Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. CORDON-CARDO C, O'BRIEN JP, BOCCIA J, CASALS D, BERTINO JR, MELAMED MR: Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J. Histochem. Cytochem.* (1990) 38:1277-1287.
2. THIEBAUT F, TSURUO T, HAMADA H, GOTTESMAN MM, PASTAN I, WILLINGHAM MC: Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. USA* (1987) 84:7735-7738.
3. FOJO AT, UEDA K, SLAMON DJ, POPLACK DG, GOTTESMAN MM, PASTAN I: Expression of a multidrug-resistance gene in human tumors and tissues. *Proc. Natl. Acad. Sci. USA* (1987) 84:265-269.
4. SUGAWARA I, KATAOKA I, MORISHITA Y *et al.*: Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK 16. *Cancer Res.* (1988) 48:1926-1929.
5. KIM RB, FROMM MF, WANDEL C *et al.*: The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J. Clin. Invest.* (1998) 101:289-294.
6. MAYER U, WAGENAAR E, BEIJNEN JH *et al.*: Substantial excretion of digoxin via the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by the mdr 1a P-glycoprotein. *Br. J. Pharmacol.* (1996) 119:1038-1044.
7. NAKAMURA Y, IKEDA S, FURUKAWA T *et al.*: Function of P-glycoprotein expressed in placenta and mole. *Biochem. Biophys. Res. Commun.* (1997) 235:849-853.
8. SCHINKEL AH, WAGENAAR E, MOL CA, VAN DEEMTER L: P-glycoprotein in the blood–brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J. Clin. Invest.* (1996) 97:2517-2524.
9. CORDON-CARDO C, O'BRIEN JP, CASALS D *et al.*: Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. *Proc. Natl. Acad. Sci. USA* (1989) 86:695-698.
10. VAN DE WATER FM, MASEREEUW R, RUSSEL FG: Function and regulation of multidrug resistance proteins (MRPs) in the renal elimination of organic anions. *Drug Metab. Rev.* (2005) 37:443-471.
11. SUZUKI H, SUGIYAMA Y: Single nucleotide polymorphisms in multidrug resistance associated protein 2 (MRP2/ABCC2): its impact on drug disposition. *Adv. Drug Deliv. Rev.* (2002) 54:1311-1331.

• **This review describes the basic physiological and pharmacological functions of MRP2 and changes in the functions by genetic variations.**

12. BUCHLER M, KONIG J, BROM M *et al.*: cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats. *J. Biol. Chem.* (1996) 271:15091-15098.
13. PAULUSMA CC, BOSMA PJ, ZAMAN GJ *et al.*: Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. *Science* (1996) 271:1126-1128.
14. SCHAUB TP, KARTENBECK J, KONIG J *et al.*: Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. *J. Am. Soc. Nephrol.* (1999) 10:1159-1169.
15. MOTTINO AD, HOFFMAN T, JENNES L, VORE M: Expression and localization of multidrug resistant protein mrp2 in rat small intestine. *J. Pharmacol. Exp. Ther.* (2000) 293:717-723.
16. CERVENAK J, ANDRIKOVICS H, OZVEGY-LACZKA C *et al.*: The role of the human ABCG2 multidrug transporter and its variants in cancer therapy and toxicology. *Cancer Lett.* (2006) 234:62-72.
17. MALIEPAARD M, SCHEFFER GL, FANEYTE IF *et al.*: Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res.* (2001) 61:3458-3464.
18. KRUIJTZER CM, BEIJNEN JH, SCHELLENS JH: Improvement of oral drug treatment by temporary inhibition of drug transporters and/or cytochrome P450 in the gastrointestinal tract and liver: an overview. *Oncologist* (2002) 7:516-530.
19. TAIPALENSUU J, TORNBLOM H, LINDBERG G *et al.*: Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. *J. Pharmacol. Exp. Ther.* (2001) 299:164-170.
20. STAUD F, PAVEK P: Breast cancer resistance protein (BCRP/ABCG2). *Int. J. Biochem. Cell Biol.* (2005) 37:720-725.
21. XU J, LIU Y, YANG Y, BATES S, ZHANG JT: Characterization of oligomeric human half-ABC transporter ATP-binding cassette G2. *J. Biol. Chem.* (2004) 279:19781-19789.
22. DOYLE AL, YANG W, ABRUZZO LV *et al.*: A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc. Natl. Acad. Sci. USA* (1998) 95:15665-15670.
23. KAGE K, TSUKAHARA S, SUGIYAMA T *et al.*: Dominant-negative inhibition of breast cancer resistance protein as drug efflux pump through the inhibition of S-S dependent homodimerization. *Int. J. Cancer* (2002) 97:626-630.
24. ROSS DD: Novel mechanisms of drug resistance in leukemia. *Leukemia* (2000) 14:467-473.
25. KULLAK-UBLICK GA, HAGENBUCH B, STIEGER B *et al.*: Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology* (1995) 109:1274-1282.
26. SMITH LH, LEE W, KIM RB: Differential expression of OATP drug uptake transporters in human liver, intestine, and kidney. *Drug Metab. Rev.* (2003) 35:73.
27. DRESSER GK, KIM RB, BAILEY DG: Effect of grapefruit juice volume on the reduction of fexofenadine bioavailability: possible role of organic anion transporting polypeptides. *Clin. Pharmacol. Ther.* (2005) 77:170-177.

•• **This paper represents a new mechanistic insight into drug interaction.**

28. TAMAI I, NEZU J, UCHINO H *et al.*: Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem. Biophys. Res. Commun.* (2000) 273:251-260.
29. ABE T, KAKYO M, TOKUI T *et al.*: Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J. Biol. Chem.* (1999) 274:17159-17163.

30. HSIANG B, ZHU Y, WANG Z *et al.*: A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J. Biol. Chem.* (1999) 274:37161-37168.

31. KONIG J, CUI Y, NIES AT, KEPPLER D: Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J. Biol. Chem.* (2000) 275:23161-23168.

32. NOZAWA T, IMAI K, NEZU J, TSUJI A, TAMAI I: Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *J. Pharmacol. Exp. Ther.* (2004) 308:438-445.

33. KULLAK-UBLICK GA, ISMAIR MG, STIEGER B *et al.*: Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* (2001) 120:525-533.

34. SEKINE T, WATANABE N, HOSOYAMADA M, KANAI Y, ENDOU H: Expression cloning and characterization of a novel multispecific organic anion transporter. *J. Biol. Chem.* (1997) 272:18526-18529.

35. DRESSER MJ, LEABMAN MK, GIACOMINI KM: Transporters involved in the elimination of drugs in the kidney: organic anion transporters and organic cation transporters. *J. Pharm. Sci.* (2001) 90:397-421.

36. MIYAZAKI H, SEKINE T, ENDOU H: The multispecific organic anion transporter family: properties and pharmacological significance. *Trends Pharmacol. Sci.* (2004) 25:654-662.

• **This review describes the basic function of OATs in humans.**

37. ENDOU H: Recent advances in molecular mechanisms of nephrotoxicity. *Toxicol. Lett.* (1998) 102-103:29-33.

38. CIHLAR T, LIN DC, PRITCHARD JB, FULLER MD, MENDEL DB, SWEET DH: The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol. Pharmacol.* (1999) 56:570-580.

39. HO ES, LIN DC, MENDEL DB, CIHLAR T: Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. *J. Am. Soc. Nephrol.* (2000) 11:383-393.

40. JARIYAWAT S, SEKINE T, TAKEDA M *et al.*: The interaction and transport of β-lactam antibiotics with the cloned rat renal organic anion transporter 1. *J. Pharmacol. Exp. Ther.* (1999) 290:672-677.

41. SIMONSON GD, VINCENT AC, ROBERG KJ, HUANG Y, IWANIJ V: Molecular cloning and characterization of a novel liver-specific transport protein. *J. Cell Sci.* (1994) 107:1065-1072.

42. SEKINE T, CHA SH, TSUDA M *et al.*: Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Lett.* (1998) 429:179-182.

43. NAGATA Y, KUSUHARA H, ENDOU H, SUGIYAMA Y: Expression and functional characterization of rat organic anion transporter 3 (rOat3) in the choroid plexus. *Mol. Pharmacol.* (2002) 61:982-988.

44. SWEET DH, MILLER DS, PRITCHARD JB, FUJIWARA Y, BEIER DR, NIGAM SK: Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 (Slc22a8)) knockout mice. *J. Biol. Chem.* (2002) 277:26934-26943.

45. JONKER JW, SCHINKEL AH: Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). *J. Pharmacol. Exp. Ther.* (2004) 308:2-9.

46. BURCKHARDT G, WOLFF NA: Structure of renal organic anion and cation transporters. *Am. J. Physiol. Renal Physiol.* (2000) 278:F853-F866.

47. ZHANG L, DRESSER MJ, GRAY AT, YOST SC, TERASHITA S, GIACOMINI KM: Cloning and functional expression of a human liver organic cation transporter. *Mol. Pharmacol.* (1997) 51:913-921.

48. GORBOULEV V, ULZHEIMER JC, AKHOUNDOVA A *et al.*: Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol.* (1997) 16:871-881.

49. VERHAAGH S, SCHWEIFER N, BARLOW DP, ZWART R: Cloning of the mouse and human solute carrier 22a3 (Slc22a3/SLC22A3) identifies a conserved cluster of three organic cation transporters on mouse chromosome 17 and human 6q26-q27. *Genomics* (1999) 55:209-218.

50. SCHWAB M, EICHELBAUM M, FROMM MF: Genetic polymorphisms of the human MDR1 drug transporter. *Ann. Rev. Pharmacol. Toxicol.* (2003) 43:285-307.

• **A comprehensive review of the *ABCB1* polymorphism.**

51. FROMM MF: The influence of *MDR1* polymorphisms on P-glycoprotein expression and function in humans. *Adv. Drug Deliv. Rev.* (2002) 54:1295-1310.

52. KIM RB: Drugs as P-glycoprotein substrates, inhibitors, and inducers. *Drug Metab. Rev.* (2002) 34:47-54.

53. WACHER VJ, WU CY, BENET LZ: Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog.* (1995) 13:129-134.

54. KIM RB, WANDEL C, LEAKE B *et al.*: Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm. Res.* (1999) 16:408-414.

55. JEDLITSCHKY G, LEIER I, BUCHHOLZ U, BARNOUIN K, KURZ G, KEPPLER D: Transport of glutathione, glucuronate, and sulfate conjugates by the MRP gene-encoded conjugate export pump. *Cancer Res.* (1996) 56:988-994.

56. PRIEBE W, KRAWCZYK M, KUO MT, YAMANE Y, SAVARAJ N, ISHIKAWA T: Doxorubicin- and daunorubicin-glutathione conjugates, but not unconjugated drugs, competitively inhibit leukotriene C4 transport mediated by MRP/GS-X pump. *Biochem. Biophys. Res. Commun.* (1998) 247:859-863.

57. BAKOS E, EVERS R, SINKO E, VARADI A, BORST P, SARKADI B: Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. *Mol. Pharmacol.* (2000) 57:760-768.

58. ISHIKAWA T: The ATP-dependent glutathione *S*-conjugate export pump. *Trends Biochem. Sci.* (1992) 17:463-468.

59. LOE DW, ALMQUIST KC, DEELEY RG, COLE SP: Multidrug resistance protein (MRP)-mediated transport of leukotriene C4 and chemotherapeutic agents in membrane vesicles. Demonstration of glutathione-dependent vincristine transport. *J. Biol. Chem.* (1996) 271:9675-9682.

60. JONKER JW, SMIT JW, BRINKHUIS RF *et al.*: Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *J. Natl. Cancer Inst.* (2000) 92:1651-1656.

61. SUZUKI M, SUZUKI H, SUGIMOTO Y, SUGIYAMA Y: ABCG2 transports sulfated conjugates of steroids and xenobiotics. *J. Biol. Chem.* (2003) 278:22644-22649.

62. BORST P, EVERS R, KOOL M, WIJNHOLDS J: A family of drug transporters: the multidrug resistance-associated proteins. *J. Natl. Cancer Inst.* (2000) 92:1295-1302.

63. LITMAN T, BRANGI M, HUDSON E *et al.*: The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). *J. Cell Sci.* (2000) 113:2011-2021.

64. ECKHARDT U, SCHROEDER A, STIEGER B *et al.*: Polyspecific substrate uptake by the hepatic organic anion transporter Oatp1 in stably transfected CHO cells. *Am. J. Physiol.* (1999) 276:G1037-G1042.

65. CVETKOVIC M, LEAKE B, DROMM MF, WILKINSON GR, KIM RB: OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab. Dispos.* (1999) 27:866-871.

66. GAO B, HAGENBUCH B, KULLAK-UBLICK GA, BENKE D, AGUZZI A, MEIER PJ: Organic anion-transporting polypeptides mediate transport of opioid peptides across blood–brain barrier. *J. Pharmacol. Exp. Ther.* (2000) 294:73-79.

67. CUI Y, KONIG J, LEIER I, BUCHHOLZ U, KEPPLER D: Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. *J. Biol. Chem.* (2001) 276:9626-9630.

68. NAKAI D, NAKAGOMI R, FURUTA Y *et al.*: Human liver-specific organic anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. *J. Pharmacol. Exp. Ther.* (2001) 297:861-867.

69. CHUNG JY, CHO JY, YU KS *et al.*: Effect of OATP1B1 (SLCO1B1) variant alleles on the pharmacokinetics of pitavastatin in healthy volunteers. *Clin. Pharmacol. Ther.* (2005) 78:342-350.

70. ABE T, UNNO M, ONOGAWA T *et al.*: LST-2, a human liver-specific organic anion transporter, determines methotrexate sensitivity in gastrointestinal cancers. *Gastroenterology* (2001) 120:1689-1699.

71. UWAI Y, OKUDA M, TAKAMI K, HASHIMOTO Y, INUI K: Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. *FEBS Lett.* (1998) 438:321-324.

72. APIWATTANAKUL N, SEKINE T, CHAIROUNGDUA A *et al.*: Transport properties of nonsteroidal anti-inflammatory drugs by organic anion transporter 1 expressed in *Xenopus laevis* oocytes. *Mol. Pharmacol.* (1999) 55:847-854.

73. MORITA N, KUSUHARA H, SEKINE T, ENDOU H, SUGIYAMA Y: Functional characterization of rat organic anion transporter 2 in LLC-PK1 cells. *J. Pharmacol. Exp. Ther.* (2001) 298:1179-1184.

74. GOTTESMAN MM, HRYCYNA CA, SCHOENLEIN PV, GERMANN UA, PASTAN I: Genetic analysis of the multidrug transporter. *Ann. Rev. Genet.* (1995)29:607-649.

75. BODOR M, KELLY EJ, HO RJ: Characterization of the human *MDR1* gene. *AAPS J.* (2005) 7:E1-E5.

76. MICKLEY LA, LEE JS, WENG Z *et al.*: Genetic polymorphism in *MDR-1*: a tool for examining allelic expression in normal cells, unselected and drug-selected cell lines, and human tumors. *Blood* (1998) 91:1749-1756.

77. HOFFMEYER S, BURK O, VON RICHTER O *et al.*: Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc. Natl. Acad. Sci. USA* (2000) 97:3473-3478.

• **This paper represents the first evidence of the functional significance of the *ABCB1* gene polymorphism.**

78. TANABE M, IEIRI I, NAGATA N *et al.*: Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J. Pharmacol. Exp. Ther.* (2001) 297:1137-1143.

79. ITO S, IEIRI I, TANABE M, SUZUKI A, HIGUCHI S, OTSUBO K: Polymorphism of the ABC transporter genes, MDR1, MRP1 and MRP2/cMOAT, in healthy Japanese subjects. *Pharmacogenetics* (2001) 11:175-184.

80. CASCORBI I, GERLOFF T, JOHNE A *et al.*: Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin. Pharmacol. Ther.* (2001) 69:169-174.

81. KIM RB, LEAKE BF, CHOO EF *et al.*: Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin. Pharmacol. Ther.* (2001) 70:189-199.

82. IEIRI I, TAKANE H, OTSUBO K: The *MDR1* (*ABCB1*) gene polymorphism and its clinical implications. *Clin. Pharmacokinet.* (2004) 43:553-576.

• **This review describes the roles of the *ABCB1* polymorphism in human tissue expression, its pharmacokinetic/pharmacodynamic impact, as well as the inter-racial variability of allelic frequencies.**

83. TANG K, NGOI SM, GWEE PC *et al.*: Distinct haplotype profiles and strong linkage disequilibrium at the *MDR1* multidrug transporter gene locus in three ethnic Asian populations. *Pharmacogenetics* (2002) 12:437-450.

• **Haplotype assessment of the *ABCB1* gene polymorphism.**

84. CHOWBAY B, CUMARASWAMY S, CHEUNG YB, ZHOU Q, LEE EJ: Genetic polymorphisms in *MDR1* and *CYP3A4* genes in Asians and the influence of *MDR1* haplotypes on cyclosporin disposition in heart transplant recipients. *Pharmacogenetics* (2003) 13:89-95.

85. KROETZ DL, PAULI-MAGNUS C, HODGES LM *et al.*; PHARMACOGENETICS OF MEMBRANE TRANSPORTERS INVESTIGATORS: Sequence diversity and haplotype structure in the human *ABCB1* (*MDR1*, multidrug resistance transporter) gene. *Pharmacogenetics* (2003) 13:481-494.

86. SAI K, KANIWA N, ITODA M *et al.*: Haplotype analysis of *ABCB1*/*MDR1* blocks in a Japanese population reveals genotype-dependent renal clearance of irinotecan. *Pharmacogenetics* (2003) 13:741-757.

87. AMEYAW MM, REGATEIRO F, LI T *et al.*: MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics* (2001) 11:217-221.

88. LINDHOLM A, WELSH M, ALTON C, KAHAN BD: Demographic factors influencing cyclosporine pharmacokinetic parameters in patients with uremia: racial

differences in bioavailability. *Clin. Pharmacol. Ther.* (1992) 52:359-371.

89. MANCINELLI LM, FRASSETTO L, FLOREN LC *et al.*: The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. *Clin. Pharmacol. Ther.* (2001) 69:24-31.

90. ELMORE JG, MOCERI VM, CARTER D, LARSON EB: Breast carcinoma tumor characteristics in black and white women. *Cancer* (1998) 83:2509-2515.

91. TSUJII H, KONIG J, ROST D, STOCKEL B, LEUSCHNER U, KEPPLER D: Exon-intron organization of the human multidrug-resistance protein 2 (MRP2) gene mutated in Dubin–Johnson syndrome. *Gastroenterology* (1999) 117:653-660.

92. TOH S, WADA M, UCHIUMI T *et al.*: Genomic structure of the canalicular multispecific organic anion-transporter gene (*MRP2/cMOAT*) and mutations in the ATP-binding-cassette region in Dubin–Johnson syndrome. *Am. J. Hum. Genet.* (1999) 64:739-746.

93. WADA M: Single nucleotide polymorphisms in *ABCC2* and *ABCB1* genes and their clinical impact in physiology and drug response. *Cancer Lett.* (2006) 234:40-50.

• **This review summarises *ABCC2* gene variants in DJS.**

94. ALLIKMETS R, SCHRIML LM, HUTCHINSON A, ROMANO-SPICA V, DEAN M: A human placenta-specific ATP-binding cassette gene (*ABCP*) on chromosome 4q22 that is involved in multidrug resistance. *Cancer Res.* (1998) 58:5337-5339.

95. IIDA A, SAITO S, SEKINE A *et al.*: Catalog of 605 single-nucleotide polymorphisms (SNPs) among 13 genes encoding human ATP-binding cassette transporters: *ABCA4*, *ABCA7*, *ABCA8*, *ABCD1*, *ABCD3*, *ABCD4*, *ABCE1*, *ABCF1*, *ABCG1*, *ABCG2*, *ABCG4*, *ABCG5*, and *ABCG8*. *J. Hum. Genet.* (2002) 47:285-310.

96. ZAMBER CP, LAMBA JK, YASUDA K *et al.*: Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics* (2003) 13:19-28.

97. KOBAYASHI D, IEIRI I, HIROTA T *et al.*: Functional assessment of *ABCG2* (BCRP) gene polymorphisms to protein expression in human placenta. *Drug Metab. Dispos.* (2005) 33:94-101.

98. BACKSTROM G, TAIPALENSUU J, MELHUS H *et al.*: Genetic variation in the ATP-binding cassette transporter gene *ABCG2* (BCRP) in a Swedish population. *Eur. J. Pharm. Sci.* (2003) 18:359-364.

99. IIDA A, SAITO S, SEKINE A *et al.*: Catalog of 258 single-nucleotide polymorphisms (SNPs) in genes encoding three organic anion transporters, three organic anion-transporting polypeptides, and three NADH:ubiquinone oxidoreductase flavoproteins. *J. Hum. Genet.* (2001) 46:668-683.

100. LEE W, GLAESER H, SMITH LH *et al.*: Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry. *J. Biol. Chem.* (2005) 280:9610-9617.

101. TIRONA RG, LEAKE BF, MERINO G, KIM RB: Polymorphisms in *OATP-C*: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J. Biol. Chem.* (2001) 276:35669-35675.

102. NIEMI M, SCHAEFFELER E, LANG T *et al.*: High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, *SLCO1B1*). *Pharmacogenetics* (2004) 14:429-440.

103. NISHIZATO Y, IEIRI I, SUZUKI H *et al.*: Polymorphisms of OATP-C (*SLC21A6*) and OAT3 (*SLC22A8*) genes: consequences for pravastatin pharmacokinetics. *Clin. Pharmacol. Ther.* (2003) 73:554-565.

• **First evidence of *in vivo* (human) function of the *SLCO1B1* gene polymorphism.**

104. MWINYI J, JOHNE A, BAUER S, ROOTS I, GERLOFF T: Evidence for inverse effects of OATP-C (*SLC21A6*) 5 and 1b haplotypes on pravastatin kinetics. *Clin. Pharmacol. Ther.* (2004) 75:415-421.

• **Inverse effects of **5* and **1b* allele on pravastatin pharmacokinetics.**

105. TAKANE H, MIYATA M, BURIOKA N *et al.*: Pharmacogenetic determinants of variability in lipid-lowering response to pravastatin therapy. *J. Hum Genet.* (2006) (In Press).

106. FUJITA T, BROWN C, CARLSON EJ *et al.*: Functional analysis of polymorphisms in the organic anion transporter, *SLC22A6* (OAT1). *Pharmacogenet. Genomics* (2005) 15:201-209.

107. XU G, BHATNAGAR V, WEN G, HAMILTON BA, ERALY SA, NIGAM SK: Analyses of coding region polymorphisms in apical and basolateral human organic anion transporter (OAT) genes [*OAT1* (*NKT*), *OAT2*, *OAT3*, *OAT4*, *URAT* (*RST*)]. *Kidney Int.* (2005) 68:1491-1499.

108. ITODA M, SAITO Y, MAEKAWA K *et al.*: Seven novel single nucleotide polymorphisms in the human *SLC22A1* gene encoding organic cation transporter 1 (OCT1). *Drug Metab. Pharmacokinet.* (2004) 19:308-312.

109. SHU Y, LEABMAN MK, FENG B *et al.*; PHARMACOGENETICS OF MEMBRANE TRANSPORTERS INVESTIGATORS: Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. *Proc. Natl. Acad. Sci. USA* (2003) 100:5902-5907.

110. KERB R, BRINKMANN U, CHATSKAIA N *et al.*: Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences. *Pharmacogenetics* (2002) 12:591-595.

111. FUKUSHIMA-UESAKA H, MAEKAWA K, OZAWA S *et al.*: Fourteen novel single nucleotide polymorphisms in the *SLC22A2* gene encoding human organic cation transporter (OCT2). *Drug Metab. Pharmacokinet.* (2004) 19:239-244.

112. LEABMAN MK, HUANG CC, KAWAMOTO M *et al.*; PHARMACOGENETICS OF MEMBRANE TRANSPORTERS INVESTIGATORS: Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. *Pharmacogenetics* (2002) 12:395-405.

113. SAITO S, IIDA A, SEKINE A *et al.*: Catalog of 238 variations among six human genes encoding solute carriers (hSLCs) in the Japanese population. *J. Hum. Genet.* (2002) 47:576-584.

114. SAKAEDA T: MDR1 genotype-related pharmacokinetics: fact or fiction? *Drug Metab. Pharmacokinet.* (2005) 20:391-414.

115. PAULI-MAGNUS C, KROETZ DL: Functional implications of genetic polymorphisms in the multidrug resistance gene *MDR1* (*ABCB1*). *Pharm. Res.* (2004) 21:904-913.

• **A balanced overview of the *ABCB1* gene polymorphism.**

116. MARZOLINI C, PAUS E, BUCLIN T, KIM RB: Polymorphisms in human *MDR1* (P-glycoprotein): recent advances and clinical relevance. *Clin. Pharmacol. Ther.* (2004) 75:13-33.

• **A comprehensive review of the *ABCB1* polymorphism including discussion of the possible reasons for conflicting results among studies.**

117. SAKAEDA T, NAKAMURA T, HORINOUCHI M *et al.*: MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm. Res.* (2001) 18:1400-1404.

118. KURATA Y, IEIRI I, KIMURA M *et al.*: Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin. Pharmacol. Ther.* (2002) 72:209-219.

119. JOHNE A, KOPKE K, GERLOFF T *et al.*: Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein *MDR1* gene. *Clin. Pharmacol. Ther.* (2002) 72:584-594.

120. WANG D, JOHNSON AD, PAPP AC, KROETZ DL, SADEE W: Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C > T affects mRNA stability. *Pharmacogenet. Genomics* (2005) 15:693-704.

121. HIROTA T, IEIRI I, TAKANE H *et al.*: Allelic expression imbalance of the human *CYP3A4* gene and individual phenotypic status. *Hum. Mol. Genet.* (2004) 13:2959-2969.

122. WOJNOWSKI L, BROCKMOLLER J: Single nucleotide polymorphism characterization by mRNA expression imbalance assessment. *Pharmacogenetics* (2004) 14:267-269.

123. BRUNNER M, LANGER O, SUNDER-PLASSMANN R *et al.*: Influence of functional haplotypes in the drug transporter gene *ABCB1* on central nervous system drug distribution in humans. *Clin. Pharmacol. Ther.* (2005) 78:182-190.

124. HENDRIKSE NH, DE VRIES EG, ERIKS-FLUKS L *et al.*: A new *in vivo* method to study P-glycoprotein transport in tumors and the blood–brain barrier. *Cancer Res.* (1999) 59:2411-2416.

125. SPARREBOOM A, GELDERBLOM H, MARSH S *et al.*: Diflomotecan pharmacokinetics in relation to *ABCG2* 421C > A genotype. *Clin. Pharmacol. Ther.* (2004) 76:38-44.

• **First evidence of *in vivo* (human) function of the *ABCG2* gene polymorphism.**

126. IMAI Y, NAKANE M, KAGE K *et al.*: C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. *Mol. Cancer Ther.* (2002) 1:611-616.

127. KONDO C, SUZUKI H, ITODA M *et al.*: Functional analysis of SNPs variants of BCRP/ABCG2. *Pharm. Res.* (2004) 21:1895-1903.

128. SPARREBOOM A, LOOS WJ, BURGER H *et al.*: Effect of *ABCG2* genotype on the oral bioavailability of topotecan. *Cancer Biol. Ther.* (2005) 4:650-658.

129. DE JONG FA, MARSH S, MATHIJSSEN RH *et al.*: *ABCG2* pharmacogenetics: ethnic differences in allele frequency and assessment of influence on irinotecan disposition. *Clin. Cancer Res.* (2004) 10:5889-5894.

130. OLOMBO S, SORANZO N, ROTGER M *et al.*: Influence of *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* heplotypes on the cellular exposure of nelfinavir *in vivo*. *Pharmacogenet. Genomics* (2005) 15:599-608.

131. SEKINO H, ONOSHI T, SEKINO H: Phase I study of ZD-4522 (rosuvastatin), a new HMG-CoA reductase inhibitor-evaluation of tolerance and pharmacokinetics in healthy adult male volunteers after single and repeated oral administration. *J. Clin. Ther. Med.* (2005) 21:187-203.

132. WARWICK MJ, DANE AL, RAZA A, ACHNECK DW: Single and multiple-dose pharmacokinetics and safety of the new HMG-CoA reductase inhibitor ZD-4522 [abstract]. *Atherosclerosis* (2000) 151:39.

133. MARTIN PD, MITCHELL PD, SCHNECK DW: Pharmacodynamic effects and pharmacokinetics of a new HMG-CoA reductase inhibitor, rosuvastatin, after morning or evening administration in healthy volunteers. *Br. J. Clin. Pharmacol.* (2002) 54:472-477.

134. SIMONSON SG, RAZA A, MARTIN PD *et al.*: Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. *Clin. Pharmacol. Ther.* (2004) 76:167-177.

135. LEE E, RYAN S, BIRMINGHAM B *et al.*: Rosuvastatin pharmacokinetics and pharmacogenetics in white and Asian subjects residing in the same environment. *Clin. Pharmacol. Ther.* (2005) 78:330-341.

136. TIRONA RG: Ethnic differences in statin disposition. *Clin. Pharmacol. Ther.* (2005) 78:311-316.

137. NIEMI M, KIVISTO KT, HOFMANN U, SCHWAB M, EICHELBAUM M, FROMM MF: Fexofenadine pharmacokinetics are associated with a polymorphism of the *SLCO1B1* gene (encoding OATP1B1). *Br. J. Clin. Pharmacol.* (2005) 59:602-604.

138. NIEMI M, BACKMAN JT, KAJOSAARI LI *et al.*: Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin. Pharmacol. Ther.* (2005) 77:468-478.

139. MAEDA K, IEIRI I, YASUDA K *et al.*: Effects of OATP1B1 haplotype on pharmacokinetics of pravastatin, valsartan and temocapril. *Clin. Pharmacol. Ther.* (2006) 79:427-439.

140. KAMEYAMA Y, YAMASHITA K, KOBAYASHI K, HOSOKAWA M, CHIBA K: Functional characterization of *SLCO1B1* (*OATP-C*) variants, *SLCO1B1*5*, *SLCO1B1*15* and *SLCO1B1*15+C1007G*, by using transient expression systems of HeLa and HEK293 cells. *Pharmacogenet. Genomics* (2005) 15:513-522.

141. IWAI M, SUZUKI H, IEIRI I, OTSUBO K, SUGIYAMA Y: Functional analysis of single nucleotide polymorphisms of hepatic organic anion transporter OATP1B1 (OATP-C). *Pharmacogenet. Genomics* (2004) 14:749-757.

142. LEABMAN M, BROWN C, CHUNG J *et al.*: Heritability of metformin renal clearance. *Clin. Pharmacol. Ther* (2005) 77:P61.

143. FELLAY J, MARZOLINI C, MEADEN ER *et al.*: Swiss HIV Cohort Study. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* (2002) 359:30-36.

144. HAAS DW, SMEATON LM, SHAFER RW *et al.*: Pharmacogenetics of long-term responses to antiretroviral regimens containing efavirenz and/or nelfinavir: an Adult Aids Clinical Trials Group Study. *J. Infect. Dis.* (2005) 192:1931-1942.

145. NASI M, BORGHI V, PINTI M *et al.*: MDR1 C3435T genetic polymorphism does not influence the response to antiretroviral therapy in drug-naive HIV-positive patients. *AIDS* (2003) 17:1696-1698.

146. WINZER R, LANGMANN P, ZILLY M *et al.*: No influence of the P-glycoprotein polymorphisms MDR1 G2677T/A and C3435T on the virological and immunological response in treatment naive HIV-positive patients. *Ann. Clin. Microbiol. Antimicrob.* (2005) 4:1-7.

147. LOSCHER W, POTSCHKA H: Role of multidrug transporters in pharmacoresistance to antiepileptic drugs. *J. Pharmacol. Exp. Ther.* (2002) 301:7-14.

148. TISHLER DM, WEINBERG KI, HINTON DR, BARBARO N, ANNETT GM, RAFFEL C: *MDR1* gene expression in brain of patients with medically intractable epilepsy. *Epilepsia* (1995) 36:1-6.

149. SISODIYA SM, LIN WR, SQUIER MV, THOM M: Multidrug-resistance protein 1 in focal cortical dysplasia. *Lancet* (2001) 357:42-43.

150. SISODIYA SM, LIN WR, HARDING BN, SQUIER MV, THOM M: Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. *Brain* (2002) 125:22-31.

151. DOMBROWSKI SM, DESAI SY, MARRONI M *et al.*: Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. *Epilepsia* (2001) 42:1501-1506.

152. SIDDIQUI A, KERB R, WEALE MR *et al.*: Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene *ABCB1*. *N. Engl. J. Med.* (2003) 348:1442-1448.

153. TAN NC, HERON SE, SCHEFFER IE *et al.*: Failure to confirm association of a polymorphism in *ABCB1* with multidrug-resistant epilepsy. *Neurology* (2004) 63:1090-1092.

154. SILLS GJ, MOHANRAJ R, BUTLER E *et al.*: Lack of association between the C3435T polymorphism in the human multidrug resistance (*MDR1*) gene and response to antiepileptic drug treatment. *Epilepsia* (2005) 46:643-647.

155. ILLMER T, SCHULER US, THIEDW C *et al.*: *MDR1* gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res.* (2002) 62:4955-4962.

156. VAN DEN HEUVEL-EIBRINK MM, WIEMER EA, DE BOEVERE MJ *et al.*: *MDR1* gene-related clonal selection and P-glycoprotein function and expression in relapsed or refractory acute myeloid leukemia. *Blood* (2001) 97:3605-3611.

157. EFFERTH T, SAUERBREY A, STEINBACH D *et al.*: Analysis of single nucleotide polymorphism C3435T of the multidrug resistance gene *MDR1* in acute lymphoblastic leukemia. *Int. J. Oncol.* (2003) 23:509-517.

158. GOREVA OB, GRISHANOVA AY, MUKHIN OV, DOMNIKOVA NP, LYAKHOVICH VV: Possible prediction of the efficiency of chemotherapy in patients with lymphoproliferative diseases based on *MDR1* gene G2677T and C3435T polymorphisms. *Bull. Exp. Biol. Med.* (2003) 136:183-185.

159. ISLA D, SARRIES C, ROSELL R *et al.*: Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. *Ann. Oncol.* (2004) 15:1194-1203.

160. PLASSCHAERT SL, GRONINGER E, BOEZEN M *et al.*: Influence of functional polymorphisms of the *MDR1* gene on vincristine pharmacokinetics in childhood acute lymphoblastic leukemia. *Clin. Pharmacol. Ther.* (2004) 76:220-229.

161. YAMAUCHI A, IEIRI I, KATAOKA Y *et al.*: Neurotoxicity induced by tacrolimus after liver transplantation: relation to genetic polymorphisms of the *ABCB1* (*MDR1*) gene. *Transplantation* (2002) 74:571-572.

162. HAUSER IA, SCHAEFFELER E, GAUER S *et al.*: *ABCB1* genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. *J. Am. Soc. Nephrol.* (2005) 16:1501-1511.

163. DROZDZIK M, MYSLIWIEC K, LEWINSKA-CHELSTOWSKA M, BANACH J, DROZDZIK A, GRABAREK J: P-glycoprotein drug transporter *MDR1* gene polymorphism in renal transplant patients with and without gingival overgrowth. *J. Clin. Periodontol.* (2004) 31:758-763.

164. FURUNO T, LANDI MT, CERONI M *et al.*: Expression polymorphism of the blood–brain barrier component P-glycoprotein (MDR1) in relation to Parkinson's disease. *Pharmacogenetics* (2002) 12:529-534.

165. DROZDZIK M, BIALECKA M, MYSLIWIEC K, HONCZARENKO K, STANKIEWICZ J, SYCH Z: Polymorphism in the P-glycoprotein drug transporter MDR1 gene: a possible link between environmental and genetic factors in Parkinson's disease. *Pharmacogenetics* (2003) 13:259-263.

166. TAN EK, DROZDZIK M, BIALECKA M *et al.*: Analysis of *MDR1* haplotypes in Parkinson's disease in a white population. *Neurosci. Lett.* (2004) 372:240-244.

167. TAN EK, CHAN DK, NG PW *et al.*: Effect of *MDR1* haplotype on risk of Parkinson's disease. *Arch. Neurol.* (2005) 62:460-464.

168. SCHWAB M, SCHAEFFELER E, MARX C *et al.*: Association between the C3435T *MDR1* gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology* (2003) 124:26-33.

169. CROUCHER PJ, MASCHERETTI S, FOELSCH UR, HAMPE J, SCHREIBER S: Lack of association between the C3435T *MDR1* gene polymorphism and inflammatory bowel disease in two independent Northern European populations. *Gastroenterology* (2003) 125:1919-1920.

170. BRANT SR, PANHUYSEN CI, NICOLAE D *et al.*: *MDR1* Ala893 polymorphism is associated with inflammatory bowel disease. *Am. J. Hum. Genet.* (2003) 73:1282-1292.

171. GAZOULI M, ZACHARATOS P, GORGOULIS V, MANTZARIS G, PAPALAMBROS E, IKONOMOPOULOS J: The C3435T *MDR1* gene polymorphism is not associated with susceptibility for ulcerative colitis in Greek population. *Gastroenterology* (2004) 126:367-369.

172. GLAS J, TOROK HP, SCHIEMANN U, FOLWACZNY C: *MDR1* gene polymorphism in ulcerative colitis. *Gastroenterology* (2004) 126:367.

173. POTOCNIK U, FERKOLJ I, GLAVAC D, DEAN M: Polymorphisms in multidrug resistance 1 (*MDR1*) gene are associated with refractory Crohn disease and ulcerative colitis. *Genes Immun.* (2004) 5:530-539.

174. HO GT, NIMMO ER, TENESA A *et al.*: Allelic variations of the multidrug resistance gene determine susceptibility and disease

behavior in ulcerative colitis. *Gastroenterology* (2005) **128**:288-296.

175. URCELAY E, MENDOZA JL, MARTIN MC *et al.*: *MDR1* gene: susceptibility in Spanish Crohn's disease and ulcerative colitis patients. *Inflamm. Bowel Dis.* (2006) **12**:33-37.
176. JAMROZIAK K, MLYNARSKI W, BALCERCZAK E *et al.*: Functional C3435T polymorphism of *MDR1* gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. *Eur. J. Haematol.* (2004) 72:314-321.
177. STANULLA M, SCHAFFELER E, ARENS S *et al.*: *GSTP1* and *MDR1* genotypes and central nervous system relapse in childhood acute lymphoblastic leukemia. *Int. J. Hematol.* (2005) **81**:39-44.
178. POTOCNIK U, RAVNIK-GLAVAC M, GOLOUH R, GLAVAC D: Naturally occurring mutations and functional polymorphisms in multidrug resistance 1 gene: correlation with microsatellite instability and lymphoid infiltration in colorectal cancers. *J. Med. Genet.* (2002) 39:340-346.
179. KURZAWSKI M, DROZDZIK M, SUCHY J *et al.*: Polymorphism in the P-glycoprotein drug transporter *MDR1* gene in colon cancer patients. *Eur. J. Clin. Pharmacol.* (2005) **61**:389-394.
180. HUMENY A, RODEL F, RODEL C *et al.*: *MDR1* single nucleotide polymorphism C3435T in normal colorectal tissue and colorectal carcinomas detected by MALDI-TOF mass spectrometry. *Anti-Cancer Res.* (2003) 23:2735-2740.
181. SIEGSMUND M, BRINKMANN U, SCHAFFELER E *et al.*: Association of the P-glycoprotein transporter *MDR1*(C3435T) polymorphism with the susceptibility to renal epithelial tumors. *J. Am. Soc. Nephrol.* (2002) **13**:1847-1854.
182. MILLER KL, KELSEY KT, WIENCKE JK *et al.*: The C3435T polymorphism of *MDR1* and susceptibility to adult glioma. *Neuroepidemiology* (2005) 25:85-90.
183. KIMURA Y, SELMI C, LEUNG PS *et al.*: Genetic polymorphisms influencing xenobiotic metabolism and transport in patients with primary biliary cirrhosis. *Hepatology* (2005) **41**:55-63.
184. PAWLIK A, WRZESNIEWSKA J, FIEDOROWICZ-FABRYCY I, GAWRONSKA-SZKLARZ B: The *MDR1* 3435 polymorphism in patients with rheumatoid arthritis. *Int. J. Clin. Pharmacol. Ther.* (2004) 42:496-503.
185. KIVISTO KT, NIEMI M, SCHAEFFELER E *et al.*: *CYP3A5* genotype is associated with diagnosis of hypertension in elderly patients: data from the DEBATE Study. *Am. J. Pharmacogenomics* (2005) 5:191-195.
186. LENNERNAS H, FAGER G: Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences. *Clin. Pharmacokinet.* (1997) 32:403-425.
187. TOBERT JA: Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nat. Rev. Drug Discov.* (2003) 2:517-526.
188. TACHIBANA-IIMORI R, TABARA Y, KUSUHARA H *et al.*: Effect of genetic polymorphism of OATP-C (*SLCO1B1*) on lipid-lowering response to HMG-CoA reductase inhibitors. *Drug Metab. Pharmacokinet.* (2004) 19:375-380.
189. NIEMI M, NEUVONEN PJ, HOFMANN U *et al.*: Acute effects of pravastatin on cholesterol synthesis are associated with *SLCO1B1* (encoding OATP1B1) haplotype **17*. *Pharmacogenet. Genomics* (2005) 15:303-309.
- **Pharmacodynamic consequence of *SLCO1B1* variants on cholesterol synthesis in humans.**
190. HIRANO M, MAEDA M, MATSUSHIMA S, NOZAKI Y, KUSUHARA H, SUGIYAMA Y: Involvement of BCRP (ABCG2) in the biliary excretion of pitavastatin. *Mol. Pharmacol.* (2005) 68:800-807.
191. VERSTUYFT C, SCHWAB M, SCHAEFFELER E *et al.*: Digoxin pharmacokinetics and *MDR1* genetic polymorphisms. *Eur. J. Clin. Pharmacol.* (2003) 58:809-812.
192. GERLOFF T, SCHAEFER M, JOHNE A *et al.*: *MDR1* genotypes do not influence the absorption of a single oral dose of 1 mg digoxin in healthy white males. *Br. J. Clin. Pharmacol.* (2002) 54:610-616.
193. YI SY, HONG KS, LIM HS *et al.*: A variant 2677A allele of the *MDR1* gene affects fexofenadine disposition. *Clin. Pharmacol. Ther.* (2004) 76:418-427.
194. DRESCHER S, SCHAEFFELER E, HITZL M *et al.*: *MDR1* gene polymorphisms and disposition of the P-glycoprotein substrate fexofenadine. *Br. J. Clin. Pharmacol.* (2002) 53:526-534.
195. VON AHSEN N, RICHTER M, GRUPP C, RINGE B, OELLERICH M, ARMSTRONG VW: No influence of the *MDR-1* C3435T polymorphism or a CYP3A4 promoter polymorphism (CYP3A4-V allele) on dose-adjusted cyclosporin A trough concentrations or rejection incidence in stable renal transplant recipients. *Clin. Chem.* (2001) 47:1048-1052.
196. MIN DI, ELLINGROD VL: C3435T mutation in exon 26 of the human *MDR1* gene and cyclosporine pharmacokinetics in healthy subjects. *Ther. Drug Monit.* (2002) 24:400-404.
197. YATES CR, ZHANG W, SONG P *et al.*: The effect of *CYP3A5* and *MDR1* polymorphic expression on cyclosporine oral disposition in renal transplant patients. *J. Clin. Pharmacol.* (2003) 43:555-564.
198. BONHOMME-FAIVRE L, DEVOCELLE A, SALIBA F *et al.*: *MDR-1* C3435T polymorphism influences cyclosporine a dose requirement in liver-transplant recipients. *Transplantation* (2004) 78:21-25.
199. MACPHEE IA, FREDERICKS S, TAI T *et al.*: Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome P4503A5 and P-glycoprotein correlate with dose requirement. *Transplantation* (2002) 74:1486-1489.
200. ZHENG H, WEBBER S, ZEEVI A *et al.*: Tacrolimus dosing in pediatric heart transplant patients is related to *CYP3A5* and *MDR1* gene polymorphisms. *Am. J. Transplant.* (2003) 3:477-483.
201. HAUFROID V, MOURAD M, VAN KERCKHOVE V *et al.*: The effect of *CYP3A5* and *MDR1* (*ABCB1*) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics* (2004) 14:147-154.
202. SIEGMUND W, LUDWIG K, GIESSMANN T *et al.*: The effects of the human MDR1 genotype on the expression of duodenal P-glycoprotein and disposition of the probe drug talinolol. *Clin. Pharmacol. Ther.* (2002) 72:572-583.
203. ZHANG WX, CHEN GL, ZHANG W *et al.*: MDR1 genotype do not influence the absorption of a single oral dose of 100 mg talinolol in healthy Chinese males. *Clin. Chim. Acta.* (2005) 359:46-52.
204. RODRIGUEZ NOVOA S, BARREIRO P, RENDON A *et al.*: Plasma levels of

atazanavir and the risk of hyperbilirubinemia are predicted by the 3435C→T polymorphism at the multidrug resistance gene 1. *Clin. Infect. Dis.* (2006) 42:291-295.

205. PAULI-MAGNUS C, FEINER J, BRETT C *et al.*: No effect of *MDR1* C3435T variant on loperamide disposition and central nervous system effects. *Clin. Pharmacol. Ther.* (2003) 74:487-498.

206. PUTNAM WS, WOO JM, HUANG Y, BENET LZ: Effect of the *MDR1* C3435T variant and P-glycoprotein induction on dicloxacillin pharmacokinetics. *J. Clin. Pharmacol.* (2005) 45:411-421.

207. KERB R, AYNACIOGLU AS, BROCKMOLLER J *et al.*: The predictive value of *MDR1*, *CYP2C9*, and *CYP2C19* polymorphisms for phenytoin plasma levels. *Pharmacogenomics J.* (2001) 1:204-210.

• **This paper offers a new approach, the gene-network trial (multiple gene analysis), for the prediction of individual phenytoin disposition.**

208. YASUI-FURUKORI N, MIHARA K, TAKAHATA T *et al.*: Effects of various factors on steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone: lack of impact of *MDR-1* genotypes. *Br. J. Clin. Pharmacol.* (2004) 57:569-575.

209. HORINOUCHI M, SAKAEDA T, NAKAMURA T *et al.*: Significant genetic linkage of *MDR1* polymorphisms at positions 3435 and 2677: functional relevance to pharmacokinetics of digoxin. *Pharm. Res.* (2002) 19:1581-1585.

210. ANGLICHEAU D, VERSTUYFT C, LAURENT-PUIG P *et al.*: Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. *J. Am. Soc. Nephrol.* (2003) 14:1889-1896.

211. ROBERTS RL, JOYCE PR, MULDER RT, BEGG EJ, KENNEDY MA: A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. *Pharmacogenomics J.* (2002) 2:191-196.

212. BRUMME ZL, DONG WW, CHAN KJ *et al.*: Influence of polymorphisms within the *CX3CR1* and *MDR-1* genes on initial antiretroviral therapy response. *AIDS* (2003) 17:201-208.

213. ALONSO-VILLAVERDE C, COLL B, GOMEZ F *et al.*: The efavirenz-induced increase in HDL-cholesterol is influenced by the multidrug resistance gene 1 C3435T polymorphism. *AIDS* (2005) 19:341-342.

214. ZHENG H, WEBBER S, ZEEVI A *et al.*: The *MDR1* polymorphisms at exons 21 and 26 predict steroid weaning in pediatric heart transplant patients. *Hum. Immunol.* (2002) 63:765-770.

215. KOTRYCH K, DOMANSKI L, GORNIK W, DROZDZIK M: *MDR1* gene polymorphism in allogeenic kidney transplant patients with tremor. *Pharmacol. Rep.* (2005) 57:241-245.

216. ZHENG HX, ZEEVI A, MCCURRY K *et al.*: The impact of pharmacogenomic factors on acute persistent rejection in adult lung transplant patients. *Transpl. Immunol.* (2005) 14:37-42.

217. KAFKA A, SAUER G, JAEGER C *et al.*: Polymorphism C3435T of the *MDR-1* gene predicts response to preoperative chemotherapy in locally advanced breast cancer. *Int. J. Oncol.* (2003) 22:1117-1121.

218. BABAOGLU MO, BAVAR B, AYNACIOGLU AS *et al.*: Association of the *ABCB1* 3435C > T polymorphism with antiemetic efficacy of 5-hydroxytryptamine type 3 antagonists. *Clin. Pharmacol. Ther.* (2005) 78:619-626.

219. YASUI-FURUKORI N, SAITO M, NAKAGAMI T, KANEDA A, TATEISHI T, KANEKO S: Association between multidrug resistance 1 (*MDR1*) gene polymorphisms and therapeutic response to bromperidol in schizophrenic patients: a preliminary study. *Prog. Neuropsychopharmacol. Biol. Psychiatry* (2006) 30(2):286-291.

220. MORIMOTO K, UEDA S, SEKI N *et al.*: Candidate gene approach for the study of genetic factors involved in HMG-CoA reductase inhibitor-induced rhabdomyolysis. *18th JSSX annual meeting.* Sapporo, Japan. 8PE-32 (2003).

Affiliation

Ichiro Ieiri[†1] PhD, Hiroshi Takane[2] PhD, Takeshi Hirota[3] PhD, Kenji Otsubo[4] PhD & Shun Higuchi[5] PhD

[†]Author for correspondence

[1]Associate Professor, Kyushu University, Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Maidashi 3-1-1, Higashi-ku, Fukuoka, 812-8582, Japan

Tel: +81 92 642 6657; Fax: +81 92 642 6660; E-mail: ieiri-ttr@umin.ac.jp

[2]Chief Pharmacist, Tottori University Hospital, Department of Clinical Pharmacy, Yonago, 683-8504, Japan

[3]Assistant Professor, Kyushu University, Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Maidashi 3-1-1, Higashi-ku, Fukuoka, 812-8582, Japan

[4]Professor, Tottori University Hospital, Department of Clinical Pharmacy, Yonago, 683-8504, Japan

[5]Professor, Kyushu University, Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Maidashi 3-1-1, Higashi-ku, Fukuoka, 812-8582, Japan

Pharmacogenetic Characterization of Sulfasalazine Disposition Based on *NAT2* and *ABCG2* (BCRP) Gene Polymorphisms in Humans

Y Yamasaki[1], I Ieiri[1], H Kusuhara[2], T Sasaki[1], M Kimura[3], H Tabuchi[1], Y Ando[1], S Irie[3], JA Ware[4], Y Nakai[5], S Higuchi[1] and Y Sugiyama[2]

The role of breast cancer resistance protein (BCRP), an efflux ABC transporter, in the pharmacokinetics of substrate drugs in humans is unknown. We investigated the impact of genetic polymorphisms of *ABCG2* (421C>A) and *NAT2* on the pharmacokinetics of sulfasalazine (SASP), a dual substrate, in 37 healthy volunteers, taking 2,000 mg of conventional SASP tablets. In *ABCG2*, SASP AUC_{0-48} of C/C, C/A, and A/A subjects was 171 ± 85, 330 ± 194, and 592 ± 275 μg h/ml, respectively, with significant differences among groups. In contrast, AUC_{0-48} of sulfapyridine (SP) tended to be lower in subjects with the *ABCG2-A* allele as homozygosity. In *NAT2*, AUC_{AcSP}/AUC_{SP} was significantly higher in rapid than in intermediate and slow acetylator (SA) genotypes. We successfully described the pharmacokinetics of SASP, SP, and *N*-acetylsulfapyridine (AcSP) simultaneously by nonlinear mixed-effects modeling (NONMEM) analysis with regard to both gene polymorphisms. The data indicate that SASP is a candidate probe of BCRP, particularly in its role in intestinal absorption.

INTRODUCTION

Sulfasalazine (SASP) has long been used in the treatment of inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, and rheumatoid disease.[1,2] After oral administration, SASP breaks down into sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA) by bacterial azo reductases in the colon and cecum (Figure 1).[3,4] 5-ASA is suggested to be effective for inflammatory bowel diseases, while SASP and SP are effective for rheumatoid disease.[5–7] In contrast to 5-ASA, the systemic uptake of SP is virtually complete, and it is further metabolized by polymorphic *N*-acetyltransferase 2 (NAT2) into *N*-acetylsulfapyridine (AcSP) in the liver.[7–10]

The *NAT2* gene, located on chromosome 8p22, demonstrates large interindividual variability in acetylating activity by genetic polymorphism in humans.[11–15] At present, at least 35 variants have been identified,[16] and some of these mutant alleles show low acetylating activities, leading to adverse reactions of SP, such as blood disorders, including thrombocytopenia and reticulocytosis, and hepatic disorders.[17–19] *NAT2*5B*, *NAT2*6A*, and *NAT2*7B* are principally important mutant alleles, and the Japanese population can be stratified by genotyping tests into three groups: rapid acetylators (RAs), intermediate acetylators (IAs), and slow acetylators (SAs).[12–14] Low acetylating activities of SP to AcSP cause higher plasma concentrations of SP, which increase the possibility of adverse reactions in SA compared to RA; higher incidences of nausea/vomiting and hepatic disorders were reported in SA subjects.[17–19] Association between *NAT2* polymorphism (*NAT2*7B*) and Crohn's disease has also been reported in Japanese patients.[20] Although genotyping of *NAT2* provides useful information about the individual metabolic capacity of SP, the reason(s) for substantial interindividual variabilities in pharmacokinetic/pharmacodynamic profiles of SASP, a parent drug of SP, have not yet been elucidated.[10] The individual variability of bacterial azo reductase activity is speculated to affect the degradation of SASP.[3,4,17]

Breast cancer resistance protein (BCRP, *ABCG2* gene) is an efflux ABC transporter, which is expressed at the apical membrane in the placenta (trophoblast cells), liver (bile

[1]Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan; [3]Kyushu Clinical Pharmacology Research Clinic, Fukuoka, Japan; [4]Department of Pharmacokinetics, Dynamics, and Metabolism, Pfizer Global Research and Development, Michigan Labs, Ann Arbor, Michigan, USA; [5]Department of Pharmacokinetics, Dynamics, and Metabolism, Pfizer Global Research and Development, Nagoya Labs, Taketoyo, Japan.
Correspondence: Y Sugiyama (sugiyama@mol.f.u-tokyo.ac.jp)

Received 5 July 2007; accepted 25 October 2007; advance online publication 2 January 2008. doi:10.1038/sj.clpt.6100459

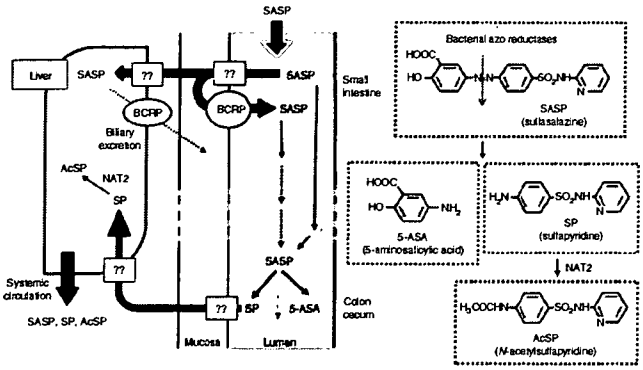

Figure 1 The possible biological fates of orally administered sulfasalazine (SASP) (in a conventional tablet form) in humans. After absorption from the luminal side, SASP is excreted into the luminal side again by breast cancer resistance protein (BCRP), which is expressed at the apical membrane of enterocytes (BCRP restricts intestinal SASP absorption). The remaining SASP travels down the small intestine to the lower intestinal tract where its diazo-bond is cleaved by colonic bacteria with the liberation of sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA). SP is almost totally absorbed from the colon and cecum, and then metabolized into *N*-acetylsulfapyridine (AcSP) by *N*-acetyltransferase 2 (NAT2), predominantly in the liver.

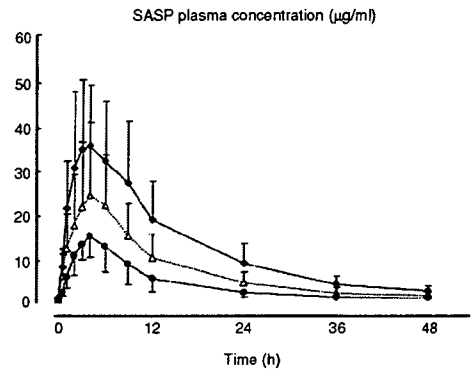

Figure 2 Effect of *ABCG2* genotype on pharmacokinetics of sulfasalazine (SASP). Plasma concentration-time profiles of SASP after oral administration of a 2,000 mg conventional SASP tablet to 421C/C subjects (closed circles, $n = 12$), 421C/A subjects (open triangles, $n = 16$), and 421A/A subjects (closed diamonds, $n = 9$).

canalicular membrane of hepatocytes), kidney, and intestine (enterocytes),[21–25] and is involved in the absorption and excretion of various drugs such as topotecan,[26] diflomotecan,[27] and rosuvastatin.[28] Recently, Zaher *et al.*[29] reported that BCRP (*ABCG2*) is an important determinant of oral bioavailability and the elimination of SASP in mice, and that SASP has the potential to be utilized as a specific *in vivo* probe of BCRP. They showed that after oral administration of SASP, the area under the plasma concentration (AUC) time profile in $BCRP^{-/-}$ mice was >100 times higher than that in **FVB** wild-type (WT) mice, and that after intravenous administration, the AUC of SASP in $BCRP^{-/-}$ mice was ~13-fold higher than that in WT mice.

Similar to the *NAT2* gene, different variants have been identified in the *ABCG2* gene. Among them, two frequent nonsynonymous variants, 34G>A (12Val>Met in exon 2) and 421C>A (141Gln>Lys in exon 5), are of interest. Pharmacokinetic profiles of topotecan,[26] diflomotecan,[27] and rosuvastatin[28] are significantly different among *ABCG2* genotyping groups. Recent clinical studies demonstrated that *ABCG2* 421C>A polymorphism was associated with gefitinib accumulation at steady state,[30] leading to an increased risk of diarrhea.[31] In contrast to these drugs, however, no remarkable change in pitavastatin pharmacokinetics has recently been reported,[32] suggesting that the effect of the *ABCG2* polymorphism underlies the substrate-dependent activity.

In this study, we demonstrate the synchronized contribution of drug transporter and metabolism gene polymorphisms to the pharmacokinetics of SASP in healthy subjects, who were selected from our study panels based on their genotypes of *ABCG2* (421C>A) and *NAT2* (*NAT2**4, *5B, *6A, and *7B).

RESULTS

No clinically undesirable signs and symptoms possibly attributed to the administration of SASP were recognized throughout the study. All subjects completed the study successfully according to the protocol.

SASP pharmacokinetics in relation to *ABCG2* and *NAT2* genotypic status

After oral administration, the mean plasma concentrations of SASP were significantly higher ($P < 0.01$) in *ABCG2-A/A* subjects than in *ABCG2-C/C* subjects, and *ABCG2-C/A* subjects had values between those in *ABCG2-A/A* and *ABCG2-C/C* subjects at all observation points except 0.5 h (**Figure 2**). The mean (± SD) AUC_{0-48} of SASP in *ABCG2-C/C* ($n = 12$), *ABCG2-C/A* ($n = 16$), and *ABCG2-A/A* ($n = 9$) subjects was 171 ± 85, 330 ± 194, and 592 ± 275 μg h/ml, respectively, and significant differences ($P < 0.05$) were observed among all three groups (**Table 1**). Similarly, the mean peak plasma concentration (Cmax) in *ABCG2-C/C*, *ABCG2-C/A*, and *ABCG2-A/A* subjects was 15.4 ± 5.2, 26.4 ± 16.1, and 40.7 ± 13.5 μg/ml, respectively, with significant differences among all three groups. In contrast, the mean CL_{total}/F in *ABCG2-C/C*, *ABCG2-C/A*, and *ABCG2-A/A* subjects was 13.7 ± 5.8, 7.3 ± 3.0, and 3.7 ± 1.3 l/h, respectively.

For combined *ABCG2* and *NAT2* genotypes, the number of patients within each combination of genotypes and pharmacokinetic parameters are summarized in **Table 2**. The mean AUC_{0-48} of SASP in groups RA-C/C, C/A, and A/A was 146 ± 43, 282 ± 78, and 742 ± 469, and in groups IA-C/C, C/A, and A/A was 171 ± 80, 310 ± 151, and 517 ± 113 μg h/ml, respectively; the mean value in *ABCG2-A/A* subjects was significantly higher than that in subjects C/A and C/C in both RA and IA. A similar trend was observed in C_{max} values. In contrast, mean CL_{total}/F in groups RA-C/C, C/A, and A/A was 14.4 ± 4.5, 7.3 ± 2.1, and 3.3 ± 1.9, and in groups IA-C/C, C/A, and A/A was 13.6 ± 6.8, 7.6 ± 3.8, and 3.9 ± 1.0 l/h, respectively; the mean value in *ABCG2-A/A* subjects was significantly lower than that in C/C subjects in both RA and IA.

The mean AUC_{0-48} of SASP in groups RA-C/C, IA-C/C, and SA-C/C was 146 ± 43, 171 ± 80, and 237, and in groups RA-C/A, IA-C/A, and SA-C/A was 282 ± 78, 310 ± 151, and 387 ± 289 μg h/ml, respectively; no significant intergenotypic

Table 1 Pharmacokinetic parameters of SASP after single oral dose of SASP with regard to the polymorphism of *ABCG2* gene

Genotype *ABCG2*	*n*	AUC_{0-48} (μg h/ml)	K_e (h^{-1})	CL_{total}/F (l/h)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/ml)
C/C	12	171 ± 85 (123 ~ 220)	0.08 ± 0.03 (0.07 ~ 0.10)	13.7 ± 5.8 (10.4 ~ 16.9)	9.8 ± 4.5 (7.2 ~ 12.3)	4.3 ± 0.9 (3.8 ~ 4.7)	15.4 ± 5.2 (12.4 ~ 18.4)
C/A	16	330 ± 194* (235 ~ 425)	0.07 ± 0.01 (0.06 ~ 0.07)	7.3 ± 3.0* (5.8 ~ 8.8)	10.5 ± 2.0 (9.5 ~ 11.5)	4.8 ± 1.6 (4.1 ~ 5.6)	26.4 ± 16.1* (18.5 ~ 34.3)
A/A	9	592 ± 275*,** (412 ~ 771)	0.07 ± 0.01 (0.06 ~ 0.07)	3.7 ± 1.3*,** (2.9 ~ 4.6)	10.3 ± 1.5 (9.4 ~ 11.3)	3.9 ± 2.2 (2.5 ~ 5.3)	40.7 ± 13.5*,** (31.8 ~ 49.5)

Each value represents the mean ± SD (95% confidence interval).

SASP, sulfasalazine (salazosulfapyridine).

*Significantly different from values in C/C subjects as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$). **Significantly different from values in C/A subjects as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$).

Table 2 Pharmacokinetic parameters of SASP after single oral dose of SASP with regard to the polymorphism of *ABCG2* and *NAT2* genes

Genotype *NAT2*	*ABCG2*	*n*	AUC_{0-48} (μg h/ml)	K_e (h^{-1})	CL_{total}/F (l/h)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/ml)
RA	C/C	5	146 ± 43 (108 ~ 183)	0.08 ± 0.02 (0.06 ~ 0.10)	14.4 ± 4.5 (10.5 ~ 18.3)	9.8 ± 4.3 (6.0 ~ 13.5)	3.8 ± 0.5 (3.4 ~ 4.2)	13.6 ± 2.6 (11.3 ~ 15.9)
	C/A	5	282 ± 78 (215 ~ 350)	0.07 ± 0.01 (0.06 ~ 0.08)	7.3 ± 2.1* (5.4 ~ 9.2)	10.5 ± 2.3 (8.5 ~ 12.4)	4.4 ± 1.5 (3.1 ~ 5.7)	23.4 ± 8.3 (16.1 ~ 30.7)
	A/A	3	742 ± 469*,** (212 ~ 1272)	0.07 ± 0.01 (0.06 ~ 0.08)	3.3 ± 1.9* (1.2 ~ 5.5)	10.4 ± 1.1 (9.1 ~ 11.6)	4.7 ± 3.8 (0.4 ~ 9.0)	44.4 ± 24.5*,** (16.7 ~ 72)
IA	C/C	5	171 ± 80 (101 ~ 241)	0.08 ± 0.03 (0.06 ~ 0.11)	13.6 ± 6.8 (7.6 ~ 19.5)	10.0 ± 5.9 (4.8 ~ 15.3)	4.4 ± 0.9 (3.6 ~ 5.2)	15.9 ± 6.4 (10.2 ~ 21.5)
	C/A	5	310 ± 151 (178 ~ 442)	0.07 ± 0.01 (0.06 ~ 0.07)	7.6 ± 3.8 (4.3 ~ 10.9)	10.5 ± 1.1 (9.5 ~ 11.5)	4.6 ± 1.3 (3.4 ~ 5.8)	22.6 ± 10.7 (13.2 ~ 32.0)
	A/A	6	517 ± 113*,** (426 ~ 607)	0.07 ± 0.01 (0.06 ~ 0.08)	3.9 ± 1.0* (3.1 ~ 4.7)	10.3 ± 1.7 (8.9 ~ 11.7)	3.2 ± 1.3 (2.1 ~ 4.2)	38.8 ± 6.3*,** (33.7 ~ 43.9)
SA	C/C	2	237	0.08	12	9.1	5	18.7
	C/A	6	387 ± 289 (156 ~ 618)	0.07 ± 0.01 (0.06 ~ 0.08)	6.9 ± 3.5 (4.1 ~ 9.8)	10.5 ± 2.5 (8.5 ~ 12.6)	4.8 ± 2.0 (3.2 ~ 6.5)	31.1 ± 24.0 (12.0 ~ 50.3)

Each value represents the mean ± SD (95% confidence interval).

IA, intermediate acetylator [*4/*6A ($n = 10$), *4/*7B ($n = 6$)]; RA, rapid acetylator (*4/*4); SA, slow acetylator [*6A/*6A ($n = 4$), *6A/*7B ($n = 4$)]; SASP, sulfasalazine (salazosulfapyridine).

*Significantly different from values in C/C subjects within the same NAT2 genotyping group as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$).
**Significantly different from values in C/A subjects within the same NAT2 genotyping group as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$).

difference was observed. Similar trends were observed in mean CL_{total}/F and C_{max} values, suggesting that *NAT2* polymorphism is not a determinant of the pharmacokinetics of SASP.

No intergenotypic differences were observed in mean elimination rate constant (K_e) and time to reach the maximum concentration (T_{max}) values.

SP and AcSP pharmacokinetics in relation to *ABCG2* and *NAT2* genotypic status

Pharmacokinetic parameters of SP after a single oral dose of SASP are summarized in Table 3. In contrast to SASP, the mean AUC_{0-48} of SP tended to be lower in *ABCG2-A/A* subjects; the order was *ABCG2-C/C* > C/A > A/A in both RA and IA types of the *NAT2* gene. A similar trend was observed in C_{max} values. The mean AUC_{0-48}, $t_{1/2}$, and C_{max} values of SP were significantly different among three *ABCG2-C/A* matched *NAT2* genotyping subjects; the order was SA > IA > RA, suggesting that pharmacokinetic profiles of SP depend both on *NAT2* and *ABCG2* polymorphisms.

Although the mean AUC_{0-48} and C_{max} of AcSP tended to be lower in *ABCG2-A/A* subjects, there were no significant differences in any of the pharmacokinetics parameters of AcSP among all *ABCG2* genotyping groups (Table 4).

With regard to the *NAT2* polymorphism alone, the mean AUC ratio of AcSP to SP (AUC_{AcSP}/AUC_{SP}) in RA, IA, and SA subjects was 3.1 ± 0.6, 1.9 ± 0.5, and 0.5 ± 0.4, respectively, with significant differences among all groups; however, the mean renal clearance (CL_r) value was comparable among all groups for both SP and AcSP (Table 5).

Simultaneous modeling and NONMEM analysis of SASP, SP, and AcSP in relation to *ABCG2* and *NAT2* polymorphisms

There were significant correlations between individual post-hoc prediction values and observed values (i.e., actual plasma

Table 3 Pharmacokinetic parameters of SP after single oral dose of SASP with regard to the polymorphism of *ABCG2* and *NAT2* genes

Genotype							
NAT2	*ABCG2*	*n*	AUC_{0-48} (μg h/ml)	K_e (h^{-1})	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/ml)
RA	C/C	5	140 ± 35 (109 ~ 171)	0.10 ± 0.01 (0.09 ~ 0.11)	7.2 ± 0.8 (6.5 ~ 7.9)	10.8 ± 2.7 (8.5 ~ 13.2)	6.3 ± 1.1 (5.3 ~ 7.3)
	C/A	5	134 ± 32* (106 ~ 162)	0.10 ± 0.02* (0.07 ~ 0.11)	7.9 ± 2.0* (6.2 ~ 9.7)	13.8 ± 5.8 (8.7 ~ 18.9)	5.7 ± 1.1* (4.7 ~ 6.6)
	A/A	3	84 ± 28 (53 ~ 116)	0.08 ± 0.03 (0.05 ~ 0.11)	9.2 ± 2.8 (6.1 ~ 12.3)	20.0 ± 6.9 (12.2 ~ 27.8)	3.7 ± 1.3**,*** (2.2 ~ 5.2)
IA	C/C	5	189 ± 55 (140 ~ 237)	0.06 ± 0.03 (0.04 ~ 0.09)	12.6 ± 5.0 (8.2 ~ 16.9)	21.6 ± 10 (12.8 ~ 30.4)	7.7 ± 3.1 (5.0 ~ 10.4)
	C/A	5	171 ± 82* (99 ~ 243)	0.08 ± 0.02* (0.06 ~ 0.09)	9.4 ± 1.6* (8.0 ~ 10.8)	13.8 ± 5.8 (8.7 ~ 18.9)	7.5 ± 3.5* (4.4 ~ 10.5)
	A/A	6	140 ± 58 (93 ~ 187)	0.07 ± 0.03 (0.04 ~ 0.09)	12.5 ± 6.0 (7.7 ~ 17.3)	18.0 ± 6.6 (12.7 ~ 23.3)	5.6 ± 2.3 (3.8 ~ 7.5)
SA	C/C	2	170	0.07	10.1	12.0	6.6
	C/A	6	357 ± 99 (278 ~ 437)	0.04 ± 0.02 (0.03 ~ 0.05)	19.4 ± 7.5 (13.4 ~ 25.4)	20.0 ± 6.2 (15.0 ~ 25.0)	12.7 ± 2.5 (10.7 ~ 14.7)

Each value represents the mean ± SD (95% confidence interval).

IA, intermediate acetylator [*4/*6A ($n = 10$), *4/*7B ($n = 6$)]; RA, rapid acetylator (*4/*4); SA, slow acetylator [*6A/*6A ($n = 4$), *6A/*7B ($n = 4$)]; SASP, sulfasalazine (salazosulfapyridine); SP, sulfapyridine.

*Significantly different from values in *SA-ABCG2-C/A* subjects as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$). **Significantly different from values in C/A subjects within the same *NAT2* genotyping group as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$). ***Significantly different from values in C/C subjects within the same *NAT2* genotyping group as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$).

Table 4 Pharmacokinetic parameters of AcSP after single oral dose of SASP with regard to the polymorphism of *ABCG2* and *NAT2* genes

Genotype							
NAT2	*ABCG2*	*n*	AUC_{0-48} (μg h/ml)	K_e (h^{-1})	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/ml)
RA	C/C	5	425 ± 148 (295 ~ 555)	0.09 ± 0.02 (0.07 ~ 0.10)	8.2 ± 1.8 (6.6 ~ 9.9)	24.0	16.3 ± 5.4 (11.5 ~ 21.1)
	C/A	5	445 ± 139*,** (324 ~ 566)	0.07 ± 0.02* (0.06 ~ 0.09)	10.2 ± 3.1* (7.5 ~ 13.0)	21.6 ± 5.4 (16.9 ~ 26.3)	17.6 ± 5.1*,** (13.2 ~ 22.1)
	A/A	3	250 ± 42 (202 ~ 298)	0.06 ± 0.04 (0.01 ~ 0.10)	15.9 ± 8.3 (6.5 ~ 25.3)	24.0	9.8 ± 1.8 (7.7 ~ 11.8)
IA	C/C	5	345 ± 146 (217 ~ 473)	0.05 ± 0.03 (0.02 ~ 0.08)	23.2 ± 21.0 (4.8 ~ 41.6)	28.8 ± 6.6 (23.0 ~ 34.6)	12.6 ± 5.2 (8.0 ~ 17.2)
	C/A	5	281 ± 129 (168 ~ 393)	0.06 ± 0.01* (0.05 ~ 0.07)	12.0 ± 2.4* (9.9 ~ 14.1)	21.6 ± 5.4 (16.9 ~ 26.3)	10.5 ± 4.6* (6.5 ~ 14.5)
	A/A	6	270 ± 109 (182 ~ 357)	0.05 ± 0.03 (0.03 ~ 0.08)	19.3 ± 12.8 (9.0 ~ 29.5)	24.0 ± 7.6 (17.9 ~ 30.1)	9.3 ± 3.4 (6.6 ~ 12.0)
SA	C/C	2	48.0	0.1	8.7	24.0	2.1
	C/A	6	146 ± 45 (110 ~ 182)	0.02 ± 0.01 (0.01 ~ 0.03)	50.1 ± 34.2 (22.2 ~ 77.5)	26.0 ± 4.9 (22.1 ~ 29.9)	5.1 ± 1.7 (3.7 ~ 6.4)

Each value represents the mean ± SD (95% confidence interval).

AcSP, *N*-acetylsulfapyridine; SASP, sulfasalazine (salazosulfapyridine).

*Significantly different from values in *SA-ABCG2-C/A* subjects as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$). **Significantly different from values in *IA-ABCG2-C/A* subjects as determined by ANOVA with Fisher's least significant difference test ($P < 0.05$).

Table 5 Pharmacokinetic parameters of SP and AcSP after single oral dose of SASP in three *NAT2* genotyping groups

			SP		AcSP	
Genotype	*n*	AUC_{AcSP}/AUC_{SP}	CL_r (l/h)	48-h Cumulative urinary excretion (%)	CL_r (l/h)	48-h Cumulative urinary excretion (%)
RA	13	3.1 ± 0.6 (2.8 ~ 3.5)	0.52 ± 0.09 (0.47 ~ 0.57)	5.2 ± 2.0 (4.2 ~ 6.3)	1.6 ± 0.3 (1.4 ~ 1.8)	41.5 ± 12.5 (34.7 ~ 48.3)
IA	16	1.9 ± 0.5* (1.7 ~ 2.1)	0.55 ± 0.07 (0.52 ~ 0.59)	7.3 ± 3.1 (5.8 ~ 8.8)	1.9 ± 0.4 (1.7 ~ 2.0)	36.7 ± 13.6 (30.0 ~ 43.3)
SA	8	0.5 ± 0.4*,** (0.3 ~ 0.8)	0.50 ± 0.06 (0.46 ~ 0.54)	12.7 ± 6.3*,** (8.3 ~ 17.1)	2.0 ± 0.6 (1.6 ~ 2.4)	15.9 ± 6.8*,** (11.2 ~ 20.6)

Each value represents the mean ± SD (95% confidence interval).

AcSP, *N*-acetylsulfapyridine; CLr, renal clearance; IA, intermediate acetylator; RA, rapid acetylator; SA, slow acetylator; SASP, sulfasalazine (salazosulfapyridine); SP, sulfapyridine.

*Significantly different from values in RA subjects as determined by ANOVA with Fisher's least significant difference test ($P < 0.001$). **Significantly different from values in IA subjects as determined by ANOVA with Fisher's least significant difference test ($P < 0.001$).

concentrations) for SASP, SP, and AcSP (Figure 3). Individual fitting curves of the three compounds in typical subjects are presented in Figure 4. Nonlinear mixed-effects modeling (NONMEM) analysis confirmed that individual *ABCG2* and *NAT2* genotyping status was a statistically significant predictor of SASP, and SP and AcSP pharmacokinetics, respectively (Figures 3 and 4, Table 6). Incorporating the effect of *ABCG2* genotype on SASP relative bioavailability (Fr, assumed 1.0 for *ABCG2-C/C* subjects), Ka, and CL/F (CL_{SASP}/F), and of *NAT2* genotype on CL_{SP} (formation CL of AcSP) was associated with significant

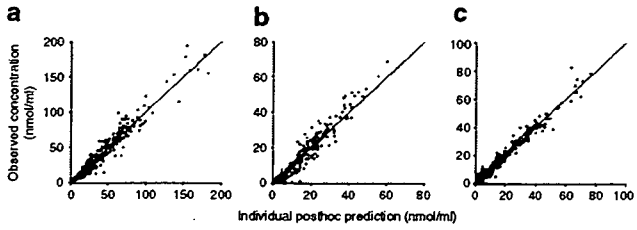

Figure 3 Scatter plots of observed plasma concentrations vs. individual posthoc prediction values of (**a**) sulfasalazine, (**b**) sulfapyridine, and (**c**) *N*-acetylsulfapyridine. The solid line is the line of identity.

Table 6 Population pharmacokinetic parameters and their variabilities for SASP, SP, and AcSP in basic and final models estimated by NONMEM with regard to *ABCG2* and *NAT2* gene polymorphisms

		Population mean (SE) (95%, confidence interval)		Variability (CV% (SE))	
Parameter/effect		Basic model	Final model	Basic model	Final model
SASP	K_a (h^{-1})	0.62 (0.16)	0.42 (0.08)	40.7 (25.1)	42.7 (26.5)
	CL/F (l/h)	5.3 (0.87)	9.3 (1.7)	125 (92.6)	111 (71.1)
	V_c/F (l)	47.8 (8.3)	74.4 (12.3)	55.7 (35.3)	50.5 (30.1)
	Q/F (l/h)	1.1 (0.36)	1.9 (0.43)	0 Fixed (–)	0 Fixed (–)
	V_p/F (l)	62.0 (28.3)	159 (67.3)	125 (78.4)	150 (95.2)
	Tlag (h)	0.25 (0.05)	0.25 (0.05)	42.8 (42.1)	68.5 (73.3)
SP	K_{TR} (h^{-1})	0.31 (0.05)	0.32 (0.02)	34.5 (19.4)	28.5 (16.0)
	CL_{SP} (l/h)	1.6 (0.36)	3.6 (0.49)	52.6 (31.0)	9.5 (8.4)
	V_{SP} (l/h)	26.7 (5.9)	32.7 (4.9)	41.3 (28.6)	70.9 (43.0)
AcSP	CL_{AcSP} (l/h)	1.3 (0.33)	1.3 (0.19)	72.6 (50.9)	3.0 (9.9)
	V_{AcSP} (l)	7.4 (2.8)	6.3 (1.0)	46.1 (31.0)	19.3 (12.0)
Genotyping effect					
	CL_{SP} (IA)	–	0.69 (0.04) [0.61–0.77]		
	CL_{SP} (SA)	–	0.18 (0.03) [0.12–0.24]		
	Fr (C/A)	–	1.7 (0.25) [1.2–2.2]		
	Fr (A/A)	–	2.1 (0.41) [1.3–3.0]		
	K_a (A/A)	–	1.8 (0.32) [1.2–2.4]		
	CL_{SASP}/F (C/A and A/A)	–	0.84 (0.02) [0.79–0.89]		
	OFV	4,168.282	3,771.129		

ABCG2 genotype effect was described as the following function: $P = P_{wildtype} \times \theta^{421C/A} \times \theta^{421A/A}$. NAT2 genotype effect was described as the following function: $P = P_{RA} \times \theta^{IA} \times \theta^{SA}$.

A/A, *ABCG2-A/A*; AcSP, *N*-acetylsulfapyridine; C/A, *ABCG2-C/A*; CL/F, apparent clearance; CL_{AcSP}, clearance of AcSP; CL_{SP}, formation clearance to AcSP; IA, intermediate acetylator; K_a, absorption rate constant; K_{TR}, transfer rate constant for Erlang's distribution; NONMEM, nonlinear mixed-effects modeling; OFV, objective function value estimated by NONMEM; Q/F, intercompartment clearance; SA, slow acetylator; SASP, sulfasalazine; SP, sulfapyridine; Tlag, absorption lag time; V_{AcSP}, volume of distribution of AcSP; Vc/F, apparent volume of distribution of central compartment; Vp/F, volume of distribution of peripheral compartment; V_{SP}, volume of distribution of SP.

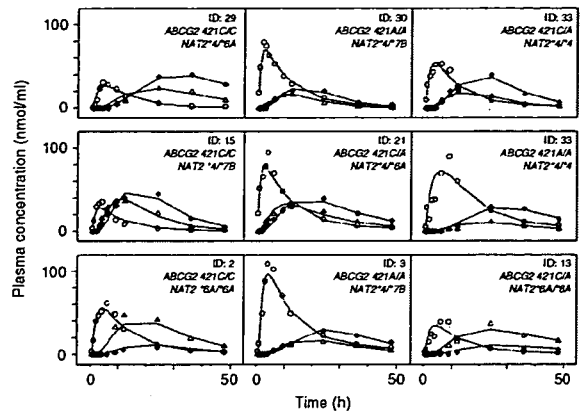

Figure 4 Observed plasma concentrations and post-hoc prediction curves in typical subjects with their *NAT2* and *ABCG2* genotyping results: sulfasalazine (open circles), sulfapyridine (open triangles), and *N*-acetylsulfapyridine (closed diamonds).

reduction in the objective function value (4,168.282→3,771.129). SASP Fr and Ka were increased, and CL_{SASP}/F was decreased in subjects with the *ABCG2-A* allele(s); the relative value of Fr in *ABCG2-C/C*, C/A, and A/A subjects was 1:1.7:2.1. The relative Ka and CL_{SASP}/F values in *ABCG2-C/C*, C/A, and A/A subjects were 1:1:1.8 and 1:0.84:0.84, respectively. CL_{SP} values were lower in IA and SA subjects than in RA subjects; the relative value of CL_{SP} in RA, IA, and SA was 1:0.7:0.18. No confidence intervals were >1.0, indicating that the contribution of the genotype to each parameter was significant. In **PPK** analysis, we fixed the interindividual variability of Q/F to zero; however, this treatment did not interfere with predictive performance (data not shown).

DISCUSSION

The aim of this study was to evaluate whether the polymorphism of drug metabolism and transporter genes is simultaneously involved in the overall pharmacokinetic profile of SASP in humans. The important findings were that: (1) significant differences in AUC of SASP were observed among subjects with different *ABCG2* genotypes; (2) AUC of SP tended to be lower in *ABCG2-A/A* subjects except for SA; (3) As with previous studies, significant differences in AUC_{AcSP}/AUC_{SP} were observed among subjects with different *NAT2* genotypes; (4) NONMEM analysis also supported the contribution of both gene polymorphisms to the pharmacokinetics of SASP, SP, and AcSP.

In this study, we demonstrated that BCRP plays an important role in the pharmacokinetics of SASP in humans. After oral administration, the mean AUC_{0-48} and C_{max} of SASP were significantly higher, and the mean CL_{total}/F was significantly lower in subjects with at least one *ABCG2-A* mutant allele (Table 1). Similar results were observed when we considered the combination of *ABCG2* and *NAT2* genotyping results, suggesting that NAT2 is not involved in the pharmacokinetics of SASP (Table 2). Previous *in vivo* studies demonstrated that *ABCG2* 421C>A polymorphism was associated with changes in the pharmacokinetics of certain clinically important drugs, such as topotecan,[26]

diflomotecan,[27] rosuvastatin,[28] and gefitinib.[30,31] Available data indicate that the *ABCG2-A* allele was associated with remarkably decreased BCRP expression compared with WT cells and human placental samples.[33,34] Since BCRP is highly expressed at the apical membrane of enterocytes and hepatocytes,[22,25] impaired transport activity caused by the *ABCG2-A* allele may lead to increased absorption at the intestinal epithelium, and/or to decreased biliary excretion of substrates, thereby resulting in elevated plasma concentrations in subjects with *ABCG2-A* allele(s) (**Figure 1**). Thus, the raised AUC of SASP in *ABCG2-A* subjects may be due to increased oral availability (F) and/or decreased hepatic clearance. Nevertheless, we have recently demonstrated no significant effect of the *ABCG2 421C>A* polymorphism on the pharmacokinetics of pitavastatin, a good substrate for BCRP, suggesting that the pharmacogenomic effect of the *ABCG2* polymorphism appeared to depend on the substrate.[32]

The raised AUC of SASP in *ABCG2-A* subjects is in accord with the findings in a $BCRP^{-/-}$ mouse study that, after oral administration, AUC of SASP in $BCRP^{-/-}$ was ~111-fold higher than that in FVB-WT mice.[29] Interestingly, after intravenous administration, the AUC in $BCRP^{-/-}$ was 13-fold higher than that in FVB-WT mice. The absolute F for SASP varied between 4 and 37% in FVB-WT and $BCRP^{-/-}$ mice, respectively. These results show that BCRP (Abcg2) plays a major role in the intestinal absorption of SASP in mice.[29] In this study, we could not determine F due to the lack of an intravenous formulation of SASP, making it difficult to describe how extent *ABCG2* polymorphism influences the intestinal absorption, intestinal secretion, and/or the biliary excretion of SASP. However, since total urinary recovery (4.5% of the dose) and biliary recovery (2.5% of the dose, about half the value of urinary recovery) of SASP have been reported to be very low in humans, the contribution of BCRP to the biliary excretion of SASP is thought to be small.[35,36]

In contrast to SASP, mean AUC_{0-48} and C_{max} values of SP tended to be lower in *ABCG2-A/A* compared with those in *ABCG2-C/C* subjects, except for SA subjects (**Table 3**). After oral administration, most SASP travels down the small intestine to the lower intestinal tract (i.e., colon and cecum) where its diazo-bond is cleaved by colonic bacteria with the liberation of SP and 5-ASA (**Figure 1**).[7] SP is almost totally absorbed from the colon and cecum, and then metabolized into AcSP by NAT2, predominantly in the liver.[7–10] The amount of SASP in the lower intestinal tract may be lower in *ABCG2-A/A* subjects than in *ABCG2-C/C* subjects due to high BCRP expression on the brush border of the small intestinal enterocytes in *ABCG2-C/C* subjects (i.e., reduction in the intestinal secretion of SASP into the gut lumen in *ABCG2-A/A* subjects), leading to lower conversion in the distal intestine to SP in *ABCG2-A/A* subjects. Individual variability of bacterial azo reductase as a result of gastrointestinal disease(s) and/or antibiotics therapy may alter the production of SP; however, all subjects were healthy volunteers who had not taken antibiotics for at least 2 months before or during this investigation. Moreover, similar findings were observed in previous $BCRP^{-/-}$ and WT mice.[29]

The mean $t_{1/2}$ and C_{max} of SP were also significantly different among three *ABCG2-C/A*-matched *NAT2* genotyping subjects; the order was SA > IA > RA (**Table 3**). Generally, C_{max} is determined by dose (the amount of SP in the colon and cecum in this case), K_e, and K_a (absorption rate constant). Since the dose can be assumed to be similar among the same *ABCG2* genotyping groups, and since K_a was comparable among these three *ABCG2-C/A*-matched *NAT2* genotyping subjects (data not shown), C_{max} may be dependent on the *NAT2* genotyping-dependent K_e value.

Interestingly, no changes in the pharmacokinetics of SP were recognized in SA subjects. The reason for the discrepancy with the data obtained from RA and IA subjects is presently unclear.

In this study, we also genotyped *ABCG2* 34G>A, another relatively frequent polymorphism among the Japanese population,[33] and similar phenotyping–genotyping analysis was attempted. The allelic frequency of 34A in our subjects was 14.9% (26 subjects were 34G/G and 11 were 34G/A). The mean AUC_{0-48} and CL_{total}/F of SASP in *ABCG2-34G/G421C/C* ($n = 5$) and *ABCG2-34G/A421C/C* ($n = 7$) were 170 ± 85 and 173 ± 92 μg h/ml, and 14.3 ± 7.2 and 13.2 ± 5.1 l/h, respectively. Similar trends were observed in the pharmacokinetic parameters of three compounds with other genotyping patterns. These results suggest that *ABCG2* 34G>A does not play an important role in the overall pharmacokinetics of SASP; however, since we had no homozygote for the 34A allele in this study, further study is necessary to confirm these observations.

The mean AUC ratio of AcSP to SP (AUC_{AcSP}/AUC_{SP}) and 48-h cumulative urinary excretion of AcSP were significantly higher in RA than in SA subjects (**Table 5**). We confirmed previous studies that *NAT2* genotypes are well-correlated with plasma concentrations and urinary excretions of SP and AcSP.[7,17,37] In view of the pharmacodynamics, the occurrence of certain adverse events of SASP has been speculated to be dependent on *NAT2* genotyping; higher incidences of nausea/vomiting and hepatic disorders were reported in SA subjects.[17–19]

Since overall pharmacokinetic profiles of SASP would be determined not only by *NAT2* but also by *ABCG2* genotyping results, it is of interest whether the risk of SASP-induced adverse events and the clinical efficacy of SASP can be partially attributed to the *ABCG2*. Until now, the action mechanism of SASP in ulcerative colitis and Crohn's disease has not been well understood. The most prevalent scenario is that SASP serves as a vehicle to deliver SP and 5-ASA to the colon and cecum in higher concentrations than can be achieved by oral administration of these metabolites alone.[7,8,36,38,39] If these hypotheses are correct, subjects with the *ABCG2-A* allele are expected to exhibit reduced clinical efficacy owing to lower SASP exposure in the lower intestinal tract (which, in turn, may be associated with lower SP and 5-ASA formations in this tract), despite high plasma concentrations and AUC of SASP. Similar to ulcerative colitis and Crohn's disease, questions still exist as to whether SASP itself or its metabolites, SP and 5-ASA, are responsible for specific antirheumatic effects of SASP; however, there is evidence that SP is also the active moiety of SASP in rheumatoid arthritis.[5,6] The effect of *ABCG2* gene polymorphism on the clinical efficacy and adverse events of SASP will be the subject of further investigation.

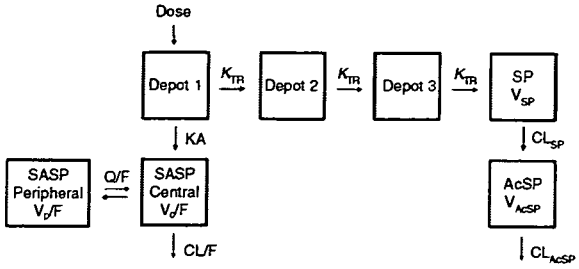

Figure 5 Scheme of the pharmacokinetic compartment model for simultaneous description of sulfasalazine (SASP), sulfapyridine (SP), and *N*-acetylsulfapyridine (AcSP) plasma concentrations.

Previous studies demonstrated that SASP concentrations in serum and urine did not differ before and after colectomy and ileostomy, while the concentration of SP and its metabolites decreased markedly in patients with inflammatory bowel disease.[7,36] Similarly, in two patients with transverse colostomy, serum and urine concentrations of SASP did not differ from those of patients with ileostomy.[36] Although BCRP has been reported to be expressed in the epithelium of the colon,[21–25] these observations suggest that the upper intestinal tract (i.e., small intestine) is the major site of SASP absorption.[7] Thus, in order to detect the effect of BCRP on the pharmacokinetic profiles of SASP more precisely, we used a conventional tablet as the test drug in this study. Whether *ABCG2* genotype-dependent changes in the pharmacokinetics of SASP occur with the enteric-coated formulation is of interest as well.

As shown in **Figures 3** and **4**, it is notable that NONMEM analysis with Erlang's distribution model gave good descriptions of all three compounds, indicating that the present structural model (**Figure 5**) is suitable to describe the three compounds simultaneously. A significant contribution of *ABCG2* and *NAT2* polymorphisms to Fr, Ka, and CL_{SASP}/F, and CL_{SP}, respectively, was detected. The relative value of Fr in *ABCG2-C/C*, C/A, and A/A subjects was 1:1.7:2.1, indicating that the bioavailability of SASP is higher in subjects with at least one *ABCG2-A* allele as compared with that in *ABCG2-C/C* subjects. A similar influence was previously suggested for various substrate drugs.[26–28] Of note, relative CL_{SASP}/F in *ABCG2-C/C*, C/A, and A/A subjects was 1:0.84:0.84. These results suggest that CL in subjects with at least the *ABCG2-A* allele was 16% lower than that in *ABCG2-C/C* subjects.

In conclusion, this study shows that the overall pharmacokinetics of SASP are highly dependent on interplay with NAT2 and BCRP; polymorphisms in both genes are associated with individual variability in pharmacokinetic profiles. In spite of the small sample studied here, it is suggested that SASP is a candidate probe for BCRP in humans and that BCRP restricts the oral absorption and/or biliary excretion of substrate drugs. Obviously, the number of subjects is a drawback of our study. Since the frequencies of SA and *ABCG2-A/A* types are low in Japanese,[15,17,33] the study subjects were recruited from a population of >100 male Japanese volunteers whose *NAT2* and *ABCG2* genotypes were prescreened. Nevertheless, it is clear that the current observation warrants confirmation and further investigation of SASP and other substrates for BCRP in large populations involving different ethnic groups.

METHODS

Subjects and genotyping of *ABCG2* and *NAT2*. This study was approved by the Ethics Review Boards of Kyushu University and Kyushu Pharmacology Research Clinic, and written informed consent was obtained from all participants before the study. Thirty-seven unrelated healthy male volunteers (age: 20–24 years; weight: 49.5–79.7 kg) were selected from our study panels based on their genotyping for *ABCG2* and *NAT2*. They were recruited from a population of >100 male Japanese volunteers whose genotypes were prescreened after written informed consent was obtained. Each subject was physically normal and had no antecedent history of significant medical illness or hypersensitivity to any drugs, and each had a body mass index between 17.6 and 25.5 kg/m^2. Their health status was judged to be normal on the basis of a physical examination with a screening of blood chemistry, complete blood count, urinalysis, electrocardiogram, and chest X-ray before the study. None had taken any drugs for at least 1 week before the study.

Genomic DNA was isolated from blood samples using the Toyobo blood kit on a Toyobo HMX-2000 robot (Toyobo, Osaka, Japan). In the *ABCG2* gene, 421C>A variant was identified using TaqMan primers and probes for the Sequence Detection System (ABI PRISM 7000; Applied Biosystems, Foster, CA). The following primers and probes were developed: forward primer, GGGCACTCTGACGGTGAGA; reverse primer, CATAGTTGTTGCAAGCCGAAGAG; probe 1 (C allele), VIC-TGAGAACTGTAAGTTTT; probe 2 (A allele), FAM-TGCTGA GAACTTTAAG. In the *NAT2* gene, we identified *NAT2*5B*, *6A, and *7B mutant alleles using commercially available TaqMan Genotyping Assay (Drug Metabolism) kits (Applied Biosystems). All subjects were classified into three groups according to their *NAT2* genotypes: RA genotype (RA, a homozygote for *NAT2*4*), intermediate (IA, a heterozygote for mutant alleles) and SA genotypes (SA, a combination of mutant alleles). As only four alleles (*NAT2*4*, *5B, *6A, and *7B) have been reported to date in Japanese subjects,[12] identification of these three variants is sufficient to define almost all NAT2 alleles in a homozygous Japanese population.[13,17] All real-time polymerase chain reaction assays were carried out according to the manufacturer's instructions.

In this study, we selected the following genotypings with regard to the combination of two genes of interest: *RA-ABCG2-C/C* (i.e., a homozygote for *ABCG2-C* allele and RA genotype, $n = 5$), *RA-C/A* ($n = 5$), *RA-A/A* ($n = 3$), *IA-C/C* ($n = 5$), *IA-C/A* ($n = 5$), *IA-A/A* ($n = 6$), *SA-C/C* ($n = 2$), and *SA-C/A* ($n = 6$).

Study protocol. The participants came to the clinic after an overnight fast. They were required to abstain from alcohol for 2 days before drug administration and during the period of hospitalization, and were served standard meals on the study day. Each subject received a single oral dose of 2,000 mg of SASP (Salazopyrin conventional tablet, Pfizer, Tokyo, Japan) with 200 ml of water. Food was given 4 h after the ingestion of SASP. Venous blood samples (5 ml each) to determine SASP, SP, and AcSP concentrations were obtained just before and 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 36, and 48 h after dosing. Plasma samples were immediately separated after centrifugation. Urine samples (20 ml each) were collected cumulatively in two fractions: 0 to 24 and 24 to 48 h. The amount and pH of urine were measured. Both plasma and urine samples were stored at −20 °C until analyzed.

Quantification of SASP, SP, and AcSP. The concentrations of SASP, SP, and AcSP in plasma and urine were measured by high-performance liquid chromatography as described previously.[37,40,41] High-performance liquid chromatography was equipped with a system controller (SCL-10A, Shimadzu, Kyoto, Japan), a variable wavelength ultraviolet detector (SPD-10A, Shimadzu) adjusted to 254 nm for SASP, 260 nm for SP and AcSP, and a data processor (C-R7Ae plus, Shimadzu). The stationary

phase was a reversed phase Chemcobond 5-ODS-H column (4.6 mm i.d. × 250 mm, particle size 5 μm, Chemco, Osaka, Japan).

Pharmacokinetic and statistical analysis

Non compartmental analysis. C_{max} and T_{max} were obtained directly from the data. The AUC_{0-48} was calculated by the linear trapezoidal rule. We calculated the apparent oral clearance (CL_{tot}/F) as follows: $CL_{tot}/F = Dose/AUC_{0-48}$. The K_e was estimated using least-squares regression analysis from the terminal postdistribution phase of the concentration–time curve. CL_r was calculated by $CL_r = Ae_{0-48}/AUC_{0-48}$, where Ae_{0-48} represents the amount of compounds excreted in urine.

Nonmem. Simultaneous modeling of SASP, SP, and AcSP with regard to the polymorphism of *ABCG2* and *NAT2* genes was assessed by the nonlinear mixed-effects modeling approach with the software package NONMEM, version V, level 1.1 (NONMEM Project Group, University of California at San Francisco).[42] Throughout the analysis, the first-order method was used as the estimation method. The pharmacokinetic model for all three compounds is shown in Figure 5. The two-compartment model with lag time assumed that first-order absorption was applied for the description of SASP disposition, and the one-compartment model was applied for SP and AcSP dispositions. To describe the absorption phase of SP, Erlang's distribution model was used.[43,44] Erlang's distribution is the analytical solution for a linear chain of n identical compartments placed upstream of the central compartment and connected by an identical transfer rate constant K_{TR}, the transfer constant between two sequential delay compartments.[43,44] In the present model, the number of serial compartments n was set to 3 for all subjects, based on the objective function value and predictive performance. Interindividual variability of all parameters was modeled as the exponential error model. As the intraindividual variability model, the exponential error model was selected for SASP and the combined additive and exponential error model was used for SP and AcSP.

The contribution of body weight, body surface area, and candidates of physiological covariate to CL and V of the three compounds was tested.

The effect of the *ABCG2* 421C>A genotype on Ka, CL/F, and V/F was assessed with the following equation:

$$Pi = TVPi \times \theta^{ABCG2421C/A} \times \theta^{ABCG2421A/A}$$

where Pi is the individual value and TVPi is the population average value for *ABCG2-C/C* (i.e., a homozygote for the WT allele) subjects.

The effect of the *NAT2* genotype on CL of SP was assessed with the following equation:

$$Pi = TVPi \times \theta^{NAT21A} \times \theta^{NAT25A}$$

where TVPi is the population average value for RA subjects.

Forward addition and backward exclusion methods were used to build the final model. The significance of the genotype effect was assessed by comparing the objective functions (−2 log likelihood) for different models, assuming χ^2 distribution of their difference, and by assessing the 95% confidence interval for estimated values.

Statistical analysis. Statistical differences among the data for each group were determined by ANOVA, followed by Fisher's least significant difference test. $P < 0.05$ was considered statistically significant.

ACKNOWLEDGMENTS

This study was supported by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare for Research on Advanced Medical Technology.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

1. Peppercorn, M.A. Sulfasalazine. Pharmacology, clinical use, toxicity, and related new drug development. *Ann. Intern. Med.* **101**, 377–386 (1984).
2. Rains, C.P., Noble, S. & Faulds, D. Sulfasalazine: A review of its pharmacological properties and therapeutic efficacy in the treatment of rheumatoid arthritis. *Drugs* **50**, 137–156 (1995).
3. Houston, J.B., Day, J. & Walker, J. Azo reduction of sulphasalazine in healthy volunteers. *Br. J. Clin. Pharmacol.* **14**, 395–398 (1982).
4. Peppercorn, M.A. & Goldman, P. The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. *J. Pharmacol. Exp. Ther.* **181**, 555–562 (1972).
5. Bird, H.A. Sulphasalazine, sulphapyridine or 5-aminosalicylic acid—which is the active moiety in rheumatoid arthritis? *Br. J. Rheumatol.* **34** (suppl.2), 16–19 (1995).
6. Pullar, T., Hunter, J.A. & Capell, H.A. Which component of sulphasalazine is active in rheumatoid arthritis? *Br. Med. J.* **290**, 1535–1538 (1985).
7. Das, K.M. & Dubin, R. Clinical pharmacokinetics of sulphasalazine. *Clin. Pharmacokinet.* **1**, 406–425 (1976).
8. Das, K.M., Eastwood, M.A., McManus, J.P.A. & Sircus, W. The metabolism of salicylazosulphapyridine in ulcerative colitis I. The relationship between metabolites and the response to treatment in inpatients. *Gut* **14**, 631–636 (1973).
9. Das, K.M., Eastwood, M.A., McManus, J.P.A. & Sircus, W. The metabolism of salicylazosulphapyridine in ulcerative colitis II. The relationship between metabolites and the progress of the disease studied in outpatients. *Gut* **14**, 637–641 (1973).
10. Schroder, H. & Campbell, D.E. Absorption, metabolism, and excretion of salicylazosulfapyridine in man. *Clin. Pharmacol. Ther.* **13**, 539–551 (1972).
11. Matas, N., Thygesen, P., Stacey, M., Risch, A. & Sim, E. Mapping AAC1, AAC2 and AACP, the genes for arylamine N-acetyltransferases, carcinogen metabolising enzymes on human chromosome 8p22, a region frequently deleted in tumours. *Cytogenet. Cell Genet.* **77**, 290–295 (1997).
12. Abe, M., Deguchi, T. & Suzuki, T. The structure and characteristics of a fourth allele of polymorphic N-acetyltransferase gene found in the Japanese population. *Biochem. Biophys. Res. Commun.* **191**, 811–816 (1993).
13. Deguchi, T., Mashimo, M. & Suzuki, T. Correlation between acetylator phenotypes and genotypes of polymorphic arylamine N-acetyltransferase in human liver. *J. Biol. Chem.* **265**, 12757–12760 (1990).
14. Parkin, D.P. *et al.* Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis. *Am. J. Respir. Crit. Care Med.* **155**, 1717–1722 (1997).
15. Okumura, K., Kita, T., Chikazawa, S., Komada, F., Iwakawa, S. & Tanigawara, Y. Genotyping of N-acetylation polymorphism and correlation with procainamide metabolism. *Clin. Pharmacol. Ther.* **61**, 509–517 (1997).
16. Human *NAT2* alleles. http://louisville.edu/medschool/pharmacology/NAT2.html
17. Tanigawara, Y. *et al.* N-acetyltransferase 2 genotype-related sulfapyridine acetylation and its adverse events. *Biol. Pharm. Bull.* **25**, 1058–1062 (2002).
18. Pounder, R.E., Craven, E.R., Henthor, J.S. & Bannatyne, J.M. Red cell abnormalities associated with sulfasalazine maintenance therapy for ulcerative colitis. *Gut* **16**, 181–185 (1975).
19. Wadelius, M., Stjernberg, E., Wiholm, B.E. & Rane, A. Polymorphisms of NAT2 in relation to sulphasalazine-induced agranulocytosis. *Pharmacogenetics* **10**, 35–41 (2000).
20. Machida, H. *et al.* Crohn's disease in Japanese is associated with a SNP-haplotype of N-acetyltransferase 2 gene. *World J. Gastroenterol.* **11**, 4833–4837 (2005).
21. Cervenak, J. *et al.* The role of the human ABCG2 multidrug transporter and its variants in cancer therapy and toxicology. *Cancer Lett.* **234**, 62–72 (2006).
22. Maliepaard, M. *et al.* Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res.* **61**, 3458–3464 (2001).
23. Kruijtzer, C.M., Beijnen, J.H. & Schellens, J.H. Improvement of oral drug treatment by temporary inhibition of drug transporters and/or cytochrome P450 in the gastrointestinal tract and liver: an overview. *Oncologist* **7**, 516–530 (2002).
24. Taipalensuu, J. *et al.* Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. *J. Pharmacol. Exp. Ther.* **299**, 164–170 (2001).
25. Doyle, L.A. & Ross, D.D. Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). *Oncogene* **22**, 7340–7358 (2003).
26. Sparreboom, A. *et al.* Effect of ABCG2 genotype on the oral bioavailability of topotecan. *Cancer Biol. Ther.* **4**, 650–653 (2005).
27. Sparreboom, A. *et al.* Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. *Clin. Pharmacol. Ther.* **76**, 38–44 (2004).
28. Zhang, W. *et al.* Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. *Clin. Chim. Acta.* **373**, 99–103 (2006).

29. Zaher, H., Khan, A.A., Palandra, J., Brayman, T.G., Yu, L. & Ware, J.A. Breast cancer resistance protein (Bcrp/abcg2) is a major determinant of sulfasalazine absorption and elimination in the mouse. *Mol. Pharm.* **3**, 55–61 (2006).
30. Li, J. *et al.* Association of variant ABCG2 and the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitors in cancer patients. *Cancer Biol. Ther.* **6**, 432–438 (2007).
31. Cusatis, G. *et al.* Pharmacogenetics of ABCG2 and adverse reactions to gefitinib. *J. Natl. Cancer Inst.* **98**, 1739–1742 (2006).
32. Ieiri, I. *et al.* SLCO1B1 (OATP1B1, an uptake transporter) and ABCG2 (BCRP, an efflux transporter) variant alleles and pharmacokinetics of pitavastatin in healthy volunteers. *Clin. Pharmacol. Ther.* **82**, 541–547 (2007).
33. Kobayashi, D. *et al.* Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug Metab. Dispos.* **33**, 94–101 (2005).
34. Kondo, C. *et al.* Functional analysis of SNPs variants of BCRP/ABCG2. *Pharm. Res.* **21**, 1895–1903 (2004).
35. Azad Khan, A.K., Truelove, S.C. & Aronson, J.K. The disposition and metabolism of sulphasalazine (salicylazosulphapyridine) in man. *Br. J. Clin. Pharmacol.* **13**, 523–528 (1982).
36. Das, K.M., Eastwood, M.A., McManus, J.P.A. & Sircus, W. The role of the colon in the metabolism of salicylazosulphapyridine. *Scand. J. Gastroenterol.* **9**, 137–141 (1974).
37. Kita, T. *et al.* N-Acetyltransferase 2 genotype correlates with sulfasalazine pharmacokinetics after multiple dosing in healthy Japanese subjects. *Biol. Pharm. Bull.* **24**, 1176–1180 (2001).
38. Goldman, P. & Peppercorn, M.A. Drug therapy-sulfasalazine. *New Eng. J. Med.* **293**, 20–23 (1975).
39. Peppercorn, M.A. & Goldman, P. Distribution studies of salicylazosulfapyridine and its metabolites. *Gastroenterology* **64**, 240–245 (1973).
40. Show, P.N., Sivner, A.L., Aarons, L. & Houston, J.B. A rapid method for the simultaneous determination of the major metabolites of sulphasalazine in plasma. *J. Chromatogr.* **274**, 393–397 (1983).
41. Chungi, V.S., Rekhi, G.S. & Shargel, L. A simple and rapid liquid chromatographic method for the determination of major metabolites of sulfasalazine in biological fluids. *J. Pharm. Sci.* **78**, 235–238 (1989).
42. Beal, S.L. & Sheiner, L.B. NONMEM User's Guides. San Francisco, CA, NONMEM Project Group, University of California at San Francisco, San Francisco, CA (1998).
43. Djebli, N. *et al.* Sirolimus population pharmacokinetic/pharmacogenetic analysis and Bayesian modelling in kidney transplant recipients. *Clin. Pharmacokinet.* **45**, 1135–1148 (2006).
44. Saint-Marcoux, F. *et al.* Patient characteristics influencing ciclosporin pharmacokinetics and accurate Bayesian estimation of ciclosporin exposure in heart, lung and kidney transplant patients. *Clin. Pharmacokinet.* **45**, 905–922 (2006).

SLCO1B1 (OATP1B1, an Uptake Transporter) and *ABCG2* (BCRP, an Efflux Transporter) Variant Alleles and Pharmacokinetics of Pitavastatin in Healthy Volunteers

I Ieiri[1], S Suwannakul[1], K Maeda[2], H Uchimaru[3], K Hashimoto[1], M Kimura[3], H Fujino[4], M Hirano[4], H Kusuhara[2], S Irie[3], S Higuchi[1] and Y Sugiyama[2]

To investigate the contribution of genetic polymorphisms of *SLCO1B1* and *ABCG2* to the pharmacokinetics of a dual substrate, pitavastatin, 2 mg of pitavastatin was administered to 38 healthy volunteers and pharmacokinetic parameters were compared among the following groups: 421C/C*1b/*1b (group 1), 421C/C*1b/*15 (group 2), 421C/C*15/*15 and 421C/A*15/*15 (group 3), 421C/A*1b/*1b (group 4), 421A/A*1b/*1b (group 5), and 421C/A*1b/*15 (group 6). In *SLCO1B1*, pitavastatin area under plasma concentration–time curve from 0 to 24 h (AUC_{0-24}) for groups 1, 2, and 3 was 81.1 ± 18.1, 144 ± 32, and 250 ± 57 ng h/ml, respectively, with significant differences among all three groups. In contrast to *SLCO1B1*, AUC_{0-24} in groups 1, 4, and 5 was 81.1 ± 18.1, 96.7 ± 35.4, and 78.2 ± 8.2 ng h/ml, respectively. Although the *SLCO1B1* polymorphism was found to have a significant effect on the pharmacokinetics of pitavastatin, a nonsynonymous *ABCG2* variant, 421C>A, did not appear to be associated with the altered pharmacokinetics of pitavastatin.

Pitavastatin is a highly potent inhibitor of 3-hydroxymethylglutaryl coenzyme A reductase and is used for the treatment of hypercholesterolemia.[1] In humans, pitavastatin is scarcely metabolized by the cytochrome P450 2C9,[2,3] and lactonization is another known metabolic pathway.[4,5] The lactone form can be reversibly converted to the parent drug.[4] Cumulative evidence has indicated that various active transport mechanisms are involved in its distribution and disposition kinetics. Pitavastatin is taken up efficiently from the circulation into hepatocytes by an organic anion-transporting polypeptide (OATP) 1B1 (formally known as OATP-C or OATP2, gene *SLCO1B1*), a sodium-independent bile-acid transporter expressed at the sinusoidal membrane of human hepatocytes responsible for the hepatocellular uptake of a variety of endogenous and foreign chemicals.[6–8] In addition to the uptake process, a recent study demonstrated that breast cancer resistance protein (BCRP, gene *ABCG2*) is involved in the biliary excretion of pitavastatin.[9] BCRP is expressed at the apical membrane in the placenta (trophoblast cells), liver (bile canalicular membrane of hepatocytes), kidney, and intestine (enterocytes).[10–13] The biliary excretion clearance of pitavastatin in Bcrp1 (−/−) mice was 10 times lower than that in control mice;[9] thus, at least two drug transporters, OATP1B1 and BCRP, contribute to hepatic uptake and efflux of pitavastatin in humans.

A number of single nucleotide polymorphisms have been identified in *SLCO1B1* and some of these single nucleotide polymorphisms are associated with a significant change in the transporter activity of OATP1B1. Two commonly occurring single nucleotide polymorphisms, 388A>G (130Asn>Asp) and 521T>C (174Val>Ala), are found to cause a remarkable change in the disposition of OATP1B1 substrates such as statins (pravastatin[14–17] and pitavastatin[18]), fexofenadine,[19] and repaglinide.[20] Interestingly, most human studies have demonstrated that subjects with haplotypes *SLCO1B1*5*, *15, or *17, all haplotypes harboring the 174Val>Ala variant, showed increased plasma levels of substrates as compared with subjects having the *SLCO1B1*1a* (130Asn174Val) or *1b (130Asp174Val) allele as homozygosity. Furthermore, recent studies reported that the

[1]Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan; [3]Kyushu Clinical Pharmacology Research Clinic, Fukuoka, Japan; [4]Tokyo New Drug Research Laboratories, Kowa Company Ltd., Tokyo, Japan. Correspondence: Y Sugiyama (sugiyama@mol.f.u-tokyo.ac.jp)

Received 19 October 2006; accepted 9 February 2007; published online 25 April 2007. doi:10.1038/sj.clpt.6100190

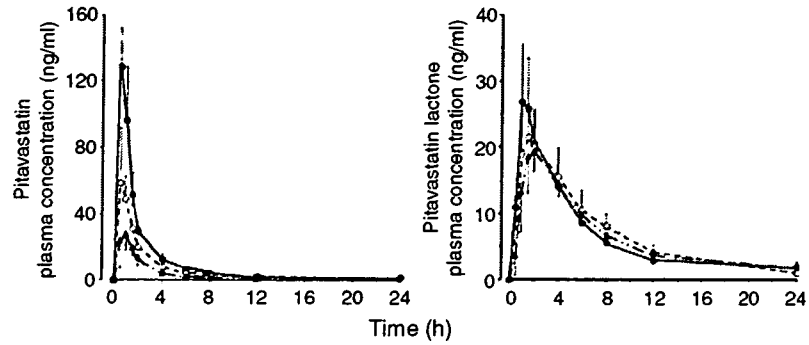

Figure 1 Effect of *SLCO1B1* haplotype on pharmacokinetics of pitavastatin. Plasma concentration–time profiles of pitavastatin and pitavastatin lactone after oral administration of 2 mg pitavastatin in 421C/C*1b/*1b subjects (closed triangles, $n = 11$), 421C/C*1b/*15 subjects (open circles, $n = 8$), and 421C/C*15/*15 and 421C/A*15/*15 subjects (closed circles, $n = 3$).

*SLCO1B1*1b* allele showed more enhanced transport activity than the *1a allele.[17,21]

Systematic mutation analysis of the *ABCG2* gene has been performed in various ethnic populations and more than 40 single nucleotide polymorphisms have been identified.[22–25] The two most frequent nonsynonymous mutations identified in humans are 34G>A (12Val>Met in exon 2) and 421C>A (141Gln>Lys in exon 5). After intravenous administration, plasma levels of diflomotecan were significantly higher in patients with the 421C/A than the 421C/C genotype.[26] These results were supported by *in vitro* experiments showing that BCRP expression of the 421C>A variant was reduced compared with the wild-type,[22,27,28] suggesting that carriers of the 421C>A variant may have decreased clearance (increased plasma levels) and/or increased bioavailability.

In view of the pharmacokinetics, at least two genes (*i.e.*, *SLCO1B1* and *ABCG2*) are of interest as candidates that may lead to large interindividual variability in the pharmacokinetics and clinical outcome of pitavastatin therapy. Recently, Chung *et al.*[18] evaluated the contribution of *SLCO1B1* haplotypes to pitavastatin pharmacokinetics and demonstrated that subjects with the *15 allele showed significantly higher dose-normalized pitavastatin plasma levels. Although these observations are similar trends to pravastatin, no homozygotes for the *15 allele participated in their study. Very recently, Zhang *et al.*[29] studied the role of *ABCG2* 421C>A variant in rosuvastatin pharmacokinetics in 14 healthy volunteers and indicated that the AUC of rosuvastatin was lower in the 421C/C group than in the (421C/A and 421A/A) group. Although all statins share a common action mechanism, they differ in terms of their chemical structures, pharmacokinetics, and pharmacodynamics.[30]

With this background in mind, we designed this study to confirm the role of *SLCO1B1* and *ABCG2* polymorphisms in the pharmacokinetics of pitavastatin in healthy volunteers. In this study, we selected volunteers from our panels based on their genotypes of *SLCO1B1* (*1b and *15) and *ABCG2* (421C>A). In addition, we investigated the importance of intestinal BCRP in the pharmacokinetics of pitavastatin using Bcrp1 (−/−) mice.

RESULTS

No clinically undesirable signs and symptoms possibly attributed to the administration of pitavastatin were recognizable throughout the study. All subjects completed the study successfully according to the protocol.

Pitavastatin pharmacokinetics in relation to *SLCO1B1* and *ABCG2* genotypic status

After oral administration, the mean plasma concentrations of pitavastatin were significantly higher ($P<0.01$) in group 3 subjects ($n=3$, homozygotes for the *SLCO1B1*15* allele, 421C/C*15/*15 ($n=2$) and 421C/A*15/*15 ($n=1$)) compared with group 1 subjects ($n=11$, homozygotes for the *SLCO1B1*1b* allele, 421C/C*1b/*1b), and group 2 subjects, heterozygotes for the *SLCO1B1*15* allele ($n=8$, 421C/C*1b/*15), had values between those in group 1 (*i.e.*, *1b/*1b) and group 3 (*15/*15) subjects at all observation points (**Figure 1**). The mean ($\pm$SD) AUC_{0-24} of pitavastatin in groups 1 (*1b/*1b), 2 (421C/C*1b/*15), 3 (*15/*15), and 6 (421C/A*1b/*15) was 81.1 ± 18.1, 144 ± 32, 250 ± 57, and 121 ± 25 ng h/ml, respectively. The mean apparent oral clearance (CLt) of pitavastatin in groups 1 (*1b/*1b), 2 (421C/C*1b/*15), 3 (*15/*15), and 6 (421C/A*1b/*15) was 0.43 ± 0.13, 0.24 ± 0.04, 0.15 ± 0.03, and 0.29 ± 0.07 l/h/kg, respectively. The group 3 (*15/*15) subjects had the highest AUC value and the lowest CLt value among all study volunteers. A similar trend was observed in peak concentration (C_{max}) values, but not in elimination rate constant (K_e) values. Although the difference did not reach the level of significance, volume of distribution/bioavailability (V_d/F) values tended to be lower in subjects with the *15 allele; the mean V_d/F in group 3 (*15/*15) was 30% of that in group 1 (*1b/*1b). In contrast to pitavastatin, no significant intergenotypic differences were observed in any mean pharmacokinetic parameters of pitavastatin lactone in this experiment (**Table 1** and **Figure 1**).

The mean plasma concentration–time curves of pitavastatin and pitavastatin lactone in relation to *ABCG2* genotypic status are shown in **Figure 2**. The pharmacokinetic parameters are also summarized in **Table 1**. There were no significant differences in any of the pharmacokinetic

Table 1 Pharmacokinetic parameters of pitavastatin and its lactone form in each genotyping group

Genotype			Pitavastatin					Pitavastatin lactone		
		n	AUC_{0-24}	CLt	C_{max}	K_e	Vd/*F*	AUC_{0-24}	C_{max}	K_e
SLCO1B1	*ABCG2*		(ng h/ml)	(l/h/kg)	(ng/ml)	(per hour)	(l/kg)	(ng h/ml)	(ng/ml)	(per hour)
*1b/*1b	421C/C	11	81.1±18.1	0.43±0.13	31.2±11.4	0.06±0.03	0.58±0.27	154±27	20.4±4.4	0.07±0.01
*1b/*15	421C/C	8	144±32[a]	0.24±0.04[a]	70.7±18.1[a]	0.06±0.02	0.27±0.09	169±38	22.3±5.4	0.08±0.02
*15/*15	421C/C 421C/A	3	250±57[a,b]	0.15±0.03[a]	129±24[a]	0.06±0.01	0.16±0.07	153±31	27.2±8.8	0.07±0.01
*1b/*1b	421C/A	7	96.7±35.4	0.37±0.13	41.7±12.4	0.06±0.03	0.46±0.25	145±38	18.9±3.1	0.06±0.02
*1b/*1b	421A/A	3	78.2±8.2	0.42±0.01	42.1±6.3	0.05±0.02	0.48±0.21	140±47	22.1±4.9	0.06±0.01
*1b/*15	421C/A	6	121±25	0.29±0.07	57.7±7.6	0.05±0.01	0.26±0.05	125±18	18.8±2.6	0.07±0.01

AUC_{0-24}, area under plasma concentration–time curve from 0 to 24 h; CL_t, total clearance; C_{max}, peak concentration; V_d/F, volume of distribution/bioavailability. Data are presented as the mean±SD. [a]Significantly different from values in *SLCO1B1*1b/*1b421C/C* subjects as determined by analysis of variance with Fisher's least significant difference test ($P<0.01$). [b]Significantly different from values in *SLCO1B1*1b/*15421C/C* subjects as determined by analysis of variance with Fisher's least significant difference test ($P<0.01$).

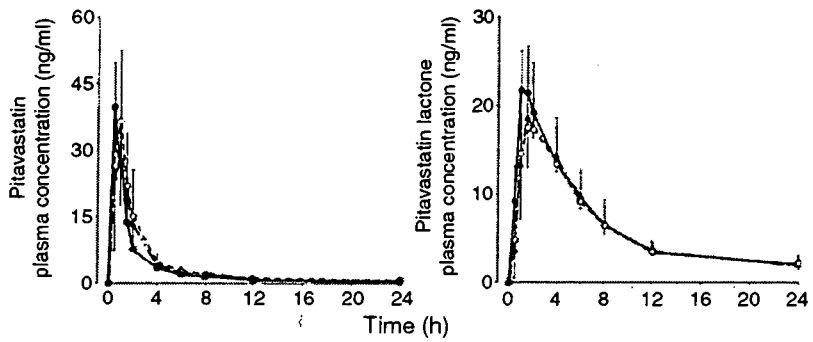

Figure 2 Effect of *ABCG2* genotype on pharmacokinetics of pitavastatin. Plasma concentration–time profiles of pitavastatin and pitavastatin lactone after oral administration of 2 mg pitavastatin in 421C/C*1b/*1b subjects (closed triangles, $n=11$), 421C/A*1b/*1b subjects (open cirdes, $n=7$), and 421A/A*1b/*1b subjects (closed circles, $n=3$).

parameters for either pitavastatin or pitavastatin lactone among the three *SLCO1B1* matched (*i.e.*, homozygotes for the *1b allele) *ABCG2* groups: group 1 ($n=11$, 421C/C*1b/*1b), group 4 ($n=7$, 421C/A*1b/*1b), and group 5 ($n=3$, 421A/A*1b/*1b). The mean AUC_{0-24} of pitavastatin in groups 1 (*i.e.*, 421C/C), 4 (421C/A*1b/*1b), 5 (421A/A), and 6 (421C/A*1b/*15) was 81.1±18.1, 96.7±35.4, 78.2±8.2, and 121±25 ng h/ml, respectively.

Role of BCRP in the intestinal absorption of pitavastatin in mice *in vivo*

To investigate the involvement of Bcrp1 in the intestinal absorption of pitavastatin, we evaluated its pharmacokinetics using control and Bcrp1 (−/−) mice. The mean AUC up to 4 h after administration was 213±13 and 221±17 ng h/ml (mean±SE, $n=4$) in control and Bcrp1 (−/−) mice, respectively ($P>0.05$). The time profiles of the plasma concentration of pitavastatin did not show any significant difference between control and Bcrp1 (−/−) mice (**Figure 3**).

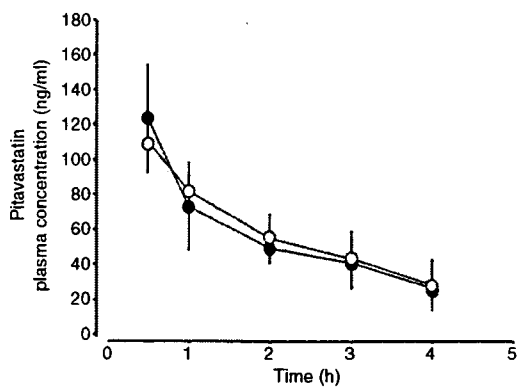

Figure 3 Time profiles of plasma concentration of pitavastatin after its oral administration (10 mg/kg) to control and Bcrp1 (−/−) mice. Closed and open circles represent the time profile of plasma concentration of pitavastatin in control and Bcrp1 (−/−) mice, respectively.

DISCUSSION

The primary objective of this study was to evaluate whether the polymorphism of two drug transporter genes contribute to large interindividual variability in the pharmacokinetics of pitavastatin, a dual substrate of OATP1B1 and BCRP. The important findings were that (1) significant differences in AUC_{0-24} and C_{max} of pitavastatin, but not in those of the lactone form, were observed among subjects with different *SLCO1B1* genotypes; and (2) in contrast to *SLCO1B1*, no significant differences in any pharmacokinetic parameters