MORPHOLOGY AND PATHOMORPHOLOGY

Cytochrome P450 2D6 Polymorphism Is a Molecular Genetic Marker of Liver Cirrhosis Progression

D. L. Nepomnyashchikh, V. A. Vavilin*, S. V. Aidagulova, S. I. Makarova*, Yu. Yu. Karavaeva, E. G. Shchepotina*, O. A. Postnikova, Zh. V. Nokhrina, E. V. Vinogradova, and V. V. Lyakhovich*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 11, pp. 568-571, November, 2011
Original article submitted September 2, 2010

Patients with infectious viral or toxic cirrhosis of the liver participated in complex clinical pathomorphological and molecular-genetic study aimed at the search for markers of predisposition to accelerated liver fibrosis, in which the xenobiotic biotransformation system is involved. The results demonstrate association between *CYP2D6 (1846G/A)* genotype and rapid cirrhosis development and indicate the necessity of studying the mechanisms underlying this association.

Key Words: *liver cirrhosis; cytochrome P450 2D6; polymorphism*

Investigations of liver cirrhosis are mainly focused on fibrogenic transformation of stellate cells [11]. However, our data on hepatotoxic effect of xenobiotics and fibrogenesis acceleration under the influence of toxic agents [3] indicate that studies of the xenobiotic transformation system including cytochrome P450 superfamily (CYP450) are promising in this respect. One of them, *CYP2D6*, constituting 2-4% of total cytochrome P450 content in the liver, is involved in the metabolism of 25% of used drugs, substantial amount of xenobiotics, and a number of endogenous physiologically active compounds [9].

Association of cytochrome P4501A1, glutathione-S-transferase M1, T1, and P1, N-acethyltransferase 2 gene polymorphisms with clinical features and predisposition to a number of chronic multifactor diseases was demonstrated [1], as well as association of glutathione-S-transferase polymorphism with peculiarities of the clinical course of acute drug poisoning in children [2]. This led to understanding of the crucial role of the xenobiotic metabolism system in predisposition and realization of the initial stages of multifactor diseases [4].

The maintenance of the balance between pro- and antifibrogenic stimuli becomes impossible because of functional incompetence and death of a part of hepatocytes caused by persistence of hepatitis C and hepatitis B viruses (HCV and HBV), particularly in combination with long-term exposure to toxic agents, which leads to activation of stellate cells and eventually results in liver fibrosis and cirrhosis. The association between cytochrome P450 gene polymorphism and fibrosis rate is analysed in only few studies [8].

Here we studied association of single nucleotide substitution 1846*G>A* in *CYP2D6* gene forming *CY-*

Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; * Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** pathol@soramn.ru. D. L. Nepomnyashchikh

0007-4888/12/1525-0633

*P2D6*4* allele cluster responsible for approximately 80% all phenotypes of slow debrisoquine hydroxylation in Caucasians with the rate of viral or toxic liver cirrhosis development.

MATERIALS AND METHODS

Samples of peripheral blood for cDNA extraction and CYP2D6 genetic polymorphism investigation were taken from 40 liver cirrhosis patients (19 males and 21 females at the age of 38-71 years). Informed consent was obtained from all patients.

Blood test revealed serological markers of HCV and/or HBV in 22 patients: HCV infection was detected in 12 patients, in 6 of them it was accompanied by alcoholic decease; HBV infection was detected in 6 patients, in 5 of them it was accompanied by alcoholic decease; HCV+HBV mixed infection was detected in 4 patients, 1 of them had alcoholic anamnesis. Among remaining 18 liver cirrhosis patients, alcoholic decease was diagnosed in 11 patients, 5 patients had primary and 1 had secondary biliary cirrhosis, and 1 female patient had cryptogenic cirrhosis. The duration of liver cirrhosis was established from clinical and anamnesis data.

HCV and HBV infection was verified by the spectrum of serological markers and PCR, including determination of replication level and HCV genotype. Duration of infection was established from the first detection of HCV or HBV infection markers.

Control group comprised Western Siberia residents, Caucasians, without clinical evidences of any disease (40 males and 110 females at the age of 23-76 years); blood samples from these individuals were obtained within the framework of investigation of the consequences of tests in Semipalatinsk nuclear test site.

PCR was carried out on a Corbett thermal cycler using oligonucleotide primers F: 5'-GCCTTCGCCAACCACTCCG-3' and R: 5'-AAATCCTGCT CTTCCGAGGC-3' [14]. A 355-b.p. product was obtained. The reaction was carried out in a total volume of 20 μl. The reaction mixture in PCR buffer contained 200 μM each deoxynucleoside triphosphate, 0.4 μM each primer, and 0.5 U Taq DNA-polymerase; the buffer contained 60 mM Tris-HCl (pH 8.5 at 25°C), 1.5 mM KCl, 10 mM 2-mercaptoethanol, 0.1% Triton X-100, and 1.5 mM $MgCl_2$. PCR (30 cycles) was performed according to the following protocol: first denaturation at 95°C for 5 min with subsequent 30-sec denaturation cycles at 94°C, primer annealing at 58°C for 15 sec and elongation at 72°C for 20 sec; to complete fragment synthesis, the samples were exposed to 72°C for 7 min in the last cycle. Hydrolysis of amplificated DNA fragment was performed by restriction endonuclease Bst2UI (SibEnzyme) for 3 h in optimal (selected by manufacturer) buffer at 60°C. Restriction products were separated using electrophoresis in 8% PAAG in Tris-borate buffer. Upon completion of electrophoresis, DNA bands were detected by ethidium bromide staining (5 μg/ml), visualized in UV light, and compared with DNA markers with known length. Odds ratio (OR) and significance of differences were estimated using exact Fisher test and EpiInfo 6 software.

RESULTS

In randomized sample of Caucasian people from Western Siberia, the incidence of *CYP2D6*4* allele was 20.3%, which did not significantly differ from the values observed in Caucasian populations [13]. The frequency of the wild-type homozygous genotype *CYP2D6 1846G/G*, heterozygous *CYP2D6 1846G/A*, and *CYP2D6 1846A/A* was 66, 30, and 5.3%, respectively, which corresponded to Hardy–Weinberg equilibrium.

In the total group of cirrhotic patients, the frequency of *CYP2D6 1846A* allele was similar to that in the control group (χ^2=0.0; p=0.966). However, accumulation of heterozygous genotypes *CYP2D6 1846G/A* was observed in the subgroup of mixed liver cirrhosis (HCV and/or HBV in combination with exposure to alcohol, Table 1). It should be noted that no accumulation of *CYP2D6 1846G/A* allele was detected in subgroups of patients with liver cirrhosis caused by only alcohol or only virus.

It is obvious that reduced activity of this cytochrome appreciably contributes to organ resistance to xenobiotic load and promotes fibrogenesis. Altered CYP2D6-mediated metabolism of endogenous substances with fibrogenous activity may also be of importance. Therefore we analyzed *CYP2D6* genotype distribution in clinical subgroups of cirrhotic patients with different disease etiology formed on the basis of presence or absence of a history of toxic alcohol exposure (Table 2). Proportion of the people with heterozygous *CYP2D6 (1846G/A)* genotype was significantly higher in groups of patients with toxic alcohol exposure.

It was previously demonstrated that liver fibrosis more rapidly develops in patients with viral hepatitis C slowly metabolizing debrisoquine [8]. Therefore detailed analysis in our clinical subgroups was of great importance (Table 3). Heterozygous *CYP2D6 1846G/A* genotype is associated with rapid liver cirrhosis development (p<0.1). Considering wide distribution of these polymorphism in Caucasian populations (19.4% according to [13]), it may be of great clinical importance.

Despite the association between *CYP2D6 1846G/A* and rapid liver cirrhosis, analysis of *CYP2D6 1846G>A* genotype distribution failed to reveal a correlation with disease severity (Table 4).

TABLE 1. Association of *CYP2D6 1846G/A* Genotype with Liver Cirrhosis Etiology

Liver cirrhosis etiology	Number of patients	Ratio of wild type homozygotes and heterozygotes	OR*
Viral (HCV and/or HBV) in association with alcoholic disease	14	7:7	3.33 (0.68-17.08) p=0.167
Alcoholic	11	7:4	1.27 (0.23-6.79) p=1.00
Viral (HCV and/or HBV)	10	9:1	0.17 (0.01-1.64) p=0.123
Primary biliary	4	3:1	0.67 (0.02-8.75) p=1.00
Secondary biliary	1	1:0	
Total	40	27:13	

Note. Here and in Tables 2-4: *odds ratio is calculated as ratio of heterozygous and wild type *CYP2D6* genotypes in the analyzed clinical subgroup to that in all other groups; *p*: significance of differences calculated using two-way Fisher exact test.

Obtained results are indicative for the association between *CYP2D6 1846G/A* genotype with rapid liver cirrhosis and point out to the necessity of studying the mechanisms underlying this association. Our previous studies showed that the duration of infection and the level of hepatitis C virus replication do not substantially affect fibrogenesis and liver cirrhosis development [5-7]. Thus, main role in fibrosis and liver cirrhosis progression is most likely played by host factors, including enzymes of xenobiotic biotransformation system.

TABLE 2. Distribution of *CYP2D6* Genotypes Depending on the Presence of Alcohol Exposure

Exposure to alcohol	Number of patients	Ratio of wild type homozygotes and heterozygotes	OR*
Presence	25	15:10	2.67 (0.50-15.76) p=0.298
Absence	15	12:3	0.38 (0.06-2.01) p=0.298
Total	40	27:13	

TABLE 3. Association of *CYP2D6 1846G/A* Genotype with Liver Cirrhosis Development Rate

Liver cirrhosis progression rate	Number of patients	Ratio of wild type homozygotes and heterozygotes	OR*
Fast	8	3:5	4.38 (0.67-31.41) p=0.099
Slow	22	16:6	0.43 (0.08-2.09) p=0.225
Moderate	7	5:2	0.69 (0.08-5.24) p=1.0
Total	37	24:13	

TABLE 4. Distribution of *CYP2D6* Genotypes According to Liver Cirrhosis Severity Assessed Using Child-Pugh Prognostic Scale

	Number of patients	Ratio of wild type homozygotes and heterozygotes	OR*
A	7	4:3	1.83 (0.25-13.22) p=0.656
A-B	5	3:2	1.53 (0.15-15.50) p=0.642
B	20	15:5	0.52 (0.10-2.57) p=0.457
B-C	4	3:1	0.70 (0.02-9.98) p=1.0
C	2	1:1	2.27 (0.03-185.4) p=0.538
Total	38	26:12	

The rate of fibrogenesis in the liver under experimental conditions can be substantially modified by exposure to different xenobiotics and by changing activity of ligand-activated PPARγ receptors (peroxisome proliferator-activated receptors), FXR (farnesoid X receptor), CB1 and CB2 (cannabinoid receptors 1 and 2) [12]. The role of xenobiotic biotransformation enzymes in the metabolism of endogenous and exogenous ligands of nuclear receptors is now intensively studied. CYP2D6 was shown to participate in the metabolism of endogenous cannabinoid anandamide (arachidonoyl ethanolamide) by catalyzing 5,6-epoxidation of its arachidonoyl residue with the formation of a potent selective cannabinoid receptor 2 agonist [15]; its activity is thought to be associated with antifibrogenic processes in the liver [10].

In accordance with the results obtained by us and S. Fishman *et al*. [8], more rapid lever fibrosis in chronic hepatitis C in slow debrisoquine metabolizers in comparison with fast metabolizers suggests that polymorphism of xenobiotic biotransformation enzymes involved in metabolism of endogenous and exogenous ligands of nuclear receptors may affect their activity and thereby fibrogenesis rate.

Generally, the obtained results demonstrate the important role of xenobiotic biotransformation system in fibrosis processes and create certain hopes in terms of prospect of therapeutic target for its treatment.

REFERENCES

1. V. A. Vavilin, S. I. Makarova, V. V. Lyakhovich, and S. M. Gavalov, *Genetika*, **38**, No. 4, 539-545 (2002).
2. V. A. Vavilin, O. G. Safronova, N. A. Manakin, *et al., Byull. Eksp. Biol. Med.*, **139**, No. 4, 424-427 (2005).
3. V. T. Ivashkin, D. L. Nepomnyashchikh, and S. V. Aidagulova, *Ros. Zhurn. Gastroenterol. Gepatol. Koloproktol.*, No. 2, 20-29 (2009).
4. V. V. Lyakhovich, V. A. Vavilin, S. I. Makarova, and A. Yu. Grishanova, *Inform. Vestn. VOGiS*, **10**, No. 3, 514-519 (2006).
5. G. I. Nepomnyashchikh, S. V. Aidagulova, and D. L. Nepomnyashchikh, *et al., Byull. Eksp. Biol. Med.*, **134**, No. 9, 356-360 (2002).
6. G. I. Nepomnyashchikh, N. P. Tolokonskaya, S. V. Aidagulova, *et al., Byull. Eksp. Biol. Med.*, **135**, No. 3, 343-348 (2003).
7. V. I. Pokrovskiy, G. I. Nepomnyashchikh, and N. P. Tolokonskaya, *Byull. Eksp. Biol. Med.*, **135**, No. 4, 364-376 (2003).
8. S. Fishman, Y. Lurie, H. Peretz, *et al., Liver International.*, **26**, 279-284 (2006).
9. F. P. Guengerich, *P450: Structure, Mechanism, and Biochemistry*, New York (2005), pp. 377-530.
10. B. Julien, P. Grenard, F. Teixeira-Clerc, *et al., Gastroenterology,* **128**, No. 3, 742-755 (2005).
11. M. Pinzani, K. Rombouts, and S. Colagrande, *J. Hepatol.*, **42**, Suppl. 1, S22-S36 (2005).
12. D. C. Rockey, *Clin. Liver Dis.*, **12**, No. 4, 939-962 (2008).
13. J. Sistonen, S. Fuselli, J. U. Palo, *et al., Pharmacogenet. Genomics*, **19**, No. 2, 170-179 (2009).
14. C. A. Smith, J. E. Moss, A. C. Gough, *et al., Environ. Health Perspect.*, **98**, 107-112 (1992).
15. N. T. Snider, V. J. Walker, and P. F. Hollenberg, *Pharmacol. Rev.*, **62**, No. 1, 136-154 (2010).