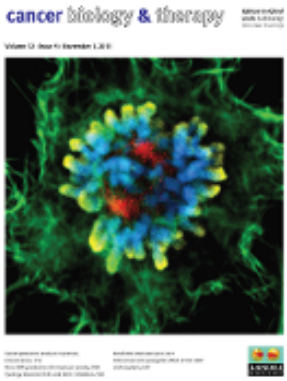

Cancer Biology & Therapy
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kcbt20

Single nucleotide polymorphisms of ABCC5 and ABCG1 transporter genes correlate to irinotecan-associated gastrointestinal toxicity in colorectal cancer patients: A DMET microarray profiling study

Maria Teresa Di Martino, Mariamena Arbitrio, Emanuela Leone, Pietro Hiram Guzzi, Maria Saveria Rotundo, Domenico Ciliberto, Vera Tomaino, Fernanda Fabiani, Danilo Talarico, Pasquale Sperlongano, Patrizia Doldo, Mario Cannataro, Michele Caraglia, Pierfrancesco Tassone & Pierosandro Tagliaferri

Published online: 01 Nov 2011.

To cite this article: Maria Teresa Di Martino, Mariamena Arbitrio, Emanuela Leone, Pietro Hiram Guzzi, Maria Saveria Rotundo, Domenico Ciliberto, Vera Tomaino, Fernanda Fabiani, Danilo Talarico, Pasquale Sperlongano, Patrizia Doldo, Mario Cannataro, Michele Caraglia, Pierfrancesco Tassone & Pierosandro Tagliaferri (2011) Single nucleotide polymorphisms of ABCC5 and ABCG1 transporter genes correlate to irinotecan-associated gastrointestinal toxicity in colorectal cancer patients: A DMET microarray profiling study, Cancer Biology & Therapy, 12:9, 780-787, DOI: 10.4161/cbt.12.9.17781

To link to this article: http://dx.doi.org/10.4161/cbt.12.9.17781

Cancer Biology & Therapy 12:9, 780-787; November 1, 2011; © 2011 Landes Bioscience

Single nucleotide polymorphisms of ABCC5 and ABCG1 transporter genes correlate to irinotecan-associated gastrointestinal toxicity in colorectal cancer patients

A DMET microarray profiling study

Maria Teresa Di Martino,[1,2,†] Mariamena Arbitrio,[3,†] Emanuela Leone,[2] Pietro Hiram Guzzi,[4] Maria Saveria Rotundo,[1] Domenico Ciliberto,[1] Vera Tomaino,[2,4] Fernanda Fabiani,[2] Danilo Talarico,[2] Pasquale Sperlongano,[5] Patrizia Doldo,[6] Mario Cannataro,[4] Michele Caraglia,[7,*] Pierfrancesco Tassone[2,8] and Pierosandro Tagliaferri[1,*]

[1]Medical Oncology Unit and [2]Referral Center for Innovative Treatments in Medical Oncology; Campus Salvatore Venuta; Magna Graecia University and Tommaso Campanella Cancer Center; Catanzaro, Italy; [3]Institute of Neurological Science-National Council of Research; UOS of Pharmacology; Catanzaro, Italy; [4]Department of Experimental and Clinical Medicine; Magna Graecia University; Catanzaro, Italy; [5]Department of Anaesthesiology and Special Surgery; Second University of Naples; Naples, Italy; [6]Gastroenterology Unit; Campus Salvatore Venuta; Magna Graecia University and Tommaso Campanella Cancer Center; Catanzaro, Italy; [7]Department of Biochemistry and Biophysics; Second University of Naples; Naples, Italy; [8]College of Science & Technology; Temple University; Philadelphia, PA USA

[†]These authors contributed equally to this work.

Keywords: irinotecan, colorectal cancer, toxicity, SNP, polymorphism, pharmacogenomics, DMET, ABCG1, ABCC5, OATP1B1/SLCO1B1

Abbreviations: CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin; CYP3A4, cytochrome P450 enzyme; SN-38, 7-Ethyl-10-hydroxycamptothecin; APC, (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin); NPC, (7-ethyl-10-[4-N-(-1-piperidino)-1-amino] carbonyloxycamptothecin); CES1/CES2, carboxylesterases enzymes; SN-38G, SN38 conjugated to glucuronic acid; UGT1A1, UDP-glucuronosyl transferase 1 family, polypeptide A1; ABCG1, ATP-binding cassette, sub-family G gene, member 1; ABCC5, ATP-binding cassette, sub-family C gene, member 5; ABCB1, ATP-binding cassette, sub-family B gene, member 1; ABCC2, ATP-binding cassette, sub-family C gene, member 2; ABCG2, ATP-binding cassette, sub-family G gene, member 2; FDA, food and drug administration; EMA, european medicines agency; CRC, colorectal cancer; mCRC, metastatic CRC; GI toxicity, gastrointestinal toxicity; SNPs, single nucleotide polymorphisms; DMET, drug-metabolizing enzyme and transporter; BRLMM, bayesan robust linear model with mahalanobis distance classifier; CNVs, copy number variations; PCR, polymerase chain reaction; MIP, molecular inversion probe

Recent findings have disclosed the role of UDP-glucuronosyltransferase (UGT) 1A1*28 on the hematological toxicity induced by irinotecan (CPT-11), a drug commonly used in the treatment of metastatic colorectal cancer (mCRC). We investigated the pharmacogenomic profile of irinotecan-induced gastrointestinal (GI) toxicity by the novel drug-metabolizing enzyme and transporter (DMET) microarray genotyping platform.

Twenty-six mCRC patients who had undergone to irinotecan-based chemotherapy were enrolled in a case [patients experiencing ≥ grade 3 gastrointestinal, (GI) toxicity] -control (matched patients without GI toxicity) study. A statistically significant difference of SNP genotype distribution was found in the case vs. control group. The homozygous genotype C/C in the (rs562) ABCC5 gene occurred in 6/9 patients with GI toxicity vs. 1/17 patients without GI toxicity ($p = 0.0022$). The homozygous genotype G/G in the (rs425215) ABCG1 was found in 7/9 patients with GI toxicity vs. 4/17 patients without GI toxicity ($p = 0.0135$). The heterozygous genotype G/A in the 388G > A (rs2306283) OATP1B1/SLCO1B1 was found in 3/9 patients with grade ≥3 GI toxicity vs. 14/17 patients without GI toxicity ($p = 0.0277$).

DNA extracted from peripheral blood cells was genotyped by DMET Plus chip on Affymetrix array system. Genotype association was calculated by Fisher exact test (two tailed) and relevant SNPs were further analyzed by direct sequencing.

We have identified three SNPs mapping in ABCG1, ABCC5 and OATP1B1/SLCO1B1 transporter genes associated with GI toxicity induced by irinotecan in mCRC patients expanding the available knowledge of irinogenomics. The DMET microarray platform is an emerging technology for easy identification of new genetic variants for personalized medicine.

*Correspondence to: Michele Caraglia and Pierosandro Tagliaferri; Email: michele.caraglia@unina2.it or michele.caraglia@alice.it and tagliaferri@unicz.it
Submitted: 07/23/11; Revised: 08/16/11; Accepted: 08/16/11
DOI:10.4161/cbt.12.9.17781

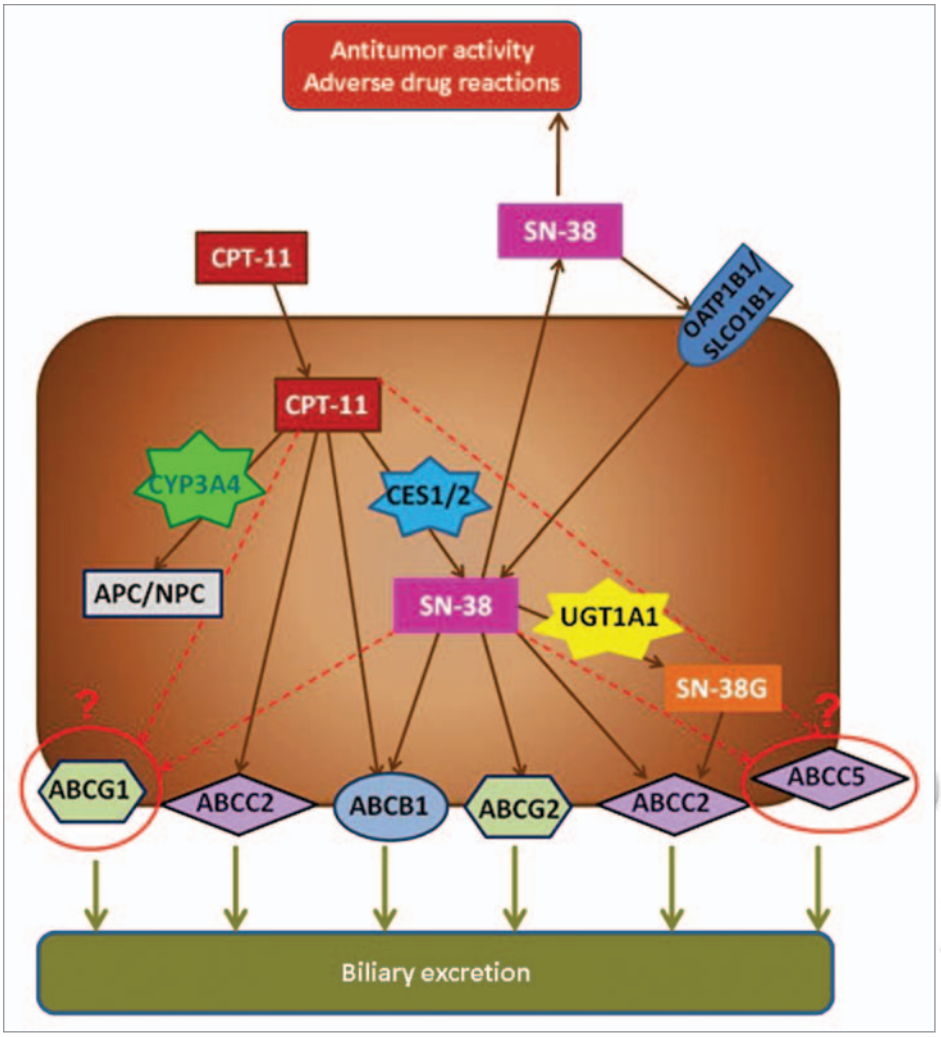

Figure 1. Irinotecan activation and disposition pathway. Findings reported in the current study are highlighted with a red circle and dotted lines. CPT-11, 7-Ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin, Camptosar; CYP3A4, Cytochrome P450 enzyme; SN-38,7-Ethyl-10-Hydroxycamptothecin; APC, (7-Ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin); NPC, (7-ethyl-10-[4-N-(-1-piperidino)-1-amino] carbonyloxycamptothecin); CES1/CES2 Carboxylesterases enzymes; SN-38G, SN38 conjugated to glucuronic acid; UGT1A1, UDP-glucuronosyl transferase 1 family, polypeptide A1; ABCG1, ATP-binding cassette, sub-family G gene, member 1; ABCC5, ATP-binding cassette, sub-family C gene, member 5; ABCB1, ATP-binding cassette, sub-family B gene, member 1; ABCC2, ATP-binding cassette, sub-family C gene, member 2; ABCG2, ATP-binding cassette, sub-family G gene, member 2.

Introduction

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin, CPT-11, Camptosar) is a US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved drug for the treatment of metastatic colorectal cancer (mCRC) patients. At present, irinotecan is used either as single agent or in combination with other chemotherapeutic agents (i.e., fluorouracil, bevacizumab and oxaliplatin) in first- or second-line treatment of mCRC patients.[1-3] Its use is limited by severe toxicities such as neutropenia and acute or delayed-type diarrhea that require close monitoring and immediate treatment. The toxicity profile of irinotecan is dependent on drug dose and schedule, but in all regimens severe diarrhea and neutropenia are the most important dose-limiting toxicities.[4] Irinotecan is detoxified by cytochrome P450 CYP3A4 to APC [7-ethyl-10-(4-N-[5-aminopentanoicacid]-1-piperidino)carbonyloxycamptothecin] and NPC [7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin] and converted by carboxylesterases (CES1, CES2) to its active metabolite SN-38. The latter is further conjugated to glucuronic acid in SN-38G by UDP-glucuronosyl transferase, UDP-glucuronosyltransferase 1A1 (UGT1A1) and possibly other isoforms, that also catalyzes bilirubin glucuronidation. Impaired glucuronidating activity of the UGT1A1 enzyme has been linked to elevated serum levels of SN-38 and bilirubin, leading to toxicity. Members of ATP-binding cassette transporter family regulate the efflux of the irinotecan metabolism products (Fig. 1). Large interindividual variability in the pharmacokinetic of irinotecan and of SN-38 as well as genetic polymorphisms of the UGT1A1 enzyme, involved in SN-38 glucuronidation, have been reported.[5-7] This variability is associated with significant differences in treatment outcome and severe unpredictable toxicity in some patients. Patients homozygous for the 7/TA allele (UGT1A1*28) which involves an extra TA repeat in the UGT1A1 promoter region show the highest variability in irinotecan pharmacokinetics and higher toxicities, specifically neutropenia.[7] In 2005, this finding led to the revision of the irinotecan label by FDA to include consideration of a dose reduction in patients homozygous for UGT1A1*28. Genetic test was approved to identify patient's genotypes in order to drive physicians to the optimal irinotecan dosage. Moreover, as population studies have associated different UGT1A1 alleles with toxicity to irinotecan and the irinogenomics include several different gene variants, innovative approaches are eagerly required to shed new light in this important area of anticancer drug pharmacogenetics and to drive drug usage.

In this study, by the use of an innovative microarray platform, we explored the pharmacogenomic profile of all currently identified genes involved in drug metabolism, in a series of mCRC patients with irinotecan-induced GI toxicity compared with matched controls non experiencing GI toxicity. DMET Plus platform in fact allows to interrogate all known polymorphisms

in Absorption, Distribution, Metabolism and Elimination (ADME)-related enzymes on a single array. The power of the DMET Plus Assay has previously been demonstrated with regard to several different drugs.[8-11]

Results

Demographics. The study was based on a case-control design and was approved by the Ethical Committee of our University Hospital. Informed consent was obtained from all patients in accordance with the Recommendations of the Declaration of Helsinki for biomedical research involving human subjects. Twenty-six mCRC patients (15 males and 11 females) with an histologic diagnosis of adenocarcinoma, stage IV according to TNM classification, were enrolled in the study. All patients underwent to first or second/line irinotecan-based chemotherapy and presented with metastatic involvement of liver and/or lung and homogeneous pathological characteristics. We enrolled 9 patients (cases) with ≥3 grade GI toxicity [median age 60 y (range: 40–66)] and 17 matched controls without GI toxicity [median age 62 y (range: 40–70)]. All patients received Irinotecan-based chemotherapy. Courses were repeated every 2 weeks for a maximum of 12 cycles. Gastrointestinal events and toxicity which occurred on II°–III° cycle were graded for severity according to WHO criteria. All cases experienced a grade ≥3 GI toxicity according to WHO criteria and irinotecan-doses were reduced by 20–25% and were not increased at subsequent cycles.

Pharmacogenomic profile with DMET plus assay. A total of 26 mCRC patients were genotyped by DMET Plus Assay. Clinical and pathological features of patients included in the study are summarized in **Table 1**.

Pharmacogenomic profiles were generated for all patients with call rate ≥98%. All alleles are in Hardy-Weinberg equilibrium (**Table 2**). Analysis of the genotype frequencies in cases vs. controls revealed a significant differential distribution ($p \leq 0.05$) of 3 SNPs: rs562, rs425215 and rs2306283 (**Table 2**). The rs562 SNP maps on the chromosome 3 in the 3'UTR sequence of the ATP-binding cassette, sub-family C gene (ABCC5), the rs425215 SNP maps on chromosome 21 in the intronic sequence of the ATP-binding cassette, sub-family G gene (ABCG1) and the rs2306283, on chromosome 12, introduces a missense mutation (388G > A) in the solute carrier organic anion transporter family, member 1B1 (OATP1B1/SLCO1B1).

Association between SNPs and gastrointestinal toxicity. The homozygous genotype C/C of the rs562 SNP showed the strongest association with GI toxicity of ≥3 grade being genotyped in 6/9 cases vs. 1/17 control patients ($p = 0.0022$, OR = 32.00, 95% CI, 2.76–370.81). The homozygous genotype G/G of the rs425215 SNP, showed a significant association with grade ≥3 GI toxicity ($p = 0.0135$, OR = 11.38, 95% CI, 1.65–78.38). This SNP was found in 7/9 cases and 4/17 controls. The SNP rs2306283 heterozygous genotype G/A was found in 3/9 patients with grade ≥3 GI vs. 14/17 patients without GI toxicity ($p = 0.0277$, OR = 2.33, 95% CI, 0.36–15.05) (**Table 3**).

The association of ABCC5 and ABCG1 polymorphisms and irinotecan toxicity has been never previously reported, while recent findings suggest a role for OATP1B1/SLCO1B1*1B in regulating the pharmacokinetics of irinotecan.[4,12] The genetic variations of the two ATP-binding transporter genes ABCC5 and ABCG1 were confirmed by direct sequencing. Importantly, concordance rates between the two technologies (DMET vs. direct sequencing) were 100%. **Figure 2** shows a representative profile of reference homozygous, heterozygous and variant homozygous sequences of the two ABC transporter genes.

Moreover, we evaluated the UGT1A1*28 genotype in all patients enrolled in the study vs. irinotecan-induced hematological toxicity, such as neutropenia. We identify 7/TA repeats genotype in 20/26 patients of which 6 carrying UGT1A1*28/*28 genotype. We observed ≥2 grade neutropenia in 4/26 genotyped patients 3 (75%) of which harbor homozygous UGT1A1*28 genotype, further supporting its predictive role for irinotecan hematological toxicity (data not shown). It has to be considered that the study was designed for GI toxicity investigation only.

Table 1. Patient characteristics

Characteristics	Number of patients %
Sex	
Male	13/26 (50.0)
Female	13/26 (50.0)
Age (y)	45–70 (61 y)
Primary site	
Colon	18/26 (69.2)
Rectum	08/26 (30.8)
Grading	
G1	2/26 (7.7)
G2	9/26 (34.6)
G3	15/26 (57.7)
Metastatic localization	
Liver	15/26 (57.7)
Lung	11/26 (42. 3)
Chemotherapy cycle	
1°	13/26 (50.0)
2°	13/26 (50.0)
WHO/ECOG performance status	
0	20/26 (76.9)
1	6/26 (23.1)

Discussion

Drug-metabolizing enzymes are involved in the activation or detoxification of anticancer drugs in colon cancer and some of the genetic variations identified in genes encoding thymidylate synthase, dihydropyrimidine dehydrogenase, glutathione S-transferase and uridine diphosphate glucosyltransferase 1A1 seem to be promising predictors of drug efficacy and/or toxicity.[13]

Allelic variants are present with a variable frequency in different populations around the world and have an important role in modifying the therapeutic index of many drugs as Irinotecan. We have undergone a genetic screening using the Affymetrix DMET

Table 2. Polymorphisms associated with gastrointestinal irinotecan toxicity

	RefSNP	Genotype frequencies			Allele frequencies	
Polymorphism Ref > Var	Alleles (A//B)	Ref	HT	Var	A	B
ABCC5_94336 > (rs562)	T//C	7	14	5	0.519	0.481
ABCG1 > rs425215	G//C	11	12	3	0.650	0.350
OATP1B1/SLCO1B1 (rs2306283)	G//A	6	17	3	0.480	0.520

Polymorphisms are reported as Reference (Ref), Heterozygous (HT) and Variant (Var). Allele frequencies were calculated in the population included in the study using the Hardy-Weinberg equation.

Table 3. Alleles and genotype frequencies of polymorphisms in advanced CRC patients

SNP and variants	Gene	Alleles distribution		p	Genotype	Genotypes distribution		p
		cases	controls			cases (%)	controls (%)	
rs562	**ABCC5**							
C		13/18	15/34	0.0799	CC	6/9 (66.6)	1/17 (5.88)	0.0022
T		5/18	19/34		TC	1/9 (11.1)	13/17 (76.4)	
					TT	2/9 (22.2)	3/17 (17.6)	
rs425215	**ABCG1**							
C		2/18	16/34	0.0136	GG	7/9 (77.7)	4/17 (23.5)	0.01350
G		16/18	18/34		CG	2/9 (22.2)	10/17 (58.8)	
					CC	0/9 (0.00)	3/17 (17.6)	
rs2306283	**OATP1B1/SLCO1B1**							
C		11/18	18/34	0.77	GG	4/9 (44.4)	2/17 (11.7)	
T		7/18	16/34		GA	3/9 (33.3)	14/17 (86.6)	0.0277
					AA	2/9 (22.2)	1/17 (5.88)	

Allelic and genotype distribution between case and control groups. SNPs are numbered as RS number used in the human SNP database. The p value was calculated by two-tailed Fisher exact test.

Plus Assay to explore whether additional polymorphisms of drug-metabolizing enzymes might correlate with irinotecan-induced GI toxicity in mCRC patients. Our results achieved by a novel approach indicate that genetic variants of two ABC transporter superfamily genes, ABCG1 and ABCC5, are associated to irinotecan GI adverse events in patients with mCRC and confirms a role for OATP1B1/SLCO1B1 388A > G genotype in the irinotecan GI toxicity. Our pharmacogenomic study, which was performed applying DMET methodology to mCRC patients who had undergone irinotecan-based chemotherapy, provides new information on the genetic bases of the GI toxicity exerted by irinotecan. The drug's activation/detoxification mechanism allows for many potential genetic sources of variability, and a number of clinical studies have revealed significant association between UGT1A1*28 genotype and irinotecan toxicity.[7] The major route of SN-38 elimination is via glucuronidation by the UGT1A enzymes, predominantly by hepatic UGT1A1. UGT1A1*28 is a common allele with 7/TA repeats in a TATA box of the promoter of UGT1A1 while the wild-type allele (UGT1A1*1) has 6/TA repeats. The allelic frequency of *28 in white population is approximately 39% and the frequency of the homozygous variant genotype (*28/*28) is approximately 10%.[14,15] 7/TA allele status in UGT1A1 is associated with decreased transcription and expression of the gene and reduced enzyme activity compared with 6/TA. Patients homozygous for UGT1A1*28 show reduced glucuronidation of SN-38 and an elevated risk of neutropenia compared with patients with one or two wild-type alleles.[15] It is likely that other factors, genetic and non-genetic, might contribute to a patient's risk of irinotecan-induced toxicity.[4] Many studies have investigated the involvement of other polymorphisms in genes that regulate the pharmacokinetics and pharmacodynamics of irinotecan in the efficacy and safety of the drug.[7,12,15-21] Polymorphisms in CYP3A4 and CES2 were been also identified to be correlated with an alteration of irinotecan.[22]

Irinotecan and its metabolites are transported by several members of the ATP-binding cassette (ABC) transporter superfamily that facilitate the cellular efflux of various molecules and are expressed in a wide variety of normal tissues.[23] Mutations in genes encoding human ABC transporters have been linked to disorders displaying mendelian inheritance.[24] The ABC transporters play an important role in the development of multi-drug resistance in cancer cells.[25] In addition, polymorphic variation in ABC transporter genes are known to influence the disposition of drugs for their involvement in intestinal absorption and biliary and renal excretion mechanisms.[26] Variable ABC gene product activity is therefore an important factor for inter-patient variability in the clearance of chemotherapeutic agents. The efflux proteins of irinotecan and its metabolites are encoded by different genes: ABCB1, ABCC1, ABCC2 and ABCG2.[4,26] Recent evidence suggests also that the solute carriers proteins (SLCO) mediate the

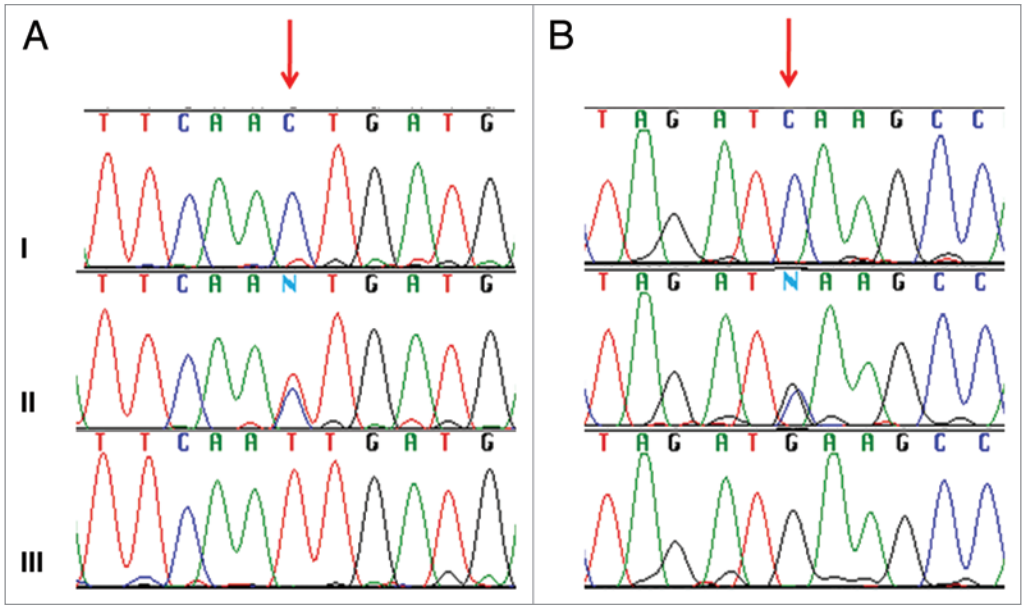

Figure 2. rs562 (A) and rs425215 (B) direct sequencing. Representative electropherogram of direct sequencing analysis of a homozygote reference allele (I), heterozygote (II) and homozygote variant allele (III). The red arrow indicates the SNP position.

cellular uptake of irinotecan and its metabolites.[27,28] In particular, the organic anion transporting polypeptide 1B1 (OATP1B1), better known as SLCO1B1, plays a role in the pharmacokinetics of irinotecan and different polymorphisms in this gene have reported to influence the irinotecan AUC.[29] It is well known that the UGT1A1*28 homozygous status is strongly associated with irinotecan-induced neutropenia. Moreover, polymorphisms in the transporting peptides ABCB1 and OATP1B1/SLCO1B1 have also been associated with GI toxicity. In particular, it has been shown that the expression of ABCB1 on the apical surface of epithelial cell of the lower gastrointestinal tract (jejunum, ileum and colon) influences intestinal drug absorption. ABCCs are members of the ABC drug transporter super family and have the capacity to mediate transmembrane transport of many conjugated drugs and other compounds.[30] Pharmacogenetic analysis of 6 ABCC2 SNPs showed that patients with the ABCC2*2 haplotype had a lower risk of severe irinotecan-induced diarrhea, if they did not carry a UGT1A1*28 allele.[26]

In the present study we found that the homozygous C/C genotype in the rs562 SNP which maps in the 3'UTR region of the ABCC5 gene is correlated ($p = 0.0022$) to grade ≥3 GI toxicity. In fact the homozygous genotype was detected in 6/9 cases while only one of the matched controls harbored this genotype. This gene is differently expressed along the gastrointestinal tract, depending by the segments of the colon.[31] This transport protein enhances the cellular efflux of cyclic nucleotides,[32] which are important signaling molecules in the regulation of cell proliferation, differentiation and apoptosis, particularly in hematopoietic development.[33,34] Park and coworkers suggested that expression profiling of ABC transporters may help to predict the clinical response to neoadjuvant chemotherapy in human breast cancer cells.[35]

Moreover, 7/9 patients with grade ≥3 GI toxicity were found to harbor the G/G homozygous genotype in rs425215 SNP of the ABCG1 gene vs. 4/17 control patients. This latter gene is highly expressed in the ileum, liver, lung, spleen and kidney.[36] It appears to be involved in the transport of cholesterol and phospholipids in macrophages and plays a critical role in lipid homeostasis in a variety of organs.[37] Recent experimental evidence indicated that an ABCG1 deletion in macrophages from ABCG1(-/-) mice produces an increased inflammatory activity, thereby inducing acceleration of pulmonary disease causing a striking inflammatory phenotype and promoting the onset of pulmonary lipidosis in mice.[38] Thus a critical role for macrophage ABCG1 in lung inflammation and homeostasis has been proposed and even small increases in intracellular sterol levels might be sufficient to induce inflammatory mediators.[30] Probably, also in the colon mucosa a variation in the protein activity could create a milieu predisposing to inflammation playing a potential role in drug toxicity. At our knowledge, our results represent the first evidence for a role of genetic variants in ABCG1 gene in the GI toxicity of irinotecan. Genetic ABCG1 variants are associated to pancreatic β-cell dysfunction,[39] obesity, atherosclerosis and other pathologies correlated with an altered cholesterol and phospholipid transport.[40]

Finally, we found a weak correlation of irinotecan toxicity and G/A heterozygous genotype in the rs2306283 SNP mapping in the OATP1B1/SLCO1B1 (388A > G) gene. We have identified the heterozygous genotype in 14/17 patients without GI toxicity besides only 3/9 patients with ≥3 grade GI toxicity show this genotype ($p \leq 0.0277$). There is evidence of an association of OATP1B1/SLCO1B1 polymorphism and inter-individual variability in response to drug treatment, for example pravastatin,[41] methotrexate and rifampin.[42] Noteworthy, Nozawa et al.[27] demonstrated that a single polymorphism in OATP1B1/SLCO1B1 affects the in vitro transport of irinotecan and its glucuronide conjugate metabolite SN-38G.[27] Moreover, Han et al.[31] has demonstrated in patients with advanced Non Small Cell Lung Cancer

Table 4. Genes included in DMET plus platform (225 total genes) are: 47 phase I enzymes, 80 phase II enzymes, 52 transporters and 46 other genes

Phase I enzymes			Phase II enzymes			Transporters			Other	
CYP1A1	*CYP4F2*	*ADH1A*	*DPYD*	*NAT1*	*UGT2A1*	*ABCB1*	*SLC22A1*	*SLCO2B1*	*ABP1*	*ORM1*
CYP1A2	*CYP4F3*	*ADH1B*	*FMO1*	*NAT2*	*UGT2B4*	*ABCB4*	*SLC22A11*	*SLCO3A1*	*AHR*	*ORM2*
CYP1B1	*CYP4F8*	*ADH1C*	*FMO2*	*NNMT*	*UGT2B7*	*ABCB7*	*SLC22A12*	*SLCO4A1*	*AKAP9*	*PNMT*
CYP2A6	*CYP4F11*	*ADH4*	*FMO3*	*NQO1*	*UGT2B11*	*ABCB11*	*SLC22A14*	*SLCO5A1*	*ALB*	*PON1*
CYP2A7	*CYP4F12*	*ADH5*	*FMO4*	*SULT1A1*	*UGT2B15*	*ABCC1*	*SLC22A2*		*AOX1*	*PON2*
CYP2A13	*CYP4Z1*	*ADH6*	*FMO5*	*SULT1A2*	*UGT2B17*	*ABCC2*	*SLC22A3*		*ARNT*	*PON3*
CYP2B6	*CYP7A1*	*ADH7*	*FMO6*	*SULT1A3*	*UGT2B28*	*ABCC3*	*SLC22A4*		*ARSA*	*POR*
CYP2B7	*CYP7B1*	*ALDH1A1*	*GSTA1*	*SULT1B1*	*UGT8*	*ABCC4*	*SLC22A5*		*CBR1*	*PPARD*
CYP2B7P1	*CYP8B1*	*ALDH2*	*GSTA2*	*SULT1C1*		*ABCC5*	*SLC22A6*		*CBR3*	*PPARG*
CYP2C8	*CYP11A1*	*ALDH3A1*	*GSTA3*	*SULT1C2*		*ABCC6*	*SLC22A7*		*CDA*	*PTGIS*
CYP2C9	*CYP11B1*	*ALDH3A2*	*GSTA4*	*SULT1E1*		*ABCC8*	*SLC22A8*		*CES2*	*RALBP1*
CYP2C18	*CYP11B2*	*CHST1*	*GSTA5*	*SULT2A1*		*ABCC9*	*SLC28A1*		*CROT*	*RPL13*
CYP2C19	*CYP17A1*	*CHST2*	*GSTM1*	*SULT2B1*		*ABCG1*	*SLC28A2*		*DCK*	*RXRA*
CYP2D6	*CYP19A1*	*CHST3*	*GSTM2*	*SULT4A1*		*ABCG2*	*SLC28A3*		*EPHX1*	*SEC15L1*
CYP2E1	*CYP20A1*	*CHST4*	*GSTM3*	*TPMT*		*ATP7A*	*SLC29A1*		*EPHX2*	*SERPINA7*
CYP2F1	*CYP21A2*	*CHST5*	*GSTM4*	*UGT1A1*		*ATP7B*	*SLC29A2*		*FAAH*	*SETD4*
CYP2J2	*CYP24A1*	*CHST6*	*GSTM5*	*UGT1A3*		*SLCA13*	*SLC5A6*		*G6PD*	*SPG7*
CYP2S1	*CYP26A1*	*CHST7*	*GSTO1*	*UGT1A4*		*SLC10A1*	*SLC6A6*		*HMGCR*	*TBXAS1*
CYP3A4	*CYP27A1*	*CHST8*	*GSTP1*	*UGT1A5*		*SLC10A2*	*SLC7A5*		*HNMT*	*TPSG1*
CYP3A5	*CYP27B1*	*CHST9*	*GSTT1*	*UGT1A6*		*SLC13A1*	*SLC7A7*		*MAT1A*	*TYMS*
CYP3A7	*CYP39A1*	*CHST10*	*GSTT2*	*UGT1A7*		*SLC15A1*	*SLC7A8*		*METTL1*	*VKORC1*
CYP3A43	*CYP46A1*	*CHST11*	*GSTZ1*	*UGT1A8*		*SLC15A2*	*SLCO1A2*		*NR1I2*	*XDH*
CYP4A11	*CYP51A1*	*CHST13*	*MAOA*	*UGT1A9*		*SLC16A1*	*SLCO1B1*		*NR1I3*	
CYP4B1		*COMT*	*MAOB*	*UGT1A10*		*SLC19A1*	*SLCO1B3*		*NR3C1*	

that the G/G genotype of the OATP1B1/SLCO1B1 (388A > G) was associated with ≥3 grade GI toxicity (p = 0.046). This result appears in agreement with our finding of association of the G allelic variant with irinotecan toxicity that we find associated significantly with G/A heterozygous genotype in patient with ≥3 grade GI toxicity (p = 0.0277).

In conclusion, our results demonstrate that genetic polymorphisms of the ABCC5 and ABCG1 transporter genes as well as of the OATP1B1/SLCO1B1 anion transporter are statistically correlated with irinotecan GI toxicity, expanding therefore the current available knowledge on irinogenomics. DMET microarray platform confirms as an useful strategy for identification of new genetic variants correlated with anticancer drug activity or toxicity. We propose the rs562 C/C and the rs425215 G/G genotypes as candidate genetic biomarker for irinotecan induced GI toxicity which warrant validation in larger series.

Material and Methods

DNA extraction and molecular profiling. DNA was extracted from peripheral blood using the Perfect Pure DNA Blood kit (5 Prime) accordingly to the manufacturer's recommendations. One μg of dsDNA, normalized by the Quanti-iT™ PicoGreen® dsDNA Assay kit (Invitrogen), was genotyped using Affymetrix DMET Plus Premier Pack as previously described in reference 43. All the genetic variants included in the array were multiplex genotyped using the molecular inversion probe (MIP) technology.[44] Genotypes were determined for each SNP site of the 1931 of all interrogated SNPs and of the 5 Copy Number Variations (CNVs) included in DMET Assay. CNV and SNP markers with call rate minor than 100% were excluded from the subsequent analysis. The genotyping profiles were generated by DMET Console software® which is based on the BRLMM (Bayesan Robust Linear Model with Mahalanobis distance classifier) algorithm.[14] DMET Plus Panel interrogates 1936 markers (1931 SNPs and 5 CNV) in 225 genes that have documented functional significance in drug disposition, including phase I and phase II metabolizing enzymes as well as drug transporters, which are reported in **Table 4**.

Genotypes were reported as homozygous wild type, heterozygous, homozygous variant or "no call." CNV markers and SNPs with call rate minor than 100% were excluded for the subsequent analysis. Direct nucleotide sequencing of the two variants mapping in the genes ABCC5 and ABCG1 was estimated from the sequence chromatogram of both the forward and reverse sequencing runs. Polymerase Chain Reaction (PCR) was performed for each variant site using the following PCR primers: ABCC5-Fw 5'-TCT CAC TCC CTT CCC AGA GA-3' and Rv 5'-TCT CTC CCC TCA AAG TCT GC-3'; ABCG1-Fw 5'-CTC TCC TGC CTC TGT GTC CT-3' and Rv 5'-AGT GTG GCC CAC TGT TGA G-3'. A 25 μl reaction

was prepared for PCR amplification. The PCR conditions consisted of the 2.5x 5PRIME MasterMix (5 Prime). This corresponds to final concentrations in the PCR reaction of 1.25 U *Taq* DNA Polymerase, 50 mM KCl, 30 mM TRIS-HCl, 1.5 mM Mg^{2+}, 0.1% Igepal®-CA360 and 200 μM of each dNTP. Genomic DNA including the SNP rs562 and rs425215 was amplified by PCR. After an initial denaturation at 94°C for 5 min, 35 cycles of amplification with denaturation at 94°C for 50 sec, annealing at 54°C for 50 sec and exstension at 72°C for 1 min was performed, followed by a final extension step at 72°C for 10 min. PCR products was purified by ExoSAP-IT™ with activation at 37°C for 15 min and inactivation at 80°C for 15 min. Direct nucleotide sequencing was performed using the Big Dye Terminator Cycle Sequencing Ready Reaction kit version 1.1 on an ABI PRISM 3100 genetic analyzer (Applied Biosystems). We sequenced a total of 21 DNA samples of the 26 included in the study.

Statistical analysis. The association between the SNP genotypes and clinical toxicity was tested by two-tailed Fisher exact test using a 2 x 2 contingency table. Results of potential interest were limited to those in which the p value was ≤ 0.05. As no adjustment was made for multiple comparisons, results are to be interpreted as hypothesis generating.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was partially supported by P.S.R.C ex art.12 LSG 502/92.

References

1. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000; 355:1041-7; PMID:10744089; DOI:10.1016/S0140-6736(00)02034-1.
2. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med 2000; 343:905-14; PMID:11006366; DOI:10.1056/NEJM200009283431302.
3. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350:2335-42; PMID:15175435; DOI:10.1056/NEJMoa032691.
4. Kweekel D, Guchelaar HJ, Gelderblom H. Clinical and pharmacogenetic factors associated with irinotecan toxicity. Cancer Treat Rev 2008; 34:656-69; PMID:18558463; DOI:10.1016/j.ctrv.2008.05.002.
5. Mathijssen RH, Verweij J, de Jonge MJ, Nooter K, Stoter G, Sparreboom A. Impact of body-size measures on irinotecan clearance: alternative dosing recommendations. J Clin Oncol 2002; 20:81-7; PMID:11773157; DOI:10.1200/JCO.20.1.81.
6. de Jong FA, Mathijssen RH, Xie R, Verweij J, Sparreboom A. Flat-fixed dosing of irinotecan: influence on pharmacokinetic and pharmacodynamic variability. Clin Cancer Res 2004; 10:4068-71; PMID:15217940; DOI:10.1158/1078-0432.CCR-03-0591.
7. Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, et al. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol 2004; 22:1382-8; PMID:15007088; DOI:10.1200/JCO.2004.07.173.
8. Deeken JF, Cormier T, Price DK, Sissung TM, Steinberg SM, Tran K, et al. A pharmacogenetic study of docetaxel and thalidomide in patients with castration-resistant prostate cancer using the DMET genotyping platform. Pharmacogenomics J 2010; 10:191-9; PMID:20038957; DOI:10.1038/tpj.2009.57.
9. Caldwell MD, Awad T, Johnson JA, Gage BF, Falkowski M, Gardina P, et al. CYP4F2 genetic variant alters required warfarin dose. Blood 2008; 111:4106-12; PMID:18250228; DOI:10.1182/blood-2007-11-122010.
10. Borgiani P, Ciccacci C, Forte V, Sirianni E, Novelli L, Bramanti P, et al. CYP4F2 genetic variant (rs2108622) significantly contributes to warfarin dosing variability in the Italian population. Pharmacogenomics 2009; 10:261-6; PMID:19207028; DOI:10.2217/14622416.10.2.261.
11. Di Martino MT, Arbitrio M, Guzzi PH, Leone E, Baudi F, Piro E, et al. A peroxisome proliferator-activated receptor gamma (PPARG) polymorphism is associated with zoledronic acid-related osteonecrosis of the jaw in multiple myeloma patients: analysis by DMET microarray profiling. Br J Haematol 2011; 154:529-33; PMID:21517810; DOI:10.1111/j.1365-2141.2011.08622.x.
12. Innocenti F, Kroetz DL, Schuetz E, Dolan ME, Ramirez J, Relling M, et al. Comprehensive pharmacogenetic analysis of irinotecan neutropenia and pharmacokinetics. J Clin Oncol 2009; 27:2604-14; PMID:19349540; DOI:10.1200/JCO.2008.20.6300.
13. Fogli S, Caraglia M. Genotype-based therapeutic approach for colorectal cancer: state of the art and future perspectives. Expert Opin Pharmacother 2009; 10:1095-108; PMID:19405786; DOI:10.1517/14656560902889775.
14. Rabbee N, Speed TP. A genotype calling algorithm for affymetrix SNP arrays. Bioinformatics 2006; 22:7-12; PMID:16267090; DOI:10.1093/bioinformatics/bti741.
15. Hoskins JM, Marcuello E, Altes A, Marsh S, Maxwell T, Van Booven DJ, et al. Irinotecan pharmacogenetics: influence of pharmacodynamic genes. Clin Cancer Res 2008; 14:1788-96; PMID:18347181; DOI:10.1158/1078-0432.CCR-07-1472.
16. Hoskins JM, McLeod HL. UGT1A and irinotecan toxicity: keeping it in the family. J Clin Oncol 2009; 27:2419-21; PMID:19364959; DOI:10.1200/JCO.2008.20.9478.
17. Carlini LE, Meropol NJ, Bever J, Andria ML, Hill T, Gold P, et al. UGT1A7 and UGT1A9 polymorphisms predict response and toxicity in colorectal cancer patients treated with capecitabine/irinotecan. Clin Cancer Res 2005; 11:1226-36; PMID:15709193.
18. Cecchin E, Innocenti F, D'Andrea M, Corona G, De Mattia E, Biason P, et al. Predictive role of the UGT1A1, UGT1A7 and UGT1A9 genetic variants and their haplotypes on the outcome of metastatic colorectal cancer patients treated with fluorouracil, leucovorin and irinotecan. J Clin Oncol 2009; 27:2457-65; PMID:19364970; DOI:10.1200/JCO.2008.19.0314.
19. de Jong FA, de Jonge MJ, Verweij J, Mathijssen RH. Role of pharmacogenetics in irinotecan therapy. Cancer Lett 2006; 234:90-106; PMID:16343744; DOI:10.1016/j.canlet.2005.04.040.
20. Innocenti F, Liu W, Chen P, Desai AA, Das S, Ratain MJ. Haplotypes of variants in the UDP-glucuronosyltransferase1A9 and 1A1 genes. Pharmacogenet Genomics 2005; 15:295-301; PMID:15864130; DOI:10.1097/01213011-200505000-00004.
21. Fujita K, Ando Y, Nagashima F, Yamamoto W, Eodo H, Araki K, et al. Genetic linkage of UGT1A7 and UGT1A9 polymorphisms to UGT1A1*6 is associated with reduced activity for SN-38 in Japanese patients with cancer. Cancer Chemother Pharmacol 2007; 60:515-22; PMID:17406868; DOI:10.1007/s00280-006-0396-1.
22. Charasson V, Bellott R, Meynard D, Longy M, Gorry P, Robert J. Pharmacogenetics of human carboxylesterase 2, an enzyme involved in the activation of irinotecan into SN-38. Clin Pharmacol Ther 2004; 76:528-35; PMID:15592324; DOI:10.1016/j.clpt.2004.08.007.
23. Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD. Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. Drug Resist Updat 2003; 6:71-84; PMID:12729805; DOI:10.1016/S1368-7646(03)00005-0.
24. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. Annu Rev Biochem 2002; 71:537-92; PMID:12045106; DOI:10.1146/annurev.biochem.71.102301.093055.
25. Szakács G, Varadi A, Ozvegy-Laczka C, Sarkadi B. The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME-Tox). Drug Discov Today 2008; 13:379-93; PMID:18468555; DOI:10.1016/j.drudis.2007.12.010.
26. de Jong FA, Scott-Horton TJ, Kroetz DL, McLeod HL, Friberg LE, Mathijssen RH, et al. Irinotecan-induced diarrhea: functional significance of the polymorphic ABCC2 transporter protein. Clin Pharmacol Ther 2007; 81:42-9; PMID:17185998; DOI:10.1038/sj.clpt.6100019.
27. Nozawa T, Minami H, Sugiura S, Tsuji A, Tamai I. Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. Drug Metab Dispos 2005; 33:434-9; PMID:15608127; DOI:10.1124/dmd.104.001909.
28. Xiang X, Jada SR, Li HH, Fan L, Tham LS, Wong CI, et al. Pharmacogenetics of SLCO1B1 gene and the impact of *1b and *15 haplotypes on irinotecan disposition in Asian cancer patients. Pharmacogenet Genomics 2006; 16:683-91; PMID:16906022; DOI:10.1097/01.fpc.0000230420.05221.71.
29. Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, et al. Influence of the organic anion-transporting polypeptide 1B1 (OATP1B1) polymorphisms on irinotecan-pharmacokinetics and clinical outcome of patients with advanced non-small cell lung cancer. Lung Cancer 2008; 59:69-75; PMID:17766002; DOI:10.1016/j.lungcan.2007.07.019.
30. Tarr PT, Tarling EJ, Bojanic DD, Edwards PA, Baldan A. Emerging new paradigms for ABCG transporters. Biochim Biophys Acta 2009; 1791:584-93; PMID:19416657.

31. Zimmermann C, Gutmann H, Hruz P, Gutzwiller JP, Beglinger C, Drewe J. Mapping of multidrug resistance gene 1 and multidrug resistance-associated protein isoform 1 to 5 mRNA expression along the human intestinal tract. Drug Metab Dispos 2005; 33:219-24; PMID:15523049; DOI:10.1124/dmd.104.001354.
32. Jedlitschky G, Burchell B, Keppler D. The multidrug resistance protein 5 functions as an ATP-dependent export pump for cyclic nucleotides. J Biol Chem 2000; 275:30069-74; PMID:10893247; DOI:10.1074/jbc.M005463200.
33. Kim SN, Ahn YH, Kim SG, Park SD, Cho-Chung YS, Hong SH. 8-Cl-cAMP induces cell cycle-specific apoptosis in human cancer cells. Int J Cancer 2001; 93:33-41; PMID:11391618; DOI:10.1002/ijc.1308.
34. Tortora G, Tagliaferri P, Clair T, Colamonici O, Neckers LM, Robins RK, et al. Site-selective cAMP analogs at micromolar concentrations induce growth arrest and differentiation of acute promyelocytic, chronic myelocytic and acute lymphocytic human leukemia cell lines. Blood 1988; 71:230-3; PMID:2825845.
35. Park S, Shimizu C, Shimoyama T, Takeda M, Ando M, Kohno T, et al. Gene expression profiling of ATP-binding cassette (ABC) transporters as a predictor of the pathologic response to neoadjuvant chemotherapy in breast cancer patients. Breast Cancer Res Treat 2006; 99:9-17; PMID:16752223; DOI:10.1007/s10549-006-9175-2.
36. Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, et al. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab 2005; 1:121-31; PMID:16054053; DOI:10.1016/j.cmet.2005.01.002.
37. Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, et al. ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci USA 2000; 97:817-22; PMID:10639163; DOI:10.1073/pnas.97.2.817.
38. Wojcik AJ, Skaflen MD, Srinivasan S, Hedrick CC. A critical role for ABCG1 in macrophage inflammation and lung homeostasis. J Immunol 2008; 180:4273-82; PMID:18322240.
39. Vergeer M, Brunham LR, Koetsveld J, Kruit JK, Verchere CB, Kastelein JJ, et al. Carriers of loss-of-function mutations in ABCA1 display pancreatic beta-cell dysfunction. Diabetes Care 2010; 33:869-74; PMID:20067955; DOI:10.2337/dc09-1562.
40. Fitzgerald ML, Mujawar Z, Tamehiro N. ABC transporters, atherosclerosis and inflammation. Atherosclerosis 2010; 211:361-70; PMID:20138281; DOI:10.1016/j.atherosclerosis.2010.01.011.
41. Niemi M, Neuvonen PJ, Hofmann U, Backman JT, Schwab M, Lutjohann D, et al. Acute effects of pravastatin on cholesterol synthesis are associated with SLCO1B1 (encoding OATP1B1) haplotype *17. Pharmacogenet Genomics 2005; 15:303-9; PMID:15864131; DOI:10.1097/01213011-200505000-00005.
42. Kerb R. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. Cancer Lett 2006; 234:4-33; PMID:16504381; DOI:10.1016/j.canlet.2005.06.051.
43. Dumaual C, Miao X, Daly TM, Bruckner C, Njau R, Fu DJ, et al. Comprehensive assessment of metabolic enzyme and transporter genes using the Affymetrix Targeted Genotyping System. Pharmacogenomics 2007; 8:293-305; PMID:17324118; DOI:10.2217/14622416.8.3.293.
44. Hardenbol P, Baner J, Jain M, Nilsson M, Namsaraev EA, Karlin-Neumann GA, et al. Multiplexed genotyping with sequence-tagged molecular inversion probes. Nat Biotechnol 2003; 21:673-8; PMID:12730666; DOI:10.1038/nbt821.