こと，薬物血中濃度と薬効，すなわち，薬物動態学的作用（pharmacokinetics，PK）と薬力学的作用（pharmacodynamics，PD）の相関が得られていないものが多いことを考慮しなければならない．また，これまでに実施された検討も数少なく，*CYP2C9* 多型の影響を結論付けるにはさらなるエビデンスが必要であろう．これらの薬物について，遺伝子多型の情報を臨床現場に活用するにはもう少し時間がかかると思われるが，今後，臨床応用につながる検証的臨床研究の成績が蓄積されることを期待したい．

3．ま と め

ワーファリンとフェニトインについては，人種や試験によって絶対量の差はあるものの，*CYP2C9* 遺伝子多型の相対的な影響は認められている．今後，個人差の原因をより明らかにするために，PD 因子の遺伝子多型を考慮することが必要になってくるであろう．その他の CYP2C9 基質薬物については健康成人での評価にとどまっており，遺伝子多型が実際の患者の治療における投与量に及ぼす影響についてはいまだほとんど検討されていない．薬効や副作用など PD の情報も一致した見解が得られておらず，遺伝子多型情報に基づいた個別化治療を実現するには，これらの点を検討することが必須となる．

文　献

1) Ingelman-Sundberg M, Daly A, Nebert D. Human cytochrome P450 (CYP) allele numenclature committee (web site). 2005. http://www.imm.ki.se/CYPalleles/.
2) Echizen H. ワーファリンの抗凝固効果の個人間変動に関係する遺伝子多型. *血栓止血誌* 2001 ; **12** : 111-8.
3) Takahashi H, Kashima T, Nomoto S, Iwade K, Tainaka H, Shimizu T, Nomizo Y, Muramoto N, Kimura S, Echizen H. Comparisons between in-vitro and in-vivo metabolism of (S)-warfarin : catalytic activities of cDNA-expressed CYP2C9, its Leu359 variant and their mixture versus unbound clearance in patients with the corresponding CYP2C9 genotypes. *Pharmacogenetics* 1998 ; **8**(5) : 365-73.
4) Takahashi H, Wilkinson GR, Caraco Y, Muszkat M, Kim RB, Kashima T, Kimura S, Echizen H. Population differences in S-warfarin metabolism between CYP2C9 genotype-matched Caucasian and Japanese patients. *Clin Pharmacol Ther* 2003 ; **73**(3) : 253-63.
5) Taube J, Halsall D, Baglin T. Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. *Blood* 2000 ; **96**(5) : 1816-9.
6) Peyvandi F, Spreafico M, Siboni SM, Moia M, Mannucci PM. CYP2C9 genotypes and dose requirements during the induction phase of oral anticoagulant therapy. *Clin Pharmacol Ther* 2004 ; **75**(3) : 198-203.
7) Palareti G, Leali N, Coccheri S, et al. Bleeding complications of oral anticoagulant treatment : an inception-cohort, prospective collaborative study (ISCOAT). Italian Study on Complications of Oral Anticoagulant Therapy. *Lancet* 1996 ; **348**(9025) : 423-8.
8) Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, Rettie AE. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 2002 ; **287**(13) : 1690-8.
9) Steward DJ, Haining RL, Henne KR, Davis G, Rushmore TH, Trager WF, Rettie AE. Genetic association between sensitivity to warfarin and expression of CYP2C9*3. *Pharmacogenetics* 1997 ; **7**(5) : 361-7.
10) Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999 ; **353**(9154) : 717-9.
11) Ogg MS, Brennan P, Meade T, Humphries SE. CYP2C9*3 allelic variant and bleeding complications. *Lancet* 1999 ; **354**(9184) : 1124.
12) Loebstein R, Yonath H, Peleg D, Almog S, Rotenberg M, Lubetsky A, Roitelman J, Harats D, Halkin H, Ezra D. Interindividual variability in sensitivity to warfarin—Nature or nurture? *Clin Pharmacol Ther* 2001 ; **70**(2) : 159-64.
13) Shikata E, Ieiri I, Ishiguro S, Aono H, Inoue K, Koide T, Ohgi S, Otsubo K. Association of pharmacokinetic (CYP2C9) and pharmacodynamic (factors II, VII, IX, and X ; proteins S and C ; and gamma-glutamyl carboxylase) gene variants with warfarin sensitivity. *Blood* 2004 ; **103**(7) : 2630-5.
14) Takahashi H, Echizen H. Pharmacogenetics of CYP2C9 and interindividual variability in anticoagulant response to warfarin. *Pharmacogenomics J* 2003 ; **3**(4) : 202-14.
15) Takahashi H, Nutescu E, Morita T, Wilkinson GR, Ieiri I, Kashima T, Kimura S, Kijima S, Echizen H. Population differences in anticoagulant response to warfarin among Caucasian, African American and Japanese patients. *2nd World Congress of the Board of Pharmaceutical Sciences of FIP.* 2004 : 384 (P1E-X-018).
16) D'Andrea G, D'Ambrosio RL, Di Perna P, Chetta M, Santacroce R, Brancaccio V, Grandone E, Margaglione M. A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. *Blood* 2005 ; **105**(2) : 645-9.
17) Yuan HY, Chen JJ, Lee MT, et al. A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. *Hum Mol Genet* 2005 ; **14**(13) : 1745-51.
18) Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, Blough DK, Thummel KE, Veenstra DL, Rettie AE. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med* 2005 ; **352**(22) : 2285-93.
19) Kidd RS, Straughn AB, Meyer MC, Blaisdell J, Goldstein JA, Dalton JT. Pharmacokinetics of chlorpheniramine, phenytoin, glipizide and nifedipine in an individual homozygous for the CYP2C9*3 allele. *Pharmacogenetics* 1999 ; **9**(1) : 71-80.
20) Mamiya K, Ieiri I, Shimamoto J, Yukawa E, Imai J, Ninomiya H, Yamada H, Otsubo K, Higuchi S, Tashiro N. The effects of genetic polymorphisms of CYP2C9 and CYP2C19 on phenytoin metabolism in Japanese adult

patients with epilepsy : studies in stereoselective hydroxylation and population pharmacokinetics. *Epilepsia* 1998 ; **39**(12) : 1317-23.

21) van der Weide J, Steijns LS, van Weelden MJ, de Haan K. The effect of genetic polymorphism of cytochrome P450 CYP2C9 on phenytoin dose requirement. *Pharmacogenetics* 2001 ; **11**(4) : 287-91.

22) 日本臨床薬理学会 (編). 中枢神経作用薬の臨床薬理. 臨床薬理第 2 版. 医学書院, 2003 : 418.

23) Odani A, Hashimoto Y, Otsuki Y, Uwai Y, Hattori H, Furusho K, Inui K. Genetic polymorphism of the CYP2C subfamily and its effect on the pharmacokinetics of phenytoin in Japanese patients with epilepsy. *Clin Pharmacol Ther* 1997 ; **62**(3) : 287-92.

24) Ninomiya H, Mamiya K, Matsuo S, Ieiri I, Higuchi S, Tashiro N. Genetic polymorphism of the CYP2C subfamily and excessive serum phenytoin concentration with central nervous system intoxication. *Ther Drug Monit* 2000 ; **22**(2) : 230-2.

25) Tate SK, Depondt C, Sisodiya SM, et al. Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. *Proc Natl Acad Sci USA* 2005 ; **102**(15) : 5507-12.

26) Sekino K, Kubota T, Okada Y, Yamada Y, Yamamoto K, Horiuchi R, Kimura K, Iga T. Effect of the single CYP2C9*3 allele on pharmacokinetics and pharmacodynamics of losartan in healthy Japanese subjects. *Eur J Clin Pharmacol* 2003 ; **59**(8-9) : 589-92.

27) Shon JH, Yoon YR, Kim KA, Lim YC, Lee KJ, Park JY, Cha IJ, Flockhart DA, Shin JG. Effects of CYP2C19 and CYP2C9 genetic polymorphisms on the disposition of and blood glucose lowering response to tolbutamide in humans. *Pharmacogenetics* 2002 ; **12**(2) : 111-9.

28) Kirchheiner J, Bauer S, Meineke I, Rohde W, Prang V, Meisel C, Roots I, Brockmoller J. Impact of CYP2C9 and CYP2C19 polymorphisms on tolbutamide kinetics and the insulin and glucose response in healthy volunteers. *Pharmacogenetics* 2002 ; **12**(2) : 101-9.

29) Lee CR, Pieper JA, Hinderliter AL, Blaisdell JA, Goldstein JA. Evaluation of cytochrome P4502C9 metabolic activity with tolbutamide in CYP2C91 heterozygotes. *Clin Pharmacol Ther* 2002 ; **72**(5) : 562-71.

30) Sullivan-Klose TH, Ghanayem BI, Bell DA, Zhang ZY, Kaminsky LS, Shenfield GM, Miners JO, Birkett DJ, Goldstein JA. The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* 1996 ; **6**(4) : 341-9.

31) Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, Kivisto KT. Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther* 2002 ; **72**(3) : 326-32.

32) Kirchheiner J, Brockmoller J, Meineke I, Bauer S, Rohde W, Meisel C, Roots I. Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. *Clin Pharmacol Ther* 2002 ; **71**(4) : 286-96.

33) Yasar U, Forslund-Bergengren C, Tybring G, Dorado P, Llerena A, Sjoqvist F, Eliasson E, Dahl ML. Pharmacokinetics of losartan and its metabolite E-3174 in relation to the CYP2C9 genotype. *Clin Pharmacol Ther* 2002 ; **71**(1) : 89-98.

34) Uchida S, Watanabe H, Nishio S, Hashimoto H, Yamazaki K, Hayashi H, Ohashi K. Altered pharmacokinetics and excessive hypotensive effect of candesartan in a patient with the CYP2C91/3 genotype. *Clin Pharmacol Ther* 2003 ; **74**(5) : 505-8.

35) Shimamoto J, Ieiri I, Urae A, Kimura M, Irie S, Kubota T, Chiba K, Ishizaki T, Otsubo K, Higuchi S. Lack of differences in diclofenac (a substrate for CYP2C9) pharmacokinetics in healthy volunteers with respect to the single CYP2C9*3 allele. *Eur J Clin Pharmacol* 2000 ; **56**(1) : 65-8.

36) Dorado P, Berecz R, Norberto MJ, Yasar U, Dahl ML, LLerena A. CYP2C9 genotypes and diclofenac metabolism in Spanish healthy volunteers. *Eur J Clin Pharmacol* 2003 ; **59**(3) : 221-5.

37) Garcia-Martin E, Martinez C, Tabares B, Frias J, Agundez JA. Interindividual variability in ibuprofen pharmacokinetics is related to interaction of cytochrome P450 2C8 and 2C9 amino acid polymorphisms. *Clin Pharmacol Ther* 2004 ; **76**(2) : 119-27.

38) Lee CR, Pieper JA, Frye RF, Hinderliter AL, Blaisdell JA, Goldstein JA. Differences in flurbiprofen pharmacokinetics between CYP2C9*1/*1, *1/*2, and *1/*3 genotypes. *Eur J Clin Pharmacol* 2003 ; **58**(12) : 791-4.

39) Vianna-Jorge R, Perini JA, Rondinelli E, Suarez-Kurtz G. CYP2C9 genotypes and the pharmacokinetics of tenoxicam in Brazilians. *Clin Pharmacol Ther* 2004 ; **76**(1) : 18-26.

40) Kirchheiner J, Kudlicz D, Meisel C, Bauer S, Meineke I, Roots I, Brockmoller J. Influence of CYP2C9 polymorphisms on the pharmacokinetics and cholesterol-lowering activity of (−)-3S,5R-fluvastatin and (+)-3R,5S-fluvastatin in healthy volunteers. *Clin Pharmacol Ther* 2003 ; **74**(2) : 186-94.

41) Vormfelde SV, Engelhardt S, Zirk A, Meineke I, Tuchen F, Kirchheiner J, Brockmoller J. CYP2C9 polymorphisms and the interindividual variability in pharmacokinetics and pharmacodynamics of the loop diuretic drug torsemide. *Clin Pharmacol Ther* 2004 ; **76**(6) : 557-66.

42) Kirchheiner J, Meineke I, Muller G, Bauer S, Rohde W, Meisel C, Roots I, Brockmoller J. Influence of CYP2C9 and CYP2D6 polymorphisms on the pharmacokinetics of nateglinide in genotyped healthy volunteers. *Clin Pharmacokinet* 2004 ; **43**(4) : 267-78.

CYP2A6 polymorphisms are associated with nicotine dependence and influence withdrawal symptoms in smoking cessation

T Kubota[1,4], C Nakajima-Taniguchi[2,4], T Fukuda[1], M Funamoto[3], M Maeda[1], E Tange[1], R Ueki[1], K Kawashima[1], H Hara[2], Y Fujio[1] and J Azuma[1]

[1]Department of Clinical Evaluation of Medicines and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, Yamadaoka, Suita City, Osaka, Japan; [2]Osaka Regional Taxation Bureau's Clinic, Otemae, Chuo-ku, Osaka 540-0008, Japan and [3]Funamoto Clinic, Urakaze-cho, Koshien, Nishinomiya City, Hyogo 663-8165, Japan

Correspondence:
Professor J Azuma, Department of Clinical Evaluation of Medicines and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita City, Osaka 565-0871, Japan.
E-mail: azuma@phs.osaka-u.ac.jp

CYP2A6 is the main enzyme that catalyzes nicotine into cotinine. Interindividual differences in nicotine metabolism result at least partially from polymorphic variation of CYP2A6 gene. In this study, we evaluated the influence of CYP2A6 polymorphisms on clinical phenotypes of smoking, such as smoking habit and withdrawal symptoms. Japanese smokers ($n = 107$) were genotyped for CYP2A6*1, *4 and *9. Consistent with the previous reports, CYP2A6 genotypes have a tendency to correlate with the number of cigarettes per day and with daily intake of nicotine. Interestingly, CYP2A6 high-activity group (CYP2A6*1/*1, *1/*9, *1/*4, *9/*9) smoked the first cigarette of the day earlier than low-activity group (CYP2A6*4/*9, *4/*4), indicating more remarkable nicotine dependence. Furthermore, nicotine withdrawal symptoms were more serious in smoking cessation in CYP2A6 high-activity group. Collectively, CYP2A6 genotypes are related with nicotine dependence, influencing smoking habits and withdrawal symptoms in quitting smoking. It is proposed that individualized smoking cessation program could be designed based on CYP2A6 genotypes.
The Pharmacogenomics Journal (2006) 6, 115–119. doi:10.1038/sj.tpj.6500348; published online 10 January 2006

Introduction

Smoking is one of the most important risk factors for serious diseases, including cancers, chronic obstructive pulmonary diseases and cardiovascular diseases. It is strongly recommended that smokers should cease smoking for good health. However, there is an interindividual diversity in the difficulties in quitting smoking, mainly due to nicotine dependence. Therefore, to carry out smoking cessation program effectively, individual status of smoking should be estimated and, more importantly, predicted, based on nicotine dependence.

Nicotine is metabolized to cotinine, an inactive metabolite, principally by CYP2A6.[1] Several CYP2A6 gene polymorphisms have been identified so far, and three alleles, *1, *4 and *9, are shown to be the major polymorphisms in Japanese. CYP2A6*1 is a wild-type allele with normal enzyme activity. CYP2A6*4 is a whole deletion type of the CYP2A6 gene.[2,3] CYP2A6*9 has a single-nucleotide polymorphism in TATA box, T-48G substitution, which impairs the transcriptional activities[4] and, consequently, its enzymatic activity.[5,6] It has been clearly demonstrated that the pharmacokinetics of nicotine is influenced by CYP2A6 polymorphisms.

In the present study, as pharmacokinetic changes in plasma nicotine concentration are considered to be related with craving for nicotine,[7] we hypothesized that CYP2A6 polymorphisms might affect smoking status. And we evaluated the relation between CYP2A6 genotypes and smoking habits, including nicotine withdrawal symptoms, from the point of view of nicotine dependence. The data presented here provide insights into individualized smoking cessation program based on CYP2A6 genotypes.

Results

First, we confirmed the relationship between the CYP2A6 genotypes and the number of cigarettes in the subjects analyzed in the present study (Figure 1a). In vivo enzymatic activity of nicotine metabolism decreases in order, CYP2A6*1/*1, *1/*9, *1/*4, *9/*9, *4/*9 and *4/*4.[5] CYP2A6 genotype, which determines the enzyme activity in vivo, had a tendency to be associated with the number of cigarettes smoked per day, as reported previously.[8–10] Next, the amounts of daily nicotine intake were also examined. As shown in Figure 1b, CYP2A6 genotype is likely to be linked with daily nicotine intake, proposing the possible association between CYP2A6 genotypes and nicotine dependence.

Association between CYP2A6 genotypes and smoking habits, such as the number of the cigarettes per day and

the daily nicotine uptake, was statistically analyzed. The subjects were divided into the high- and low-activity group, based on their CYP2A6 genotypes, according to the previous study.[5] The subjects with the *1/*1, *1/*9, *1/*4 and *9/*9 genotypes, whose metabolic activities of nicotine are more than 70% of those of the subjects with *1/*1, were defined as high-activity group, whereas the subjects with the *4/*9 and *4/*4 genotypes with less than 50% of metabolic activities of the subjects with *1/*1 as low-activity group. It was found that the associations of CYP2A6 genotypes with the number of cigarettes or with the nicotine uptake approached statistical significance ($P = 0.09$ or 0.06, respectively).

To evaluate nicotine dependence more directly, we analyzed the relation between CYP2A6 genotypes and the time to the first cigarette as described in Materials and methods. As shown in Figure 2, proportion of subjects who smoked the first cigarette within 5 min of waking up was significantly higher in CYP2A6 high-activity group than in low-activity group (36.8%, $n = 95$ and 8.3%, $n = 12$, respectively, $P < 0.05$), suggesting that the subjects with high CYP2A6 activity show the severer nicotine dependence than those with low activity.

Fagerstrom Test for Nicotine Dependence (FTND) is commonly performed to estimate nicotine dependence. Thus, the relationship between CYP2A6 genotypes and nicotine dependence was evaluated according to FTND. Consistent with the results shown in Figure 2, there was significant association between the total score of FTND and CYP2A6 activity (3.95 ± 1.45 in high-activity group, 3.17 ± 0.94 in low-activity group, $P < 0.05$) (Figure 3).

Finally, nicotine dependence was diagnosed according to the severity of withdrawal symptoms observed during smoking cessation. In the population of smokers who tried to quit smoking, withdrawal syndrome was categorized into
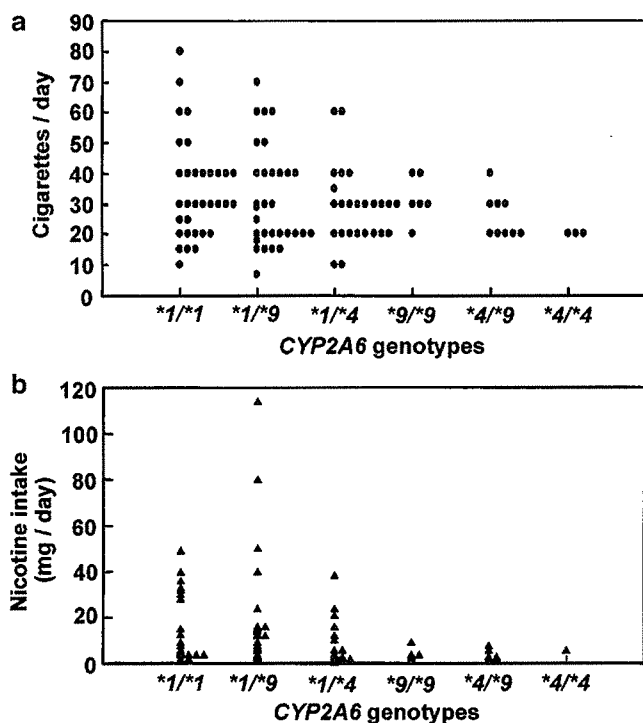

Figure 1 Relationship between the CYP2A6 genotypes and the number of cigarettes smoked per day (a) or the amount of daily nicotine intake (b). CYP2A6 genotypes had a tendency to be associated with the number of cigarettes smoked per day ($n = 107$, $P = 0.09$) and the amount of daily nicotine intake ($n = 70$, $P = 0.06$).
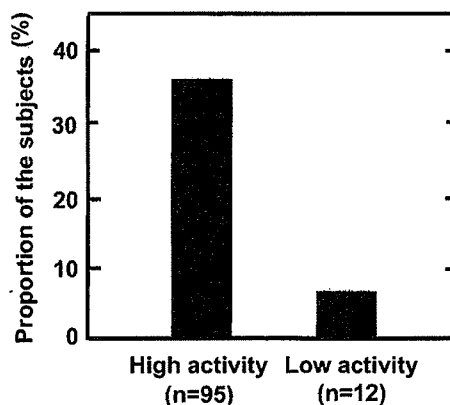

Figure 2 CYP2A6 genotypes were related to time to the first cigarette of the day. The proportion of subjects who smoked the first cigarette within 5 min of waking up was calculated, as an index for nicotine dependence. The proportion of subjects was significantly higher in CYP2A6 high-activity group than in low-activity group. CYP2A6 high-activity group consists of subjects carrying CYP2A6*1/*1, *1/*9, *1/*4 and *9/*9. Low-activity group consists of subjects carrying CYP2A6*4/*9 and *4/*4.
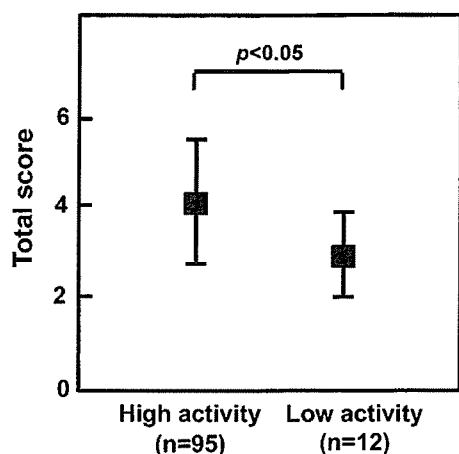
Figure 3 CYP2A6 genotypes are associated with nicotine dependence, analyzed by Fagerstrom Test for Nicotine Dependence. A total score for nicotine dependence was calculated by total scores on self-reported number of cigarettes smoked per day and time to the first cigarette of the day. The score was significantly high in CYP2A6 high-activity group, compared to low-activity group. Data are shown as mean±s.d.
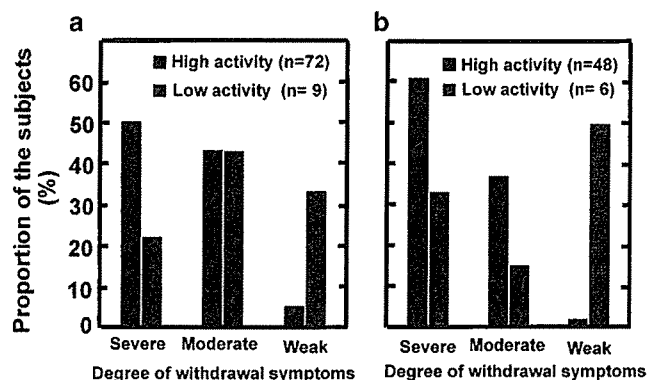

Figure 4 Impacts of CYP2A6 genotypes on withdrawal symptoms in the subpopulation that tried to quit smoking. Degree of withdrawal symptoms was evaluated using a questionnaire. (a) In CYP2A6 high-activity group, the withdrawal symptoms were significantly more serious than the low-activity group. (b) Degree of withdrawal symptoms and CYP2A6 genotypes in the population that tried to quit smoking by receiving nicotine replacement therapy. Among the population that tried to quit smoking by receiving nicotine replacement therapy, severe withdrawal symptoms were more remarkable in CYP2A6 high-activity group than in low activity group.

three groups: severe, moderate and weak. The proportion of subjects with severe withdrawal symptoms was higher in CYP2A6 high-activity group than in low-activity group (Figure 4a). The proportion of subjects was 50.0, 44.4 and 5.6% for severe, moderate and weak withdrawal symptoms, respectively, in high-activity group $(n = 72)$, and 22.2, 44.4 and 33.3%, respectively, in low-activity group $(n = 9)$ (χ^2 test; $P < 0.05$). Furthermore, as nicotine replacement therapy affects the withdrawal symptoms, the degree of withdrawal symptoms was compared in the subpopulation that tried to quit smoking by receiving nicotine replacement therapy. The proportion of subjects was 60.4, 37.5 and 2.1% for severe, moderate and weak symptom, respectively, in high-activity group $(n = 48)$, and 33.3, 16.7 and 50.0%, respectively, in low-activity group $(n = 6)$ (χ^2 test; $P < 0.01$; Figure 4b). To clarify the association between the CYP2A6 activities and nicotine dependence, the odds ratios (ORs) with 95% confidence intervals (CIs) were estimated relatively to the subjects with the weak withdrawal symptoms. In the total subjects, the ORs (95% CIs) were 6.0 (0.97–37.12, $P = 0.128$) for the moderate and 13.5 (1.71–106.56, $P = 0.025$) for the severe, respectively. In the subpopulation, they are 54.0 (2.61–1116.96, $P = 0.0089$) for the moderate and 43.5 (2.99–633.62, $P = 0.00341$) for the severe, respectively. Collectively, the relation between severities of withdrawal symptoms and CYP2A6 genotypes is more clearly demonstrated in this subpopulation.

Discussion

In the present study, we have demonstrated that CYP2A6 mutant allele with impaired or null enzyme activity was a negative risk factor for habit of smoking, especially nicotine dependence.

First, the number of cigarettes per day was likely to be associated with the activity of CYP2A6. Relation between the number of cigarettes and CYP2A6 genotype has been analyzed in several studies, with inconsistent results. Some studies have also shown that subjects who possessed CYP2A6 mutant allele smoked fewer cigarettes,[8-10] as is the case with the present study, whereas others reported that CYP2A6 genotypes are not associated with cigarettes consumption in Japanese,[11-13] Chinese[14] and Caucasians.[15] As smoking behavior is also influenced by environmental factors, these conflicting results might be due to interindividual differences in the environmental factors including lifestyles. In this study, a majority of the subjects were working as 'white collar workers', so difference in environmental influence was expected to be minimized.

To our knowledge, this is the first report that evaluated the relationship between the time to the first cigarette of the day and CYP2A6 genotypes. And it is revealed that the subjects with high-activity alleles of CYP2A6 smoke the first cigarette earlier than those with low activity. Importantly, as the time to the first cigarette of the day is considered to be influenced by nicotine dependence, it is possible that CYP2A6 activity is related with nicotine dependence. To address this possibility, nicotine dependence was quantified by calculating the score on the number of cigarettes per day and the time to the first cigarette of the day, according to FTND score. These two items are most important factors of FTND score,[16] and are generally used in smoking cessation program. As a result, nicotine dependence was more remarkable in the subjects with CYP2A6 high activity than in those with low activity.

Finally, we investigated the relationship between nicotine withdrawal symptoms and CYP2A6 genotypes. It was revealed that the subjects in CYP2A6 high-activity group

exhibited manifest withdrawal symptoms, which are clinical phenotypes derived from nicotine dependence in smoking cessation. Moreover, the correlation between CYP2A6 genotypes and withdrawal symptoms is more remarkable in subjects who received nicotine replacement therapy. Recent studies have provided molecular and cellular aspects of nicotine abuse. From the neuroscientific point of view, withdrawal symptom is considered to be the process of the nicotinic acetylcholine receptor from desensitization/inactivation states to functional states.[17] Importantly, low concentrations of nicotine cause desensitization of its receptors. Therefore, the smokers with high CYP2A6 activity might maintain a low level of nicotine that may inactivate a larger number of nicotinic receptors, compared with those with low activity. As a result, after many hours of abstinence, an excessive number of desensitized/inactivated nicotine receptors may begin to recover to functional states in the smokers with high CYP2A6 activity, resulting in the severe withdrawal symptoms.

In the process of smoking cessation, a number of smokers receive nicotine replacement therapy. High dose of nicotine is administered, for example, with nicotine patch, at the starting point and the subjects gradually weaned themselves from nicotine by reducing the dosage according to the generalized cessation protocol. Considering that the subjects with high CYP2A6 activity are prone to nicotine dependence, it might be beneficial to individualize the protocol for nicotine replacement therapy. Theoretically, by reducing the dosage of nicotine more deliberately in the subjects with high activity than in those with low activity, the success rate in quitting smoking would be improved. At the same time, we have also noticed the limitation of the individualized program for smoking cessation based on CYP2A6 genotypes alone. It is likely that other gene polymorphisms, in addition to CYP2A6, might be involved in nicotine dependence, because interindividual differences were not completely canceled by classifying the subjects based on CYP2A6 genotypes. Further investigation may be required to understand the genetic background of the susceptibility to nicotine dependence.

In conclusion, we found that CYP2A6 genotypes affect smoking habit, nicotine dependence, and withdrawal symptoms during smoking cessation. It could be proposed that CYP2A6 genotyping may be a novel pharmacogenomic strategy for smoking cessation program as an individualized health care.

Materials and methods

Subjects

This study is designed as a multicenter trial. The study subjects consisted of 107 Japanese smokers who attended to a clinic for their health care. The patients with life-threatening diseases, including cancer, heart failure and symptomatic chronic obstructive pulmonary diseases, were excluded. All subjects gave their informed consent to participate in this study.

Table 1 Scoring for the degree of nicotine dependence, analyzed by the Fagerstrom Test for Nicotine Dependence (FTND)

Score	0	1	2	3
Number of cigarettes/day	~10	11–20	21–30	31~
First cigarette of the day (min)	61~	31–60	6–30	~5

~ = from XX to XY.

This study was approved by the institutional review committee of Osaka University.

Estimation of smoking status

All subjects were interviewed about their smoking habits such as the number of cigarettes per day, the nicotine content of the cigarettes, which they usually smoke, and time to the first cigarette of the day, which is generally accepted as a clinical index for nicotine dependence. Daily nicotine intake was calculated by multiplying the number of cigarettes per day by nicotine content of cigarette.

The total score for nicotine dependence was calculated by summing scores on two items that were extracted from FTND: 'the number of cigarettes smoked per day' and 'time to the first cigarette of the day' (Table 1).

In the subpopulation that tried to quit smoking ($n = 81$), the degree of withdrawal symptoms was evaluated using a questionnaire. The degree of withdrawal symptoms was categorized into three groups: severe, moderate and weak.

Genotyping

Genomic DNA was extracted from blood using the QIAamp Blood Kit according to the manufacturer's protocol (Qiagen). The genotyping of CYP2A6*4 was carried out by the PCR-RFLP method, according to the previous report.[18] The primers used for the PCR were as follows: forward – CAC CGA AGT GTT CCC TAT GCT G; reverse – TGT AAA ATG GGC ATG AAC GCC C. Genomic DNA samples (45 ng) were added to the 25-μl PCR mixtures that consisted of 0.2 μM each primer, PCR Gold Buffer, 2.5 mM MgCl$_2$, 0.4 mM dNTPs and 1.25 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR was performed with an initial step at 94°C for 5 min, followed by 40 cycles at 95°C for 5 min, at 56°C for 1 min and at 72°C for 2 min, with a final extension at 72°C for 7 min. The PCR product was digested with Eco81I. The digestion patterns were analyzed by electrophoresis with 2% agarose gel. Mutation allele was identified from the fragment with 728 bp, whereas the wild-type allele was from that with 789 bp.

CYP2A6*9 alleles were genotyped by the allele-specific PCR method reported previously,[5] with minor modification. The primers used for the PCR were as follows: forward – GAT TCC TCT CCC CTG GAA C, reverse-wild type: GGC TGG GGT GGT TTG CCT TTA; reverse-mutant type – GGC TGG GGT GGT TTG CCT TTC. The PCR reaction was performed in 25 μl PCR reaction mixtures containing 45 ng genomic DNA, 0.4 μM each primer, PCR Gold Buffer, 2 mM MgCl$_2$, 0.2 mM each dNTP and 1.25 U AmpliTaq Gold DNA

polymerase. PCR was performed with an initial step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, at 66°C for 30 s and at 72°C for 25 s, with a final extension at 72°C for 7 min. Under these conditions, amplification of DNA fragments produced single product. The PCR product was detected with ABI PRISM 7700 Sequence detector (Applied Biosystems) using fluorescent dye SYBR Green I (Molecular Probes).

Owing to the low frequency, the alleles, except CYP2A6*4 and *9, were defined as CYP2A6*1.

Statistical analysis

According to the genotypes, subjects were divided into two groups, high- and low-activity group, as described previously.[5] In brief, subjects with the *4/*9 and *4/*4 genotypes were considered to have less than 50% of the enzyme activity of *1/*1 and, therefore, defined as CYP2A6 low-activity group, whereas those with the *1/*1, *1/*9, *1/*4 and *9/*9 genotypes were defined as CYP2A6 high-activity group. All comparisons were carried out between CYP2A6 high-activity group and CYP2A6 low-activity group. Differences in the number of cigarettes, daily nicotine intake and score for nicotine dependence were tested using Mann–Whitney U-test. The χ^2 test was used to assess the time to the first cigarette of the day. The frequencies of withdrawal symptoms were also analyzed with χ^2 test. To assess the association of the CYP2A6 genotypes with the withdrawal symptoms, we calculated ORs and their 95% CIs. An association was reported as statistically significant if the respective null hypothesis of OR = 1 was rejected at $P < 0.05$ or when the respective 95% CIs did not include the value 1.

Duality of interest

None declared.

Acknowledgments

References

1 Benowitz NL, Jacob III P. Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clin Pharmacol Ther 1994; 56: 483–493.

2 Nunoya K, Yokoi T, Kimura K, Inoue K, Kodama T, Funayama et al. A new deleted allele in the human cytochrome P450 2A6 (CYP2A6) gene found in individuals showing poor metabolic capacity to coumarin and (+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride (SM-12502). Pharmacogenetics 1998; 8: 239–249.

3 Nunoya KI, Yokoi T, Kimura K, Kainuma T, Satoh K, Kinoshita M et al. A new CYP2A6 gene deletion responsible for the in vivo polymorphic metabolism of (+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride in humans. J Pharmacol Exp Ther 1999; 289: 437–442.

4 Pitarque M, von Richter O, Oke B, Berkkan H, Oscarson M, Ingelman-Sundberg M. Identification of a single nucleotide polymorphism in the TATA box of the CYP2A6 gene: impairment of its promoter activity. Biochem Biophys Res Commun 2001; 284: 455–460.

5 Yoshida R, Nakajima M, Nishimura K, Tokudome S, Kwon JT, Yokoi T. Effects of polymorphism in promoter region of human CYP2A6 gene (CYP2A6*9) on expression level of messenger ribonucleic acid and enzymatic activity in vivo and in vitro. Clin Pharmacol Ther 2003; 74: 69–76.

6 Kiyotani K, Yamazaki H, Fujieda M, Iwano S, Matsumura K, Satarug S et al. Decreased coumarin 7-hydroxylase activities and CYP2A6 expression levels in humans caused by genetic polymorphism in CYP2A6 promoter region (CYP2A6*9). Pharmacogenetics 2003; 13: 689–695.

7 Jarvik ME, Madsen DC, Olmstead RE, Iwamoto-Schaap PN, Elins JL, Benowitz NL. Nicotine blood levels and subjective craving for cigarettes. Pharmacol Biochem Behav 2000; 66: 553–558.

8 Fujieda M, Yamazaki H, Saito T, Kiyotani K, Gyamfi MA, Sakurai M et al. Evaluation of CYP2A6 genetic polymorphisms as determinants of smoking behavior and tobacco-related lung cancer risk in male Japanese smokers. Carcinogenesis 2004; 25: 2451–2458.

9 Schoedel KA, Hoffmann EB, Rao Y, Sellers EM, Tyndale RF. Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. Pharmacogenetics 2004; 14: 615–626.

10 Minematsu N, Nakamura H, Iwata M, Tateno H, Nakajima T, Takahashi S et al. Association of CYP2A6 deletion polymorphism with smoking habit and development of pulmonary emphysema. Thorax 2003; 58: 623–628.

11 Ando M, Hamajima N, Ariyoshi N, Kamataki T, Matsuo K, Ohno Y. Association of CYP2A6 gene deletion with cigarette smoking status in Japanese adults. J Epidemiol 2003; 13: 176–181.

12 Yang M, Kunugita N, Kitagawa K, Tateno H, Nakajima T, Takahashi S et al. Individual differences in urinary cotinine levels in Japanese smokers: relation to genetic polymorphism of drug-metabolizing enzymes. Cancer Epidemiol Biomarkers Prev 2001; 10: 589–593.

13 Zhang X, Amemo K, Ameno S, Iwahashi K, Kinoshita H, Kubota T et al. Lack of association between smoking and CYP2A6 gene polymorphisms in a Japanese population. Nihon Arukoru Yakubutsu Igakkai Zasshi 2001; 36: 486–490.

14 Tan W, Chen GF, Xing DY, Song CY, Kadlubar FF, Lin DX. Frequency of CYP2A6 gene deletion and its relation to risk of lung and esophageal cancer in the Chinese population. Int J Cancer 2001; 95: 96–101.

15 Loriot MA, Rebuissou S, Oscarson M, Cenee S, Miyamoto M, Ariyoshi N et al. Genetic polymorphisms of cytochrome P450 2A6 in a case–control study on lung cancer in a French population. Pharmacogenetics 2001; 11: 39–44.

16 John U, Meyer C, Schumann A, Hapke U, Rumpf HJ, Adam C et al. A short form of the Fagerstrom Test for Nicotine Dependence and the Heaviness of Smoking Index in two adult population samples. Addict Behav 2004; 29: 1207–1212.

17 Dani JA, Heinemann S et al. Molecular and cellular aspects of nicotine abuse. Neuron 1996; 16: 905–908.

18 Ariyoshi N, Takahashi Y, Miyamoto M et al. Structural characterization of a new variant of the CYP2A6 gene (CYP2A6*1B) apparently diagnosed as heterozygotes of CYP2A6*1A and CYP2A6*4C. Pharmacogenetics 2000; 10: 687–693.

Warfarin dose requirement for patients with both VKORC1 3673A/A and CYP2C9*3/*3 genotypes

To the Editor:

Recently, interindividual variation in the maintenance dose of warfarin has been accounted for by several genetic factors, including cytochrome P450 (CYP) 2C9 and vitamin K epoxide reductase complex subunit 1 (VKORC1), according to excellent articles reported by Aquilante et al[1] and Lee et al[2] in this journal.

We have encountered a 69-year-old female patient (weight, 79.8 kg) with atrial fibrillation whose maintenance dose of warfarin was quite low, 0.5 mg/d (international normalized ratio [INR], 1.93; target INR, 1.5-2.0). For that reason, we tried to analyze her genotypes and measure the plasma concentration of S- and R-warfarin by HPLC[3] after receiving informed consent from her. Surprisingly, we found that she is homozygous for CYP2C9*3 and for 3673A of VKORC1 (VKORC1A/A). In addition, the data of 14 patients were further interpreted with regard to the relationship between the maintenance dose of warfarin and their genotypes (Fig 1). Four patients with VKORC1A/G and CYP2C9*1/*1 were identified as taking the 4 highest doses among all patients, consistent with several reports.[1] The plasma concentration of S-warfarin (286 ng/mL) in the patient with CYP2C9*3/*3 (VKORC1A/A) was rather high compared with that in patients with CYP2C9*1/*1 and VKORC1A/A (mean [± SD], 140 ± 50 ng/mL; range, 96-257 ng/mL), although the dose (0.5 mg/d) and R-warfarin concentration (132 ng/mL) were the lowest of the patients (mean, 451 ± 127 ng/mL; range, 214-684 ng/mL). This study was approved by the institutional ethical board of Osaka University, Osaka, Japan.

Patients with both CYP2C9*3/*3 and VKORC1A/A are rare, especially among the white population, because VKORC1G/G is the major genotype in white persons. However, these patients should be treated carefully, because they are expected to require the lowest dose of warfarin. So far, 2 cases have been reported concerning the maintenance dose of warfarin in patients with CYP2C9*3/*3 in a Japanese population. One patient re-
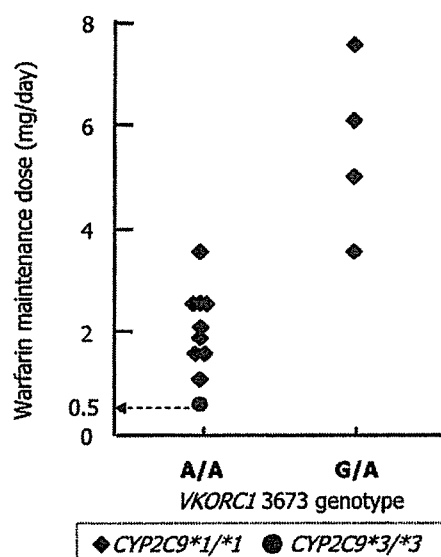

Fig 1. Relationship between warfarin maintenance dose and genotypes. *Diamonds, CYP2C9*1/*1; circles, CYP2C9*3/*3.*

ceived 0.4 mg/d of warfarin,[3] whereas in another patient, identified from a sample of 828 Japanese patients, the symptoms seemed to be controlled by 2.0 mg/d of warfarin.[4] The maintenance dose of the second patient was similar to the mean dose (2.0 mg/d) for patients with CYP2C9*1/*3 and VKORC1A/A. According to data in white subjects,[5] those with CYP2C9*3/*3 required a much lower dose (1.6 ± 0.81 mg/d, n = 5) than those with CYP2C9*1/*3 (3.3 ± 0.94 mg/d, n = 18), although most patients probably have the VKORC1G/G genotype. This result implies that the patient reported by Mushiroda et al[4] may have received an overdose of warfarin or that this patient's treatment must have been influenced by other factors leading to an increased requirement for warfarin, as pointed out in the article by Aquilante et al.[1] Unfortu-

nately, the medical condition of the patient, including weight, INR, target INR, smoking status, and so on, was not detailed in the article by Mushiroda et al. Although they suggested that patients with *CYP2C9*3/*3* and *CYP2C9*1/*3* together with *VKORC1A/A* were classified as "index 0," for whom the recommended dose was 2.0 mg/d, the maintenance dose for patients with *CYP2C9*3/*3* and *VKORC1A/A* might be around 0.5 mg/d according to our observation, as well as the report by Takahashi et al.[3]

In general, it is difficult to obtain reliable evidence for phenotyping patients with a rare genotype; therefore these data should be accumulated to comprise accurate evidence. From this viewpoint, our observation may provide practical information about the maintenance dose in patients with *CYP2C9*3/*3* and *VKORC1A/A*, who have a high potential risk of bleeding as a result of warfarin overdose.

Tsuyoshi Fukuda, PhD
Tomoko Tanabe, MS
Masako Ohno, PhD
Katsuhiko Tougou, MS
Yasushi Fujio, MD, PhD
Junichi Azuma, MD
Department of Clinical Evaluation of Medicines and
Therapeutics
Graduate School of Pharmaceutical Sciences
Osaka University

Isamu Yamamoto, MD, PhD
Akira Takeda, MD, PhD
Kita Osaka Police Hospital
Osaka, Japan
E-mail: *azuma@phs.osaka-u.ac.jp*

The authors have no conflict of interest.

References

1. Aquilante CL, Langaee TY, Lopez LM, Yarandi HN, Tromberg JS, Mohuczy D, et al. Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. Clin Pharmacol Ther 2006;79:291-302.
2. Lee SC, Ng SS, Oldenburg J, Chong PY, Rost S, Guo JY, et al. Interethnic variability of warfarin maintenance requirement is explained by VKORC1 genotype in an Asian population. Clin Pharmacol Ther 2006;79:197-205.
3. Takahashi H, Kashima T, Nomoto S, Iwade K, Tainaka H, Shimizu T, et al. Comparisons between in-vitro and in-vivo metabolism of (S)-warfarin: catalytic activities of cDNA-expressed CYP2C9, its Leu359 variant and their mixture versus unbound clearance in patients with the corresponding CYP2C9 genotypes. Pharmacogenetics 1998;8:365-73.
4. Mushiroda T, Ohnishi Y, Saito S, Takahashi A, Kikuchi Y, Shimomura H, et al. Association of VKORC1 and CYP2C9 polymorphisms with warfarin dose requirements in Japanese patients. J Hum Genet 2006;51:249-53.
5. Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, et al. Association between CYP2C9

genetic variants and anticoagulation-related outcomes during warfarin therapy. JAMA 2002;287:1690-8.

フォーラム

遺伝子多型情報に基づく投与指針作成に向けて
～CYP2C19～

田　邊　智　子[*1]　　大　野　雅　子[*1]　　福　田　剛　史[*1]　　景　山　　　茂[*2]
東　　　純　一[*1]

1．はじめに

　薬の応答性の個体差と薬物代謝酵素の遺伝子多型との関係が多くの薬物で検討されている．今回は，CYP2Cファミリーの中でCYP2C9と並んで薬物に対する影響が検討されているCYP2C19について述べる．

2．CYP2C19の遺伝子多型と表現型

　CYP2C19 に関しては，現在**1B* から**21* までのアレルが報告されている（2006年2月28日更新）[1]．このうち，酵素活性との関連が明らかになっている遺伝子多型で，日本人で重要なのは**2* と**3* で，アレル頻度はそれぞれ約30%，約10%である．両者で日本人の poor metabolizer（PM）のほとんどを説明できる．遺伝子型と表現型とはよく一致し，**2* および**3* などのアレルを有さず代謝の速い homozygous extensive metabolizer（homoEM），**2* もしくは**3* のいずれかを有する中間型の heterozygous extensive metabolizer（heteroEM），および両アレルが**2* もしくは**3* であり代謝の遅い PM の3群に分けられる．日本人では，CYP2C19酵素活性が低下しているPMが約20%で[2]，欧米人に比べてPMの割合が有意に高く，臨床的に重要である．

　CYP2C19の基質薬物として，プロトンポンプ阻害薬（proton pump inhibitor, PPI）や抗不安薬（diazepam）などが知られている．本稿では，遺伝子多型のみならず表現型としての血中動態や薬効との関連がよく検討されているPPIについて最初に取り上げる．

3．PPIの臨床効果とCYP2C19の遺伝子多型

　現在日本で認可されているPPIは omeprazole（OPZ），lansoprazole（LPZ）および rabeprazole（RPZ）である．いずれの代謝にも，CYP2C19が関与しているが，その経口クリアランスに対するCYP2C19活性の寄与度は異なる．たとえば，これら3種類のPPIを健康成人に8日間連続投与した際，血中濃度（pharmacokinetics, PK）と胃内pH（pharmacodynamics, PD）とに対する *CYP2C19* 遺伝子多型の影響は，OPZ（20 mg）＞LPZ（30 mg）＞RPZ（20 mg）の順である[3,4]．*CYP2C19* 遺伝子多型が薬効に及ぼす影響についての報告を，以下に取り上げる．

1）*Helicobacter pylori*（*H. pylori*）除菌

　現在，日本ヘリコバクター学会のガイドラインによると，*H. pylori* 除菌治療の第一選択はPPIと抗生物質2剤の3剤併用療法である[5]．NCBIが提供するPubMedを利用し，「proton pump inhibitor」「CYP2C19」「pylori」のキーワードで検索した．そのうち，日本人での *H. pylori* 除菌に関するものでかつ1998年以降のものは13件であった．これらの論文を網羅するとともに，必要に応じて海外文献なども参照した．

　Table 1-Aに示すように，PPI通常用量による除菌療法では，*H. pylori* の除菌率に *CYP2C19* 遺伝子型が影響すると報告されている[6~11]．PMの少ない海外（白人におけるPMの頻度：3～5%）でも多型の影響が観察されている[11]．HomoEMとheteroEMとにおける除菌失敗の要因の1つとしてPPIの用量不足が考えられる．すなわち，LPZを常用量の30 mg×2回から30 mg×4回に増量して投与すると，homoEMでも胃酸分泌が十分に抑制されることが報告されている[4]．さらに，3剤併用療法で除菌に失敗したhomoEMおよびheteroEMの日本人患者に対して，PPIを増量して再除菌を試みたところ，ほぼ100%の

Table 1　*H. pylori* 感染の除菌率と *CYP2C19* 遺伝子型

A：*CYP2C19* 遺伝子多型と除菌率に有意な関係が観察された研究

対象 (*H. pylori* 陽性)	処方	効果判定時期	*CYP2C19* 遺伝子型別の除菌率			文献
			homoEM	heteroEM	PM	
胃潰瘍・十二指腸潰瘍 （日本人 n＝62）	2剤療法（OPZ 20 mg×1/AMPC 500 mg×4） 2週間　のち OPZ 20 mg×1　4～6週間	4週	28.6%	60.0%	100%	6)
胃炎・胃潰瘍 （日本人 n＝21）	2剤療法（OPZ 40 mg/AMPC 2 g） 1週間	4週	33.3% (3/9)	30.0% (3/10)	100% (2/2)	7)
胃炎・胃潰瘍 （日本人 n＝44）	3剤療法（OPZ 40 mg/AMPC 2000 mg/CAM 800 mg）1週間	4週	81.3% (13/16)	94.7% (18/19)	100% (4/4)	
胃炎 （日本人 n＝97）	2剤療法（RPZ 10 mg×2/AMPC 500 mg×3） 2週間	4週	60.6%	91.7%	93.8%	8)
胃潰瘍・十二指腸潰瘍・胃炎 （日本人 n＝261）	3剤療法（OPZ 20 mg or LPZ 30 mg×2/AMPC 500 mg×3/CAM 200 mg×3）1週間 のち OPZ 20 mg or LPZ 30 mg×1　5～7週間	4週	72.7%	92.1%	97.8%	9)
胃炎・胃潰瘍 （日本人 n＝26）	2剤療法（OPZ 20 mg×2/AMPC 500 mg×4） 1週間	4週 以降	40.0% (4/10)	41.7% (5/12)	100% (4/4)	10)
胃炎・胃潰瘍 （日本人 n＝57）	3剤療法（OPZ 20 mg×2/AMPC 500 mg×4/ CAM 200 mg×4）1週間		75.0% (15/20)	88.5% (23/26)	100% (11/11)	
上部消化管異常（内視鏡・症状） （白人 n＝131）	4剤療法（LPZ 30 mg×2/AMPC 1 g×2/CAM 250 mg×2/MNZ 400 mg×2）5日間	4～12 週	*80.2%	*97.8%	*100%	11)

OPZ：omeprazole, RPZ：rabeprazole, LPZ：lansoprazole,
AMPC：amoxicillin, CAM：clarithromycin, MNZ：metronidazole
*CYP2C9*2 のみ判定

除菌率が得られたと報告されている（Table 2）[8,9,12~14]．HeteroEM で再除菌率が 25% と低値であった報告[14]は，OPZ の用量が他の報告と比較して少ないことが一因である．欧米人を対象とした検討でも，除菌失敗患者に対し高用量の OPZ（40 mg×4）と amoxicillin（AMPC/750 mg×4）で 2 週間治療を行ったときの除菌率は 83.8% で，2 剤併用療法の平均的な除菌率より高い効果が得られている[15]．本報告では，遺伝子多型は調べられていないが，欧米人での PM の頻度を考慮すると，大半の患者が EM であった可能性が高い．なお，高用量投与時の副作用に関しては，単純に比較することはできないものの，日本人では問題とされてはいないが，海外の報告[15]では下痢，嘔気，頭痛などが軽微としながらも言及されている．

一方，*H. pylori* の除菌率に対する *CYP2C19* 遺伝子多型の関連が明らかではないとする報告もある（Table 1-B）[16~23]．また，homoEM の再除菌で，高用量 RPZ を含む 2 剤療法（RPZ 20 mg×2/AMPC 1,000 mg×2：2 週間）を用いた場合でも，除菌率が約 60% にとどまったという報告[24]もある．この報告に対して Furuta ら[25]は，homoEM の除菌には投与回数も影響し，同じ「RPZ 1 日量 40 mg」でも，1 日 2 回投与よりも 1 日 4 回（10 mg×4）投与のほうが有効率が高いことを報告し，試験デザインの差異を指摘している．また，RPZ 処方時には除菌率に対する CYP2C19 の関与が小さい可能性も指摘される（Table 1-B[17~19,21,22]）．これは，RPZ の代謝への CYP2C19 の寄与が低いことによるものと考えられ，胃内 pH に対する効果は *CYP2C19* 遺伝子多型間で有意な差がなかったとの報告と一致する[26]．以上のように，*H. pylori* 除菌に対する *CYP2C19* 遺伝子多型の影響については明らかでないものも散見される．しかし，明らかでないとする報告でも EM の除菌率は 80% 前後であり効果不十分であること，PPI 増量による除菌成功の報告（Table 2）があることから，EM に対して 2 倍程度増量することにより，EM における治療効果が上昇する可能性が高い．

また，*CYP2C19* 遺伝子多型以外の除菌率に影響する因子として，*H. pylori* 菌の抗生物質耐性の有無[9,19,20,27]がある．初回除菌治療時の耐性菌の存在は，clarithromycin の乱用が背景として挙げられる．そのほか，喫煙者では除菌率が低くなっており[28]，非喫

Table 1 （つづき）

B：CYP2C19 遺伝子多型と除菌率に有意な関係が観察されていない研究

対象 (H. pylori 陽性)	処方	効果判定時期	CYP2C19 遺伝子型別の除菌率・備考			文献
			homoEM	heteroEM	PM	
消化性潰瘍・非潰瘍性消化不良 (日本人 n=150)	3剤療法 (OPZ 20 mg×2/AMPC 500 mg×3/CAM 200 mg×2) 1週間	4～8週	89.3%			16)
			本文中で遺伝子多型間に有意差なしとあるが，遺伝子型別の除菌率の記載なし			
胃潰瘍 (日本人 n=92)	3剤療法 (RPZ 10 mg×1 or 10 mg×2 or 20 mg×2/AMPC 750 mg×2/CAM 200 mg×2) 1週間　のち RPZ 10 mg 7週間	6週	86.5%		76.9%	17)
消化性潰瘍 (日本人 n=174)	2剤療法 (OPZ 20 mg or RPZ 10 mg×2/AMPC 500 mg×3) 2週間 [非喫煙者のみ]	4～8週	72.4% [85.0%]	72.3% [87.5%]	78.8% [100%]	18)
消化性潰瘍・胃炎 (日本人 n=138)	3剤療法 (LPZ 30 mg×2 or RPZ 10 mg×2 or RPZ 20 mg×2/AMPC 1000 mg×2/CAM 400 mg×2) 1週間	4週以降	88.6%	88.9%	77.3%	19)
消化性潰瘍・非潰瘍性消化不良 (日本人 n=61)	3剤療法 (LPZ 30 mg×2/AMPC 750 mg×2/CAM 200 mg×2) 1週間	4～8週	76.2%	78.6%	91.7%	20)
慢性胃炎 (日本人 n=164)	3剤療法 (OPZ 20 mg or RPZ 20 mg×2/AMPC 750 mg×2/CAM 400 mg×2) 1週間	3ヵ月	73.3%	86.1%	85.0%	21)
			81.0%	82.9%	87.5%	
			上段：OPZ　下段：RPZ			
消化性潰瘍 (日本人 n=173)	3剤療法 (LPZ 30 mg×2 or RPZ 10 mg×2/AMPC 750 mg×2/CAM 400 mg×2) 1週間 のち LPZ 30 mg×1 or RPZ 10 mg×1 4週間 (一部の患者)	6週	73%	74%	83%	22)
			87%	81%	60%	
			上段：LPZ　下段：RPZ 多型の影響　LPZ で傾向あり			
消化性潰瘍 (日本人 n=116)	3剤療法 (OPZ 20 mg or LPZ 30 mg or RPZ 10 mg×2/AMPC 500 mg×3/CAM 200 mg×3) 1週間	4～6ヵ月	76.2%	88.9%	90.0%	23)
			90.0%	89.7%	88.9%	
			62.5%	87.1%	87.5%	
			上段：OPZ　中段：LPZ　下段：RPZ 多型の影響　OPZ 傾向あり 　　　　　　RPZ 有意差あり 治療効果が LPZ＞RPZ となり試験中止			

OPZ：omeprazole，RPZ：rabeprazole，LPZ：lansoprazole，
AMPC：amoxicillin，CAM：clarithromycin

煙者で CYP2C19 遺伝子多型の影響が見られたという報告[18]もある．喫煙による胃酸分泌の変化などが除菌率に影響していた可能性も考えられる．これらの要因が，CYP2C19 遺伝子多型の除菌率に対する影響が明らかではないとする報告の一因になっている可能性がある．さらに，胃酸分泌抑制に関係する IL-1β や薬物輸送にかかわる MDR1 の遺伝子多型が影響しているという報告[29~31]もあり，CYP2C19 遺伝子多型に加えてこれらの因子についても今後検討する必要があろう．

2）逆流性食道炎

PPI は，逆流性食道炎および胃食道逆流症 (GERD) の治療にも用いられる．GERD の治療効果に対する CYP2C19 遺伝子多型の影響に関しても見解は分かれており，影響するという報告[32,33]と影響しない[34]という報告がある．H. pylori 陰性の健康成人を対象に RPZ による夜間胃酸分泌の抑制を検討した報告[35]では，homoEM と heteroEM の場合，GERD の治療に必要とされる胃内 pH 4 未満時間 16.7% 以下を達成するのに，従来の投与量では不十分であり，増

Table 2 *H. pylori* 再除菌療法と *CYP2C19* 遺伝子多型

除菌を失敗したときの処方	多型 患者背景	再除菌のときの処方 PPI＋AMPC による 2 剤療法	再除菌 成功率	文献
3 回除菌失敗 ① LPZ 30 mg×2＋CAM 200 mg×3 　＋AMPC 500 mg×3　1 週間 ② LPZ 30 mg×2＋CAM 200 mg×4 　＋AMPC 500 mg×4　1 週間 ③ LPZ 30 mg×2＋minocycline 200 mg×2 　＋cefaclor 250 mg×3　2 週間	homoEM CAM 耐性	OPZ 40 mg×3, AMPC 750 mg×3 2 週間	100% (1 症例)	12)
RPZ 10 mg×2＋AMPC 500 mg×3 2 週間	homoEM (n=10) heteroEM (n=2)	RPZ 10 mg×4　AMPC 500 mg×4 2 週間	100% (12/12)	8)
OPZ 20 mg or LPZ 30 mg×2 AMPC 500 mg×3 CAM 200 mg×3 1 週間	homoEM (n=24) heteroEM (n=7)	LPZ 30 mg×4　AMPC 500 mg×4 2 週間	96.9% (31/32)	9)
	PM (n=1)	LPZ 30 mg×2　AMPC 500 mg×4 2 週間		
OPZ 40 mg＋AMPC 1500 mg ＋CAM 800 mg or LPZ 60 mg or RPZ 20 mg＋AMPC 1500 mg ＋CAM 600 mg　1 週間	homoEM (n=12) heteroEM (n=5)	RPZ 10 mg×4　AMPC 500 mg×4 2 週間	100% (17/17)	13)
PPI＋AMPC＋CAM※ 5〜14 日間	CAM と MNZ 耐性あり homoEM (n=2)	OPZ 120 mg/day (for homoEM) or OPZ 40 mg/day (for heteroEM) AMPC 1000 mg×2/day 2 週間	100% (2/2)	14)
	heteroEM (n=4)		25% (1/4)	

OPZ：omeprazole, RPZ：rabeprazole, LPZ：lansoprazole,
AMPC：amoxicillin, CAM：clarithromycin, MNZ：metronidazole
※詳細な処方は記載なし

量および分割投与が有効であるとしている．この報告では，RPZ を PM には 20 mg/day，heteroEM には 20 mg×2/day または 10 mg×4/day，homoEM には 10 mg×4/day 投与することが推奨されている．

PPI の副作用で重篤なものは少ないが，胃潰瘍や長期投与可能な逆流性食道炎に対する OPZ 投与により，homoEM と比較して heteroEM や PM では，血清ガストリン濃度の上昇，および血清ビタミン B_{12} の減少が報告[34,36,37]されている．現在のところ，これらの濃度変化と具体的な副作用との関連性は報告されていない．しかし，体内成分の二次的な濃度変化に伴う副作用の原因となる可能性も否定できないため，heteroEM や PM での長期投与では注意深く観察するのが望ましい．

3）小児における PPI 使用の現状

PPI は小児においても重要な医薬品であるが，日本では小児に対する投与は承認されていない．一方，海外では小児を対象とした検討が行われており，米国で LPZ，欧州で OPZ が小児適応を取得している．小児における *CYP2C19* 遺伝子多型の影響を検討したものとしては，これまでに 2 つの報告[38,39]がある．いずれの検討でも *CYP2C19* の PM では，EM に比較して PPI（OPZ[38]または pantoprazole[39]）の血中濃度下面積が 6〜11 倍増加している．しかし，PM の人数がそれぞれ 1 人および 3 人と非常に少ないため，これらの検討だけでその影響を結論付けることはできない．日本では，小児で *CYP2C19* 遺伝子多型を考慮した試験はまだ実施されておらず，PM の多い日本での検討が望まれる．著者らは，大阪府立母子保健総合医療センターでの検討を開始した（ClinicalTrials. gov Identifier：NCT 00299845）．

日本では PPI は小児においても *H. pylori* 除菌療法および逆流性食道炎に対する有力な治療手段と認められており[40]，*H. pylori* 除菌療法に関して小児用のガイドライン[41]が作成されている段階である．小児に対しても PPI を処方する際に，*CYP2C19* 遺伝子多型

Table 3　ベンゾジアゼピン系薬物に対する *CYP2C19* 遺伝子多型の影響

薬物	対象・投与量など	PKパラメータ（EMに対するPMの比）未変化体			代謝物	PD	備考	文献
		CL	$T_{1/2}$	AUC				
Diazepam	健康成人・白人（n＝16）10 mg　単回経口投与	0.47倍	2.2倍		CL　0.45倍 $T_{1/2}$　2.2倍	—	・phenotyping（mephenytoin）	42)
	健康成人・韓国人（n＝17）8 mg　単回経口投与	0.55倍	1.5倍	1.8倍	$T_{1/2}$　2.2倍 AUC　1.9倍	—	・phenotyping（mephenytoin） ・メフェニトイン代謝能と「未」の $T_{1/2}$, CL 相関あり ・「未」と「代」の $T_{1/2}$ 相関あり	43)
	健康成人・中国人（n＝16）5 mg　単回経口投与	差なし	差なし		濃度（7日後以降）1.3～2.9倍 $T_{1/2}$　1.4倍	—	・phenotyping（mephenytoin） ・人種差について言及	44)
	健康成人・中国人（n＝18）5 mg　単回経口投与	0.14倍	4.2倍	6.1倍	$T_{1/2}$　1.8倍 AUC　2.4倍	—	・genotyping（*2のみ） ・*CYP2C19*2/*2* との比較	45)
	健康成人・日本人（n＝15）0.1 mg/kg　単回静脈注射	0.52倍	1.6倍	1.8倍	AUC　1.4倍	—	・phenotyping（mephenytoin） ・OPZ により EM で「未」の PK パラメータ変化（PM では変化なし）	46)
	健康成人・日本人（n＝13）2 mg　単回経口投与	0.65倍	1.7倍	1.4倍	AUC　1.4倍	×	・genotyping（*2, *3）	47)
Flunitrazepam	健康成人・白人/中国人/他（n＝16）1 mg　単回経口投与		差なし		2つの代謝物の AUC と OPZ 代謝能に相関あり	—	・genotyping（*2, *3）/phenotyping（OPZ） ・CYP2C19 の寄与は minor と結論	48)
Quazepam	健康成人・日本人（n＝20）20 mg　単回経口投与	C_{max} 2.8倍		2.4倍	C_{max}　差なし AUC　差なし	×	・genotyping（*2, *3） ・喫煙の影響もみられている	49)
Clobazam	患者・日本人（n＝16）0.28 mg/kg/day（EM の平均：多型間で差なし）4週間以上服用量安定	C_{ss}　差なし			C_{ss}　8.2倍 代/未　gene dose effect*あり	△	・genotyping（*2, *3） ・CYP3A4 誘導作用のある併用薬で，血中濃度/投与量の割合が低下する等の影響あり	50)

PK（薬物動態学）……CL：クリアランス　$T_{1/2}$：半減期　AUC：血中濃度下面積　C_{ss}：定常状態血中濃度　未：未変化体　代：代謝物
　　OPZ：omeprazole
PD（薬力学）作用への影響………—：記述なし　×：*CYP2C19* 多型の影響なし　△：PM で副作用1例報告あり
*gene dose effect：変異アレルの保有数が多いほど遺伝子多型の影響が大きくなること

の影響を考慮することは，より個人に合わせた処方を可能にすると考えられ，そのためのエビデンスの確立が必要である．

3. ベンゾジアゼピン系薬物への *CYP2C19* 遺伝子多型の影響

　CYP2C19 遺伝子多型とベンゾジアゼピン系薬物との関連もいくつか報告されている．Diazepam に関する検討は，健康成人を対象にした単回投与の試験において，表現型または遺伝子型に基づいた群分けをして PK パラメータを比較したものである（Table 3)[42~50]．報告により若干異なるが，遺伝子多型間で PK パラメータに次のような差異が認められている．すなわち，EM に比し PM では，未変化体のクリアランスが低下し，半減期の延長や AUC の増加がみられる．また，代謝物の AUC が PM では EM の1.4～2.4倍に増加していることから，代謝物も CYP2C19 の基質であることが示唆される．その他，人種差について言及している報告もある[44,51]．Diazepam は治療域が比較的広い薬物とされているが，これまでに得られている報告は健康成人での単回投与による検討である．実際の患者での PD 作用への影響を明らかにするために，患者を対象とした検討の実施が望まれる．

　Diazepam 以外のベンゾジアゼピン系薬物については Table 3 にまとめた．いずれの薬物も未変化体および代謝物が薬理活性を有する．PK パラメータには *CYP2C19* 遺伝子多型の影響が見られるが，PD への影響に関しては，遺伝子多型の関与を明らかに示した

報告はない.

このほか, cyclophosphamide の代謝に CYP2C19 が関与しているという新たな報告[52]があり, 今後の研究が待たれる.

4. まとめ

CYP2C ファミリーの代謝に関与する薬物の中で, 遺伝子多型情報に基づいた個別化治療実現に最も近いのは, 今回取り上げた H. pylori 除菌療法における PPI である. 治療対象患者の CYP2C19 遺伝子多型をあらかじめ判定し, 遺伝子多型によって治療法を個別化するというプロスペクティブな臨床研究がすでに開始されており[53], 遺伝子多型に基づく投与指針の作成まであと一歩という段階である.

一方, ベンゾジアゼピン系薬物に関しては, 遺伝子多型の PD への影響や患者における投与量や副作用との関係が検討されておらず, 現時点では多型別の投与量を論ずる段階ではない. PPI の小児に対する使用や, ベンゾジアゼピン系薬物と CYP2C19 遺伝子多型との関係に関しては, エビデンスが不十分であり今後の検討課題である.

文　献

1) Ingelman-Sundberg M, Daly A, Nebert D. Human cytochrome p 450 (CYP) allele numenclature committee (web site). http://www.imm.ki.se/CYPalleles

2) Horai Y, Nakano M, Ishizaki T, Ishikawa K, Zhou HH, Zhou BI, Liao CL, Zhang LM. Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects : Japanese versus mainland Chinese. *Clin Pharmacol Ther* 1989 ; **46**(2) : 198-207.

3) Shirai N, Furuta T, Moriyama Y, et al. Effects of CYP2C19 genotypic differences in the metabolism of omeprazole and rabeprazole on intragastric pH. *Aliment Pharmacol Ther* 2001 ; **15**(12) : 1929-37.

4) Furuta T, Shirai N, Xiao F, Ohashi K, Ishizaki T. Effect of high-dose lansoprazole on intragastic pH in subjects who are homozygous extensive metabolizers of cytochrome P 4502 C 19. *Clin Pharmacol Ther* 2001 ; **70**(5) : 484-92.

5) 日本ヘリコバクター学会. *Helicobacter pylori* 感染の診断と治療のガイドライン 改訂版. 2003.

6) Furuta T, Ohashi K, Kamata T, Takashima M, Kosuge K, Kawasaki T, Hanai H, Kubota T, Ishizaki T, Kaneko E. Effect of genetic differences in omeprazole metabolism on cure rates for Helicobacter pylori infection and peptic ulcer. *Ann Intern Med* 1998 ; **129**(12) : 1027-30.

7) Aoyama N, Tanigawara Y, Kita T, Sakai T, Shirakawa K, Shirasaka D, Kodama F, Okumura K, Kasuga M. Sufficient effect of 1-week omeprazole and amoxicillin dual treatment for Helicobacter pylori eradication in cytochrome P450 2C19 poor metabolizers. *J Gastroenterol* 1999 ; **34** Suppl 11 : 80-3.

8) Furuta T, Shirai N, Takashima M, Xiao F, Hanai H, Nakag-awa K, Sugimura H, Ohashi K, Ishizaki T. Effects of genotypic differences in CYP2C19 status on cure rates for Helicobacter pylori infection by dual therapy with rabeprazole plus amoxicillin. *Pharmacogenetics* 2001 ; **11**(4) : 341-8.

9) Furuta T, Shirai N, Takashima M, Xiao F, Hanai H, Sugimura H, Ohashi K, Ishizaki T, Kaneko E. Effect of genotypic differences in CYP2C19 on cure rates for Helicobacter pylori infection by triple therapy with a proton pump inhibitor, amoxicillin, and clarithromycin. *Clin Pharmacol Ther* 2001 ; **69** : 158-68.

10) Tanigawara Y, Aoyama N, Kita T, Shirakawa K, Komada F, Kasuga M, Okumura K. CYP2C19 genotype-related efficacy of omeprazole for the treatment of infection caused by Helicobacter pylori. *Clin Pharmacol Ther* 1999 ; **66** : 528-34.

11) Schwab M, Schaeffeler E, Klotz U, Treiber G. CYP2C19 polymorphism is a major predictor of treatment failure in white patients by use of lansoprazole-based quadruple therapy for eradication of Helicobacter pylori. *Clin Pharmacol Ther* 2004 ; **76** : 201-9.

12) Furuta T, Takashima M, Shirai N, Xiao F, Hanai H, Ohashi K, Ishizaki T. Cure of refractory duodenal ulcer and infection caused by Helicobacter pylori by high doses of omeprazole and amoxicillin in a homozygous CYP2C19 extensive metabolizer patient. *Clin Pharmacol Ther* 2000 ; **67** : 684-9.

13) Furuta T, Shirai N, Xiao F, Takashita M, Sugimoto M, Kajimura M, Ohashi K, Ishizaki T. High-dose rabeprazole/amoxicillin therapy as the second-line regimen after failure to eradicate H. pylori by triple therapy with the usual doses of a proton pump inhibitor, clarithromycin and amoxicillin. *Hepatogastroenterology* 2003 ; **50** : 2274-8.

14) Miwa H, Nagahara A, Kurosawa A, Ohkusa T, Ohkura R, Hojo M, Enomoto N, Sato N. Is antimicrobial susceptibility testing necessary before second-line treatment for Helicobacter pylori infection? *Aliment Pharmacol Ther* 2003 ; **17** : 1545-51.

15) Miehlke S, Kirsch C, Schneider-Brachert W, et al. A prospective, randomized study of quadruple therapy and high-dose dual therapy for treatment of Helicobacter pylori resistant to both metronidazole and clarithromycin. *Helicobacter* 2003 ; **8** : 310-9.

16) Miwa H, Misawa H, Yamada T, Nagahara A, Ohtaka K, Sato N. Clarithromycin resistance, but not CYP2C-19 polymorphism, has a major impact on treatment success in 7-day treatment regimen for cure of H. pylori infection : a multiple logistic regression analysis. *Dig Dis Sci* 2001 ; **46** : 2445-50.

17) Hokari K, Sugiyama T, Kato M, et al. Efficacy of triple therapy with rabeprazole for Helicobacter pylori infection and CYP2C19 genetic polymorphism. *Aliment Pharmacol Ther* 2001 ; **15** : 1479-84.

18) Miyoshi M, Mizuno M, Ishiki K, et al. A randomized open trial for comparison of proton pump inhibitors, omeprazole versus rabeprazole, in dual therapy for Helicobacter pylori infection in relation to CYP2C19 genetic polymorphism. *J Gastroenterol Hepatol* 2001 ; **16** : 723-8.

19) Miki I, Aoyama N, Sakai T, et al. Impact of clarithromycin resistance and CYP2C19 genetic polymorphism on treatment efficacy of Helicobacter pylori infection with lansoprazole-or rabeprazole-based triple therapy in Japan. *Eur J Gastroenterol Hepatol* 2003 ; **15** : 27-33.

20) Isomoto H, Inoue K, Furusu H, Nishiyama H, Shikuwa S, Omagari K, Mizuta Y, Murase K, Murata I, Kohno S.

Lafutidine, a novel histamine H 2-receptor antagonist, vs lansoprazole in combination with amoxicillin and clarithromycin for eradication of Helicobacter pylori. *Helicobacter* 2003 ; 8 : 111-9.

21) Dojo M, Azuma T, Saito T, Ohtani M, Muramatsu A, Kuriyama M. Effects of CYP2C19 gene polymorphism on cure rates for Helicobacter pylori infection by triple therapy with proton pump inhibitor (omeprazole or rabeprazole), amoxycillin and clarithromycin in Japan. *Dig Liver Dis* 2001 ; 33 : 671-5.

22) Kawabata H, Habu Y, Tomioka H et al. Effect of different proton pump inhibitors, differences in CYP2C19 genotype and antibiotic resistance on the eradication rate of Helicobacter pylori infection by a 1-week regimen of proton pump inhibitor, amoxicillin and clarithromycin. *Aliment Pharmacol Ther* 2003 ; 17 : 259-64.

23) Inaba T, Mizuno M, Kawai K, Yokota K, Oguma K, Miyoshi M, Take S, Okada H, Tsuji T. Randomized open trial for comparison of proton pump inhibitors in triple therapy for Helicobacter pylori infection in relation to CYP2C19 genotype. *J Gastroenterol Hepatol* 2002 ; 17 : 748-53.

24) Isomoto H, Inoue K, Furusu H, et al. High-dose rabeprazole-amoxicillin versus rabeprazole-amoxicillin-metronidazole as second-line treatment after failure of the Japanese standard regimen for Helicobacter pylori infection. *Aliment Pharmacol Ther* 2003 ; 18 : 101-7.

25) Furuta T, Shirai N, Sugimoto M, Ohashi K, Ishizaki T. Retreatment of H. pylori with dual therapy using high doses of rabeprazole or lansoprazole can be effective. *Aliment Pharmacol Ther* 2003 ; 18 : 1175-6.

26) Adachi K, Katsube T, Kawamura A, Takashima T, Yuki M, Amano K, Ishihara S, Fukuda R, Watanabe M, Kinoshita Y. CYP2C19 genotype status and intragastric pH during dosing with lansoprazole or rabeprazole. *Aliment Pharmacol Ther* 2000 ; 14 : 1259-66.

27) Realdi G, Dore MP, Piana A, et al. Pretreatment antibiotic resistance in Helicobacter pylori infection : results of three randomized controlled studies. *Helicobacter* 1999 ; 4 : 106-12.

28) Treiber G, Wittig J, Ammon S, Walker S, van Doorn LJ, Klotz U. Clinical outcome and influencing factors of a new short-term quadruple therapy for Helicobacter pylori eradication : a randomized controlled trial (MACLOR study). *Arch Intern Med* 2002 ; 162 : 153-60.

29) Take S, Mizuno M, Ishiki K, et al. Interleukin-1 beta genetic polymorphism influences the effect of cytochrome P 2 C 19 genotype on the cure rate of 1-week triple therapy for Helicobacter pylori infection. *Am J Gastroenterol* 2003 ; 98 : 2403-8.

30) Furuta T, Shirai N, Xiao F, El-Omar EM, Rabkin CS, Sugimura H, Ishizaki T, Ohashi K. Polymorphism of interleukin-1 beta affects the eradication rates of Helicobacter pylori by triple therapy. *Clin Gastroenterol Hepatol* 2004 ; 2 : 22-30.

31) Gawronska-Szklarz B, Wrzesniewska J, Starzynska T, Pawlik A, Safranow K, Ferenc K, Drozdzik M. Effect of CYP2C19 and MDR 1 polymorphisms on cure rate in patients with acid-related disorders with Helicobacter pylori infection. *Eur J Clin Pharmacol* 2005 ; 61 : 375-9.

32) Kawamura M, Ohara S, Koike T, Iijima K, Suzuki J, Kayaba S, Noguchi K, Hamada S, Noguchi M, Shimosegawa T. The effects of lansoprazole on erosive reflux oesophagitis are influenced by CYP2C19 polymorphism. *Aliment Pharmacol*

Ther 2003 ; 17 : 965-73.

33) Furuta T, Shirai N, Watanabe F, et al. Effect of cytochrome P 4502 C 19 genotypic differences on cure rates for gastroesophageal reflux disease by lansoprazole. *Clin Pharmacol Ther* 2002 ; 72 : 453-60.

34) Ohkusa T, Maekawa T, Arakawa T, et al. Effect of CYP2C 19 polymorphism on the safety and efficacy of omeprazole in Japanese patients with recurrent reflux oesophagitis. *Aliment Pharmacol Ther* 2005 ; 21 : 1331-9.

35) Sugimoto M, Furuta T, Shirai N, Kajimura M, Hishida A, Sakurai M, Ohashi K, Ishizaki T. Different dosage regimens of rabeprazole for nocturnal gastric acid inhibition in relation to cytochrome P 450 2 C 19 genotype status. *Clin Pharmacol Ther* 2004 ; 76 : 290-301.

36) Sagar M, Janczewska I, Ljungdahl A, Bertilsson L, Seensalu R. Effect of CYP2C19 polymorphism on serum levels of vitamin B 12 in patients on long-term omeprazole treatment. *Aliment Pharmacol Ther* 1999 ; 13 : 453-8.

37) Sagar M, Bertilsson L, Stridsberg M, Kjellin A, Mardh S, Seensalu R. Omeprazole and CYP2C19 polymorphism : effects of long-term treatment on gastrin, pepsinogen I, and chromogranin A in patients with acid related disorders. *Aliment Pharmacol Ther* 2000 ; 14 : 1495-502.

38) Kearns GL, Andersson T, James LP, Gaedigk A, Kraynak RA, Abdel-Rahman SM, Ramabadran K, van den Anker JN. Omeprazole disposition in children following single-dose administration. *J Clin Pharmacol* 2003 ; 43 : 840-8.

39) Kearns GL, Ferron GM, James LP, Blumer JL, Gaedigk A, Mayer P, Abel M, Getsy JA, Leeder JS and Paul J, Pantoprazole disposition in pediatrics. *Clin Pharmacol Ther* 2003 ; 73 : 38.[abstract P II-30]

40) 豊田茂. 厚生科学研究班「小児等特殊患者群に対する医薬品の用法・用量の確立に関する研究」平成 14 年度報告書.

41) 加藤晴一. Helicobacter pylori 感染の診断と治療のガイドライン. 小児科臨床 2002 ; 55 : 1335-40.

42) Bertilsson L, Henthorn TK, Sanz E, Tybring G, Sawe J, Villen T. Importance of genetic factors in the regulation of diazepam metabolism : relationship to S-mephenytoin, but not debrisoquin, hydroxylation phenotype. *Clin Pharmacol Ther* 1989 ; 45 : 348-55.

43) Sohn DR, Kusaka M, Ishizaki T, Shin SG, Jang IJ, Shin JG, Chiba K. Incidence of S-mephenytoin hydroxylation deficiency in a Korean population and the interphenotypic differences in diazepam pharmacokinetics. *Clin Pharmacol Ther* 1992 ; 52 : 160-9.

44) Zhang YA, Reviriego J, Lou YQ, Sjoqvist F, Bertilsson L. Diazepam metabolism in native Chinese poor and extensive hydroxylators of S-mephenytoin : interethnic differences in comparison with white subjects. *Clin Pharmacol Ther* 1990 ; 48 : 496-502.

45) Qin XP, Xie HG, Wang W, He N, Huang SL, Xu ZH, Ou-Yang DS, Wang YJ, Zhou HH. Effect of the gene dosage of CgammaP 2 C 19 on diazepam metabolism in Chinese subjects. *Clin Pharmacol Ther* 1999 ; 66 : 642-6.

46) Ishizaki T, Chiba K, Manabe K, Koyama E, Hayashi M, Yasuda S, Horai Y, Tomono Y, Yamato C, Toyoki T. Comparison of the interaction potential of a new proton pump inhibitor, E 3810, versus omeprazole with diazepam in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation. *Clin Pharmacol Ther* 1995 ; 58 : 155-64.

47) Kosuge K, Jun Y, Watanabe H, Kimura M, Nishimoto M, Ishizaki T, Ohashi K. Effects of CYP3A4 inhibition by dilti-

azem on pharmacokinetics and dynamics of diazepam in relation to CYP2C19 genotype status. *Drug Metab Dispos* 2001 ; **29** : 1284-9.

48) Gafni I, Busto UE, Tyndale RF, Kaplan HL, Sellers EM. The role of cytochrome P450 2C19 activity in flunitrazepam metabolism in vivo. *J Clin Psychopharmacol* 2003 ; **23** : 169-75.

49) Fukasawa T, Yasui-Furukori N, Aoshima T, Suzuki A, Tateishi T, Otani K. Single oral dose pharmacokinetics of quazepam is influenced by CYP2C19 activity. *Ther Drug Monit* 2004 ; **26** : 529-33.

50) Kosaki K, Tamura K, Sato R, Samejima H, Tanigawara Y, Takahashi T. A major influence of CYP2C19 genotype on the steady-state concentration of N-desmethylclobazam. *Brain Dev* 2004 ; **26** : 530-4.

51) Caraco Y, Tateishi T, Wood AJ. Interethnic difference in omeprazole's inhibition of diazepam metabolism. *Clin Pharmacol Ther* 1995 ; **58** : 62-72.

52) Timm R, Kaiser R, Lotsch J, Heider U, Sezer O, Weisz K, Montemurro M, Roots I, Cascorbi I. Association of cyclophosphamide pharmacokinetics to polymorphic cytochrome P450 2C19. *Pharmacogenomics J* 2005 ; **5** : 365-73.

53) 古田隆久，白井直人，杉本光繁，堤箸幸子，江頭徹，植田幸治，米山政男，大橋京一，菱田明，石崎高志．H. pylori のクラリスロマイシン耐性，並びに CYP2C19 の SNP 検査に基づくテーラーメイドの除菌療法．臨床薬理 2004 ; 35 : S184.

Original

Determination of Single Nucleotide Polymorphisms in N-Acetyltransferase2 Gene Using an Electrochemical DNA Chip and an Automated DNA Detection System

Naoko NAKAMURA[*1], *Keiko ITO*[*2], *Sadato HONGO*[*3], *Kouji HASHIMOTO*[*4],

Miyuki FURUTSUKA[*5], *Ryuuji KUBOTA*[*6], *Tsuyoshi FUKUDA*[*7], *Masako OHNO*[*8],

Junichi AZUMA[*9] and *Nobuhiro GEMMA*[*10]

An electrochemical DNA chip using an electrochemically active intercalator and DNA probe immobilized on a gold electrode has been developed for genetic analysis. In this study, N-acetyltransferase2 (NAT2) gene polymorphisms (C481T G590A G857A) were determined by the electrochemical DNA chip and the automated DNA detection system that performs hybridization reaction, washing, detection, and data analysis. Human genomic DNAs were extracted from blood and DNA fragments containing the three polymorphisms were amplified by the polymerase chain reaction (PCR) method. Double-stranded PCR products were treated with T7 exonuclease and single-stranded target DNAs were obtained. A sample containing the single-stranded target DNAs was injected into a cassette including the electrochemical DNA chip and set in an automated system. The turnaround time for genotyping with this system was 90 min. A total of 38 samples were automatically genotyped by an SNP determination algorithm. The results of genotype were completely consistent with those determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Consequently, this method requires no labeling step and has the advantage of realizing a compact and automatic system, and so the system is expected to contribute to personalized medicine based on genotype.

[Rinsho Byori 55 : 216~223, 2007]

[Key Words] electrochemical DNA chip, automated DNA detection system, single nucleotide polymorphisms, N-acetyltransferase2

I. Introduction

Among several types of genetic variation, single nucleotide polymorphisms (SNPs) are the most important and basic form of variation in the genome. They are responsible for individual differences in disease susceptibility and drug response. If an individual's genomic information is identified in advance of drug treatment, safe and effective treatment, so-called "personalized medicine," is achieved.

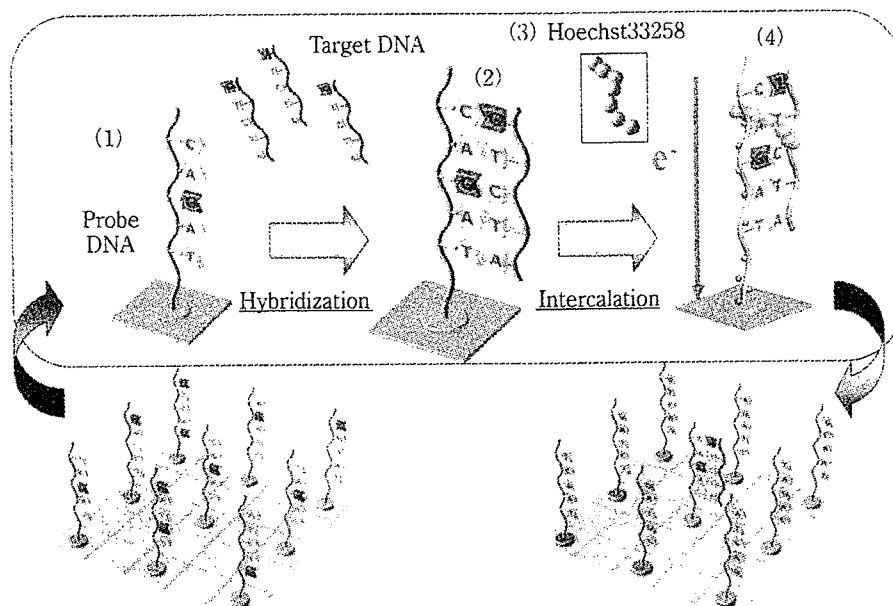A DNA chip, a device in which DNA probes are located with high density on glass or silicon, is widely used

Figure 1 Principal of electrochemical DNA chip.

(1) Immobilized DNA probe on the gold electrode

(2) Hybridization and washing of target DNA

(3) Adding the electrochemically active intercalator, Hoechst 33258

(4) Detecting an anodic current by electrochemical measurement

for gene expression and SNP analysis. In regard to the DNA chip, fluorescence-based detection methods such as those applied by Affimetrix Inc. are the most general[1]~[3]. However, these methods need complicated fluorochrome labeling and expensive fluorescence analysis equipment. Therefore, the use of the methods is limited to research work in laboratories. In order to solve such problems, some electrochemical-based detection methods have been reported[4]~[8]. Among these methods, we have been developing those using the electrochemically active intercalator, Hoechst 33258 (**Fig. 1**)[9][10], and have reported DNA chips for determination of SNPs in MxA and MBL gene associated with the efficacy of interferon therapy for Hepatitis C patient[11] and SNPs in MDR1 gene that encode the drug transport protein[12].

The electrochemical DNA detection method is advantageous for realizing an automated system, because the reaction part and the detection part are unified and simple. Moreover, large and expensive signal transduction equipment is unnecessary. Exploiting these advantages, we have developed a small detection system that automatically performs the process after hybridization. In this study, we have demonstrated the feasibility of this automated system by performing accurate genotyping of NAT2 C481T, G590A, and G857A polymorphisms, which relate to the hepatotoxicity of anti-tuberculous drug, isoniazid[13].

II. Materials and Methods

A. Human genomic DNA

Human genomic DNAs were purchased from Coriell Cell Repositories (Camden, NJ, USA) and were used for determination of genotyping condition by the DNA chip. Peripheral blood was drawn from 38 tuberculosis patients into EDTA-containing tube, and genomic DNA was extracted from whole blood using QIAamp DNA blood kit (QIAGEN, Hilden, Germany). All of the genomic DNAs were genotyped by PCR-RFLP analysis in order to confirm the accuracy of the DNA chip.

B. Preparation of a target sample

The enzyme activity of NAT2 in Japanese is mostly predicted by determination of *NAT2*4, NAT2*5,*

NAT2*6, NAT2*7[13) 14)]. In this study, we detect the polymorphisms of C481T, G590A and G857A for these alleles determination[15)]. G590A and G857A were designed to be located on the same fragment of PCR product. The target for C481T and the target for G590A and G857A were coamplified simultaneously in a single-tube according to the reaction condition shown in **Table1 (1)**. Amplification of the PCR products was checked by electrophoresis (**Fig. 2 (2)**). These PCR products were treated with T7 gene6 exonuclease (USB, Cleveland, USA) and the single-stranded target DNA samples were obtained to increase hybridization efficiency between probes and target DNAs. A technique for preparing single-stranded target DNAs has been described previously[12)]. The protocol of target DNA preparation is shown in **Fig. 2 (1)**.

C. PCR-RFLP conditions

PCR was performed under the reaction conditions shown in **Table 1 (1)**. The restriction enzymes used for RFLP and the digested patterns are shown in **Table 1 (2)**. The results of PCR-RFLP analysis for three polymorphisms are shown in **Fig. 3**.

D. Preparation of the DNA chip

The DNA chip substrates used in this study were prepared as described previously[10)]. The substrate consists of 40 working electrodes (200μm diameter), a reference electrode, and a counter electrode. Oligonucleotide probes with a thiol group at the 5' or 3' end were obtained as custom synthesis products from Greiner Japan (Tokyo, Japan). Each working electrode was spotted with 0.1μl of the probe solution containing the 40μg/ml oligonucleotide probe and 400mmol/l sodium chloride by use of a spotter (Microsys™ 4100 manufactured by Cartesian Technology, Irvine, CA, USA). The DNA chip was covered with a reaction chamber (50μl, Grace Bio-Labs, Bend, OR, USA) to prevent drying and kept for 1 h at room temperature. After washing with distilled water, the chip was stored at 4℃.

E. Genotyping by the automated detection system

Fig. 4 shows the DNA chip cassette including the probe-immobilized DNA chip substrate and the automated detection system. The system consists of a temperature control part, an electrochemical analyzing part and a reagent sending part. After setting a cassette with a sample, hybridization reaction, washing and electrochemical detection are performed automatically. Specially designed software for SNP analysis is installed on a computer connected to the system and SNPs are automatically genotyped using voltammetric results. The process operated in the system is described below.

45μl of 2×SSC solution (300mmol/l sodium chloride, 30mmol/l sodium citrate) containing the single-stranded target DNAs was reacted with DNA probes on the electrodes. The hybridization reaction was carried out at 35℃ for 40 min. After the reaction, the chip was washed at 35℃ for 40 min with 0.2×SSC to remove non-specific hybridized DNA. The chip was reacted with phosphate buffer (20mmol/l, pH7.0) containing 50μmol/l Hoechst 33258 (Wako Pure Chemicals, Osaka, Japan) and 100mmol/l sodium chloride at 25℃ for 10 min. Then, the anodic current derived from Hoechst 33258 was measured by linear sweep voltammetry. Anodic peak current (Ipa) values were measured from the voltammogram of Hoechst 33258.

III. Results

In order to carry out simultaneous genotyping of the NAT2 3SNPs, two types of probes, wild type and mutant type were prepared for each polymorphism. The sequences of 6 probes for polymorphisms and 2 probes for control are shown in **Table 1 (3)**. **Fig. 5 (A)** shows voltammograms for G590A polymorphisms with the automated system. The 590-G probe, the 590-A probe, and the 5'SH control probe were immobilized on separate electrodes. The target DNA amplified from genomic DNA (590G/G, 590A/A or 590G/A) was reacted with these probes, respectively. The Ipa values on the 590-G probe, the 590-A probe, and the 5'SH control probe for target 590G/G were (a) 41nA, (b) 22nA, and (c) 20nA, respectively. In the case of target 590A/A and target 590G/A, the Ipa values on the 590-G probe, the 590-A probe, and the 5'SH control probe were (d) 24nA, (e) 49nA, (f) 20nA, and (g) 34nA, (h) 37nA, (i) 17nA, respectively. A signal-to-noise