174) Borucki, K., Weikert, C., Fisher, E., Jakubiczka, S., Luley, C., Westphal, S. and Dierkes, J.: Haplotypes in the UGT1A1 gene and their role as genetic determinants of bilirubin concentration in healthy German volunteers. *Clin. Biochem.*, **42**: 1635–1641 (2009).
175) Köhle, C., Möhrle, B., Münzel, P. A., Schwab, M., Wernet, D., Badary, O. A. and Bock, K. W.: Frequent co-occurrence of the TATA box mutation associated with Gilbert's syndrome (UGT1A1*28) with other polymorphisms of the UDP-glucuronosyltransferase-1 locus (UGT1A6*2 and UGT1A7*3) in Caucasians and Egyptians. *Biochem. Pharmacol.*, **65**: 1521–1527 (2003).
176) Biondi, M. L., Turri, O., Dilillo, D., Stival, G. and Guagnellini, E.: Contribution of the TATA-box genotype (Gilbert syndrome) to serum bilirubin concentrations in the Italian population. *Clin. Chem.*, **45**: 897–898 (1999).
177) Oliveira, E., Marsh, S., van Booven, D. J., Amorim, A., Prata, M. J. and McLeod, H. L.: Pharmacogenetically relevant polymorphisms in Portugal. *Pharmacogenomics*, **8**: 703–712 (2007).
178) Seco, M. L., del Río, E., Barceló, M. J., Remacha, A., Ginovart, G., Moliner, E. and Baiget, M.: Interest in the study of genetic variants of the promoter region of the UGT1A1 gene in neonatal jaundice. *An. Esp. Pediatr.*, **56**: 139–143 (2002).
179) Tsezou, A., Tzetis, M., Giannatou, E., Gennatas, C., Pampanos, A., Kanavakis, E. and Kitsiou-Tzeli, S.: Genetic polymorphisms in the UGT1A1 gene and breast cancer risk in Greek women. *Genet. Test.*, **11**: 303–306 (2007).
180) Vítek, L., Novotná, M., Lenícek, M., Novotný, L., Eberová, J., Petrásek, J. and Jirsa, M.: Serum bilirubin levels and UGT1A1 promoter variations in patients with schizophrenia. *Psychiatry Res.*, **178**: 449–450 (2010).
181) Shatalova, E. G., Loginov, V. I., Braga, E. A., Kazubskaia, T. P., Sudomoina, M. A., Blanchard, R. L. and Favorova, O. O.: Association of polymorphisms in SULT1A1 and UGT1A1 Genes with breast cancer risk and phenotypes in Russian women. *Mol. Biol. (Mosk.)*, **40**: 263–270 (2006).
182) Innocenti, F., Grimsley, C., Das, S., Ramírez, J., Cheng, C., Kuttab-Boulos, H., Ratain, M. J. and Di Rienzo, A.: Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. *Pharmacogenetics*, **12**: 725–733 (2002).
183) Innocenti, F., Liu, W., Chen, P., Desai, A. A., Das, S. and Ratain, M. J.: Haplotypes of variants in the UDP-glucuronosyltransferase1A9 and 1A1 genes. *Pharmacogenet. Genomics*, **15**: 295–301 (2005).
184) Lampe, J. W., Bigler, J., Horner, N. K. and Potter, J. D.: UDP-glucuronosyltransferase (UGT1A1*28 and UGT1A6*2) polymorphisms in Caucasians and Asians: relationships to serum bilirubin concentrations. *Pharmacogenetics*, **9**: 341–349 (1999).
185) Ménard, V., Girard, H., Harvey, M., Pérusse, L. and Guillemette, C.: Analysis of inherited genetic variations at the UGT1 locus in the French-Canadian population. *Hum. Mutat.*, **30**: 677–687 (2009).
186) Fertrin, K. Y., Gonçalves, M. S., Saad, S. T. and Costa, F. F.: Frequencies of UDP-glucuronosyltransferase 1 (UGT1A1) gene promoter polymorphisms among distinct ethnic groups from Brazil. *Am. J. Med. Genet.*, **108**: 117–119 (2002).
187) Guillemette, C., Millikan, R. C., Newman, B. and Housman, D. E.: Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 and association with breast cancer among African Americans. *Cancer Res.*, **60**: 950–956 (2000).
188) Akaba, K., Kimura, T., Sasaki, A., Tanabe, S., Ikegami, T., Hashimoto, M., Umeda, H., Yoshida, H., Umetsu, K., Chiba, H., Yuasa, I. and Hayasaka, K.: Neonatal hyperbilirubinemia and mutation of the bilirubin uridine diphosphate-glucuronosyltransferase gene: a common missense mutation among Japanese, Koreans and Chinese. *Biochem. Mol. Biol. Int.*, **46**: 21–26 (1998).
189) Lin, R., Wang, X., Wang, Y., Zhang, F., Wang, Y., Fu, W., Yu, T., Li, S., Xiong, M., Huang, W. and Jin, L.: Association of polymorphisms in four bilirubin metabolism genes with serum bilirubin in three Asian populations. *Hum. Mutat.*, **30**: 609–615 (2009).
190) Sutomo, R., Talib, N. A., Yusoff, N. M., Van Rostenberghe, H. and Sadewa, A. H.: Sunarti., Sofro, A.S., Yokoyama, N., Lee, M.J., Matsuo, M. and Nishio, H.: Screening for G71R mutation of the UGT1A1 gene in the Javanese-Indonesian and Malay-Malaysian populations. *Pediatr. Int.*, **46**: 565–569 (2004).
191) Jada, S. R., Lim, R., Wong, C. I., Shu, X., Lee, S. C., Zhou, Q., Goh, B. C. and Chowbay, B.: Role of UGT1A1*6, UGT1A1*28 and ABCG2 c.421C>A polymorphisms in irinotecan-induced neutropenia in Asian cancer patients. *Cancer Sci.*, **98**: 1461–1467 (2007).
192) Thomas, S. S., Li, S. S., Lampe, J. W., Potter, J. D. and Bigler, J.: Genetic variability, haplotypes, and htSNPs for exons 1 at the human UGT1A locus. *Hum. Mutat.*, **27**: 717 (2006).
193) Hein, D. W.: Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. *Mutat. Res.*, **506-507**: 65–77 (2002).
194) Hein, D. W., Doll, M. A., Fretland, A. J., Leff, M. A., Webb, S. J., Xiao, G. H., Devanaboyina, U. S., Nangju, N. A. and Feng, Y.: Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol. Biomarkers Prev.*, **9**: 29–42 (2000).
195) Zang, Y., Zhao, S., Doll, M. A., States, J. C. and Hein, D. W.: The T341C (Ile114Thr) polymorphism of N-acetyltransferase 2 yields slow acetylator phenotype by enhanced protein degradation. *Pharmacogenetics*, **14**: 717–723 (2004).
196) Sabbagh, A., Langaney, A., Darlu, P., Gérard, N., Krishnamoorthy, R. and Poloni, E. S.: Worldwide distribution of NAT2 diversity: implications for NAT2 evolutionary history. *BMC Genet.*, **9**: 21 (2008).
197) Martínez, C., Agúndez, J. A., Olivera, M., Martín, R., Ladero, J. M. and Benítez, J.: Lung cancer and mutations at the polymorphic NAT2 gene locus. *Pharmacogenetics*, **5**: 207–214 (1995).
198) Agúndez, J. A., Olivera, M., Martínez, C., Ladero, J. M. and Benítez, J.: Identification and prevalence study of 17 allelic variants of the human NAT2 gene in a white population. *Pharmacogenetics*, **6**: 423–428 (1996).
199) Agúndez, J. A., Golka, K., Martínez, C., Selinski, S., Blaszkewicz, M. and García-Martín, E.: Unraveling ambiguous NAT2 genotyping data. *Clin. Chem.*, **54**: 1390–1394 (2008).
200) Okumura, K., Kita, T., Chikazawa, S., Komada, F., Iwakawa, S. and Tanigawara, Y.: Genotyping of N-acetylation polymorphism and correlation with procainamide metabolism. *Clin. Pharmacol. Ther.*, **61**: 509–517 (1997).
201) Ohno, M., Yamaguchi, I., Yamamoto, I., Fukuda, T., Yokota, S., Maekura, R., Ito, M., Yamamoto, Y., Ogura, T., Maeda, K., Komuta, K., Igarashi, T. and Azuma, J.: Slow N-acetyltransferase 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity. *Int. J. Tuberc. Lung Dis.*, **4**: 256–261 (2000).
202) Sekine, A., Saito, S., Iida, A., Mitsunobu, Y., Higuchi, S., Harigae, S. and Nakamura, Y.: Identification of single-nucleotide polymorphisms (SNPs) of human N-acetyltransferase genes NAT1, NAT2, AANAT, ARD1 and L1CAM in the Japanese population. *J. Hum. Genet.*, **46**: 314–319 (2001).
203) Machida, H., Tsukamoto, K., Wen, C. Y., Shikuwa, S., Isomoto, H., Mizuta, Y., Takeshima, F., Murase, K., Matsumoto, N., Murata, I., Kohno, S. and Wen, C. Y.: Crohn's disease in Japanese is associated with a SNP-haplotype of N-acetyltransferase 2 gene. *World J. Gastroenterol.*, **11**: 4833–4837 (2005).

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204) Lee, S. Y., Lee, K. A., Ki, C. S., Kwon, O. J., Kim, H. J., Chung, M. P., Suh, G. Y. and Kim, J. W.: Complete sequencing of a genetic polymorphism in NAT2 in the Korean population. *Clin. Chem.*, **48**: 775–777 (2002).
205) Lee, K. M., Park, S. K., Kim, S. U., Doll, M. A., Yoo, K. Y., Ahn, S. H., Noh, D. Y., Hirvonen, A., Hein, D. W. and Kang, D.: N-acetyltransferase (NAT1, NAT2) and glutathione S-transferase (GSTM1, GSTT1) polymorphisms in breast cancer. *Cancer Lett.*, **196**: 179–186 (2003).
206) Rothman, N., Hayes, R. B., Bi, W., Caporaso, N., Broly, F., Woosley, R. L., Yin, S., Feng, P., You, X. and Meyer, U. A.: Correlation between N-acetyltransferase activity and NAT2 genotype in Chinese males. *Pharmacogenetics*, **3**: 250–255 (1993).
207) Lu, J. F., Cao, X. M., Liu, Z. H., Cao, W., Guo, L. Q., Zhuo, H. T., Ling, S. S., Chen, Y. L., Zhao, Q., Wang, W. P. and Li, F. Q.: Genetic analysis of N-acetyltransferase polymorphism in a Chinese population. *Zhongguo Yao Li Xue Bao*, **19**: 347–351 (1998).
208) Ma, Q. W., Lin, G. F., Chen, J. G., Xiang, C. Q., Guo, W. C., Golka, K. and Shen, J. H.: Polymorphism of N-acetyltransferase 2 (NAT2) gene polymorphism in shanghai population: occupational and non-occupational bladder cancer patient groups. *Biomed. Environ. Sci.*, **17**: 291–298 (2004).
209) Chen, B., Zhang, W. X. and Cai, W. M.: The influence of various genotypes on the metabolic activity of NAT2 in a Chinese population. *Eur. J. Clin. Pharmacol.*, **62**: 355–359 (2006).
210) Lin, H. J., Han, C. Y., Lin, B. K. and Hardy, S.: Ethnic distribution of slow acetylator mutations in the polymorphic N-acetyltransferase (NAT2) gene. *Pharmacogenetics*, **4**: 125–134 (1994).
211) Huang, C. C., Chien, W. P., Wong, R. H., Cheng, Y. W., Chen, M. C., Chou, M. C. and Lee, H.: NAT2 fast acetylator genotype is associated with an increased risk of colorectal cancer in Taiwan. *Dis. Colon Rectum*, **50**: 981–989 (2007).
212) Kukongviriyapan, V., Prawan, A., Tassaneyakul, W., Aiemsa-Ard, J. and Warasiha, B.: Arylamine N-acetyltransferase-2 genotypes in the Thai population. *Br. J. Clin. Pharmacol.*, **55**: 278–281 (2007).
213) Cavaco, I., Asimus, S., Peyrard-Janvid, M., Ferreira, P. E., Veiga, M. I., Hai, T. N., Ribeiro, V., Ashton, M. and Gil, J. P.: The Vietnamese Khin population harbors particular N-acetyltransferase 2 allele frequencies. *Clin. Chem.*, **53**: 1977–1979 (2007).
214) Bechtel, Y. C., Bechtel, P. R., Lelouët, H., Choisy, H. and Dy, N. R.: The acetylator polymorphism in a Khmer population: clinical consequences. *Therapie*, **56**: 409–413 (2001).
215) Zhao, B., Lee, E. J., Wong, J. Y., Yeoh, P. N. and Gong, N. H.: Frequency of mutant CYP1A1, NAT2 and GSTM1 alleles in normal Indians and Malays. *Pharmacogenetics*, **5**: 275–280 (1995).
216) Lee, E. J., Zhao, B., Moochhala, S. M. and Ngoi, S. S.: Frequency of mutant CYPIA1, NAT2 and GSTM1 alleles in a normal Chinese population. *Pharmacogenetics*, **4**: 355–358 (1994).
217) Yuliwulandari, R., Sachrowardi, Q., Nishida, N., Takasu, M., Batubara, L., Susmiarsih, T. P., Rochani, J. T., Wikaningrum, R., Miyashita, R., Miyagawa, T., Sofro, A. S. and Tokunaga, K.: Polymorphisms of promoter and coding regions of the arylamine N-acetyltransferase 2 (NAT2) gene in the Indonesian population: proposal for a new nomenclature. *J. Hum. Genet.*, **53**: 201–209 (2008).
218) Pandey, S. N., Modi, D. R., Choudhuri, G. and Mittall, B.: Slow acetylator genotype of N-acetyl transferase2 (NAT2) is associated with increased susceptibility to gallbladder cancer: the cancer risk not modulated by gallstone disease. *Cancer Biol. Ther.*, **6**: 91–96 (2007).
219) Bakayev, V. V., Mohammadi, F., Bahadori, M., Sheikholslami, M., Javeri, A., Masjedi, M. R. and Velayati, A. A.: Arylamine N-acetyltransferase 2 slow acetylator polymorphisms in unrelated Iranian individuals. *Eur. J. Clin. Pharmacol.*, **60**: 467–471 (2004).
220) Torkaman-Boutorabi, A., Hoormand, M., Naghdi, N., Bakhshayesh, M. and Milanian, I.: Genotype and allele frequencies of N-acetyltransferase 2 and glutathione S-transferase in the Iranian population. *Clin. Exp. Pharmacol. Physiol.*, **34**: 1207–1211 (2007).
221) Rabstein, S., Unfried, K., Ranft, U., Illig, T., Kolz, M., Rihs, H. P., Mambetova, C., Vlad, M., Brüning, T. and Pesch, B.: Variation of the N-acetyltransferase 2 gene in a Romanian and a Kyrgyz population. *Cancer Epidemiol. Biomarkers Prev.*, **15**: 138–141 (2006).
222) Al-Moundhri, M. S., Al-Kindi, M., Al-Nabhani, M., Al-Bahrani, B., Burney, I. A., Al-Madhani, A., Ganguly, S. S. and Tanira, M.: NAT2 polymorphism in Omani gastric cancer patients-risk predisposition and clinicopathological associations. *World J. Gastroenterol.*, **13**: 2697–2702 (2007).
223) Okkels, H., Sigsgaard, T., Wolf, H. and Autrup, H.: Arylamine N-acetyltransferase 1 (NAT1) and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. *Cancer Epidemiol. Biomarkers Prev.*, **6**: 225–231 (1997).
224) Johnson, N., Bell, P., Jonovska, V., Budge, M. and Sim, E.: NAT gene polymorphisms and susceptibility to Alzheimer's disease: identification of a novel NAT1 allelic variant. *BMC Med. Genet.*, **5**: 6 (2004).
225) Smith, C. A., Wadelius, M., Gough, A. C., Harrison, D. J., Wolf, C. R. and Rane, A.: A simplified assay for the arylamine N-acetyltransferase 2 polymorphism validated by phenotyping with isoniazid. *J. Med. Genet.*, **34**: 758–760 (1997).
226) Lemos, M. C. and Regateiro, F. J.: N-acetyltransferase genotypes in the Portuguese population. *Pharmacogenetics*, **8**: 561–564 (1998).
227) Gajecka, M., Rydzanicz, M., Jaskula-Sztul, R., Kujawski, M., Szyfter, W. and Szyfter, K.: CYP1A1, CYP2D6, CYP2E1, NAT2, GSTM1 and GSTT1 polymorphisms or their combinations are associated with the increased risk of the laryngeal squamous cell carcinoma. *Mutat. Res.*, **574**: 112–123 (2005).
228) Klimčáková, L., Habalová, V., Sivoňová, M., Nagy, V., Šalagovič, J. and Židzik, J.: Effect of NAT2 gene polymorphism on bladder cancer risk in Slovak population. *Mol. Biol. Rep.*, **38**: 1287–1293 (2011).
229) Bell, D. A., Taylor, J. A., Butler, M. A., Stephens, E. A., Wiest, J., Brubaker, L. H., Kadlubar, F. F. and Lucier, G. W.: Genotype/phenotype discordance for human arylamine N-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. *Carcinogenesis*, **14**: 1689–1692 (1993).
230) Probst-Hensch, N. M., Haile, R. W., Ingles, S. A., Longnecker, M. P., Han, C. Y., Lin, B. K., Lee, D. B., Sakamoto, G. T., Frankl, H. D., Lee, E. R. and Lin, H. J.: Acetylation polymorphism and prevalence of colorectal adenomas. *Cancer Res.*, **55**: 2017–2020 (1995).
231) Martínez, C., Agúndez, J. A., Olivera, M., Llerena, A., Ramirez, R., Hernández, M. and Benítez, J.: Influence of genetic admixture on polymorphisms of drug-metabolizing enzymes: analyses of mutations on NAT2 and C gamma P2E1 genes in a mixed Hispanic population. *Clin. Pharmacol. Ther.*, **63**: 623–628 (1998).
232) Whalen, R. and Boyer, T. D.: Human glutathione S-transferases. *Semin. Liver Dis.*, **18**: 345–358 (1998).
233) Landi, S.: Mammalian class theta GST and differential susceptibility to carcinogens: a review. *Mutat. Res.*, **463**: 247–283 (2000).
234) Goekkurt, E., Al-Batran, S. E., Hartmann, J. T., Mogck, U., Schuch, G., Kramer, M., Jaeger, E., Bokemeyer, C., Ehninger, G. and Stoehlmacher, J.: Pharmacogenetic analyses of a phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil and leucovorin plus either oxaliplatin or cisplatin: a study of the arbeitsgemeinschaft internistische onkologie. *J. Clin. Oncol.*,

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27: 2863–2873 (2009).

235) Boige, V., Mendiboure, J., Pignon, J. P., Loriot, M. A., Castaing, M., Barrois, M., Malka, D., Trégouët, D. A., Bouché, O., Le Corre, D., Miran, I., Mulot, C., Ducreux, M., Beaune, P. and Laurent-Puig, P.: Pharmacogenetic assessment of toxicity and outcome in patients with metastatic colorectal cancer treated with LV5FU2, FOLFOX, and FOLFIRI: FFCD 2000-05. *J. Clin. Oncol.*, **28**: 2556–2564 (2010).

236) McLeod, H. L., Sargent, D. J., Marsh, S., Green, E. M., King, C. R., Fuchs, C. S., Ramanathan, R. K., Williamson, S. K., Findlay, B. P., Thibodeau, S. N., Grothey, A., Morton, R. F. and Goldberg, R. M.: Pharmacogenetic predictors of adverse events and response to chemotherapy in metastatic colorectal cancer: results from North American Gastrointestinal Intergroup Trial N9741. *J. Clin. Oncol.*, **28**: 3227–3233 (2010).

237) Watanabe, I., Tomita, A., Shimizu, M., Sugawara, M., Yasumo, H., Koishi, R., Takahashi, T., Miyoshi, K., Nakamura, K., Izumi, T., Matsushita, Y., Furukawa, H., Haruyama, H. and Koga, T.: A study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with type 2 diabetes mellitus. *Clin. Pharmacol. Ther.*, **73**: 435–455 (2003).

238) Lucena, M. I., Andrade, R. J., Martínez, C., Ulzurrun, E., García-Martín, E., Borraz, Y., Fernández, M. C., Romero-Gomez, M., Castiella, A., Planas, R., Costa, J., Anzola, S. and Agúndez, J. A.: Spanish Group for the Study of Drug-Induced Liver Disease.: Glutathione S-transferase m1 and t1 null genotypes increase susceptibility to idiosyncratic drug-induced liver injury. *Hepatology*, **48**: 588–596 (2008).

239) Kihara, M., Kihara, M. and Noda, K.: Lung cancer risk of GSTM1 null genotype is dependent on the extent of tobacco smoke exposure. *Carcinogenesis*, **15**: 415–418 (1994).

240) Oyama, T., Kawamoto, T., Mizoue, T., Nishida, K., Osaki, T., Sugio, K., Yasumoto, K. and Mitsudomi, T.: p53 mutations of lung cancer are not significantly affected by CYP1A1 or GSTM1 polymorphisms. *Int. J. Oncol.*, **11**: 305–309 (1997).

241) Morinobu, A., Kanagawa, S., Koshiba, M., Sugai, S. and Kumagai, S.: Association of the glutathione S-transferase M1 homozygous null genotype with susceptibility to Sjögren's syndrome in Japanese individuals. *Arthritis Rheum.*, **42**: 2612–2615 (1999).

242) Inoue, H., Kiyohara, C., Shinomiya, S., Marugame, T., Tsuji, E., Handa, K., Hayabuchi, H., Eguchi, H., Fukushima, Y. and Kono, S.: Glutathione S-transferase polymorphisms and risk of colorectal adenomas. *Cancer Lett.*, **163**: 201–206 (2001).

243) Naoe, T., Takeyama, K., Yokozawa, T., Kiyoi, H., Seto, M., Uike, N., Ino, T., Utsunomiya, A., Maruta, A., Jin-nai, I., Kamada, N., Kubota, Y., Nakamura, H., Shimazaki, C., Horiike, S., Kodera, Y., Saito, H., Ueda, R., Wiemels, J. and Ohno, R.: Analysis of Genetic Polymorphism in *NQO1, GST-M1, GST-T1*, and *CYP3A4* in 469 Japanese Patients with Therapy-related Leukemia/Myelodysplastic Syndrome and *de novo* Acute Myeloid Leukemia1. *Clin. Cancer Res.*, **6**: 4091–4095 (2000).

244) Murata, M., Watanabe, M., Yamanaka, M., Kubota, Y., Ito, H., Nagao, M., Katoh, T., Kamataki, T., Kawamura, J., Yatani, R. and Shiraishi, T.: Genetic polymorphisms in cytochrome P450 (CYP) 1A1, CYP1A2, CYP2E1, glutathione S-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. *Cancer Lett.*, **165**: 171–177 (2001).

245) Hishida, A., Terakura, S., Emi, N., Yamamoto, K., Murata, M., Nishio, K., Sekido, Y., Niwa, T., Hamajima, N. and Naoe, T.: GSTT1 and GSTM1 deletions, NQO1 C609T polymorphism and risk of chronic myelogenous leukemia in Japanese. *Asian Pac. J. Cancer Prev.*, **6**: 251–255 (2005).

246) Tamaki, Y., Arai, T., Sugimura, H., Sasaki, T., Honda, M., Muroi, Y., Matsubara, Y., Kanno, S., Ishikawa, M., Hirasawa, N. and Hiratsuka, M.: Association between Cancer Risk and Drug Metabolizing Enzyme Gene (CYP2A6, CYP2A13, CYP4B1, SULT1A1, GSTM1, and GSTT1) Polymorphisms in Japanese Cases of Lung Cancer. *Drug Metab. Pharmacokinet.*, **26**: 516–522 (2011).

247) Hong, Y. S., Chang, J. H., Know, O. J., Ham, Y. A. and Choi, J. H.: Polymorphism of the CYP1A1 and glutathione-S-transferase genes in Korean lung cancer patients. *Exp. Mol. Med.*, **30**: 192–198 (1998).

248) Park, S. K., Yoo, K. Y., Lee, S. J., Kim, S. U., Ahn, S. H., Noh, D. Y., Choe, K. J., Strickland, P. T., Hirvonen, A. and Kang, D.: Alcohol consumption, glutathione S-transferase M1 and T1 genetic polymorphisms and breast cancer risk. *Pharmacogenetics*, **10**: 301–309 (2000).

249) Kim, W. J., Lee, H. L., Lee, S. C., Kim, Y. T. and Kim, H.: Polymorphisms of N-acetyltransferase 2, glutathione S-transferase mu and theta genes as risk factors of bladder cancer in relation to asthma and tuberculosis. *J. Urol.*, **164**: 209–213 (2000).

250) Choi, S. C., Yun, K. J., Kim, T. H., Kim, H. J., Park, S. G., Oh, G. J., Chae, S. C., Oh, G. J., Nah, Y. H., Kim, J. J. and Chung, H. T.: Prognostic potential of glutathione S-transferase M1 and T1 null genotypes for gastric cancer progression. *Cancer Lett.*, **195**: 169–175 (2003).

251) Cho, H. J., Lee, S. Y., Ki, C. S. and Kim, J. W.: GSTM1, GSTT1 and GSTP1 polymorphisms in the Korean population. *J. Korean Med. Sci.*, **20**: 1089–1092 (2005).

252) Piao, J. M., Shin, M. H., Kweon, S. S., Kim, H. N., Choi, J. S., Bae, W. K., Shim, H. J., Kim, H. R., Park, Y. K., Choi, Y. D. and Kim, S. H.: Glutathione-S-transferase (GSTM1, GSTT1) and the risk of gastrointestinal cancer in a Korean population. *World J. Gastroenterol.*, **15**: 5716–5721 (2009).

253) Kwon, D. D., Lee, J. W., Han, D. Y., Seo, I. Y., Park, S. C., Jeong, H. J., Yang, Y. S., Chae, S. C., Na, K. S., Mo, K. J., Kim, J. J. and Rim, J. S.: Relationship between the Glutathione-S-Transferase P1, M1, and T1 Genotypes and Prostate Cancer Risk in Korean Subjects. *Korean J. Urol*, **52**: 247–252 (2011).

254) Setiawan, V. W., Zhang, Z. F., Yu, G. P., Li, Y. L., Lu, M. L., Tsai, C. J., Cordova, D., Wang, M. R., Guo, C. H., Yu, S. Z. and Kurtz, R. C.: GSTT1 and GSTM1 null genotypes and the risk of gastric cancer: a case-control study in a Chinese population. *Cancer Epidemiol. Biomarkers Prev.*, **9**: 73–80 (2000).

255) Chen, S., Xue, K., Xu, L., Ma, G. and Wu, J.: Polymorphisms of the CYP1A1 and GSTM1 genes in relation to individual susceptibility to lung carcinoma in Chinese population. *Mutat. Res.*, **458**: 41–47 (2001).

256) Mu, L. N., Lu, Q. Y., Yu, S. Z., Jiang, Q. W., Cao, W., You, N. C., Setiawan, V. W., Zhou, X. F., Ding, B. G., Wang, R. H., Zhao, J., Cai, L., Rao, J. Y., Heber, D. and Zhang, Z. F.: Green tea drinking and multigenetic index on the risk of stomach cancer in a Chinese population. *Int. J. Cancer*, **116**: 972–983 (2005).

257) Liu, L., Li, C., Gao, J., Li, K., Gao, L. and Gao, T.: Genetic polymorphisms of glutathione S-transferase and risk of vitiligo in the Chinese population. *J. Invest. Dermatol.*, **129**: 2646–2652 (2009).

258) Zhong, S. L., Zhou, S., Chen, X. and Huang, M.: Rapid determination of common mutations in glutathione S-transferase gene by PCR-based methods in healthy Chinese. *Clin. Chim. Acta*, **364**: 205–208 (2006).

259) Guo, X., O'Brien, S. J., Zeng, Y., Nelson, G. W. and Winkler, C. A.: GSTM1 and GSTT1 gene deletions and the risk for nasopharyngeal carcinoma in Han Chinese. *Cancer Epidemiol. Biomarkers Prev.*, **17**: 1760–1763 (2008).

260) Lee, Y. L., Hsiue, T. R., Lee, Y. C., Lin, Y. C. and Guo, Y. L.: The association between glutathione S-transferase P1, M1

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polymorphisms and asthma in Taiwanese schoolchildren. *Chest*, **128**: 1156–1162 (2005).

261) Fujihara, J., Yasuda, T., Iida, R., Takatsuka, H., Fujii, Y. and Takeshita, H.: Cytochrome P450 1A1, glutathione S-transferases M1 and T1 polymorphisms in Ovambos and Mongolians. *Leg. Med. (Tokyo)*, **11** (Suppl 1): S408–S410 (2009).

262) Agusa, T., Iwata, H., Fujihara, J., Kunito, T., Takeshita, H., Minh, T. B., Trang, P. T., Viet, P. H. and Tanabe, S.: Genetic polymorphisms in glutathione S-transferase (GST) superfamily and arsenic metabolism in residents of the Red River Delta, Vietnam. *Toxicol. Appl. Pharmacol.*, **242**: 352–362 (2010).

263) Rimando, M. G., Chua, M. N., Yuson, E., de Castro-Bernas, G. and Okamoto, T.: Prevalence of GSTT1, GSTM1 and NQO1 (609C>T) in Filipino children with ALL (acute lymphoblastic leukaemia). *Biosci. Rep.*, **28**: 117–124 (2008).

264) Pakakasama, S., Mukda, E., Sasanakul, W., Kadegasem, P., Udomsubpayakul, U., Thithapandha, A. and Hongeng, S.: Polymorphisms of drug-metabolizing enzymes and risk of childhood acute lymphoblastic leukemia. *Am. J. Hematol.*, **79**: 202–205 (2005).

265) Tiwawech, D., Srivatanakul, P., Karalak, A. and Ishida, T.: Glutathione S-transferase M1 gene polymorphism in Thai nasopharyngeal carcinoma. *Asian Pac. J. Cancer Prev.*, **6**: 270–275 (2005).

266) Sangrajrang, S., Jedpiyawongse, A. and Srivatanakul, P.: Genetic polymorphisms of CYP2E1 and GSTM1 in a Thai population. *Asian Pac. J. Cancer Prev.*, **7**: 415–419 (2006).

267) Klinchid, J., Chewaskulyoung, B., Saeteng, S., Lertprasertsuke, N., Kasinrerk, W. and Cressey, R.: Effect of combined genetic polymorphisms on lung cancer risk in northern Thai women. *Cancer Genet. Cytogenet.*, **195**: 143–149 (2009).

268) Amtha, R., Ching, C. S., Zain, R., Razak, I. A., Basuki, B., Roeslan, B. O., Gautama, W. and Purwanto, D.: GSTM1, GSTT1 and CYP1A1 polymorphisms and risk of oral cancer: a case-control study in Jakarta, Indonesia. *Asian Pac. J. Cancer Prev.*, **10**: 21–26 (2009).

269) Zhao, B., Lee, E. J., Wong, J. Y., Yeoh, P. N. and Gong, N. H.: Frequency of mutant CYP1A1, NAT2 and GSTM1 alleles in normal Indians and Malays. *Pharmacogenetics*, **5**: 275–280 (1995).

270) Zhao, B., Seow, A., Lee, E. J. and Poh, W. T.: the, M., Eng, P., Wang, Y.T., Tan, W.C., Yu, M.C. and Lee, H.P.: Dietary isothiocyanates, glutathione S-transferase -M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. *Cancer Epidemiol. Biomarkers Prev.*, **10**: 1063–1067 (2001).

271) Lee, E. J., Wong, J. Y., Yeoh, P. N. and Gong, N. H.: Glutathione S transferase-theta (GSTT1) genetic polymorphism among Chinese, Malays and Indians in Singapore. *Pharmacogenetics*, **5**: 332–334 (1995).

272) Buch, S. C., Notani, P. N. and Bhisey, R. A.: Polymorphism at GSTM1, GSTM3 and GSTT1 gene loci and susceptibility to oral cancer in an Indian population. *Carcinogenesis*, **23**: 803–807 (2002).

273) Mishra, D. K., Kumar, A., Srivastava, D. S. and Mittal, R. D.: Allelic variation of GSTT1, GSTM1 and GSTP1 genes in North Indian population. *Asian Pac. J. Cancer Prev.*, **5**: 362–365 (2004).

274) Konwar, R., Manchanda, P. K., Chaudhary, P., Nayak, V. L., Singh, V. and Bid, H. K.: Glutathione S-transferase (GST) gene variants and risk of benign prostatic hyperplasia: a report in a North Indian population. *Asian Pac. J. Cancer Prev.*, **11**: 1067–1072 (2010).

275) Sreeja, L., Syamala, V., Hariharan, S., Madhavan, J., Devan, S. C. and Ankathil, R.: Possible risk modification by CYP1A1, GSTM1 and GSTT1 gene polymorphisms in lung cancer susceptibility in a South Indian population. *J. Hum. Genet.*, **50**: 618–627 (2005).

276) Vettriselvi, V., Vijayalakshmi, K., Solomon, F.D.P. and Venkatachalam, P.: Genetic variation of GSTM1, GSTT1 and GSTP1 genes in a South Indian population. *Asian Pac. J. Cancer Prev.*, **7**: 325–328 (2006).

277) Hou, S. M., Ryberg, D., Fält, S., Deverill, A., Tefre, T., Børresen, A. L., Haugen, A. and Lambert, B.: GSTM1 and NAT2 polymorphisms in operable and non-operable lung cancer patients. *Carcinogenesis*, **21**: 49–54 (2000).

278) Garte, S., Gaspari, L., Alexandrie, A. K., Ambrosone, C., Autrup, H., Autrup, J. L., Baranova, H., Bathum, L., Benhamou, S., Boffetta, P., Bouchardy, C., Breskvar, K., Brockmoller, J., Cascorbi, I., Clapper, M. L., Coutelle, C., Daly, A., Dell'Omo, M., Dolzan, V., Dresler, C. M., Fryer, A., Haugen, A., Hein, D. W., Hildesheim, A., Hirvonen, A., Hsieh, L. L., Ingelman-Sundberg, M., Kalina, I., Kang, D., Kihara, M., Kiyohara, C., Kremers, P., Lazarus, P., Le Marchand, L., Lechner, M. C., van Lieshout, E. M., London, S., Manni, J. J., Maugard, C. M., Morita, S., Nazar-Stewart, V., Noda, K., Oda, Y., Parl, F. F., Pastorelli, R., Persson, I., Peters, W. H., Rannug, A., Rebbeck, T., Risch, A., Roelandt, L., Romkes, M., Ryberg, D., Salagovic, J., Schoket, B., Seidegard, J., Shields, P. G., Sim, E., Sinnet, D., Strange, R. C., Stücker, I., Sugimura, H., To-Figueras, J., Vineis, P., Yu, M. C. and Taioli, E.: Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol. Biomarkers Prev.*, **10**: 1239–1248 (2001).

279) Bu, H., Rosdahl, I., Holmdahl-Källen, K., Sun, X. F. and Zhang, H.: Significance of glutathione S-transferases M1, T1 and P1 polymorphisms in Swedish melanoma patients. *Oncol. Rep.*, **17**: 859–864 (2007).

280) Risch, A., Wikman, H., Thiel, S., Schmezer, P., Edler, L., Drings, P., Dienemann, H., Kayser, K., Schulz, V., Spiegelhalder, B. and Bartsch, H.: Glutathione-S-transferase M1, M3, T1 and P1 polymorphisms and susceptibility to non-small-cell lung cancer subtypes and hamartomas. *Pharmacogenetics*, **11**: 757–764 (2001).

281) Schneider, J., Bernges, U., Philipp, M. and Woitowitz, H. J.: GSTM1, GSTT1, and GSTP1 polymorphism and lung cancer risk in relation to tobacco smoking. *Cancer Lett.*, **208**: 65–74 (2004).

282) Kabesch, M., Hoefler, C., Carr, D., Leupold, W., Weiland, S. K. and von Mutius, E.: Glutathione S transferase deficiency and passive smoking increase childhood asthma. *Thorax*, **59**: 569–573 (2004).

283) Abbas, A., Delvinquiere, K., Lechevrel, M., Lebailly, P., Gauduchon, P., Launoy, G. and Sichel, F.: GSTM1, GSTT1, GSTP1 and CYP1A1 genetic polymorphisms and susceptibility to esophageal cancer in a French population: different pattern of squamous cell carcinoma and adenocarcinoma. *World J. Gastroenterol.*, **10**: 3389–3393 (2004).

284) Alberti, G., Oguni, M., Podgor, M., Sperduto, R. D., Tomarev, S., Grassi, C., Williams, S., Kaiser-Kupfer, M., Maraini, G. and Hejtmancik, J. F.: Glutathione S-transferase M1 genotype and age-related cataracts. Lack of association in an Italian population. *Invest. Ophthalmol. Vis. Sci.*, **37**: 1167–1173 (1996).

285) Boccia, S., Sayed-Tabatabaei, F. A., Persiani, R., Gianfagna, F., Rausei, S., Arzani, D., La Greca, A., D'Ugo, D., La Torre, G., van Duijn, C. M. and Ricciardi, G.: Polymorphisms in metabolic genes, their combination and interaction with tobacco smoke and alcohol consumption and risk of gastric cancer: a case-control study in an Italian population. *BMC Cancer*, **7**: 206 (2007).

286) García-Closas, M., Malats, N., Silverman, D., Dosemeci, M., Kogevinas, M., Hein, D. W., Tardón, A., Serra, C., Carrato, A., García-Closas, R., Lloreta, J., Castaño-Vinyals, G., Yeager, M., Welch, R., Chanock, S., Chatterjee, N., Wacholder, S., Samanic, C., Torà, M., Fernández, F., Real, F. X. and Rothman, N.: NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. *Lancet*, **366**: 649–659 (2005).

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287) Varela-Lema, L., Ruano-Ravina, A., Juiz, Crespo, M. A., Kelsey, K. T., Loidi, L. and Barros-Dios, J. M.: CYP1A1, mEH, and GSTM1 Polymophisms and Risk of Oral and Pharyngeal Cancer: A Spanish Case-Control Study. *J. Oncol.*, **2008**: 741310 (2008).
288) Conde, A. R., Martins, G., Saraiva, C., Rueff, J. and Monteiro, C.: Association of p53 genomic instability with the glutathione S-transferase null genotype in gastric cancer in the Portuguese population. *Mol. Pathol.*, **52**: 131–134 (1999).
289) Dialyna, I. A., Miyakis, S., Georgatou, N. and Spandidos, D. A.: Genetic polymorphisms of CYP1A1, GSTM1 and GSTT1 genes and lung cancer risk. *Oncol. Rep.*, **10**: 1829–1835 (2003).
290) Georgiou, I., Filiadis, I. F., Alamanos, Y., Bouba, I., Giannakopoulos, X. and Lolis, D.: Glutathione S-transferase null genotypes in transitional cell bladder cancer: a case-control study. *Eur. Urol.*, **37**: 660–664 (2000).
291) Binková, B., Smerhovský, Z., Strejc, P., Boubelík, O., Stávková, Z., Chvátalová, I. and Srám, R. J.: DNA-adducts and atherosclerosis: a study of accidental and sudden death males in the Czech Republic. *Mutat. Res.*, **501**: 115–128 (2002).
292) Toncheva, D. I., Von Ahsen, N., Atanasova, S. Y., Dimitrov, T. G., Armstrong, V. W. and Oellerich, M.: Identification of NQO1 and GSTs genotype frequencies in Bulgarian patients with Balkan endemic nephropathy. *J. Nephrol.*, **17**: 384–389 (2004).
293) Cote, M. L., Kardia, S. L., Wenzlaff, A. S., Land, S. J. and Schwartz, A. G.: Combinations of glutathione S-transferase genotypes and risk of early-onset lung cancer in Caucasians and African Americans: a population-based study. *Carcinogenesis*, **26**: 811–819 (2005).
294) Millikan, R., Pittman, G., Tse, C. K., Savitz, D. A., Newman, B. and Bell, D.: Glutathione S-transferases M1, T1, and P1 and breast cancer. *Cancer Epidemiol. Biomarkers Prev.*, **9**: 567–573 (2000).
295) Van Emburgh, B. O., Hu, J. J., Levine, E. A., Mosley, L. J., Perrier, N. D., Freimanis, R. I., Allen, G. O., Rubin, P., Sherrill, G. B., Shaw, C. S., Carey, L. A., Sawyer, L. R. and Miller, M. S.: Polymorphisms in CYP1B1, GSTM1, GSTT1 and GSTP1, and susceptibility to breast cancer. *Oncol. Rep.*, **19**: 1311–1321 (2008).
296) Adams, C. H., Werely, C. J., Victor, T. C., Hoal, E. G., Rossouw, G. and van Helden, P. D.: Allele frequencies for glutathione S-transferase and N-acetyltransferase 2 differ in African population groups and may be associated with oesophageal cancer or tuberculosis incidence. *Clin. Chem. Lab. Med.*, **41**: 600–605 (2003).
297) Katoh, T., Nagata, N., Kuroda, Y., Itoh, H., Kawahara, A., Kuroki, N., Ookuma, R. and Bell, D. A.: Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. *Carcinogenesis*, **17**: 1855–1859 (1996).
298) Niemi, M., Pasanen, M. K. and Neuvonen, P. J.: Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol. Rev.*, **63**: 157–181 (2011).
299) Kalliokoski, A. and Niemi, M.: Impact of OATP transporters on pharmacokinetics. *Br. J. Pharmacol.*, **158**: 693–705 (2009).
300) Niemi, M., SchaeŠeler, E., Lang, T., Fromm, M. F., Neuvonen, M., Kyrklund, C., Backman, J. T., Kerb, R., Schwab, M., Neuvonen, P. J., Eichelbaum, M. and Kivisto, K. T.: High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1). *Pharmacogenetics*, **14**: 429–440 (2004).
301) Niemi, M., Neuvonen, P. J., Hofmann, U., Backman, J. T., Schwab, M., Lutjohann, D., von Bergmann, K., Eichelbaum, M. and Kivisto, K. T.: Acute efects of pravastatin on cholesterol synthesis are associated with SLCO1B1 (encoding OATP1B1) haplotype *17. *Pharmacogenet. Genomics*, **15**: 303–309 (2005).
302) Nozawa, T., Nakajima, M., Tamai, I., Noda, K., Nezu, J., Sai, Y., Tsuji, A. and Yokoi, T.: Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis. *J. Pharmacol. Exp. Ther.*, **302**: 804–813 (2002).
303) Nishizato, Y., Ieiri, I., Suzuki, H., Kimura, M., Kawabata, K., Hirota, T., Takane, H., Irie, S., Kusuhara, H., Urasaki, Y., Urae, A., Higuchi, S., Otsubo, K. and Sugiyama, Y.: Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin. Pharmacol. Ther.*, **73**: 554–565 (2003).
304) Kim, S. R., Saito, Y., Sai, K., Kurose, K., Maekawa, K., Kaniwa, N., Ozawa, S., Kamatani, N., Shirao, K., Yamamoto, N., Hamaguchi, T., Kunitoh, H., Ohe, Y., Yamada, Y., Tamura, T., Yoshida, T., Minami, H., Ohtsu, A., Saijo, N. and Sawada, J.: Genetic variations and frequencies of major haplotypes in SLCO1B1 encoding the transporter OATP1B1 in Japanese subjects: SLCO1B1*17 is more prevalent than *15. *Drug Metab. Pharmacokinet.*, **22**: 456–461 (2007).
305) Kim, E. Y., Cho, D. Y., Shin, H. J., Lee, S. S., Shon, J. H., Shin, J. G. and Shin, S. G.: Duplex pyrosequencing assay of the 388A>G and 521T>C SLCO1B1 polymorphisms in three Asian populations. *Clin. Chim. Acta*, **388**: 68–72 (2008).
306) Choi, J. H., Lee, M. G. and Cho, J. Y.: Lee. J.E., Kim, K.H. and Park, K.: Influence of OATP1B1 genotype on the pharmacokinetics of rosuvastatin in Koreans. *Clin. Pharmacol. Ther.*, **83**: 251–257 (2008).
307) Xu, L. Y., He, Y. J., Zhang, W., Deng, S., Li, Q., Zhang, W. X., Liu, Z. Q., Wang, D., Huang, Y. F., Zhou, H. H. and Sun, Z. Q.: Organic anion transporting polypeptide-1B1 haplotypes in Chinese patients. *Acta Pharmacol. Sin.*, **28**: 1693–1697 (2007).
308) Jada, S. R., Xiaochen, S., Yan, L. Y., Xiaoqiang, X., Lal, S., Zhou, S. F., Ooi, L. L. and Chowbay, B.: Pharmacogenetics of SLCO1B1: haplotypes, htSNPs and hepatic expression in three distinct Asian populations. *Eur. J. Clin. Pharmacol.*, **63**: 555–563 (2007).
309) Lee, E., Ryan, S., Birmingham, B., Zalikowski, J., March, R., Ambrose, H., Moore, R., Lee, C., Chen, Y. and Schneck, D.: Rosuvastatin pharmacokinetics and pharmacogenetics in white and Asian subjects residing in the same environment. *Clin. Pharmacol. Ther.*, **78**: 330–341 (2005).
310) Ho, W. F., Koo, S. H., Yee, J. Y. and Lee, E. J.: Genetic variations of the SLCO1B1 gene in the Chinese, Malay and Indian populations of Singapore. *Drug Metab. Pharmacokinet.*, **23**: 476–482 (2008).
311) Srivastava, A., Srivastava, A., Srivastava, N., Choudhuri, G. and Mittal, B.: Organic anion transporter 1B1 (SLCO1B1) polymorphism and gallstone formation: High incidence of Exon4 CA genotype in female patients in North India. *Hepatol. Res.*, **41**: 71–78 (2011).
312) Mwinyi, J., Köpke, K., Schaefer, M., Roots, I. and Gerloff, T.: Comparison of SLCO1B1 sequence variability among German, Turkish, and African populations. *Eur. J. Clin. Pharmacol.*, **64**: 257–266 (2008).
313) Pasanen, M. K., Backman, J. T., Neuvonen, P. J. and Niemi, M.: Frequencies of single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide 1B1 SLCO1B1 gene in a Finnish population. *Eur. J. Clin. Pharmacol.*, **62**: 409–415 (2006).
314) Pasanen, M. K., Neuvonen, P. J. and Niemi, M.: Global analysis of genetic variation in SLCO1B1. *Pharmacogenomics*, **9**: 19–33 (2008).
315) Rohrbacher, M., Kirchhof, A., Skarke, C., Geisslinger, G. and

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Lötsch, J.: Rapid identification of three functionally relevant polymorphisms in the OATP1B1 transporter gene using Pyrosequencing. *Pharmacogenomics*, **7**: 167–176 (2006).

316) Tirona, R. G., Leake, B. F., Merino, G. and Kim, R. B.: Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J. Biol. Chem.*, **276**: 35669–35675 (2001).

317) Boivin, A. A., Cardinal, H., Barama, A., Pichette, V., Hébert, M. J. and Roger, M.: Organic anion transporting polypeptide 1B1 (OATP1B1) and OATP1B3: genetic variability and haplotype analysis in white Canadians. *Drug Metab. Pharmacokinet.*, **25**: 508–515 (2010).

318) Ni, Z., Bikadi, Z., Rosenberg, M. F. and Mao, Q.: Structure and function of the human breast cancer resistance protein (BCRP/ABCG2). *Curr. Drug Metab.*, **11**: 603–617 (2010).

319) Cusatis, G. and Sparreboom, A.: Pharmacogenomic importance of ABCG2. *Pharmacogenomics*, **9**: 1005–1009 (2008).

320) Cusatis, G., Gregorc, V., Li, J., Spreafico, A., Ingersoll, R. G., Verweij, J., Ludovini, V., Villa, E., Hidalgo, M., Sparreboom, A. and Baker, S. D.: Pharmacogenetics of ABCG2 and adverse reactions to gefitinib. *J. Natl. Cancer Inst.*, **98**: 1739–1742 (2006).

321) Kim, I. S., Kim, H. G., Kim, D. C., Eom, H. S., Kong, S. Y., Shin, H. J., Hwang, S. H., Lee, E. Y. and Lee, G. W.: ABCG2 Q141K polymorphism is associated with chemotherapy-induced diarrhea in patients with diffuse large B-cell lymphoma who received frontline rituximab plus cyclophosphamide/doxorubicin/vincristine/prednisone chemotherapy. *Cancer Sci.*, **99**: 2496–2501 (2008).

322) Zamber, C. P., Lamba, J. K., Yasuda, K., Farnum, J., Thummel, K., Schuetz, J. D. and Schuetz, E. G.: Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics*, **13**: 19–28 (2003).

323) Kobayashi, D., Ieiri, I., Hirota, T., Takane, H., Maegawa, S., Kigawa, J., Suzuki, H., Nanba, E., Oshimura, M., Terakawa, N., Otsubo, K., Mine, K. and Sugiyama, Y.: Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug Metab. Dispos.*, **33**: 94–101 (2005).

324) Imai, Y., Nakane, M., Kage, K., Tsukahara, S., Ishikawa, E., Tsuruo, T., Miki, Y. and Sugimoto, Y.: 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.*, **1**: 611–616 (2002).

325) Maekawa, K., Itoda, M., Sai, K., Saito, Y., Kaniwa, N., Shirao, K., Hamaguchi, T., Kunitoh, H., Yamamoto, N., Tamura, T., Minami, H., Kubota, K., Ohtsu, A., Yoshida, T., Saijo, N., Kamatani, N., Ozawa, S. and Sawada, J.: Genetic variation and haplotype structure of the ABC transporter gene ABCG2 in a Japanese population. *Drug Metab. Pharmacokinet.*, **21**: 109–121 (2006).

326) Kim, K. A., Joo, H. J. and Park, J. Y.: ABCG2 polymorphisms, 34G>A and 421C>A in a Korean population: analysis and a comprehensive comparison with other populations. *J. Clin. Pharm. Ther.*, **35**: 705–712 (2010).

327) Lee, S. S., Jeong, H. E., Yi, J. M., Jung, H. J., Jang, J. E., Kim, E. Y., Lee, S. J. and Shin, J. G.: Identification and functional assessment of BCRP polymorphisms in a Korean population. *Drug Metab. Dispos.*, **35**: 623–632 (2007).

328) Kim, I. S., Kim, H. G., Kim, D. C., Eom, H. S., Kong, S. Y., Shin, H. J., Hwang, S. H., Lee, E. Y. and Lee, G. W.: ABCG2 Q141K polymorphism is associated with chemotherapy-induced diarrhea in patients with diffuse large B-cell lymphoma who received frontline rituximab plus cyclophosphamide/doxorubicin/vincristine/prednisone chemotherapy. *Cancer Sci.*, **99**: 2496–2501 (2008).

329) de Jong, F. A., Marsh, S., Mathijssen, R. H., King, C., Verweij, J., Sparreboom, A. and McLeod, H. L.: ABCG2 pharmacogenetics: ethnic differences in allele frequency and assessment of influence on irinotecan disposition. *Clin. Cancer Res.*, **10**: 5889–5894 (2004).

330) Wang, B., Miao, Z., Liu, S., Wang, J., Zhou, S., Han, L., Meng, D., Wang, Y., Li, C. and Ma, X.: Genetic analysis of ABCG2 gene C421A polymorphism with gout disease in Chinese Han male population. *Hum. Genet.*, **127**: 245–246 (2010).

331) Bäckström, G., Taipalensuu, J., Melhus, H., Brändström, H., Svensson, A. C., Artursson, P. and Kindmark, A.: Genetic variation in the ATP-binding cassette transporter gene ABCG2 (BCRP) in a Swedish population. *Eur. J. Pharm. Sci.*, **18**: 359–364 (2003).

332) Bosch, T. M., Kjellberg, L. M., Bouwers, A., Koeleman, B. P., Schellens, J. H., Beijnen, J. H., Smits, P. H. and Meijerman, I.: Detection of single nucleotide polymorphisms in the ABCG2 gene in a Dutch population. *Am. J. Pharmacogenomics*, **5**: 123–131 (2005).

333) Müller, P. J., Dally, H., Klappenecker, C. N., Edler, L., Jäger, B., Gerst, M., Spiegelhalder, B., Tuengerthal, S., Fischer, J. R., Drings, P., Bartsch, H. and Risch, A.: Polymorphisms in ABCG2, ABCC3 and CNT1 genes and their possible impact on chemotherapy outcome of lung cancer patients. *Int. J. Cancer*, **124**: 1669–1674 (2009).

334) Stark, K., Reinhard, W., Grassl, M., Erdmann, J., Schunkert, H., Illig, T. and Hengstenberg, C.: Common polymorphisms influencing serum uric acid levels contribute to susceptibility to gout, but not to coronary artery disease. *PLoS ONE*, **4**: e7729 (2009).

335) Fischer, S. and Lakatos, P. L.: Hungarian IBD Study Group: Lakatos, L., Kovacs, A., Molnar, T., Altorjay, I., Papp, M., Szilvasi, A., Tulassay, Z., Osztovits, J., Papp, J., Demeter, P., Schwab, R., Tordai, A. and Andrikovics, H.: ATP-binding cassette transporter ABCG2 (BCRP) and ABCB1 (MDR1) variants are not associated with disease susceptibility, disease phenotype response to medical therapy or need for surgery in Hungarian patients with inflammatory bowel diseases. *Scand. J. Gastroenterol.*, **42**: 726–733 (2007).

336) Mizuarai, S., Aozasa, N. and Kotani, H.: Single nucleotide polymorphisms result in impaired membrane localization and reduced atpase activity in multidrug transporter ABCG2. *Int. J. Cancer*, **109**: 238–246 (2004).

337) Ho, R. H., Choi, L., Lee, W., Mayo, G., Schwarz, U. I., Tirona, R. G., Bailey, D. G., Michael Stein, C. and Kim, R. B.: Effect of drug transporter genotypes on pravastatin disposition in European- and African-American participants. *Pharmacogenet. Genomics*, **17**: 647–656 (2007).

338) Steven, G. E. M., Peter, P. and Linda, D. B.: THE HLA Facts Book. pp. 14–45.

339) Tohkin, M., Ishiguro, A., Kaniwa, N., Saito, Y., Kurose, K. and Hasegawa, R.: Prediction of severe adverse drug reactions using pharmacogenetic biomarkers. *Drug Metab. Pharmacokinet.*, **25**: 122–133 (2010).

340) Hung, S. I., Chung, W. H., Jee, S. H., Chen, W. C., Chang, Y. T., Lee, W. R., Hu, S. L., Wu, M. T., Chen, G. S., Wong, T. W., Hsiao, P. F., Chen, W. H., Shih, H. Y., Fang, W. H., Wei, C. Y., Lou, Y. H., Huang, Y. L., Lin, J. J. and Chen, Y. T.: Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet. Genomics*, **16**: 297–306 (2006).

341) Ozeki, T., Mushiroda, T., Yowang, A., Takahashi, A., Kubo, M., Shirakata, Y., Ikezawa, Z., Iijima, M., Shiohara, T., Hashimoto, K., Kamatani, N. and Nakamura, Y.: Genome-wide association study identifies HLA-A*3101 allele as a genetic

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risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. *Hum. Mol. Genet.*, **20**: 1034–1041 (2011).

342) McCormack, M., Alfirevic, A., Bourgeois, S., Farrell, J. J., Kasperavičiūtė, D., Carrington, M., Sills, G. J., Marson, T., Jia, X., de Bakker, P. I., Chinthapalli, K., Molokhia, M., Johnson, M. R., O'Connor, G. D., Chaila, E., Alhusaini, S., Shianna, K. V., Radtke, R. A., Heinzen, E. L., Walley, N., Pandolfo, M., Pichler, W., Park, B. K., Depondt, C., Sisodiya, S. M., Goldstein, D. B., Deloukas, P., Delanty, N., Cavalleri, G. L. and Pirmohamed, M.: HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. *N. Engl. J. Med.*, **364**: 1134–1143 (2011).

343) Hung, S. I., Chung, W. H., Liou, L. B., Chu, C. C., Lin, M., Huang, H. P., Lin, Y. L., Lan, J. L., Yang, L. C., Hong, H. S., Chen, M. J., Lai, P. C., Wu, M. S., Chu, C. Y., Wang, K. H., Chen, C. H., Fann, C. S., Wu, J. Y. and Chen, Y. T.: HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc. Natl. Acad. Sci. USA*, **102**: 4134–4139 (2005).

344) Tassaneeyakul, W., Jantararoungtong, T., Chen, P., Lin, P. Y., Tiamkao, S., Khunarkornsiri, U., Chucherd, P., Konyoung, P., Vannaprasaht, S., Choonhakarn, C., Pisuttimarn, P., Sangviroon, A. and Tassaneeyakul, W.: Strong association between HLA-B*5801 and allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in a Thai population. *Pharmacogenet. Genomics*, **19**: 704–709 (2009).

345) Kaniwa, N., Saito, Y., Aihara, M., Matsunaga, K., Tohkin, M., Kurose, K., Sawada, J., Furuya, H., Takahashi, Y., Muramatsu, M., Kinoshita, S., Abe, M., Ikeda, H., Kashiwagi, M., Song, Y., Ueta, M., Sotozono, C., Ikezawa, Z. and Hasegawa, R.: JSAR research group: HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. *Pharmacogenomics*, **9**: 1617–1622 (2008).

346) Lonjou, C., Borot, N., Sekula, P., Ledger, N., Thomas, L., Halevy, S., Naldi, L., Bouwes-Bavinck, J. N., Sidoroff, A., de Toma, C., Schumacher, M., Roujeau, J. C., Hovnanian, A. and Mockenhaupt, M.: RegiSCAR study group: A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet. Genomics*, **18**: 99–107 (2008).

347) Itoh, Y., Mizuki, N., Shimada, T., Azuma, F., Itakura, M., Kashiwase, K., Kikkawa, E., Kulski, J. K., Satake, M. and Inoko, H.: High-throughput DNA typing of HLA-A, -B, -C, and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population. *Immunogenetics*, **57**: 717–729 (2005).

348) Saito, S., Ota, S., Yamada, E., Inoko, H. and Ota, M.: Allele frequencies and haplotypic associations defined by allelic DNA typing at HLA class I and class II loci in the Japanese population. *Tissue Antigens*, **56**: 522–529 (2000).

349) Tokunaga, K., Ishikawa, Y., Ogawa, A., Wang, H., Mitsunaga, S., Moriyama, S., Lin, L., Bannai, M., Watanabe, Y., Kashiwase, K., Tanaka, H., Akaza, T., Tadokoro, K. and Juji, T.: Sequence-based association analysis of HLA class I and II alleles in Japanese supports conservation of common haplotypes. *Immunogenetics*, **46**: 199–205 (1997).

350) Lee, K. W., Oh, D. H., Lee, C. and Yang, S. Y.: Allelic and haplotypic diversity of HLA-A, -B, -C, -DRB1, and -DQB1 genes in the Korean population. *Tissue Antigens*, **65**: 437–447 (2005).

351) Chung, H. Y., Yoon, J. A., Han, B. Y., Song, E. Y. and Park, M. H.: Allelic and haplotypic diversity of HLA-A, -B, -C, and -DRB1 genes in Koreans defined by high-resolution DNA typing. *Korean J. Lab. Med.*, **30**: 685–696 (2010).

352) Yang, G., Deng, Y. J., Hu, S. N., Wu, D. Y., Li, S. B., Zhu, J., Zhu, B. F. and Liu, Y.: HLA-A, -B, and -DRB1 polymorphism defined by sequence-based typing of the Han population in Northern China. *Tissue Antigens*, **67**: 146–152 (2006).

353) Hong, W., Fu, Y., Chen, S., Wang, F., Ren, X. and Xu, A.: Distributions of HLA class I alleles and haplotypes in Northern Han Chinese. *Tissue Antigens*, **66**: 297–304 (2005).

354) Feng, M. L., Guo, X. J., Zhang, J. Y., Xie, J. H., Chen, L., Lu, Q., Yang, J. H., Ji, Y. and Qian, K. C.: Study on the haplotypes of MICA and MICB microsatellite and HLA-B locus in the Guangzhou Han population. *Tissue Antigens*, **64**: 281–285 (2004).

355) Chen, S., Li, W., Hu, Q., Liu, Z., Xu, Y. and Xu, A.: Polymorphism of HLA class I genes in Meizhou Han population of Guangdong, China. *Int. J. Immunogenet.*, **34**: 131–136 (2007).

356) Yao, Y., Shi, L., Shi, L., Matsushita, M., Yu, L., Lin, K., Tao, Y., Huang, X., Yi, W., Oka, T., Tokunaga, K. and Chu, J.: Distribution of HLA-A, -B, -Cw, and -DRB1 alleles and haplotypes in an isolated Han population in Southwest China. *Tissue Antigens*, **73**: 561–568 (2009).

357) Middleton, D., Hawkins, B. R., Williams, F., Meenagh, A., Moscoso, J., Zamora, J. and Arnaiz-Villena, A.: HLA class I allele distribution of a Hong Kong Chinese population based on high-resolution PCR-SSOP typing. *Tissue Antigens*, **63**: 555–561 (2004).

358) Chen, S., Hong, W., Shao, H., Fu, Y., Liu, X., Chen, D. and Xu, A.: Allelic distribution of HLA class I genes in the Tibetan ethnic population of China. *Int. J. Immunogenet.*, **33**: 439–445 (2006).

359) Hong, W., Chen, S., Shao, H., Fu, Y., Hu, Z. and Xu, A.: HLA class I polymorphism in Mongolian and Hui ethnic groups from Northern China. *Hum. Immunol.*, **68**: 439–448 (2007).

360) Wen, S. H., Lai, M. J. and Yang, K. L.: Human leukocyte antigen-A, -B, and -DRB1 haplotypes of cord blood units in the Tzu Chi Taiwan Cord Blood Bank. *Hum. Immunol.*, **69**: 430–436 (2008).

361) Yang, K. L., Chen, S. P., Shyr, M. H. and Lin, P. Y.: High-resolution human leukocyte antigen (HLA) haplotypes and linkage disequilibrium of HLA-B and -C and HLA-DRB1 and -DQB1 alleles in a Taiwanese population. *Hum. Immunol.*, **70**: 269–276 (2009).

362) Yu, K. J., Gao, X., Chen, C. J., Yang, X. R., Diehl, S. R., Goldstein, A., Hsu, W. L., Liang, X. S., Marti, D., Liu, M. Y., Chen, J. Y., Carrington, M. and Hildesheim, A.: Association of human leukocyte antigens with nasopharyngeal carcinoma in high-risk multiplex families in Taiwan. *Hum. Immunol.*, **70**: 910–914 (2009).

363) Chen, P. L., Fann, C. S., Chu, C. C., Chang, C. C., Chang, S. W., Hsieh, H. Y., Lin, M., Yang, W. S. and Chang, T. C.: Comprehensive genotyping in two homogeneous Graves' disease samples reveals major and novel HLA association alleles. *PLoS ONE*, **6**: e16635 (2011).

364) Yuliwulandari, R., Sachrowardi, Q., Nakajima, H., Kashiwase, K., Hirayasu, K., Mabuchi, A., Sofro, A. S. and Tokunaga, K.: Association of HLA-A, -B, and -DRB1 with pulmonary tuberculosis in western Javanese Indonesia. *Hum. Immunol.*, **71**: 697–701 (2010).

365) Yuliwulandari, R., Kashiwase, K., Nakajima, H., Uddin, J., Susmiarsih, T. P., Sofro, A. S. and Tokunaga, K.: Polymorphisms of HLA genes in Western Javanese (Indonesia): close affinities to Southeast Asian populations. *Tissue Antigens*, **73**: 46–53 (2009).

366) Romphruk, A. V., Romphruk, A., Kongmaroeng, C., Klumkrathok, K., Paupairoj, C. and Leelayuwat, C.: HLA class I and II alleles and haplotypes in ethnic Northeast Thais. *Tissue Antigens*, **75**: 701–711 (2010).

367) Pimtanothai, N., Charoenwongse, P., Mutirangura, A. and Hurley, C. K.: Distribution of HLA-B alleles in nasopharyngeal carcinoma patients and normal controls in Thailand. *Tissue*

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Antigens, **59**: 223–225 (2002).
368) Hoa, B. K., Hang, N. T., Kashiwase, K., Ohashi, J., Lien, L. T., Horie, T., Shojima, J., Hijikata, M., Sakurada, S., Satake, M., Tokunaga, K., Sasazuki, T. and Keicho, N.: HLA-A, -B, -C, -DRB1 and -DQB1 alleles and haplotypes in the Kinh population in Vietnam. *Tissue Antigens*, **71**: 127–134 (2008).
369) Rani, R., Marcos, C., Lazaro, A. M., Zhang, Y. and Stastny, P.: Molecular diversity of HLA-A, -B and -C alleles in a North Indian population as determined by PCR-SSOP. *Int. J. Immunogenet.*, **34**: 201–208 (2007).
370) Rajalingam, R., Krausa, P., Shilling, H. G., Stein, J. B., Balamurugan, A., McGinnis, D., Cheng, N., Mehra, N. K. and Parham, P.: Distinctive KIR and HLA diversity in a panel of north Indian Hindus. *Immunogenetics*, **53**: 1009–1019 (2002).
371) Shankarkumar, U., Sridharan, B. and Pitchappan, R. M.: HLA diversity among Nadars, a primitive Dravidian caste of South India. *Tissue Antigens*, **62**: 542–547 (2003).
372) Mohyuddin, A., Ayub, Q., Khaliq, S., Mansoor, A., Mazhar, K., Rehman, S. and Mehdi, S. Q.: HLA polymorphism in six ethnic groups from Pakistan. *Tissue Antigens*, **59**: 492–501 (2002).
373) Farjadian, S., Naruse, T., Kawata, H., Ghaderi, A., Bahram, S. and Inoko, H.: Molecular analysis of HLA allele frequencies and haplotypes in Baloch of Iran compared with related populations of Pakistan. *Tissue Antigens*, **64**: 581–587 (2004).
374) Sánchez-Velasco, P., Karadsheh, N. S., García-Martín, A., Ruíz de Alegría, C. and Leyva-Cobián, F.: Molecular analysis of HLA allelic frequencies and haplotypes in Jordanians and comparison with other related populations. *Hum. Immunol.*, **62**: 901–909 (2001).
375) Darke, C., Guttridge, M. G., Thompson, J., McNamara, S., Street, J. and Thomas, M.: HLA class I (A, B) and II (DR, DQ) gene and haplotype frequencies in blood donors from Wales. *Exp. Clin. Immunogenet.*, **15**: 69–83 (1998).
376) Middleton, D., Williams, F., Meenagh, A., Daar, A. S., Gorodezky, C., Hammond, M., Nascimento, E., Briceno, I. and Perez, M. P.: Analysis of the distribution of HLA-A alleles in populations from five continents. *Hum. Immunol.*, **61**: 1048–1052 (2000).
377) Williams, F., Meenagh, A., Darke, C., Acosta, A., Daar, A. S., Gorodezky, C., Hammond, M., Nascimento, E. and Middleton, D.: Analysis of the distribution of HLA-B alleles in populations from five continents. *Hum. Immunol.*, **62**: 645–650 (2001).
378) Dunne, C., Crowley, J., Hagan, R., Rooney, G. and Lawlor, E.: HLA-A, B, Cw, DRB1, DQB1 and DPB1 alleles and haplotypes in the genetically homogenous Irish population. *Int. J. Immunogenet.*, **35**: 295–302 (2008).
379) Hidajat, H., Andrien, M., De Canck, I. and Dupont, E.: Caucasian Belgian Normal. In Terasaki, P.I. and Gjertson, D.W. (ed.): *HLA 1998*, Los Angeles, American Society for Histocompatibility, 1998, pp. 119-121.
380) Schmidt, A. H., Baier, D., Solloch, U. V., Stahr, A., Cereb, N., Wassmuth, R., Ehninger, G. and Rutt, C.: Estimation of high-resolution HLA-A, -B, -C, -DRB1 allele and haplotype frequencies based on 8862 German stem cell donors and implications for strategic donor registry planning. *Hum. Immunol.*, **70**: 895–902 (2009).
381) Ferrara, G. B., Delfino, L., Longo, A., Morabito, A., Pera, C. and Pozzi, C.: Caucasian Italian Normal. In Terasaki, P.I. and Gjertson, D.W. (ed.): *HLA 1998*, Los Angeles, American Society for Histocompatibility, 1998, pp. 166–167.
382) Comas, D., Mateu, E., Calafell, F., Pérez-Lezaun, A., Bosch, E., Martínez-Arias, R. and Bertranpetit, J.: HLA class I and class II DNA typing and the origin of Basques. *Tissue Antigens*, **51**: 30–40 (1998).
383) Ivanova, M., Rozemuller, E., Tyufekchiev, N., Michailova, A., Tilanus, M. and Naumova, E.: HLA polymorphism in Bulgarians defined by high-resolution typing methods in comparison with other populations. *Tissue Antigens*, **60**: 496–504 (2002).
384) Nowak, J., Mika-Witkowska, R., Polak, M., Zajko, M., Rogatko-Koroś, M., Graczyk-Pol, E. and Lange, A.: Allele and extended haplotype polymorphism of HLA-A, -C, -B, -DRB1 and -DQB1 loci in Polish population and genetic affinities to other populations. *Tissue Antigens*, **71**: 193–205 (2008).
385) Piancatelli, D., Canossi, A., Aureli, A., Oumhani, K., Del Beato, T., Di Rocco, M., Liberatore, G., Tessitore, A., Witter, K., El Aouad, R. and Adorno, D.: Human leukocyte antigen-A, -B, and -Cw polymorphism in a Berber population from North Morocco using sequence-based typing. *Tissue Antigens*, **63**: 158–172 (2004).
386) Ayed, K., Ayed-Jendoubi, S., Sfar, I., Labonne, M. P. and Gebuhrer, L.: HLA class-I and HLA class-II phenotypic, gene and haplotypic frequencies in Tunisians by using molecular typing data. *Tissue Antigens*, **64**: 520–532 (2004).
387) Kijak, G. H., Walsh, A. M., Koehler, R. N., Moqueet, N., Eller, L. A., Eller, M., Currier, J. R., Wang, Z., Wabwire-Mangen, F., Kibuuka, H. N., Michael, N. L., Robb, M. L. and McCutchan, F. E.: HLA class I allele and haplotype diversity in Ugandans supports the presence of a major east African genetic cluster. *Tissue Antigens*, **73**: 262–269 (2009).
388) Cao, K., Moormann, A. M., Lyke, K. E., Masaberg, C., Sumba, O. P., Doumbo, O. K., Koech, D., Lancaster, A., Nelson, M., Meyer, D., Single, R., Hartzman, R. J., Plowe, C. V., Kazura, J., Mann, D. L., Sztein, M. B., Thomson, G. and Fernández-Viña, M. A.: Differentiation between African populations is evidenced by the diversity of alleles and haplotypes of HLA class I loci. *Tissue Antigens*, **63**: 293–325 (2004).
389) Tang, J., Naik, E., Costello, C., Karita, E., Rivers, C., Allen, S. and Kaslow, R. A.: Characteristics of HLA class I and class II polymorphisms in Rwandan women. *Exp. Clin. Immunogenet.*, **17**: 185–198 (2000).
390) Ellis, J. M., Mack, S. J., Leke, R. F., Quakyi, I., Johnson, A. H. and Hurley, C. K.: Diversity is demonstrated in class I HLA-A and HLA-B alleles in Cameroon, Africa: description of HLA-A*03012, *2612, *3006 and HLA-B*1403, *4016, *4703. *Tissue Antigens*, **56**: 291–302 (2000).
391) Maiers, M., Gragert, L. and Klitz, W.: High-resolution HLA alleles and haplotypes in the United States population. *Hum. Immunol.*, **68**: 779–788 (2007).
392) Mack, S. J., Tu, B., Lazaro, A., Yang, R., Lancaster, A. K., Cao, K., Ng, J. and Hurley, C. K.: HLA-A, -B, -C, and -DRB1 allele and haplotype frequencies distinguish Eastern European Americans from the general European American population. *Tissue Antigens*, **73**: 17–32 (2009).
393) Tu, B., Mack, S. J., Lazaro, A., Lancaster, A., Thomson, G., Cao, K., Chen, M., Ling, G., Hartzman, R., Ng, J. and Hurley, C. K.: HLA-A, -B, -C, -DRB1 allele and haplotype frequencies in an African American population. *Tissue Antigens*, **69**: 73–85 (2007).
394) Klitz, W., Gragert, L., Maiers, M., Tu, B., Lazaro, A., Yang, R., Xu, Q., Masaberg, C., Ng, J. and Hurley, C. K.: Four-locus high-resolution HLA typing in a sample of Mexican Americans. *Tissue Antigens*, **74**: 508–513 (2009).

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PHARMACOKINETICS

Population pharmacokinetics of fluconazole after administration of fosfluconazole and fluconazole in critically ill patients

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SUMMARY

What is known and Objective: Fluconazole is an antifungal agent that is commonly used to treat patients with serious systemic fungal infections in intensive care units. Fosfluconazole is a phosphate prodrug of fluconazole, which was developed to reduce the volume of fluid required to administer fluconazole by intravenous injection. The objective of this study was to characterize the pharmacokinetics of the antifungal fluconazole after the intravenous administration of the prodrug fosfluconazole or fluconazole in critically ill patients with serious systemic fungal infections, by population pharmacokinetic analysis using the NONMEM software package.

Methods: Clinical biochemical data including serum fluconazole levels were obtained from 57 patients treated in the intensive care unit along with two naïve pooled patients gleaned from previous reports. The pharmacokinetic model of fluconazole was estimated using a one-compartment model. The probability that the area under the concentration–time curve is higher than 800 μg h/mL was determined by simulation.

Results: It was assumed that all the administered fosfluconazole was converted to fluconazole with an estimated fosfluconazole-fluconazole conversion rate constant of 2·05/h. The significant covariates for clearance for fluconazole (CL) and volume of distribution for fluconazole (Vd) were resulted in creatinine clearance (CLcr) and body weight (BW), respectively, in the final pharmacokinetic model equations: $CL\ (L/h) = 0{\cdot}799 \times [CLcr\ (mL/min)/92{\cdot}7]^{0{\cdot}685}$ and $Vd\ (L) = 48{\cdot}1 \times [BW\ (kg)/65]^{1{\cdot}40}$, where the interpatient variabilities in CL and Vd and the intrapatient variability were 44·8%, 79·7% and 19·8%, respectively. On the basis of the results of the Monte Carlo simulation, the probabilities of target attainment were 60%, 26% and 11% for 400 mg/day administration as fluconazole equivalent at CLcr values of 40, 70 and 100 mL/min, respectively.

What is new and Conclusion: The present population pharmacokinetic analysis strongly indicates that fosfluconazole (and fluconazole) dosage should be optimized in terms of CLcr in critically ill patients.

Keywords: critically ill patients, fluconazole, fosfluconazole, modelling and simulation, pharmacokinetics, population pharmacokinetic analysis

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WHAT IS KNOWN AND OBJECTIVE

Fungal infections cause considerable morbidity and mortality in critically ill patients in the intensive care units where the most invasive modes of treatment are administered (1). Fluconazole is an antifungal agent that is commonly used to treat patients with serious systemic fungal infections in intensive care units. The elimination half-life of fluconazole is within 31–37 h (2). The binding of fluconazole to plasma proteins has been

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reported to reach a level of 11% in healthy male subjects (3). About 80·9% of intravenously administered fluconazole is excreted in the urine (4). Fosfluconazole is a phosphate prodrug of fluconazole, which was developed to reduce the volume of fluid required to administer fluconazole by intravenous injection. Fluconazole requires administration as a high-volume (200 mg/100 mL) infusion. Fosfluconazole is useful for critically ill patients who need to regulate fluid balance because of its high water solubility (200 mg/ 2·5 mL). Less than 1% of the dose of fosfluconazole administered is excreted unchanged in the urine and 85·6% of the dose is excreted in the urine as fluconazole after fosfluconazole administration (4). Target exposure is an area under the concentration–time curves of fluconazole from 0 to 24 h (AUC) of 800 μg h/mL (5). This AUC ensures that the exposure exceeds the pharmacodynamic target with an AUC/MIC value of over 50 for *Candida* species with an MIC of 8 μg/mL at the Clinical Laboratory Standards Insitute sensitivity breakpoint (5, 6). There is little information on fluconazole pharmacokinetics after fosfluconazole administration in critically ill patients. The pharmacokinetics in critically ill patients differs from that in healthy individuals because physiological alterations such as liver and kidney dysfunctions are frequently observed in critically ill patients (7, 8). Fluconazole is considered a potential first-line option for the treatment of invasive candidiasis, according to recent clinical practice guidelines (9). However, such guidelines do not address fluconazole dosing in critically ill patients. Population pharmacokinetic analysis revealing influential factors associated with interpatient variability is considered essential for medicating critically ill patients more effectively. Once population pharmacokinetic parameters have been obtained, Bayesian dosage adjustment is applicable to predict the serum drug concentration–time curve in each patient on the basis of a limited number of drug concentration measurements (10). Moreover, the population pharmacokinetic parameters provide an effective dosing regimen depending on the attainment of the target pharmacokinetic/ pharmacodynamic indices obtained by Monte Carlo simulation (11) considering the variability in pharmacokinetic parameters in critically ill patients.

The objectives of this study were to characterize the pharmacokinetics of fluconazole after fosfluconazole or fluconazole administration in critically ill patients with serious systemic fungal infections by population pharmacokinetic analysis and to investigate the effective fosfluconazole or fluconazole dosage in critically ill patients by Monte Carlo simulation.

METHODS

Patients and data collection

Clinical biochemical data including serum fluconazole levels were obtained from 57 patients treated in the intensive care unit of Nippon Medical School Hospital and two naïve pooled patients (12) gleaned from two published studies (13, 14). The data of the naïve pooled patients were added to supplement the poor serum fluconazole level data up to 8 h after fluconazole administration. Buijk *et al.* (13) studied 14 critically ill patients who were administered fluconazole at 400 mg intravenously every 24 h with an extra dose of 400 mg intravenously after 12 h on day 1. The infusion time was assumed to be 0·5 h. The sample times after intravenous administration were prior to infusion and 0·5, 1, 2, 4, 8 and 24 h after the start of the infusion. Pooled data were extracted from figure 1 of Buijk *et al.* (13). The mean of the patients' age was 45·2 years. Information contributed one naïve pooled patient. Nicolau *et al.* (14) studied five critically ill patients who were administered fluconazole at 200 mg intravenously every 24 h. The infusion time was assumed to be 1 h. The sample times after intravenous administration were prior to infusion and 0·5, 1, 2, 3, 6, 9, 12 and 24 h after the start of the infusion. Pooled data were extracted from figure 1 of Nicolau *et al.* (14). The means of creatinine clearance (CLcr), body weight, and age were 96 mL/min, 79 kg and 53 years, respectively. Information contributed one naïve pooled patient. Graphs of concentration–time data were scanned, and all the graphed data points were extracted using the software UN-SCAN-IT (Silk Scientific, Inc., Orem, UT, USA). Two hundred and seven serum fluconazole concentrations from 34 patients administered fluconazole, 72 serum fluconazole concentrations from 23 patients administered fosfluconazole, and 16 serum fluconazole concentra-

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tions from the two naïve pooled patients administered fluconazole were used. Table 1 shows the characteristics of the patients except the two naïve pooled patients in this study. The protocol for this study was approved by the ethics committees of Nippon Medical School Hospital and Showa Pharmaceutical University. Written informed consent was obtained from the next of kin of eligible patients. The daily dose administered was determined by the clinician responsible for each patient. The daily doses ranged from 50 to 400 mg for fluconazole and from 100 to 800 mg (calculated as fluconazole equivalent) for fosfluconazole. The rates of fluconazole infusion ranged from 50 to 400 mg/h (median, 200 mg/h). The infusion time of fluconazole was 1 h. The infusion time of fosfluconazole was 1 h for only one patient. In the other patients administered fosfluconazole, fosfluconazole was administered by bolus injection. In patients administered fluconazole, the sampling times (ratio of each concentrations to all concentrations) relative to the dose were 0–6 h (2%), 6–12 h (42%), 12–18 h (25%), 18–24 h (25%), 24–48 h (1%), and 48 h after (3%). In patients administered fosfluconazole, the sampling times (ratio of each concentrations to all concentrations) relative to the dose were 0–6 h (40%), 6–12 h (6%), 12–18 h (3%), 18–24 h (28%), 24–48 h (21%), and 48 h after (3%). The ratio of each concentration to all concentrations after fluconazole dose and fosfluconazole dose prior to steady state assumed as 136 h were 59% and 65%, respectively. For each patient, demographic data such as age, gender, and weight; routine clinical biochemistry data such as alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total serum bilirubin (TBIL), serum creatinine (Scr), blood urea nitrogen (BUN), serum albumin (ALB) and total serum protein (TP) concentrations; and fluconazole or fosfluconazole dose history such as dose rate and administration time were recorded.

Table 1. Characteristics of patients administrated fluconazole and fosfluconazole

Characteristic	Median or number	Range
Number of patients (male/female)	57 (42/15)	
Body weight (kg)	65	38–100
Age (years)	57	23–99
Albumin (g/dL)	2·8	1·7–4
Total protein (g/dL)	6·1	3·6–9·8
Aspartate aminotransferase (U/L)	45	9–5555
Alanine aminotransferase (U/L)	41	5–3795
Total bilirubin (mg/dL)	1·4	0·1–28·4
Lactate dehydrogenase (U/L)	416	57–13485
Serum creatinine (mg/dL)	0·71	0·15–5·41
Blood urea nitrogen (mg/dL)	20·7	3–148·2
Creatinine clearance (mL/min)	92·7	9·0–350

Blood sampling and fluconazole assay

Blood samples were collected at the same time as blood was taken for other reasons such as diagnostic tests. Immediately after blood collection, the samples were centrifuged. The serum samples were stored at −30 °C until analysis. Serum fluconazole concentration was determined using the modified high-performance liquid chromatography (HPLC) procedure described by Koks *et al.* (15) The serum samples with phenacetin added as the internal standard were mixed after adding 5 M sodium hydrate. The mixture was subjected to extraction with chloroform and 1-propranol (400 : 100 : 5, v/v). Protein was removed by centrifugation for 10 min at 1610 *g*. The chloroform solution was transferred to another tube and dried under nitrogen atmosphere at 50 °C. The residue was dissolved in an acetonitrile/25 mM sodium acetate (20 : 80, v/v) solution and injected into an HPLC system. Chromatography was performed using a CAPCELL PAK C18 UG120 S-5 (4·6 × 150 mm; Shiseido, Tokyo, Japan) by isocratic elution with acetonitrile/25 mM sodium acetate (20 : 80, v/v) solution as the mobile phase and ultraviolet detection (Shimadzu Corp., Kyoto, Japan) at 260 nm. The flow rate was 1·0 mL/min.

Population pharmacokinetic modelling

Population analysis was carried out using the computer software package NONMEM version 6 (ICON Development Solutions, Ellicott City, MD, USA) as implemented under Wings for NONMEM

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(16). An Intel visual Fortran compiler version 10.1 (Intel Corp., Santa Clara, CA, USA) under MS Windows XP (Microsoft Corp., Seattle, WA, USA) was used to compile NONMEM. Population pharmacokinetic parameters were estimated using first-order conditional estimation and the subroutine ADVAN6 in NONMEM. Model selection was carried out on the basis of the maximum likelihood objective function, the SE% of the parameter estimates and model diagnosis plots. A decrease in the objective function value (OFV) of NONMEM by 6·63 was considered statistically significant ($P < 0{\cdot}01$). Scatter plots of observed concentration (OBS) vs. population-predicted concentration (PRED), OBS vs. individual predicted concentration (IPRED), conditional weighted residual (CWRES) vs. PRED and CWRES vs. time were used as model diagnosis plots. CWRES was calculated using Xpose 4 (17).

The pharmacokinetics of fluconazole was assumed to follow a one-compartment model with first-order elimination because the two-compartment model exhibited convergence difficulties. It was assumed that all the administrated fosfluconazole was converted to fluconazole and that the conversion rate was dependent on the first-order kinetics. The pharmacokinetic structural model is shown in Fig. 1. The equations used to describe the pharmacokinetics of fluconazole are

$$\frac{dX_1}{dt} = -k_c \cdot X_1 \qquad (1)$$

$$\frac{dX_2}{dt} = k_c \cdot X_1 - \frac{CL}{Vd} \cdot X_2 \qquad (2)$$

where X_1 and X_2 are the amounts of fosfluconazole and fluconazole, respectively. k_c is the first-order conversion rate constant, CL is the clearance for

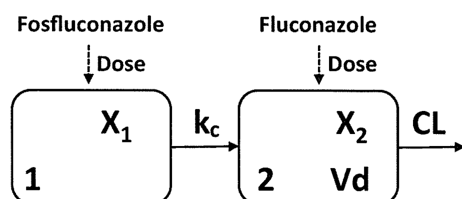

Fig. 1. Pharmacokinetic model for fluconazole after fosfluconazole and fluconazole administrations. X_1, amount of fosfluconazole; X_2, amount of fluconazole; k_c, first-order conversion rate constant; CL, clearance for fluconazole; Vd, volume of distribution for fluconazole.

fluconazole, and Vd is the volume of distribution for fluconazole. The interpatient variability in the pharmacokinetic parameter was modelled assuming a lognormal distribution described as

$$P_i = P_{pop} \cdot \exp(\eta_{pi}) \qquad (3)$$

where P_i is the pharmacokinetic parameter of the ith individual and η_{pi} is an interpatient random effect normally distributed with a mean of zero and a variance of ω_p^2. Intrapatient error was tested using an exponential error model and a combined additive and exponential error model described as

$$Y_{obs} = Y_{pred} \cdot \exp(\epsilon_1) \qquad (4)$$

$$Y_{obs} = Y_{pred} \cdot \exp(\epsilon_1) + \epsilon_2 \qquad (5)$$

where Y_{obs} is the measured serum fluconazole concentration, Y_{pred} is the predicted serum fluconazole concentration according to the model, and ϵ_1 and ϵ_2 are the normally distributed error terms with means of zero and variances of σ_1^2 and σ_2^2, respectively.

In the next step, each of the covariates was introduced individually into the base model. The covariates considered in CL were as follows: CLcr, Scr and BUN as indices of renal function; ALT, AST, TBIL, LDH, ALB and TP as indices of hepatic function; and age and body weight (BW) as indices of demographic factors. CLcr was predicted using the Cockcroft–Gault equation (18). The covariates considered in Vd were Alb and TP as indices of protein-drug binding, and age and BW as indices of demographic factors. The covariates were modelled and centred around the patients' median expressed as

$$P_{pop} = \theta_p \cdot (\mathrm{covariate}/\mathrm{median_{cov}})^{\theta\mathrm{cov}} \qquad (6)$$

where P_{pop} is the pharmacokinetic parameter, θ_p is the typical pharmacokinetic parameter for the covariate equal median, and θ_{cov} is a covariate scale factor. Statistically significant covariates were included in the base model in a forward selection manner to the final model. In covariate evaluation, the patients and naïve pooled patients with missing variables were assigned the corresponding medians shown in Table 1.

The model was evaluated by performing a prediction- and variability corrected visual predictive check (pvc-VPC) (19). pvc-VPC involved the generation of 500 simulated fluconazole concentration

sets from final population pharmacokinetic model parameters using a NONMEM software package as implemented under Perl-speaks-NONMEM (20). The 2·5th, 50th, 97·5th percentiles of the simulated concentration at each time point were overlaid in lines and shown together with the observed data for visual inspection.

Simulation

Pharmacokinetic simulation was carried out to determine the dosing regimen on the basis of the covariates in the critically ill patients. The areas under the concentration–time curves of fluconazole from 0 to 24 h (AUC) in the steady state were simulated for 1000 patients according to the dose divided by the clearances simulated by random sampling from the distribution of the interpatient variability. The probability (%) that AUC is higher than 800 μg h/mL in the steady state was calculated as the ratio of the number of simulated patients to the total number of patients. The simulation was performed using R version 2.9 software (21).

RESULTS

Pharmacokinetic modelling

Figure 2 shows the fluconazole concentrations used for the population pharmacokinetic analysis. In the first step, we estimated the basic population pharmacokinetic parameters without covariates using NONMEM. Because the variances of the interpatient variabilities in k_c were very small and could be excluded without significantly altering the parameter values and OFV, the variances of the interpatient variabilities in k_c was excluded from the rest of the steps in the model building. Modelling intrapatient variability using the exponential error model was found to be preferable to modelling using the combined additive plus exponential error model. The basic population pharmacokinetic parameter estimates obtained without covariates were k_c = 1·72/h, CL = 0·714 L/h, and Vd = 47·0 L, where the interpatient variabilities in CL and Vd and the intrapatient variability were 69·7%, 84·1% and 19·5%, respectively.

In the second step in the fixed effect modelling concerning CL and Vd, various covariates that were likely to affect the pharmacokinetics of fluconazole were added one by one and tested for statistical significance. The results obtained using the different models tested in this study are shown in Table 2. The single-covariate model showed that CL significantly depends on CLcr, Scr, BUN, TBIL and ALB; Vd significantly depends on BW and TP. In the next step, the effect of CLcr as an index of renal function on CL was retained in the model after the single-covariate search, as it provides the largest drop in OFV. The other indices of renal function were not tested in the double-covariate analysis. The double-covariate model showed that CL significantly depends on TP, ALB and TBIL; Vd significantly depends on BW and TP. In the next

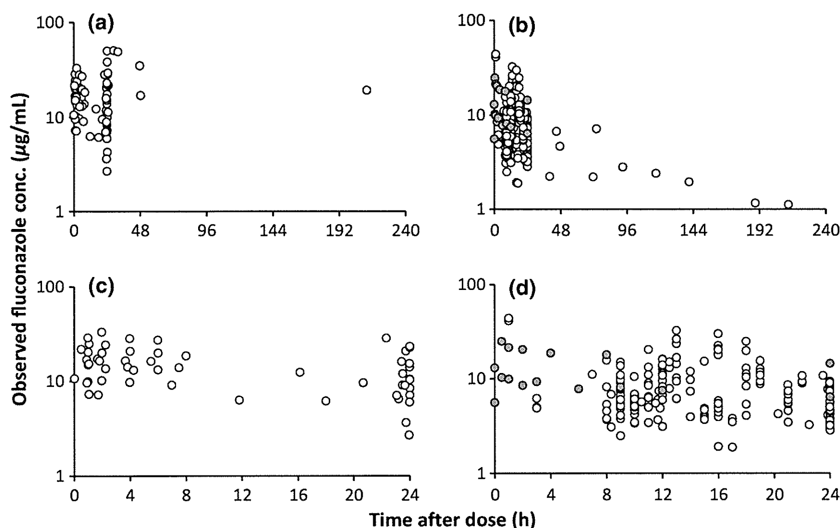

Fig. 2. Fluconazole concentration–time profile after fosfluconazole (a, c) and fluconazole (b, d) administrations. c and d show the enlargements of a and b at 24 h after dosing. The grey circles are the data gleaned from the literature.

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Table 2. Summary of covariate model building

Model no.	Model	OFV	ΔOFV
1	Basic model	956·763	–
2	Model 1 + ALB on CL	941·989	−14·774*
3	Model 1 + TP on Vd	941·657	−15·106*
4	Model 1 + BW on Vd	938·912	−17·851*
5	Model 1 + TBIL on CL	931·080	−25·683*
6	Model 1 + BUN on CL	893·504	−63·259*
7	Model 1 + Scr on CL	887·841	−68·922*
8	Model 1 + CLcr on CL	885·826	−70·937*
9	Model 8 + TP on Vd	878·638	−7·187*
10	Model 8 + TBIL on CL	877·651	−8·175*
11	Model 8 + ALB on CL	877·633	−8·192*
12	Model 8 + TP on CL	871·358	−14·468*
13	Model 8 + BW on Vd	863·595	−22·231*
14	Model 13 + TP on CL	856·085	−7·509*
15	Model 13 + TBIL on CL	854·984	−8·610*

OFV, objective function value; ΔOFV, difference in OFV between two nested models; CL, fluconazole clearance; Vd, volume of distribution for fluconazole; ALB, serum albumin; BUN, blood urea nitrogen; BW, body weight; CLcr, creatinine clearance; TBIL, total bilirubin; TP, total serum protein.
$^*P < 0{\cdot}01$.

step, covariates affecting the pharmacokinetics of fluconazole were explored using the model, including the effects of CLcr on CL and of BW on Vd as the basic model. The triple-covariate model showed that CL significantly depends on TBIL and TP. However, the SE% of θ_{cov} was large, in which the 95% confidence interval includes zero. Thus, the double-covariate model that included CLcr as the covariate for CL and BW as the covariate for Vd was designated as the final model. The population pharmacokinetic parameters estimated using the final model were summarized in Table 3. The equations of the final population pharmacokinetic model were $k_c = 2{\cdot}05$ /h, $CL = 0{\cdot}799 \times (CLcr/92{\cdot}7)^{0{\cdot}685}$ L/h, and $Vd = 48{\cdot}1 \times (BW/65)^{1{\cdot}40}$ L, where the interpatient variabilities in CL and Vd and the intrapatient variability were 44·8%, 79·7% and 19·8%, respectively.

Model evaluation

The scatter plots of OBS vs. PRED, OBS vs. IPRED, CWRES vs. PRED and CWRES vs. time in the final model are shown in Fig. 3. CWRES is uniformly distributed within an acceptable range (22, 23) (i.e. −2·6 and 3·4) in the final model. The pvc-VPC results are given in Fig. 4. In pvc-VPC, the percentile intervals describing the OBS should mostly be within the 95% confidence intervals for the percentile intervals describing the simulated concentrations.

Table 3. Population pharmacokinetic parameters of fluconazole for final model

Parameter	Estimate	SE%
k_c (/h) = θ_1		
θ_1	2·05	43
CL (L/h) = θ_2*(CLcr/92·7)$^{\theta_3}$		
θ_2	0·799	5·6
θ_3	0·685	14·5
Vd (L) = θ_4*(BW/65)$^{\theta_5}$		
θ_4	48·1	9·2
θ_5	1·4	19·6
ω^2_{CL} (CV%)	44·8	30·6
ω^2_{Vd} (CV%)	79·7	37·4
σ^2 (CV%)	19·8	20·7

k_c, first-order conversion rate constant; CL, clearance for fluconazole; Vd, volume of distribution for fluconazole; CLcr, creatinine clearance (mL/min); BW, body weight (kg); ω^2_{CL}, interpatient variability in CL; ω^2_{Vd}, interpatient variability in Vd; σ^2, intrapatient variability.

Simulation

By using the final model, simulation experiments were performed to determine the dosing strategy in patients with renal impairment because CLcr was found as a covariate for CL. AUC was calculated by dividing dose by CL simulated for 1000 patients whose CLcrs were 40, 70 and 100 mL/min. The simulations were performed for fluconazole and fosfluconazole (as fluconazole equivalent) doses ranging from 100 to 1500 mg per day. The probabilities (%) that AUC is higher than 800 μg h/mL in the steady state are shown in Fig. 5.

DISCUSSION

Pharmacokinetic analysis, which reveals influential factors associated with intrapatient variability and interpatient differences, is considered essential for medicating critically ill patients more effectively

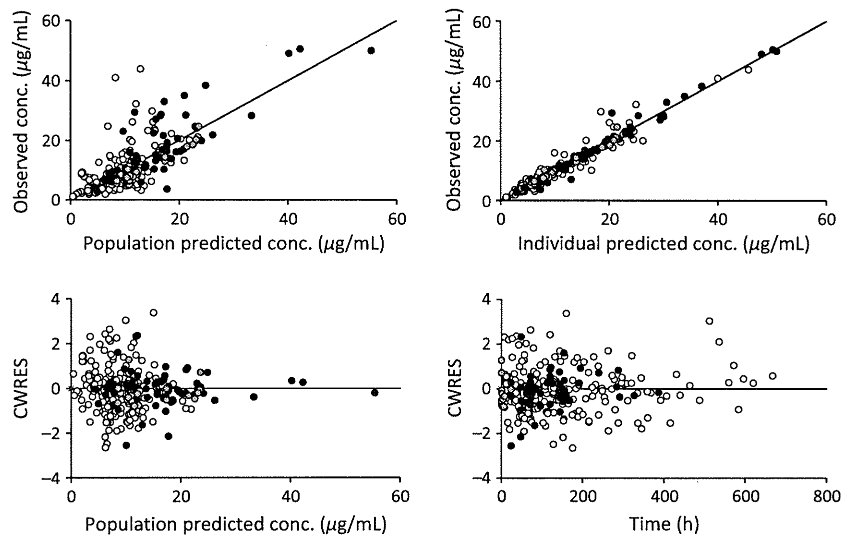

Fig. 3. Model diagnosis plots of final model. The black and white circles are the data obtained after fosfluconazole and fluconazole administrations, respectively. The grey circles are the data obtained after fluconazole administration, gleaned from the literature.

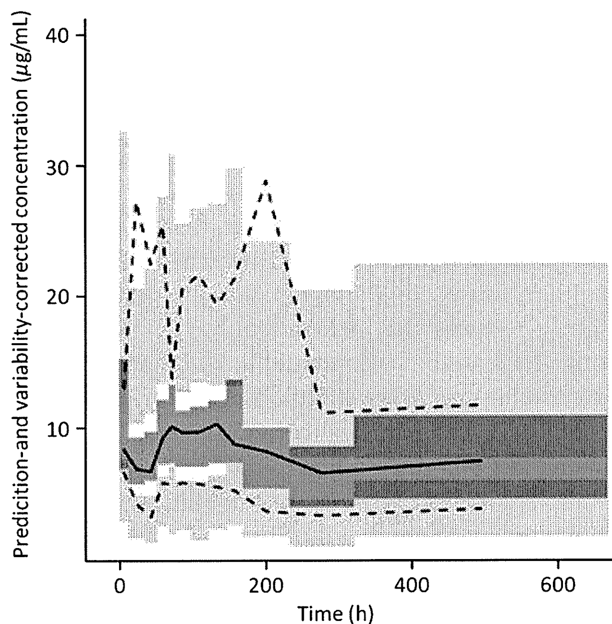

Fig. 4. Prediction and variability corrected visual predictive check of final model. The black line at the center represents the median concentrations observed and the dotted black lines represent the 2·5th and 97·5th percentiles for observations. The dark grey area represent the 95% confidence interval for the 50th percentiles prediction interval and the grey areas represent those for the 2·5th and 97·5th percentiles prediction intervals.

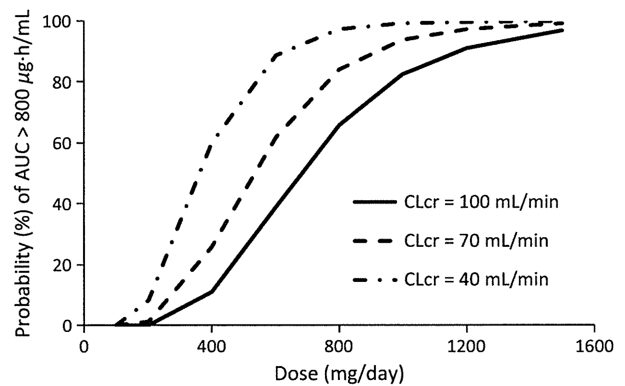

Fig. 5. Probabilities (%) that the areas under the concentration–time curve are higher than 800 μg h/mL in the steady state.

(24, 25). Despite the common use of fosfluconazole as an antifungal agent, there is little data on fluconazole pharmacokinetics after fosfluconazole administration to critically ill patients. Fungal infections cause morbidity and mortality in critically ill patients. The interpatient variabilities in CL and Vd in the basic model showed a large variability in fluconazole pharmacokinetics in critically ill patients. This variability in fluconazole pharmacokinetics indicates the need for individualized dosage regimen in critically ill patients. It was considered that fosfluconazole and fluconazole doses can be optimized using the population pharmacokinetic parameters of fluconazole after fosfluconazole or fluconazole administration.

In this study, the population pharmacokinetics of fluconazole after fosfluconazole or fluconazole administration was characterized using a one-compartment model and estimated by incorporating first-order kinetics for k_c, which represents the metabolic conversion from fosfluconazole to fluconazole. It was assumed that all the administrated fosfluconazole was converted to fluconazole. The

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pharmacokinetics of fosfluconazole after the administration of 1000–2000 mg is different from that after the administration of 50–500 mg owing to the strong protein binding and its saturation (26). In the case of the patients administered a high fosfluconazole dose and with a low serum protein concentration, the metabolic conversion rate may be different from that of other patients. A Fosfluconazole dose range from 200 to 800 mg was used in this study. It was considered that the saturation of the protein binding of fosfluconazole did not affect the pharmacokinetics of fluconazole in the critically ill patients in the dose range. The interpatient variability in the metabolic conversion rate was included in the interpatient variability in Vd in this population pharmacokinetic model that excludes the interpatient variability in k_c. However, ALB and TP were not covariates for Vd in this study. The half-life of fosfluconazole calculated from k_c was 0·34 h. The half-life of fosfluconazole was shorter than 1·5–2·5 h in healthy subjects (26). It was reported that fosfluconazole is more rapidly converted to fluconazole in hepatically impaired subjects, but that fluconazole exposure is not affected by hepatic impairment (27). The population mean Vd (BW, 65 kg) was 48 L, which was nearly equal to 57·4 L, found in HIV-positive patients (28). Hence, this population pharmacokinetic model that excluded the interpatient variability in k_c was considered to describe the data adequately. The population pharmacokinetic parameter estimates obtained using raw data set almost equal to that obtained using a data set including the data of the naïve pooled patients. However, the SE% of Vd (θ_4) obtained using the raw data set was higher than that obtained using a data set including the data of the naïve pooled patients (14·2% vs. 9·2%, data not shown). To use the data set including the data of the naïve pooled patients, Vd was estimated adequately. In covariate evaluation, the naïve pooled patients with missing variables were assigned the corresponding medians shown in Table 1, and the covariates were modelled and centred around the patients' medians. Therefore, it was thought that the effect of using the data set including the data of the naïve pooled patients for the covariate evaluation was small.

We observed high interpatient variabilities in pharmacokinetic parameters, which were only partially explained by the patients' covariates. The significant covariate for CL was CLcr reflecting the renal function, which reduces the interpatient variability in CL from 69·7% in the basic model to 44·8% in the final model. 80·9% of the fluconazole dose was excreted unchanged in the urine (4). Rajagopalan *et al.* (29) reported that CLcr affects CL in patients after enteral fluconazole administration in a surgical intensive care unit. In HIV-positive patients, BW significantly affects fluconazole CL but not CLcr (28). In this study, BW was not a significant covariate. It was considered that CLcr affects fluconazole CL more than BW and that the effect of CLcr on fluconazole CL was not significant in HIV-positive patient analysis because the number of patients with impaired renal function was small. The population mean CL were 0·80 L/h, (CLcr, 93 mL/min), 0·62 L/h (CLcr, 64 mL/min) and 0·27 L/h (CLcr, 19 mL/min) in this study. Sobue *et al.* (30) reported that the fluconaozole CL were 1·37 L/h (CLcr, 93 mL/min; BW, 65 kg), 1·40 L/h (CLcr, 64 mL/min; BW, 65 kg) and 0·43 L/h (CLcr, 19 mL/min; BW, 65 kg) in a study of patients with renal impairment. The population mean CL in ICU patients was lower than the fluconazole CL in patients with renal impairment. The significant covariate for Vd was BW, which slightly reduces the interpatient variability in Vd from 84·1% in the basic model to 79·7% in the final model. The Vd of fluconazole has been reported to be 0·65–0·76 L/kg in healthy volunteers (2). Thus, fluconazole is a drug whose Vd mirrors total body water. The high interpatient variability in Vd may be explained on the basis of the pathology related to total body water [e.g. severe burn (31)]. Owing to the lack of data, the effects of pathology that alters the pharmacokinetics of fluconazole such as burn could not be determined in this study.

In the final model, CLcr and BW were found to affect the CL and Vd of fluconazole in critically ill patients, respectively. To use CLcr, the optimal maintenance dose was explored by simulation analysis. Simulation was performed assuming that the target AUC was 800 μg h/mL in the steady state, because the exposure exceeded the pharmacodynamic target with an AUC/MIC value of over 50 for *Candida* species with an MIC of 8 μg/mL at the Clinical Laboratory Standards Institute sensitivity breakpoint (5, 6). In accordance with the decrease in CLcr that caused a decrease in the CL of fluconazole, the maintenance dose can be

determined from CLcr. Figure 5 shows the probabilities (%) that AUC is higher than 800 μg h/mL. The simulation suggests the typical maintenance doses of fosfluconazole and fluconazole in critically ill patients at various CLcrs. At a CLcr of 40 mL/min, the probability of target attainment was 60% for 400 mg/day administration as fluconazole equivalent. On the other hand, at a CLcr of 100 mL/min, the probability of target attainment was 66% for 800 mg/day administration as fluconazole equivalent. The result was in good agreement with that of a pharmacokinetic study of fluconazole in patients with renal failure, that is, the maintenance dose should be reduced by 50% for patients with CLcr $\leqq$ 50 mL/min (32). The approved dosages of fluconazole and fosfluconazole were both 400 mg/day in Japan. At a CLcr of 100 mL/min, the probabilities of target attainment were 66 and 91% for 800 and 1200 mg/day administrations as fluconazole equivalent, respectively. These results suggest that the dosage of fluconazole should be higher than that recommended by the package insert for critically ill patients with a CLcr of 100 mL/min. The safety of fluconazole is illustrated in the Infectious Diseases Society of America treatment guidelines for cryptococcal diseases, in which doses of up to 2000 mg/day are recommended (33). The simulated AUC values at a CLcr of 100 mL/min and a dose of 800 mg/day ranged from 475 to 1908 (5th to 90th percentiles) μg h/mL (data not shown). Moreover, the high interpatient variability in Vd indicates the wide time range to reach the steady state. The marked variability in fluconazole pharmacokinetics after fosfluconazole or fluconazole administration in critically ill patients shows the need for individualized dosage adjustments. Thus, dosage adjustment in terms of CLcr is useful in determining the initial dosage regimen in critically ill patients.

WHAT IS NEW AND CONCLUSION

A population pharmacokinetic model of fluconazole after fosfluconazole or fluconazole administration in critically ill patients with serious systemic fungal infections was developed. CLcr and BW were significant predictors of the CL and Vd of fluconazole, respectively, in this population. Thus, dosage adjustment in terms of CLcr is useful in determining the dosage regimen in critically ill patients.

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REFERENCES

1. Bustamante C (2005) Treatment of *Candida* infection: a view from the trenches! *Current Opinion in Infectious Diseases*, **18**, 490–495.
2. Shiba K, Saito A, Miyahara T (1989) Pharmacokinetic evaluation of fluconazole in healthy volunteers. *Japanese Journal of Antibiotics*, **42**, 17–30.
3. Humphrey M, Jevons S, Tarbit M (1985) Pharmacokinetic evaluation of UK-49,858, a metabolically stable triazole antifungal drug, in animals and humans. *Antimicrobial Agents and Chemotherapy*, **28**, 648–653.
4. Sobue S, Tan K, Layton G, Eve M, Sanderson J (2004) Pharmacokinetics of fosfluconazole and fluconazole following multiple intravenous administration of fosfluconazole in healthy male volunteers. *British Journal of Clinical Pharmacology*, **58**, 20–25.
5. Wade K, Wu D, Kaufman D *et al.* (2008) Population pharmacokinetics of fluconazole in young infants. *Antimicrobial Agents and Chemotherapy*, **52**, 4043–4049.
6. Pai M, Turpin R, Garey K (2007) Association of fluconazole area under the concentration–time curve/MIC and dose/MIC ratios with mortality in nonneutropenic patients with candidemia. *Antimicrobial Agents and Chemotherapy*, **51**, 35–39.
7. Boucher B, Wood G, Swanson J (2006) Pharmacokinetic changes in critical illness. *Critical Care Clinics*, **22**, 255–271, vi.
8. Hirata K, Matsumoto Y, Kurokawa A *et al.* (2003) Possibility of influence of midazolam sedation on the diagnosis of brain death: concentrations of active metabolites after cessation of midazolam. *Yakugaku Zasshi*, **123**, 811–815.
9. Cota J, Burgess D (2010) Antifungal dose adjustment in renal and hepatic dysfunction: pharmacokinetic and pharmacodynamic considerations. *Current Fungal Infection Reports*, **4**, 120–128.

10. Tanigawara Y, Sato R, Morita K, Kaku M, Aikawa N, Shimizu K (2006) Population pharmacokinetics of Arbekacin in patients infected with methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, **50**, 3754–3762.
11. Katsube T, Yano Y, Wajima T, Yamano Y, Takano M (2010) Pharmacokinetic/pharmacodynamic modeling and simulation to determine effective dosage regimens for doripenem. *Journal of Pharmaceutical Sciences*, **99**, 2483–2491.
12. Herd D, Anderson B, Holford N (2007) Modeling the norketamine metabolite in children and the implications for analgesia. *Paediatric Anaesthesia*, **17**, 831–840.
13. Buijk S, Gyssens I, Mouton J, Verbrugh H, Touw D, Bruining H (2001) Pharmacokinetics of sequential intravenous and enteral fluconazole in critically ill surgical patients with invasive mycoses and compromised gastro-intestinal function. *Intensive Care Medicine*, **27**, 115–121.
14. Nicolau D, Crowe H, Nightingale C, Quintiliani R (1995) Bioavailability of fluconazole administered via a feeding tube in intensive care unit patients. *Journal of Antimicrobial Chemotherapy*, **36**, 395–401.
15. Koks C, Rosing H, Meenhorst P, Bult A, Beijnen J (1995) High-performance liquid chromatographic determination of the antifungal drug fluconazole in plasma and saliva of human immunodeficiency virus-infected patients. *Journal of Chromatography. B, Biomedical Applications*, **663**, 345–351.
16. Holford N (1989) *Wings for NONMEM*. Available at: http://wfn.sourceforge.net (accessed 7 July 2011).
17. Jonsson E, Karlsson M (1999) Xpose – an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Computer Methods and Programs in Biomedicine*, **58**, 51–64.
18. Cockcroft D, Gault M (1976) Prediction of creatinine clearance from serum creatinine. *Nephron*, **16**, 31–41.
19. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO (2011) Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. *The AAPS Journal*, **13**, 143–151.
20. Lindbom L, Ribbing J, Jonsson EN (2004) Perl-speaks-NONMEM (PsN) – a Perl module for NONMEM related programming. *Computer Methods and Programs in Biomedicine*, **75**, 85–94.
21. R Development Core Team (2009) *R: A language and environment for statistical computing*. Available at: http://cran.r-project.org (accessed 7 July 2011).
22. Rousseau A, Léger F, Le Meur Y, Saint-Marcoux F, Paintaud G, Buchler M, Marquet P (2004) Population pharmacokinetic modeling of oral cyclosporin using NONMEM: comparison of absorption pharmacokinetic models and design of a Bayesian estimator. *Therapeutic Drug Monitoring*, **26**, 23–30.
23. Hooker A, Staatz C, Karlsson M (2007) Conditional weighted residuals (CWRES): a model diagnostic for the FOCE method. *Pharmaceutical Research*, **24**, 2187–2197.
24. Hirata K, Aoyama T, Matsumoto Y, Ogawa F, Yamazaki H, Kikuti A, Yamamoto Y (2007) Pharmacokinetics of antifungal agent micafungin in critically ill patients receiving continuous hemodialysis filtration. *Yakugaku Zasshi*, **127**, 897–901.
25. Hirata K, Matsumoto Y, Ohno K, Hirano M, Yamamoto Y (2005) Pharmacokinetics of midazolam in critically ill patients receiving continuous hemodiafiltration using a polymethyl methacrylate and/or polysulfone membrane. *Japanese Journal of Pharmaceutical Health Care and Science*, **31**, 99–104.
26. Sobue S, Sekiguchi K, Shimatani K, Tan K (2004) Pharmacokinetics and safety of fosfluconazole after single intravenous bolus injection in healthy male Japanese volunteers. *Journal of Clinical Pharmacology*, **44**, 284–292.
27. Sobue S, Tan K, Haug-Pihale G (2005) The effects of hepatic impairment on the pharmacokinetics of fosfluconazole and fluconazole following a single intravenous bolus injection of fosfluconazole. *British Journal of Clinical Pharmacology*, **59**, 160–166.
28. Csajka C, Décosterd L, Buclin T, Pagani J, Fattinger K, Bille J, Biollaz J (2001) Population pharmacokinetics of fluconazole given for secondary prevention of oropharyngeal candidiasis in HIV-positive patients. *European Journal of Clinical Pharmacology*, **57**, 723–727.
29. Rajagopalan P, Pelz R, Lipsett P, Swoboda S, Rinaldi M, Hendrix C (2003) Enteral fluconazole population pharmacokinetics in patients in the surgical intensive care unit. *Pharmacotherapy*, **23**, 592–602.
30. Sobue S, Keith T, Cary L, Violette L, Angelika W (2004) The effects of renal impairment on the pharmacokinetics and safety of fosfluconazole and fluconazole following a single intravenous bolus injection of fosfluconazole. *British Journal of Clinical Pharmacology*, **57**, 773–784.
31. Pittrow L, Penk A (1999) Special pharmacokinetics of fluconazole in septic, obese and burn patients. *Mycoses*, **42**(Suppl. 2), 87–90.
32. Berl T, Wilner K, Gardner M, Hansen R, Farmer B, Baris B, Henrich W (1995) Pharmacokinetics of fluconazole in renal failure. *Journal of the American Society of Nephrology*, **6**, 242–247.
33. Bergman S, Tyagi I, Ronald K (2010) Antifungal dosing in critically ill patients. *Current Fungal Infection Reports*, **4**, 78–86.

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Research Article

Cancer Prevention Research

The Histone Demethylase JMJD2B Plays an Essential Role in Human Carcinogenesis through Positive Regulation of Cyclin-Dependent Kinase 6

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Abstract

Histone methyltransferases and demethylases are known to regulate transcription by altering the epigenetic marks on histones, but the pathologic roles of their dysfunction in human diseases, such as cancer, still remain to be elucidated. Herein, we show that the histone demethylase JMJD2B is involved in human carcinogenesis. Quantitative real-time PCR showed notably elevated levels of *JMJD2B* expression in bladder cancers, compared with corresponding nonneoplastic tissues ($P < 0.0001$), and elevated protein expression was confirmed by immunohistochemistry. In addition, cDNA microarray analysis revealed transactivation of *JMJD2B* in lung cancer, and immunohistochemical analysis showed protein overexpression in lung cancer. siRNA-mediated reduction of expression of *JMJD2B* in bladder and lung cancer cell lines significantly suppressed the proliferation of cancer cells, and suppressing *JMJD2B* expression lead to a decreased population of cancer cells in S phase, with a concomitant increase of cells in G_1 phase. Furthermore, a clonogenicity assay showed that the demethylase activity of JMJD2B possesses an oncogenic activity. Microarray analysis after knockdown of *JMJD2B* revealed that JMJD2B could regulate multiple pathways which contribute to carcinogenesis, including the cell-cycle pathway. Of the downstream genes, chromatin immunoprecipitation showed that CDK6 (cyclin-dependent kinase 6), essential in G_1–S transition, was directly regulated by JMJD2B, via demethylation of histone H3-K9 in its promoter region. Expression levels of *JMJD2B* and *CDK6* were significantly correlated in various types of cell lines. Deregulation of histone demethylation resulting in perturbation of the cell cycle, represents a novel mechanism for human carcinogenesis and JMJD2B is a feasible molecular target for anticancer therapy. *Cancer Prev Res; 4(12); 2051–61. ©2011 AACR.*

Introduction

Covalent histone modifications, including acetylation, methylation, phosphorylation, ubiquitination, glycosylation, and sumoylation can modulate chromatin dynamics and affect multiple cellular functions (1–3). Among these modifications, histone methylation is associated with activated or repressed transcription (3). Five lysine residues (H3K4, H3K9, H3K27, H3K36, and H4K20), located in the N-terminal tails of histones, are reported as representative lysines which can become mono-, di-, or trimethylated. According to recent findings, H3K9, H3K27, and H4K20 methylation mainly represses transcription, whereas methylation of H3K4 and H3K36 is associated with activated transcription (3). Although histone methylation had been thought to be irreversible, lysine specific demethylase 1, LSD1, was discovered to be the first example of a demethylase that can reverse histone H3 lysine 4 methylation status (4, 5). Later, the Jumonji C (JmjC) domain containing

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protein family, which catalyzes the hydroxylation of a lysine methyl group via a radical-based mechanism, has been identified as histone demethylases which differ from LSD1 (6, 7). Although our knowledge of the physiologic functions of histone demethylases is increasing, it still remains unclear how deregulation of the enzymes is involved in human diseases such as cancer.

We previously reported that the histone methyltransferase SMYD3 plays a critical role in human carcinogenesis (8–10). Besides our research, other groups also clarified that dysfunction of histone methylation status contributes to human carcinogenesis (11–13), but the detailed relationship between abnormal histone demethylation and human carcinogenesis is unclear. To find demethylases that contribute to human carcinogenesis, we examined the expression profiles of a number of proteins containing JmjC histone demethylase domains in clinical tissues and found that expression levels of JMJD2B were significantly upregulated in cancer tissues, compared with those in corresponding normal tissues. JMJD2B, also known as KDM4B, was identified *in silico* (14) and shown to be one of the demethylases capable of removing the trimethyl group from histone H3 lysine 9 on pericentric heterochromatin in mammalian cells (15). A line of recent reports indicated that hypoxic conditions can induce the expression of some JmjC family members, including JMJD2B (16, 17). In fact, JMJD2B has been shown to harbor HIF binding sites in their promoter sequences (17). However, the significance of JMJD2B in oncogenesis and cancer progression is not fully understood so far.

Here, we showed a critical role for JMJD2B in carcinogenesis, through the regulation of cancer-related downstream genes, and suggested the possibility that JMJD2B might be a novel therapeutic target for several types of cancer, especially bladder and lung cancer.

Materials and Methods

Tissue samples and RNA preparation

Bladder tissue sampling and RNA preparation were described previously (18). Briefly, 76 surgical specimens of primary urothelial carcinoma were collected, either at cystectomy or transurethral resection of bladder tumor, and snap frozen in liquid nitrogen. Twenty specimens of normal bladder urothelial tissue were collected from areas of macroscopically normal bladder urothelium in patients with no evidence of malignancy. Vimentin is primarily expressed in mesenchymally derived cells and was used as a stromal marker. Uroplakin is a marker of urothelial differentiation and is preserved in up to 90% of epithelially derived tumors (19). Use of tissues for this study was approved by Cambridgeshire Local Research Ethics Committee (Ref 03/018).

Cell culture

NIH3T3, CCD-18Co, SW780, SCaBER, A549, H2170, SW480, HCT116, LoVo, HepG2, HeLa, HFL1, and 293T cells were from American Type Culture Collection in 2001 and 2003 and tested and authenticated by DNA profiling for polymorphic short-tandem repeat (STR) markers. RERF-LC-AI, SBC5 and Huh-7 cells were from Japanese Collection of Research Bioresources (JCRB) in 2001 and tested and authenticated by DNA profiling for polymorphic STR markers. The 253J cells were from Korean Cell Line Bank in 2001 and tested and authenticated by DNA profiling for polymorphic STR markers. ACC-LC-319 cells were from Aichi Cancer Center in 2003 and tested and authenticated by DNA profiling for SNP, mutation, and deletion analysis. All cell lines were grown in monolayers in appropriate media: Dulbecco modified Eagle medium (DMEM) for RERF-LC-AI, HepG2, Huh-7, NIH3T3, and 293T cells; Eagleminimal essential medium (EMEM) for 253J, CCD-18, SCaBER, HeLa, SCaBER, and SBC5 cells; McCoy 5A medium for HCT116 cells; Leibovitz L-15 for SW480 and SW780 cells; RPMI-1640 medium for A549, H2170, and ACC-LC-319 cells, all supplemented with 10% FBS and 1% antibiotic/antimycotic solution (Sigma). LoVo cells were cultured in Ham F-12 medium supplemented with 20% FBS and 1% antibiotic/antimycotic solution. HFL1 cells were cultures in F-12K medium supplemented with 10% FBS, 1% antibiotic/antimycotic solution, 2 mmol/L L-glutamine, and 1,500 mg/L sodium bicarbonate. Cells were maintained at 37°C in humid air with 5% CO_2 condition (RERF-LC-AI, HepG2, Huh-7, NIH3T3, 293T, HeLa, SCaBER, SBC5, HCT116, A549, H2170, and ACC-LC-319) or without CO_2 (SW480 and SW780). Cells were transfected with FuGENE6 (Roche Applied Science) according to the manufacturer protocol.

Expression profiling in cancer using cDNA microarrays

We established a genome-wide cDNA microarray with 36,864 cDNAs selected from the UniGene database of the National Center for Biotechnology Information (NCBI). This microarray system was constructed essentially as described previously (20). Briefly, the cDNAs were amplified by reverse transcriptase PCR (RT-PCR) using poly $(A)^+$ RNAs isolated from various human organs as templates; the lengths of the amplicons ranged from 200 to 1,100 bp, without any repetitive or poly (A) sequences. Many types of tumor and corresponding nonneoplastic tissues were prepared in 8-μm sections, as described previously (20). A total of 30,000 to 40,000 cancer or noncancerous cells were collected selectively using the EZ cut system (SL Microtest GmbH) according to the manufacturer protocol. Extraction of total RNA, T7-based amplification, and labeling of probes were done as described previously (20). A measure of 2.5-μg aliquots of twice amplified RNA (aRNA) from each cancerous and noncancerous tissue was then labeled, respectively, with Cy3-dCTP or Cy5-dCTP.

Quantitative real-time PCR

As described previously, we prepared 76 bladder cancer and 20 normal bladder tissues in Addenbrooke's Hospital, Cambridge UK. For quantitative RT-PCR reactions, specific primers for all *GAPDH* (housekeeping gene), *JMJD2B*, *HDAC1*, *AKT3*, and *MAP3K1* were designed (Primer sequences in Supplementary Table S1; ref. 21). PCR

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