審査会において承認、9月にJCOG運営委員会において承認を得た。現在、本研究事務局とJCOGデータセンターにおいて最終プロトコールの策定にむけ作業が進められている。

A．研究目的

切除不能胆道がん患者の予後はきわめて不良であり、その生存期間を向上するためには新しい有効な治療法の確立が必要であり、全国規模の比較試験が必須である。切除不能胆道がんに対して延命効果を証明した標準的な化学療法は確立していないが、現在国内外において、ゲムシタビン単独療法を対照群としたランダム化比較試験が進行中あるいは計画中であり、ゲムシタビン単独療法は事実上の標準治療法として位置づけられている。これらのランダム化比較試験のうち現在最も注目されているのが、ゲムシタビン単独療法とゲムシタビンとシスプラチンの併用療法との比較で、英国では第Ⅲ相試験が、本邦ではランダム化第Ⅱ相試験が進行中である。これらの試験の結果によっては、ゲムシタビンとシスプラチンの併用療法が標準治療となる可能性がある。一方、S-1は本邦で開発された新しい抗がん剤であり、切除不能胆道がんに対しても第Ⅱ相試験において良好な成績が示され適応拡大が申請され、平成19年8月に承認された。本研究では、最初にS-1単独療法とS-1とゲムシタビンの併用療法とのランダム化第Ⅱ相試験を実施し、より有用性が期待できるレジメンを慎重に選択し、続いて英国での第Ⅲ相試験後に明らかとなる標準治療法との第Ⅲ相試験を実施し、切除不能胆道がんに対する標準治療法を確立する。

S-1は切除不能胆道がんに対する2次治療薬としての期待も大きく、その有効性と安全性を明らかにするため、「ゲムシタビン耐性胆道がんにおけるS-1の臨床第Ⅱ相試験」を不随研究として行う。

B．研究方法

本研究は以下の２段階で行う。

1）S-1単独療法とS-1とゲムシタビンの併用療法とのランダム化第Ⅱ相試験

2) 英国での第Ⅲ相試験後に明らかとなる標準治療法と、1) のランダム化第Ⅱ相試験で選択される治療法とのランダム化第Ⅲ相試験

■　S-1単独療法vs. S-1とゲムシタビンの併用療法とのランダム化第Ⅱ相試験
［研究形式］多施設共同のランダム化第Ⅱ相試験、プライマリーエンドポイントは1年生存割合。
［対象症例］、切除不能胆道がんの未治療例、PS 0または1、骨髄・肝・腎などの主要臓器機能が保持され、十分な説明後に本人より文書で同意の得られた症例。
［症例の登録］JCOGデータセンターによる中央登録方式とする。
［治療内容］S-1単独療法群ではS-1をday 1-28に連日経口投与する。これを６週毎に原疾患の悪化または毒性のため中止するまで継続する。S-1とゲムシタビンの併用療法群ではゲムシタビンをday 1，8に静注投与し、S-1はday 1-14に連日経口投与する。これを３週毎に原疾患の悪化または毒性のため中止するまで継続する。
［予定症例数］症例数90例前後、症例集積期間１.5年程度となる予定。

■　英国での第Ⅲ相試験後に明らかとなる標準治療法vs. 上記ランダム化第Ⅱ相試験で選択される治療法とのランダム化第Ⅲ相試験
［研究形式］多施設共同のランダム化第III相試験、プライマリーエンドポイントは全生存期間。
［対象症例］切除不能胆道がんの未治療例、PS 0または1、骨髄・肝・腎などの主要臓器機能が保持され、十分な説明後に本人より文書で同意の得られた症例。
［症例の登録とランダム割付］JCOGデータセンターによる中央登録方式とする。電話またはFAXにてデータセンターへの症例登録を行い、適格性の確認後、治療群の割付を受ける。
［治療内容］症例登録時のランダム割付に従い、標準治療または試験治療のいずれかの治療を実施する。治療は原疾患の悪化または毒性のため中止するまで継続する。
［解析方法］安全性のモニタリングは年２回実施する。登録期間終了後、追跡を継続し、症例集積終了の１年後に最終解析を行う。
［予定症例数］予定症例数、症例集積期間は、現在進行中のランダム化第Ⅱ相試験、本研究で計画中のランダム化第Ⅱ相試験の成績を解析したのち設定する。
［実施施設］本研究の研究者の所属する施設を中心に全国20～30施設

■倫理面への配慮
本研究では、肝内胆管がん・肝外胆管がん・胆のうがん・乳頭部がんという臨床的特徴を異にする4つの疾患群を含み、胆管炎等の重篤な有害事象を併発しやすい癌腫である胆道がんを対象に、新しい抗がん剤（S-1）を用いた化学療法を実施することになるので、まずランダム化第II相試験を慎重に行い、有効性と安全性を十分に確認した上でランダム化第Ⅲ相試験を実施する。
また、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）は厚生労働省がん研究助成金指定研究5班（17指-1～5）を中心に、同計画研究班6班および厚生労働科学研究費がん臨床研究事業22研究班、計33班の任意の集合体であり、JCOG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

本研究班では、最初にS-1単独療法とS-1とゲムシタビンの併用療法とのランダム化第Ⅱ相試験を実施し、より有用性が期待できるレジメンを慎重に選択することとしたが、本年度はこのランダム化第Ⅱ相試験をJCOG試験として実施するための作業が進められた。本研究は平成19年3月に本省より承認を得たが、これに先立ち1月より本研究組織内で研究計画について議論を重ね、プロトコールコンセプトの作成作業を開始した。3月にJCOG医学審査委員による事前レビュー審査会にプロトコールコンセプトを提出。事前相談会での指摘事項をうけ、コンセプトを修正、5月に本研究組織内での合意を得たのち、7月にJCOGプロトコールレビュー審査会において承認、9月にJCOG運営委員会において承認を得た。現在、本研究事務局とJCOGデータセンターにおいて最終プロトコールの策定にむけ作業が進められている。

S-1は切除不能胆道がんに対する2次治療薬としての期待も大きく、その有効性と安全性を明らかにすることを目的として本研究班での付随研究として「ゲムシタビン耐性胆道がんに対するS-1の第Ⅱ相試験」を計画し、平成19年3月より登録を開始した。登録は順調に進められており、平成20年1月に40例中20例の登録が得られ、中間解析のため登録を一時休止した。

D．考察

我が国における胆道がん死亡数は増加傾向にあり、悪性腫瘍死亡数の第６位となっている。切除不能胆道がんに対して延命効果を証明した標準的な化学療法は確立していないが、現在国内外において、ゲムシタビン単独療法を対照群としたランダム化比較試験が進行中あるいは計画中であり、ゲムシタビン単独療法は事実上の標準治療法として位置づけられている。しかしその治療成績は生存期間中央値がわずかに8～9か月程度ときわめて不良であり、より有効な治療法の開発が切望されている。最近、本邦で開発された経口抗がん剤であるS-1が切除不能胆道がんに対し優れた抗腫瘍効果を示すことが明らかにされ、2007年8月に胆道がんへの適応拡大が承認された。

日本は国際的に見て胆道がん患者が比較的多く、治療開発を積極的に行い、世界を牽引していく必要がある。また、日本で開発された薬剤であるS-1は胆道がんに対しても大いに期待されている薬剤であり、S－１を含む治療レジメンを評価することを目的とした本研究は国際的にも非常に重要であると考えられる。

E．結論

切除不能胆道がんの予後の改善を目指し、新規抗がん剤であるS-1を用いた化学療法の有用性を検討する研究が進められており、まもなくS-1単独療法とS-1とゲムシタビンの併用療法とのランダム化第Ⅱ相試験が登録開始となる見込みである。

F．健康危険情報

なし。

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H. 知的財産権の出願・登録状況

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

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ORIGINAL REPORT

Pharmacokinetics of Gemcitabine in Japanese Cancer Patients: The Impact of a Cytidine Deaminase Polymorphism

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ABSTRACT

Purpose
Gemcitabine is rapidly metabolized to its inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU), by cytidine deaminase (CDA). We previously reported that a patient with homozygous 208A alleles of *CDA* showed severe adverse reactions with an increase in gemcitabine plasma level. This study extended the investigation of the effects of *CDA* genetic polymorphisms on gemcitabine pharmacokinetics and toxicities.

Patients and Methods
Genotyping of *CDA* was performed by a direct sequencing of DNA obtained from the peripheral blood of Japanese gemcitabine-naïve cancer patients (n = 256). The patients recruited to the association study received a 30-minute intravenous infusion of gemcitabine at a dose of either 800 or 1,000 mg/m^2, and eight blood samples were periodically collected (n = 250). Plasma levels of gemcitabine and dFdU were measured by high-performance liquid chromatography. Plasma CDA activities toward cytidine and gemcitabine were also measured (n = 121).

Results
Twenty-six genetic variations, including 14 novel ones and two known nonsynonymous single nucleotide polymorphisms (SNPs), were detected. Haplotypes harboring the nonsynonymous SNPs 79A>C (Lys27Gln) and 208G>A (Ala70Thr) were designated **2* and **3*, respectively. The allelic frequencies of the two SNPs were 0.207 and 0.037, respectively. Pharmacokinetic parameters of gemcitabine and plasma CDA activities significantly depended on the number of haplotype **3*. Haplotype **3* was also associated with increased incidences of grade 3 or higher neutropenia in the patients who were coadministered fluorouracil, cisplatin, or carboplatin. Haplotype **2* showed no significant effect on gemcitabine pharmacokinetics.

Conclusion
Haplotype **3* harboring a nonsynonymous SNP, 208G>A (Ala70Thr), decreased clearance of gemcitabine, and increased incidences of neutropenia when patients were coadministered platinum-containing drugs or fluorouracil.

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INTRODUCTION

Gemcitabine (2′,2′-difluorodeoxycytidine) is a nucleoside anticancer drug that has a broad spectrum of antitumor activity against various solid tumors, such as non–small-cell lung cancer and pancreatic cancer.[1] In a randomized clinical trial, gemcitabine was confirmed to provide a survival advantage over fluorouracil in addition to symptom-relieving benefits in patients with advanced pancreatic cancer.[2] On the basis of these results, gemcitabine has generally been accepted as a standard chemotherapeutic agent for advanced pancreatic cancer.

Gemcitabine is transported into cells by concentrative and equilibrative nucleoside transporters,[3-8] where it is phosphorylated to its monophosphate form by deoxycytidine kinase. Gemcitabine triphosphate, an active form of gemcitabine, is incorporated into an elongating DNA strand, and is followed by the addition of another deoxynucleotide that leads to the halt of DNA synthesis.[9,10] Another mode of action in solid tumors, associated with the inhibition of ribonucleotide reductase, has also been suggested.[11]

Gemcitabine is rapidly metabolized to an inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU)

Table 1. *CDA* Haplotypes Estimated in This Study

Region		5'-Flanking			Exon 1 (5'-UTR)			Exon 1	Intron 1	Exon 2		Intron 2		
SNP ID		CDA001	CDA002	CDA003	CDA004	CDA005	CDA007	CDA009	CDA010	CDA011	CDA012	CDA014	CDA016	CDA017
Nucleotide change		−451C>T	−205C>G	−182G>A	−116G>A	−92A>G	−33_−31 delC	79A>C	IVSI+37 G>A	208G>A	210T>C	IVS2 +87_+88 insTCAT	IVS2+242 A>G	IVS2+296 T>A
Amino acid change								Lys27Gln		Ala70Thr	Ala70Ala			
Haplotypes														
*1	*1a													
	*1b													
	*1c													
	*1d													
	*1e													
	*1f													
	*1g													
	*1h													
	*1i													
	*1j													
	*1k													
	*1l													
	*1m													
	*1n													
	Other *1													
*2	*2a													
	*2b													
	*2c													
	*2d													
	Other *2													
*3	*3a													
	*3b													

(continued on next page)

NOTE. The haplotypes were described as a number plus a small alphabetical letter. Four single nucleotide polymorphisms (SNPs) (CDA006, 008, 013, 015) were found only in the very rare ambiguous *1 haplotypes. Since these ambiguous haplotypes were grouped and described as "Other *1" in this table, the four SNPs are not shown in the row of nuclotide change. White, major allele; gray, minor allele.

by cytidine deaminase (CDA),[9] and most of an administered dose is recovered as dFdU in the urine.[12] CDA is expressed at varying levels in the human tissues,[13] and the rapid clearance of gemcitabine can be attributed to its plentiful occurrence in the liver.[14] Two single nucleotide polymorphisms (SNPs), 79A>C (Lys27Gln) and 435T>C (Thr145Thr), have been discovered in *CDA*, the CDA-encoding gene in humans.[15,16] The 79A>C SNP reportedly reduces the deamination activity (maximum velocity/Km) toward 1-beta-D-arabinofuranosyl cytosine (cytarabine),[15] and increases Km toward gemcitabine,[17] in vitro. A recently discovered third SNP, 208G>A (Ala70Thr) displayed a decrease in deamination activity of 60% for cytidine and 68% for cytarabine when introduced into a *CDA*-null yeast strain.[18]

Toxicity of gemcitabine is generally mild,[19,20] but unpredictable severe toxicities such as myelosuppression are occasionally experienced.[21,22] Our previous case report described a patient with homozygous 208A alleles of the *CDA* gene who showed severe adverse reactions with increased plasma gemcitabine levels.[23] In addition, there has been controversy over the relationship between cellular CDA activity and the clinical effects of cytarabine.[24-27] This study examined the relationship between *CDA* polymorphisms, and the pharmacokinetics of gemcitabine, plasma CDA activity, or adverse reactions in Japanese cancer patients.

PATIENTS AND METHODS

Gemcitabine and dFdU for analytic standards were supplied by Eli Lilly Japan K.K. (Kobe, Japan). Tetrahydrouridine, 3'-deoxy-3'-fluoro-thymidine (3'-dFT), cytidine and uridine (Sigma-Aldrich Chemical Co, St Louis, MO) were purchased. All other chemicals were of highest grade available.

Patients

The participants in this study consisted of 256 Japanese patients with carcinoma, including six patients described in a previous report,[23] at the National Cancer Center Hospital (Tokyo, Japan) and National Cancer Center Hospital East (Kashiwa, Japan). Two hundred fifty-one patients received a 30-minute intravenous infusion of gemcitabine at a dose of either 800 or 1,000 mg/m^2, and five patients received a fixed dose-rate (10 mg/m^2/min) infusion at a dose between 1,000 and 1,500 mg/m^2. The eligibility criteria for the study were as previously reported.[23] The ethics committees of the National Cancer Center and the National Institutes of Health Sciences approved this study. Written informed consent was obtained from each participant.

Table 1. *CDA* Haplotypes Estimated in This Study (continued)

Intron 3					Exon 4	Exon 4 (3'-UTR)					
CDA018	CDA019	CDA020	CDA021	CDA022	CDA023	CDA024	CDA025	CDA026	No.	Frequency	
IVS3+71 T>C	IVS3 −194_−193 insAlu	IVS3-56 G>A	IVS3-36 G>A	IVS3-23 C>T	435C>T	510 (*69) G>T	637_638 (*196_*197) insC	676 (*235) A>G			
					Thr145Thr						
									175	0.342	
									63	0.123	
									52	0.102	
									17	0.033	
									13	0.025	
									12	0.023	
									12	0.023	
									11	0.021	0.756
									8	0.016	
									5	0.010	
									4	0.008	
									4	0.008	
									2	0.004	
									1	0.002	
									8	0.016	
									84	0.164	
									11	0.021	
									5	0.010	0.207
									3	0.006	
									3	0.006	
									18	0.035	0.037
									1	0.002	
									512	1.000	1.000

Monitoring and Toxicities

A complete medical history and data on physical examinations were recorded before the gemcitabine therapy. CBC and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of gemcitabine treatment. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 2.

DNA Sequencing

All four exons and the 5'-upstream region (approximately 800 base pairs [bp] from the translation initiation codon) of *CDA* were amplified from 100 ng of DNA extracted from peripheral blood, and sequenced along both strands. Polymerase chain reaction (PCR) primers[23] and sequencing and PCR conditions[28] were described previously. For detection of an approximately 300-bp Alu insertion (IVS3-194_-193insAlu), PCR was performed using a specific primer set (5'- TTGTCATAGCAGAAGGAGGTT-3' and 5'- TCAG CTCTCCACACCATAAGG-3') and 100 ng of DNA as a template. Then, sizes of the amplified fragments were determined by 1% agarose gel electrophoresis. NT_004610.17 (GenBank, National Center for Biotechnology Information, Bethesda, MD) was used as the reference sequence.

Linkage Disequilibrium and Haplotype Analyses

Hardy-Weinberg equilibrium and linkage disequilibrium (LD) analyses were performed by SNPAlyze software (Dynacom Co, Yokohama, Japan). All of the detected variations were found to be in Hardy-Weinberg equilibrium ($P \geq .05$), except for the SNP IVS1+37G>A ($P = .002$). Some of the haplotypes were unambiguously assigned from subjects with homozygous variations at all sites or a heterozygous variation at only one site. The diplotype configurations (a combination of haplotypes) were separately inferred by LDSUPPORT software,[29] which determines the posterior probability distribution of the diplotype configuration for each subject based on the estimated haplotype frequencies. The diplotype configurations of all but 11 subjects were inferred with probability of more than 0.93. All haplotypes inferred in single subjects were gathered as the groups "Other *1" and "Other *2" in Table 1.

Pharmacokinetic Study

Five patients with fixed dose-rate infusion and one patient with interruption of infusion for more than 15 minutes were excluded from the pharmacokinetic analysis described herein. Heparinized blood was collected before administration of gemcitabine and used to measure plasma CDA activity. Five milliliters of heparinized blood was also sampled for pharmacokinetic analysis before the first gemcitabine administration, and at 0, 15, 30, 60, 90, 120, and 240 minutes after the termination of the infusion. Fifty microliters of 1% tetrahydrouridine was immediately added to these samples to prevent ex vivo deamination. Plasma levels of gemcitabine and dFdU were determined using the high-performance liquid chromatography method previously reported.[23] The area under the curve (AUC) and mean residence time from 0 to infinity, peak concentration (C_{max}), clearance (CL/m^2) and distribution volume based on the terminal phase (Vz/m^2) were calculated using WINNonlin (Scientific Consultant, Apex, NC) version 4.01 (Pharsight Corporation, Mountain View,

CA). AUC and C_{max} were corrected for dose, assuming that all patients received 1,000 mg/m^2 of gemcitabine.

CDA Activities in Plasma

Determination of CDA activities was performed using the method by Richards et al[30] with slight modifications (modifications are as follows: gemcitabine was used as a substrate as well as cytidine, internal standards for analysis [3'-dFT for gemcitabine or dFdU for cytidine] were added to the mixtures at the beginning of the reaction, and high-performance liquid chromatography was used for detection of reaction products). CDA activity was expressed by unit, and one unit of enzyme activity was defined as the concentration that produces 0.1 nmol of dFdU or uridine per minute per milliliter of plasma.[30]

Statistical Analysis

Kruskal-Wallis, Mann-Whitney, and Pearson's correlation tests were performed using the JMP software (SAS Institute Inc, Cary, NC). Two ordinally scaled categoric data were subjected to χ^2 analysis for a correlation test. A significance level of .05 was applied to all two-tailed and correlation tests. Multiplicity was adjusted by the false-discovery rate,[31] if necessary.

RESULTS

Genetic Variations and Haplotype Structures of CDA

Twenty-six (14 novel) genetic variations were detected in the 256 Japanese cancer patients enrolled onto this study (Table 2). Three of the novel variations were found in the 5'-untranslated region, one in exon 2, three in the 3'-untranslated region and seven in the introns. Three known SNPs in the coding region of *CDA* were also detected. Among these, the nonsynonymous SNPs, 79A>C (Lys27Gln) and 208G>A (Ala70Thr), exhibited allelic frequencies of 0.207 and 0.037 (Table 2), respectively, and they were comparable to those reported previously.[18] One patient was found to be homozygous for the 208A polymorphism. A novel insertion of an approximately 320-bp Alu element (IVS3-194_-193insAlu) was newly found in intron 3.

The detected variations were used to analyze LD (Fig 1). Four novel variations (IVS3-56G>A, IVS3-36G>A, IVS3-23C>T and

Table 2. Variations of the *CDA* Gene Found

SNP ID				Position				
This Study	NCBI (dbSNP)	JSNP	Location	NT_004610.17	From the Translational Initiation Site or From the Nearest Exon	Nucleotide Change and Flanking Sequences (5' to 3')	Amino Acid Change	Allele Frequency
MPJ6_CDA001	rs532545	IMS-JST008767	5'-Flanking	3739514	−451‡	TGCCTCGTGGCT**C/T**GGGATGCCGCAG		0.199
MPJ6_CDA002	rs603412	IMS-JST008768	5'-Flanking	3739760	−205‡	CACACGTAGGCA**C/G**TGTCTTACACCA		0.266
MPJ6_CDA003	rs12726436		5'-Flanking	3739783	−182‡	CACACCTGCTGA**G/A**TCCAAACCATGG		0.061
MPJ6_CDA004*			Exon 1 (5'-UTR)	3739849	−116‡	CTGAGAGCCTGC**G/A**GTCTGGCTGCAG		0.059
MPJ6_CDA005	rs602950†		Exon 1 (5'-UTR)	3739873	−92‡	GGGACACACCCA**A/G**GGGGAGGAGCTG		0.205
MPJ6_CDA006*			Exon 1 (5'-UTR)	3739884	−81‡	AAGGGGAGGAGC**T/C**GCAATCGTGTCT		0.002
MPJ6_CDA007	rs3215400	IMS-JST076939	Exon 1 (5'-UTR)	3739934	−33_−31‡	GCTCCTGTTTCCC**/**GCTGCTCTGCTG		0.451
MPJ6_CDA008*			Exon 1 (5'-UTR)	3739957	−8‡	TGCCTGCCCGGG**G/A**TACCAACATGGC		0.002
MPJ6_CDA0091	rs2072671	IMS-JST008769	Exon 1	3740043	79†	CAGGAGGCCAAGA**/C**AGTCAGCCTACT	Lys27Gln	0.207
MPJ6_CDA010	rs12059454		Intron 1	3740155	IVS1+37	CCCAGCCCAGCA**G/A**CCTGGGTGGTGG		0.184
MPJ6_CDA0111			Exon 2	3755816	208†	GCTGAACGGACC**G/A**CTATCCAGAAGG	Ala70Thr	0.037
MPJ6_CDA012*			Exon 2	3755818	210‡	TGAACGGACCGC**T/C**ATCCAGAAGGCC	Ala70Ala	0.004
MPJ6_CDA013*			Intron 2	3755932	IVS2−58§	GCCAACATCTTCC**/**TTTACACATATTA		0.002
MPJ6_CDA014*			Intron 2	3755961_3755962	IVS2+87_+88	TCATTCATTCAT**-/TCAT**CTGACATATGTT		0.135
MPJ6_CDA015			Intron 2	3756043_3756044	IVS2+169_+170§	ATAAGGAGATAA**A/GT**AAGAAATGGAG		0.00244
MPJ6_CDA016	rs10916825		Intron 2	3756116	IVS2+242	CATACAAGGGCC**A/G**GTATGCCCCTGT		0.289
MPJ6_CDA017	rs818194		Intron 2	3756170	IVS2+296	GTCCTACAAGATT**/**ATAACAGAAAGGC		0.217
MPJ6_CDA018	rs3738130	IMS-JST083844	Intron 3	3764805	IVS3+71	AGCCACGCCAAG**T/C**TGCAGGCATGGC		0.053
MPJ6_CDA019*			Intron 3	3769093_3769094	IVS3-194_-193§	CTGTTCAGTTTC**-/(Alu)**§ACAGCATTCTTT		0.293
MPJ6_CDA020*			Intron 3	3769231	IVS3-56	CAGACCCAGTCC**G/A**TCTCAGCCCCCT		0.293
MPJ6_CDA021			Intron 3	3769257	IVS3-36	GCCCTCAGCCAC**G/A**CTGTGTCTCTCA		0.293
MPJ6_CDA022*			Intron 3	3769264	IVS3-23	CTGTGTCTCTCA**C/T**GCCAGCTTTGCC		0.293
MPJ6_CDA023	rs1784627		Exon 4	3769397	435†	CCTGCAGAAGACC**/T**CAGTGACAGCCA	Thr145Ile	0.293
MPJ6_CDA024*			Exon 4 (3'-UTR)	3769472	510 (*69)‡	CTCACAGCCCTG**G/T**GGACACCTGCCC		0.002
MPJ6_CDA025			Exon 4 (3'-UTR)	3769599_3769600	637_638 (§196_197)‡	ACCGCCGCCCCC**-/C**TGCCCCACCTTT		0.293
MPJ6_CDA026*			Exon 4 (3'-UTR)	3769638	676 (*235)‡	GGGCCCTCTTTC**A/G**AAGTCCAGCCTA		0.010

*Novel variations detected in this study.
†Yue et al.[18]
‡A of the translation initiation codon ATG is numbered 1, and the number with * in parentheses indicates the position from the termination codon TGA.
§The sequence of the Alu insertion was as follows: 5' - (T)nGAGACGGAGTCTCGCTGTCGCCCAGGCTGGAGTGCAGTGGCGCAATCTCGGCTCACTGCAGGCTCCG CCCCCTGGGGTTCACGCCATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGACTACAGGCGCCCGCCACCTCGCCCGGCTAATTTTTTGTATTTTTAGTAGAGACGGGGTT TCACCGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCGTGATCCGCCCGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCGCGCCCGGCCCACTG TTCAGTTTC-3' (n = approximately 25).

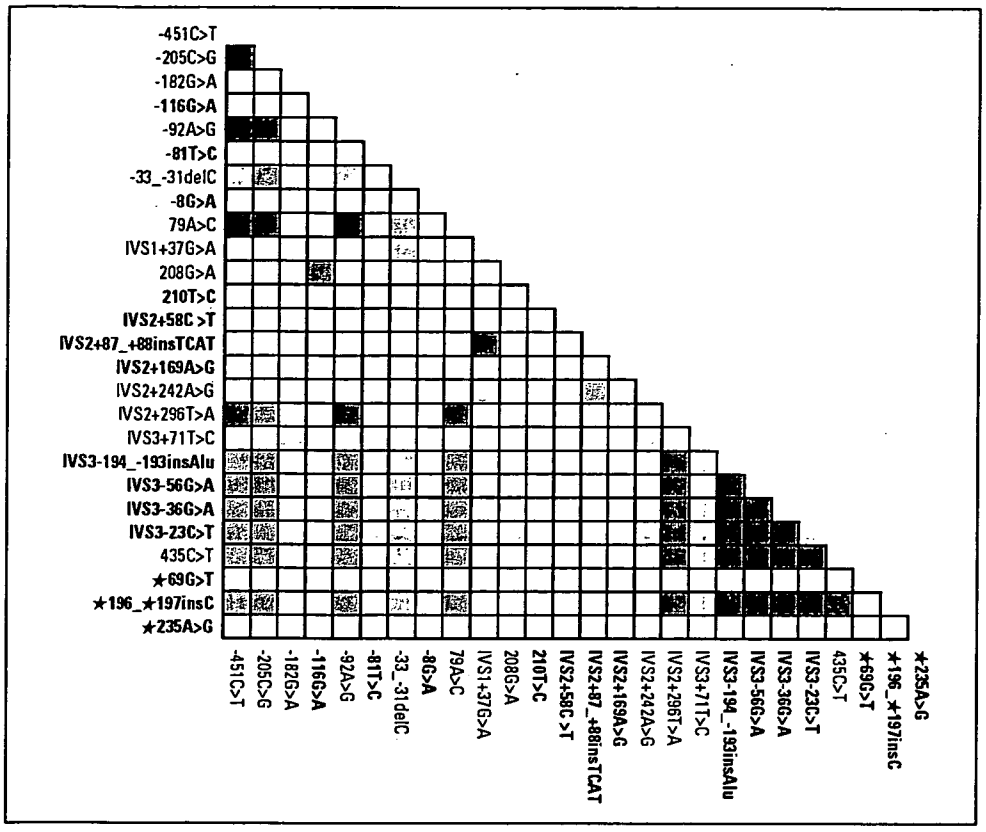

Fig 1. Linkage disequilibrium (LD) among 26 *CDA* variations. Pairwise LD as r^2 (from 0 to 1) is expressed as 10-graded blue color. The density of the blue color increases with higher linkage rates.

*196_*197insC), the Alu element insertion and a known SNP 435C>T (Thr145Thr) showed complete linkage (Fig 1) with a frequency of 0.293. Strong LD ($r^2 \geq 0.93$) was also observed among SNPs −451C>T, −92A>G, and 79A>C. Note that moderate linkages ($r^2 \geq 0.42$) were observed between the two completely and strongly linked groups (Fig 1). Because relatively close linkages were observed throughout the entire *CDA* gene spanning approximately 30 kb, the *CDA* haplotypes were analyzed as one LD block.

The haplotypes determined/inferred in this study are summarized in Table 1. Haplotypes without amino acid changes were defined as the **1* group. These harboring the nonsynonymous SNPs 79A>C and 208G>A were designated **2* and **3*, respectively. The most frequent haplotype was **1a* (frequency, 0.342), followed by **2a* (0.164), **1b* (0.123), and **1c* (0.102).

Effects of Patient Background Factors on Gemcitabine Pharmacokinetics

Characteristics of the 250 patients recruited for the pharmacokinetic study are shown in Table 3. As previously reported, the patient who was homozygous for 208A showed extraordinarily high gemcitabine and low dFdU plasma concentrations.[23] Therefore, this patient was excluded when effects of patient background factors on the pharmacokinetic parameters of gemcitabine were analyzed.

The effects of age and sex on pharmacokinetic parameters are summarized in Table 4. Vz/m^2 was significantly higher in males than in females, even after adjustments for their body surface areas (Mann-Whitney $P = .0031$). The C_{max}, AUC, CL/m^2, and Vz/m^2 of gemcitabine showed significant correlations with age ($P < .0001$ for all parameters). Values of any clinical tests, including creatinine concentration, did not correlate with pharmacokinetic parameters of gemcitabine. Although approximately 30% of patients in this study underwent combined chemotherapy, no clinically significant effects of coadministered drugs on pharmacokinetic parameter values of gemcitabine were detected.

Effects of CDA *Genetic Polymorphisms on Gemcitabine Pharmacokinetics*

Because age and sex were unbiasedly distributed among the patients, with the various genotypes compared in the following analysis (data not shown), the 250 patients were not further stratified.

After careful examination, the data did not identify any **1*, **2*, or **3* subtypes that showed statistically significant differences from each major subtype within the three groups (Table 5; unpublished data). Therefore, each subtype was combined into one group (the **1*, **2*, or **3* group) to investigate the association between pharmacokinetic parameters and genetic groups.

The relationships between the diplotype groups and the pharmacokinetic parameters of gemcitabine are shown in Figure 2 and summarized in Table 6. The data clearly showed a haplotype **3*–dependent decrease in clearance and increases in C_{max} and AUC values (χ^2 trend $P < .0001$ for all parameters). The values of C_{max}, AUC, and CL/m^2 observed in the patient bearing a homozygous 208G>A (**3/*3*) were two-fold, five-fold, and one-fifth of the means of the **1/*1* group, respectively (Table 6). In contrast, the pharmacokinetic parameters of gemcitabine except for mean residence time (data not shown) were not significantly influenced by the haplotype **2*.

Table 3. Characteristics of Patients Recruited to Pharmacokinetic Studies (N = 250)

Characteristic	
Sex	
Male	165
Female	85
Age, years	
Mean	62.6
Range	32-80
SD	9.2
Body surface area, m^2	
Mean	1.57
Range	1.18-1.99
SD	0.17
Weight, kg	
Mean	54.8
Range	34.4-80.3
SD	9.7
Performance status	
0	122
1	118
2	10
Primary tumor	
Pancreas	205
Lung	38
Mesothelium	7
Dose, mg/m^2	
1,000	246
800	4
Regimen	
Gemcitabine alone	180
Gemcitabine-based combination	70
Cisplatin	30
Carboplatin	16
Fluorouracil	14
Vinorelbine ditartrate	10
Previous treatment	
None	134
Surgery	66
Radiation	74
Chemotherapy	65

*Effect of Haplotypes *2 and *3 on Plasma CDA Activity*

Plasma CDA activities were measured in 121 patients of the 250 patients in this study. One patient in the **1/*2* group who showed extremely high plasma CDA activities to both gemcitabine and cytidine (43.04 and 29.04 units, respectively; far higher than the 99% upper confidence limits of plasma CDA activities for the **1/*2* group) was excluded as an outlier from the following statistical analysis, although his pharmacokinetic parameters were quite normal.

Haplotype **2* failed to show any significant effects on the plasma CDA activities toward both gemcitabine and cytidine. On the other hand, activity decreased depending on the number of haplotype **3* (Table 6; Fig 3). The plasma CDA activities in the homozygous **3* (208A) patient were 12% (gemcitabine) and 25% (cytidine) of the median activities for the **1/*1* patients. As shown in Figure 4, a statistically significant correlation between the plasma CDA activity toward gemcitabine and the AUC values of gemcitabine was observed ($r = -0.30$; $P = .0009$). However, the correlations were not remarkable.

*Effect of Haplotype *3 on Toxicities*

Then, associations of haplotype **3* with toxicities were analyzed. Nadir grades of neutrophil counts were compared between the patient groups with and without haplotype **3* under the individual therapeutic regimens. As shown in Table 7, there were no significant differences in incidences of grade 3 or higher neutropenia between the two groups under the gemcitabine monotherapy. However, when gemcitabine was administered with carboplatin, cisplatin, or fluorouracil, grade 3 or higher neutropenia was more frequently observed in the haplotype **3*–bearing group than in the group without haplotype **3*. The increases in incidences were statistically significant. AUC values were also increased in the group with haplotype **3* under concomitant therapeutic regimen as under the monotherapy.

DISCUSSION

The pharmacokinetic parameters summarized in Table 4 showed great similarity to those obtained with adult American patients.[32] The age-dependent decrease in gemcitabine clearance in Japanese patients in this study is in agreement with the description for Gemzar injections (Eli Lilly Japan K.K.), which is based on a population pharmacokinetic study performed outside Japan. The main route of gemcitabine elimination is its metabolism into dFdU, and there was no correlation between plasma creatinine level and gemcitabine clearance. Therefore, the aging effect on gemcitabine clearance is likely to result from a decrease in distribution volume or liver function. It is

Table 4. Effects of Patient Background Factors on Pharmacokinetic Parameters of Gemcitabine

Factor	C_{max} (μg/mL) Median	C_{max} (μg/mL) 1/4-3/4 Quantiles	AUC (hr · μg/mL) Median	AUC (hr · μg/mL) 1/4-3/4 Quantiles	CL/m^2 (L/hr/m^2) Median	CL/m^2 (L/hr/m^2) 1/4-3/4 Quantiles	Vz/m^2 (L/m^2) Median	Vz/m^2 (L/m^2) 1/4-3/4 Quantiles
Sex*								
Male	23.1	18.4-26.1	9.9	8.6-11.8	100.3	83.7-115.9	42.4	35.13-52.04
Female	24.0	19.8-28.8	10.2	9.0-11.5	97.6	86.1-111.2	38.7	32.7-43.5
Mann-Whitney *U* test	NS		NS		NS		$P < .005$	
Age								
Spearman r	0.32		0.39		−0.39		−0.39	
P value	< .0001		< .0001		< .0001		< .0001	

Abbreviations: C_{max}, peak concentration; AUC, area under the curve; CL/m^2, clearance; Vz/m^2, distribution volume based on the terminal phase.
*Significantly different from the value for female (Mann-Whitney *U* test $P = .0031$).

Table 5. Pharmacokinetic Parameters of Gemcitabine in Patients With Various *CDA* Diplotypes

Diplotype	No. of Patients	Median Gemcitabine PK Parameters				
		C_{max} (μg/mL)	AUC (hr · μg/mL)	CL/m² (L/hr/m²)	MRT (hours)	AUC Ratio (dFdU/gemcitabine)
*1a/*1a	30	22.40	10.54	94.24	0.37	8.86
*1a/*1b	17	22.75	10.08	97.91	0.35	9.08
*1b/*1b	6	20.81	9.19	108.60	0.36	9.19
P value*		0.82	0.40	0.59	0.97	0.83
*1a/*1c	23	23.23	10.87	94.31	0.35	8.73
*1d/*1c	1	25.84	16.62	60.16	0.55	8.40
P value*		0.77	0.57	0.94	0.97	0.83
*1a/*1d	7	22.05	9.07	108.30	0.36	9.04
*1d/*1d	1	26.43	9.99	100.10	0.31	7.70
P value*		0.82	0.45	0.90	0.86	0.57
*2a/*2a	8	23.94	9.34	107.20	0.33	9.70
*2a/*2b	4	23.02	9.78	100.13	0.38	8.59
*2a/*2c	2	21.50	9.22	111.63	0.36	10.99
P value†		0.66	0.98	0.76	0.077	0.46

Abbreviations: PK, pharmacokinetics; C_{max}, peak concentration; AUC, area under the curve; CL/m², clearance; MRT, mean residence time; dFdU, 2′,2′-difluorodeoxyuridine.

P* value of a correlation test among **1a/*1a*, **1a/*1b*, **1c*, or **1d*), and (1b*, **1c*, or **1d*)/(**1b*, **1c*, or **1d*). Multiplicity is adjusted by false-discovery rate.

†*P* value of a Kruskal-Wallis test among **2a/*2a*, **2a/*2b*, and **2a/*2c*.

also indicated on the label that the elimination half-life of gemcitabine was longer in females than in males in a population pharmacokinetic study using 45 Japanese non–small-cell lung cancer patients. The present study did not reveal any significant sex-based difference in clearance. However, the distribution volume was significantly smaller in females than in males.

Human CDA is involved in the salvaging of pyrimidines,[33,34] and plays a key role in detoxifying gemcitabine. Although the activities of 27Gln or 70Thr variant (the products of 79A>C or 208G>A) toward cytidine and cytarabine were reported to be lower than those of the "prototype" in a yeast expression system,[18] the decreased CDA activity in patients bearing these SNPs has not been reported. Kreis et al[35] reported that the response of leukemic patients to cytarabine correlated with the phenotype of CDA deamination determined based on the ratio of plasma concentrations of a cytarabine metabolite and cytarabine.[35] They reported that 70% of subjects were slow metabolizers. However, the relationship between genetic polymorphisms and phenotypes remained to be clarified.

In our study, the haplotype *2 harboring 79C (27Gln) did not show clear effects on the AUC and CL/m² values. In contrast, the 208A (Thr70, *3) -dependent decreases in gemcitabine clearance and plasma CDA activities were clearly demonstrated in this study. These results suggest that the *CDA* variant loses its in vivo deamination activities toward gemcitabine considerably. Moreover, the decreased plasma CDA activities toward gemcitabine and cytidine ex vivo also strongly suggest that the reduced enzymatic activity was caused by the genetic variation.

In the monotherapy group, the increased AUC in the patient with haplotype *3 did not clearly augment the incidence of toxicities including neutropenia. However, the incidences of grade 3 or higher neutropenia were higher in patients heterozygous for haplotype *3* compared with in the patients without haplotype *3* when they received concomitant chemotherapy with fluorouracil or platinum compounds. As we reported recently, one patient homozygous for haplotype *3* who received both gemcitabine and cisplatin suffered from extremely severe adverse effects including grade 3 anathema.[23] However, he experienced neither of the specific toxicities associated with cisplatin, nephrotoxicity, and neurotoxicity. Abbruzzese et al[36] reported the gemcitabine dose-dependent increase in incidence of thrombocytopenia (one of seven at 525 mg/m²/wk, three of nine at 790 mg/m²/wk, and three of six at 1,000 mg/m²/wk).[36] Therefore, we concluded that extremely high exposure to gemcitabine (AUC five times higher than the average) due to the decreased deamination activity caused the life-threatening severe toxicities in this patient. In contrast, the gemcitabine AUC of the patients with heterozygous haplotype *3* was only slightly (23% to 48%) increased from that of the patients having no haplotype *3* (Table 6). This finding coincides with the lack of life-threatening severe toxicities in the heterozygotes for *3*, although the incidences of grade 3 or higher neutropenia in the heterozygotes in combined chemotherapy groups were higher in the group without haplotype *3*.

CDA is also involved in the activation of capecitabine to its active form fluorouracil.[37] Therefore, capecitabine activation would be inefficient in patients who are homozygous for 208A. The allele frequency of the 208G>A SNP, a tagging SNP of haplotype *3*, was reported to be 0.125 in Africans, while it was not detected in Europeans.[38] The frequency of homozygous carriers of the variant could be higher in Africans than in the Japanese population. However, the frequency of 208G>A in Africans is still controversial, because it was not detected in 60 African Americans in a recent report.[17] Extra attention may be necessary for patients with the allele before treatments with gemcitabine or cytarabine are initiated, especially to *3/*3 patients, although more studies are necessary to confirm the clinical importance of this allele in the treatments using gemcitabine or cytarabine.

A number of studies have investigated the associations between cellular CDA activity and drug responses to cytarabine.[24-27,39] However, correlation between plasma CDA activity and the

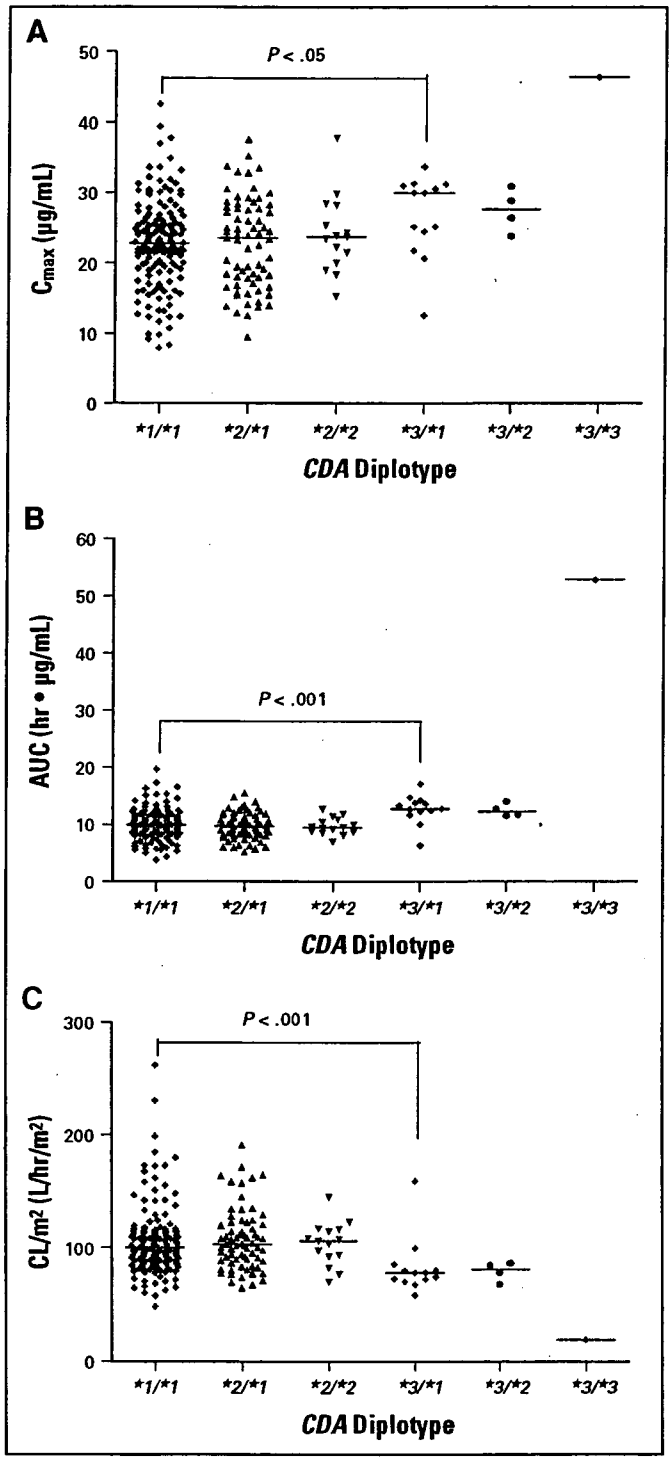

Fig 2. Effects of haplotypes **2* and **3* on the pharmacokinetic parameters of gemcitabine. (A) Peak concentration (C_{max}) and (B) area under the curve (AUC) were corrected assuming that all patients received 1,000 mg/m^2 of gemcitabine. (C) Clearance (CL/m^2). Each point corresponds to an individual patient. The bars denote the median values. *P* values are from Dunn's multiple comparison test.

pharmacokinetics of gemcitabine has not been reported. Plasma CDA activity may be a useful biomarker to screen patients with a markedly decreased metabolic CDA activity such as the patient homozygous for the **3* allele found in our study, who showed extremely low plasma CDA activity. However, a very low contribution of plasma CDA to the total clearance of gemcitabine was reported,[36] and the plasma CDA levels are increased in the inflammatory diseases.[30,40] These may account for the failure in obtaining good correlations between plasma CDA activity and the pharmacokinetic parameters of gemcitabine, as shown in Figure 4.

In conclusion, we analyzed the *CDA* genetic variations and haplotypes in Japanese cancer patients who received gemcitabine. We then investigated the associations between genetic polymorphisms and the pharmacokinetics of gemcitabine or toxicities. Depending on the haplotype **3* harboring 208A, the metabolic clearance of gemcitabine decreased, and AUC and C_{max} values were increased. Moreover, plasma CDA activities correlated well with the *CDA* genotypes. The clinical importance of the SNP 208G>A, especially of homozygotes, should be confirmed by prospective clinical studies because only one homozygous **3* patient was found in this study.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Table 6. Pharmacokinetic Parameters of Gemcitabine and Plasma CDA Activities in the Patient Groups Categorized According to Diplotypes

Diplotype	Median Gemcitabine PK Parameters				Median CDA Activity (units)		
	No. of Patients	C_{max} (μg/mL)	AUC (hr·μg/mL)	CL/m² (L/hr/m²)	No. of Patients	Gemcitabine	Cytidine
*1/*1	148	22.81	9.96	100.30	63	6.26	5.54
*2/*1	69	23.57	9.71	103.00	25	6.81	5.71
*2/*2	15	23.75	9.57	106.10	14	6.53	6.24
P value*		0.52	0.46	0.99		0.47	0.19
*3/*1	13	30.02	12.83	77.93	13	2.99	3.07
*3/*3	1	46.42	52.86	18.92	1	0.74	1.40
P value†		5.94E-04	6.66E-13	7.77E-04		9.35E-05	2.45E-04

Abbreviations: CDA, cytidine deaminase; C_{max}, peak concentration; AUC, area under the curve; CL/m², clearance.
**P* value of a correlation test among **1/*1*, **1/*2*, and **2/*2*. Multiplicity is adjusted by false-discovery rate.
†*P* value of a correlation test among **1/*1*, **1/*3*, and **3/*3*. Multiplicity is adjusted by false-discovery rate.

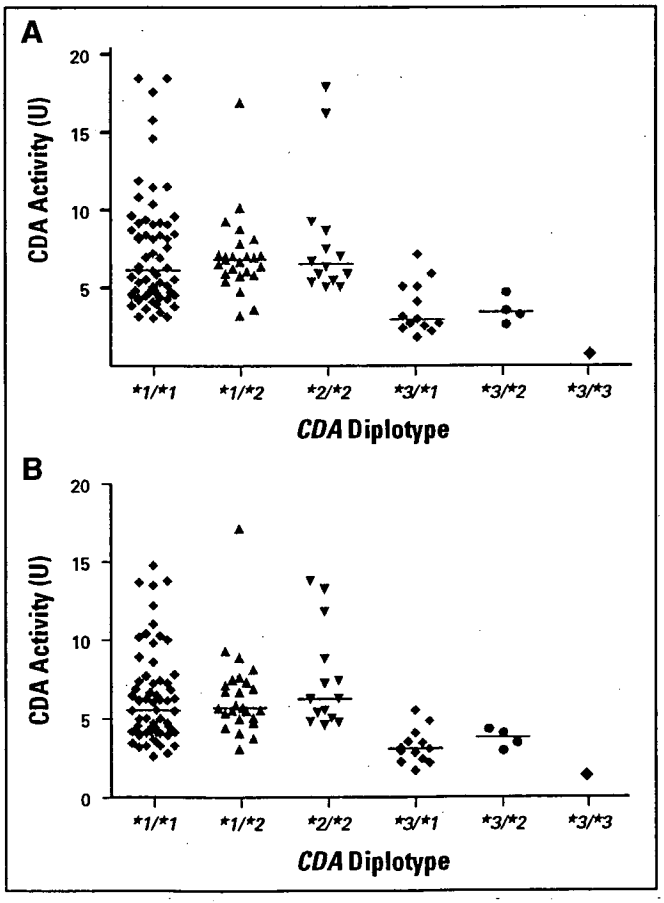

Fig 3. Effects of haplotypes **2* and **3* on plasma cytidine deaminase (CDA) activity toward gemcitabine and cytidine substrates. (A) Gemcitabine was used as a substrate, and (B) cytidine was used as a substrate. Each point corresponds to an individual patient. The bars denote the median values.

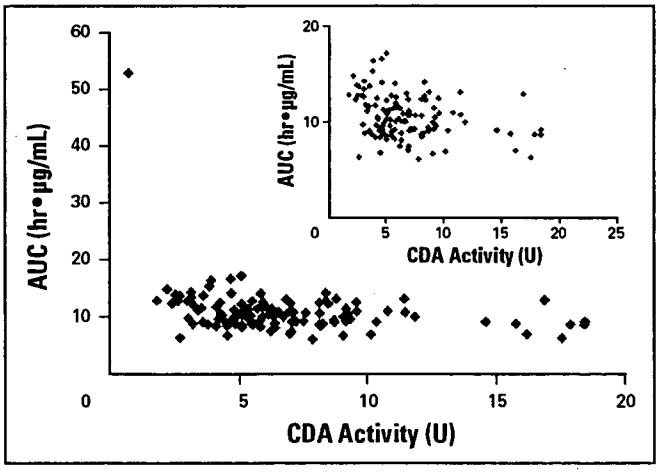

Fig 4. Correlation between plasma area under the curve (AUC) and cytidine deaminase (CDA) activity toward gemcitabine. AUC was corrected assuming that all patients received 1,000 mg/m² of gemcitabine. The inset excludes the data obtained from a homozygous **3* carrier. The correlation coefficient is −0.31 when the homozygous **3* carrier is included and −0.28 when the carrier is excluded.

Table 7. Comparison of Adverse Reaction Incidence and Pharmacokinetic Parameters of Gemcitabine Between Two Patient Groups With and Without Haplotype **3*

		Incidence of Neturopenia (nadir)*						
		≥ Grade 3			≥ Grade 4			
Chemotherapy	Genotype	No. of Cases	Total No. of Patients	Probability	No. of Cases	Total No. of Patients	Probability	AUC† (hr·μg/mL)
Monotherapy	*non *3/non *3*	66	167	0.40	8	67	0.05	9.91
	*non *3/*3*	6	10	0.60	1	10	0.10	13.13
	P			0.205			0.514	0.0017
With fluorouracil	*non *3/non *3*	3	12	0.25	2	12	0.17	8.11
	*non *3/*3*	2	2	1.00	1	2	0.50	11.98
	P			**0.029**			0.327	0.055
With carboplatin	*non *3/non *3*	9	13	0.69	1	13	0.08	9.87
	*non *3/*3*	3	3	1.00	2	3	0.67	12.48
	P			0.163			**0.033**	**0.031**
With cisplatin	*non *3/non *3*	8	28	0.29	2	28	0.07	9.53
	*non *3/*3*	1	1	1.00	0	1	0.00	11.71
	**3/*3*	1	1	1.00	1	1	1.00	52.86
	P‡			**0.030**			0.128	0.061

Note. No analyses were performed in patients who received gemcitabine with vinorelbine, because only one patient bore the haplotype **3*. Boldfacing indicates a statistically significant difference ($P < .05$).
*χ^2-test.
†Kruskal-Wallis test.
‡A *P* value for comparison between *non*3/non*3* and (*non*3/*3* + **3/*3*).

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