Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 299 (2002) 25–28
BBRC
www.academicpress.com

Linkage between the *CYP2C8* and *CYP2C9* genetic polymorphisms

Umit Yasar,[a] Stefan Lundgren,[a] Erik Eliasson,[a] Anna Bennet,[b] Björn Wiman,[c] Ulf de Faire,[b] and Anders Rane[a,*]

[a] *Department of Medical Laboratory Sciences and Technology, Division of Clinical Pharmacology, Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden*
[b] *Cardiovascular Laboratory, Department of Cardiology, Karolinska Hospital, Stockholm, Sweden and Department of Environmental Medicine, Division of Cardiovascular Epidemiology, Karolinska Institute, Stockholm, Sweden*
[c] *Department of Surgical Sciences, Division of Blood Coagulation Research, Karolinska Hospital, Stockholm, Sweden*

Received 4 October 2002

Abstract

Cytochrome P450 (CYP) 2C8 and 2C9 are polymorphic enzymes. The *CYP2C8*3* and *CYP2C9*2* are the major variant alleles in Caucasian populations. The enzymes encoded by these variant alleles have impaired function for the metabolism of several drug substrates. In the present study 1468 subjects that were used as population-based controls in the Stockholm Heart Epidemiology Program (SHEEP) were genotyped by allelic discrimination using a 5'-nuclease assay for *CYP2C8*1*, *2C8*3*, *2C9*1*, *2C9*2*, and *2C9*3* variant alleles in which the frequencies appeared to be 0.91, 0.095, 0.83, 0.11, and 0.066, respectively. Approximately, 96% of the subjects with *CYP2C8*3* allele also carried a *CYP2C9*2* and 85% of the subjects that had *CYP2C9*2* variant also carried a *CYP2C8*3*. The number of subjects carrying both of the *CYP2C8*1*3* and *CYP2C9*1*2* was 4.5-fold higher than expected. This strong association may be of importance especially for the metabolism of common substrates of CYP2C8 and CYP2C9 like arachidonic acid that produces physiologically active metabolites.

Keywords: CYP2C8; CYP2C9; Genetic linkage; Polymorphism

Human cytochrome P450 enzymes belonging to the CYP2C subfamily are responsible for the metabolism of about 20% of clinically used drugs, as well as some endogenous substances such as arachidonic acid. Genetic polymorphisms have been identified for all members of this subfamily, i.e., *CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19* [1].

Among the six reported variants of *CYP2C9* (**1–*6*) the major variant allele *CYP2C9*2* was found in 11–13% of Caucasians, 1–4% of Africans, and 0% of Orientals [1]. The *CYP2C9*2* and *CYP2C9*3* variants have been associated with impaired metabolism of many specific drug substrates like *S*-warfarin, phenytoin, losartan, and several non-steroidal anti-inflammatory drugs [2–4]. The CYP2C9 polymorphism has clinical implications for several important drugs with a narrow therapeutic window, such as *S*-warfarin, phenytoin, glipizide, and glibenclamide [1,5,6], where patients with a slow metabolism are at increased risk of adverse drug reactions.

Three different allelic variants of *CYP2C8* were recently reported [7]. The *CYP2C8*3* variant, with an allele frequency of 13% in Caucasians and 2% in Afro-Americans, was associated with markedly defective metabolism of paclitaxel and arachidonic acid, corresponding to only 15% and 35% of the *CYP2C8*1* activity, respectively [7]. We here report that the *CYP2C8*3* allelic variant appears to be linked to the *CYP2C9*2* allele.

Materials and methods

DNA samples from 1468 subjects (974 male and 494 female) were included in this study. This material constitutes the control group of participants in the Stockholm Heart Epidemiology Program (SHEEP),

* Corresponding author. Fax: +46-8-585-81070.
E-mail address: Anders.Rane@labtek.ki.se (A. Rane).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0006-291X(02)02592-5

Table 1
The primers and specific (bold capital letters) MGB-TaqMan probes for the discrimination of the **1* (labelled with VIC and a Dark quencher) and **3* (labelled with FAM and a Dark quencher) alleles

	*CYP2C8*1* and **3*	*CYP2C9*1* and **3*
Forward primer	atgtccactacttctcctcact tctg	caggaagagattgaacgt gtgatt
Reverse primer	aaagtggccagggtcaaaga	ctatgaatttggggacttc gaaa
Probe for discrimination of **1*	atgatgaca**A**agaattt	agagatac**A**ttgaccttc
Probe for discrimination of **3*	tgatgaca**G**agaattt	agatac**C**ttgaccttct

The discrimination of *CYP2C9*1* and *CYP2C9*2* was performed with pre-developed TaqMan assay reagents (PE Biosystems, Warrington, UK).

a population based case-control study aimed to investigate the effects of different risk factors for myocardial infarction in men and women. More detailed demographic characteristics of these control subjects (i.e., without myocardial infarction) were reported elsewhere [8,9]. The subjects were aged 45–70 and were selected from the Stockholm County population registry. All subjects were of Swedish descent. The DNA samples were prepared using the RapidPrep Macro Genomic DNA isolation kit (Pharmacia biotech, Sweden). The samples were genotyped to identify the *CYP2C8*1*, *CYP2C8*3*, *CYP2C9*1*, *CYP2C9*2*, and *CYP2C9*3* variants by allele discrimination using a 5'-nuclease assay [10]. The primers (CyberGene AB, Novum Research Park, Stockholm, Sweden) and specific MGB-TaqMan probes (Applied Biosystems, Cheshire, UK) are shown in Table 1. The reaction contained 450 nM of forward and reverse primers and 50 nM of each probe using standard Taqman reagents (Applied Biosystems, New Jersey, USA) in a volume of 10 μl. For the discrimination of *CYP2C9*1* and *CYP2C9*2*, pre-developed TaqMan assay reagents (PE Biosystems, Warrington, UK) were used at the concentrations recommended by the manufacturer in a total volume of 10 μl. After 10 min of denaturation at 95 °C, the samples were subjected to 40 cycles including 95 °C for 15 s followed by 60 °C for 60 s. Six DNA samples with different genotypes of *CYP2C8* and *CYP2C9* were sequenced by using an ABI Prism BigDye terminator kit (Applied Biosystems, Foster City, CA) on an ABI Prism 377 DNA sequencer in order to confirm the identity of the *CYP2C8*- or *CYP2C9*-specific amplification.

Results and discussion

The allele frequencies of *CYP2C8*1*, **3*, *CYP2C9*1*, **2*, and **3* variants in the study population ($n = 1468$) were 0.91, 0.095, 0.83, 0.11, and 0.066, respectively. This is in agreement with previously reported frequencies in Swedish subjects and other Caucasian populations [11]. The frequencies of the corresponding genotypes are given in Table 2. These frequencies were used to calculate expected number of subjects with different *CYP2C8*/*CYP2C9* genotypes (Table 2, within parentheses). Importantly, there was a clear discrepancy between observed and expected number of subjects with different genotypes. For example, the expected number of subjects with the most rare *CYP2C8*3*3*/*CYP2C9*2*2* genotype was less than one, but in fact 10 such subjects

Table 2
Observed (bold) and expected (within parenthesis) number of subjects with different genotype combinations of the *CYP2C8* and *CYP2C9* genes. *f* denotes frequency and *n* number of individuals with a specific *CYP2C8* or *CYP2C9* genotype in the study material

CYP	***2C9*1*1***	***2C9*1*2***	***2C9*2*2***	***2C9*2*3***	***2C9*1*3***	***2C9*3*3***	*n*	*f*
2C8*1*1	**991** (807)	**40** (211)	**1** (14)	**4** (18)	**160** (132)	**4** (3)	1200	*0.817*
2C8*1*3	**9** (185)	**222** (49)	**6** (3)	**18** (4)	**3** (30)	**0** (1)	258	*0.176*
2C8*3*3	**0** (8)	**0** (2)	**10** (0)	**0** (0)	**0** (1)	**0** (0)	10	*0.0068*
n	1000	262	17	22	163	4	1468	
f	*0.681*	*0.179*	*0.0116*	*0.015*	*0.111*	*0.0027*		*1.000*

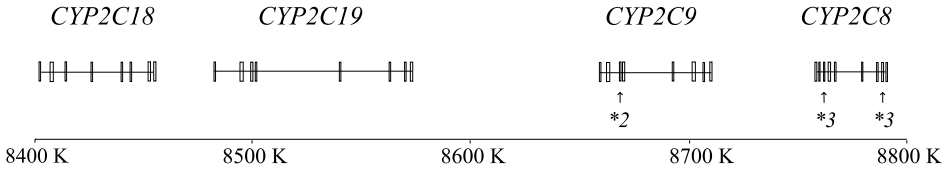

Fig. 1. Map of CYP2C cluster on the chromosome 10q24 based on the human genome map in www.ncbi.nlm.nih.gov. Arrows show the SNP(s) for the specified variant alleles.

were found in the study population (Table 2). Moreover, the number of subjects heterozygous in both genes (*CYP2C8*1*3* and *CYP2C9*1*2*) was approximately 4.5-fold higher than expected. As much as 96% of the subjects carrying a *CYP2C8*3* allele also carried a *CYP2C9*2* allele, and approximately 85% of subjects carrying a *CYP2C9*2* allele also carried a *CYP2C8*3* allele. This indicates a strong, but not complete, linkage between these two allelic variants.

Unpublished data from genotyping of Swedish subjects in previous studies [11] showed no association between *CYP2C19*2*, **3* alleles and *CYP2C9*2*, **3* variants. The samples in the present study were not analysed for the *CYP2C9*4*, *CYP2C9*5*, *CYP2C9*6*, and *CYP2C8*2* alleles because these variants have not been detected in Caucasians previously [12–14].

This is the first report showing a SNP linkage between the *CYP2C8* and *CYP2C9* genes. The *CYP2C* subfamily members are located on the 10th chromosome as a cluster that spans approximately 400 kb on the proximal part of 10q24 (Fig. 1). The *CYP2C8*3* includes two SNPs in exon 3 (G416A) and exon 8 (A1996G); coding for $Arg^{139}Lys$ and $Lys^{399}Arg$, respectively. The *CYP2C9*2* includes a SNP in exon 3 (C416T) coding for $Arg^{144}Cys$. The close distance between the localisation of these two genes favours a possible genetic linkage. A similar linkage has recently been reported between the *CYP2C18* and *CYP2C19* mutations [15,16].

This association may be potentially important especially for substrates that are common to these two enzymes such as arachidonic acid [7,17]. Studies on the physiological and pathological importance of this linkage in the synthesis of endogenous arachidonic acid metabolites like endothelium-derived hyperpolarising factor are warranted. Finally, it is tempting to speculate that for some substrates that are metabolised by both CYP2C8 and CYP2C9, an impaired clearance in vivo previously exclusively attributed to the *CYP2C9*2* variant could in part be explained by slow metabolism of the same substrate by the associated *CYP2C8*3* variant.

Acknowledgments

The project has been supported by grants from the Swedish Science Research Council (MRC 04496), the Swedish Medical Research Council (05193 and 09533), and the Swedish Lung and Heart Foundation.

References

[1] J.A. Goldstein, Clinical relevance of genetic polymorphisms in the human CYP2C subfamily, Br. J. Clin. Pharmacol. 52 (2001) 349–355.
[2] K. Takanashi, H. Tainaka, K. Kobayashi, T. Yasumori, M. Hosakawa, K. Chiba, CYP2C9 Ile359 and Leu359 variants: enzyme kinetic study with seven substrates, Pharmacogenetics 10 (2000) 95–104.
[3] U. Yasar, G. Tybring, M. Hidestrand, M. Oscarson, M. Ingelman-Sundberg, M.L. Dahl, E. Eliasson, Role of CYP2C9 polymorphism in losartan oxidation, Drug Metab. Dispos. 29 (2001) 1051–1056.
[4] J.O. Miners, D.J. Birkett, Cytochrome P4502C9: an enzyme of major importance in human drug metabolism, Br. J. Clin. Pharmacol. 45 (1998) 525–538.
[5] J. Kirchheiner, J. Brockmoller, I. Meineke, S. Bauer, W. Rohde, C. Meisel, I. Roots, Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers, Clin. Pharmacol. Ther. 71 (2002) 286–296.
[6] G.P. Aithal, C.P. Day, P.J. Kesteven, A.K. Daly, Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications, Lancet 353 (1999) 717–719.
[7] D. Dai, D.C. Zeldin, J.A. Blaisdell, B. Chanas, S.J. Coulter, B.I. Ghanayem, J.A. Goldstein, Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid, Pharmacogenetics 11 (2001) 597–607.
[8] K. Leander, J. Hallqvist, C. Reuterwall, A. Ahlbom, U. de Faire, Family history of coronary heart disease, a strong risk factor for myocardial infarction interacting with other cardiovascular risk factors: results from the Stockholm Heart Epidemiology Program (SHEEP), Epidemiology 12 (2001) 215–221.
[9] C. Reuterwall, J. Hallqvist, A. Ahlbom, U. De Faire, F. Diderichsen, C. Hogstedt, G. Pershagen, T. Theorell, B. Wiman, A. Wolk, Higher relative, but lower absolute risks of myocardial infarction in women than in men: analysis of some major risk factors in the SHEEP study, The SHEEP Study Group, J. Intern. Med. 246 (1999) 161–174.
[10] K.J. Livak, Allelic discrimination using fluorogenic probes and the 5′ nuclease assay, Genet. Anal. 14 (1999) 143–149.
[11] Ü. Yasar, E. Eliasson, M.L. Dahl, I. Johansson, M. Ingelman-Sundberg, F. Sjöqvist, Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a Swedish population [published erratum appears in Biochem. Biophys. Res. Commun. 1999 Apr;258:227], Biochem. Biophys. Res. Commun. 254 (1999) 628–631.
[12] U. Yasar, E. Aklillu, R. Canaparo, M. Sandberg, J. Sayi, H.-K. Roh, A. Wennerholm, Analysis of *CYP2C9*5* in Caucasian, Oriental and Black-African populations, Eur. J. Clin. Pharmacol. (2002), in press.
[13] R.S. Kidd, T.B. Curry, S. Gallagher, T. Edeki, J. Blaisdell, J.A. Goldstein, Identification of a null allele of CYP2C9 in an

African–American exhibiting toxicity to phenytoin, Pharmacogenetics 11 (2001) 803–808.

[14] A. Gaedigk, W.L. Casley, R.F. Tyndale, E.M. Sellers, M. Jurima-Romet, J.S. Leeder, Cytochrome P4502C9 (CYP2C9) allele frequencies in Canadian Native Indian and Inuit populations, Can. J. Physiol. Pharmacol. 79 (2001) 841–847.

[15] K. Inoue, H. Yamazaki, T. Shimada, Linkage between the distribution of mutations in the CYP2C18 and CYP2C19 genes in the Japanese and Caucasian, Xenobiotica 28 (1998) 403–411.

[16] K. Mamiya, I. Ieiri, S. Miyahara, J. Imai, H. Furuumi, Y. Fukumaki, H. Ninomiya, N. Tashiro, H. Yamada, S. Higuchi, Association of polymorphisms in the cytochrome P450 (CYP) 2C19 and 2C18 genes in Japanese epileptic patients, Pharmacogenetics 8 (1998) 87–90.

[17] I. Fleming, U.R. Michaelis, D. Bredenkotter, B. Fisslthaler, F. Dehghani, R.P. Brandes, R. Busse, Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries, Circ. Res. 88 (2001) 44–51.