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CYP1A2 polymorphism in Chinese patients with acute liver injury induced by *Polygonum multiflorum*

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ABSTRACT. The objective of this study was to evaluate the genotype and allelic frequencies of *CYP1A2* in Chinese patients with acute liver injury induced by *Polygonum multiflorum*. We examined the clinical mechanism of acute liver injury induced by *P. multiflorum*. According to the diagnostic criteria for drug-induced liver injury (DILI), 43 cases of *P. multiflorum*-induced liver injury admitted to the First Affiliated Hospital, Zhejiang University were identified between January 2008 and December 2012. An additional 43 control subjects were also chosen. Several alleles, including *1C, *1F, *2, *7, *9, and *11 of *CYP1A2* were amplified from genomic DNA and sequenced. We used the chi-square test to determine whether *CYP1A2* allele polymorphisms are associated with acute liver injury induced by *P. multiflorum*. The frequency of the *CYP1A2* *1C allele was 46.5% in *P. multiflorum*-induced DILI patients, which was significantly different from the frequency of 27.9% observed in healthy subjects. The frequency of the *CYP1A2**1F allele was 63.9% in *P. multiflorum*-induced DILI patients, compared to 57.0% in healthy controls; the difference was not significant. The allelic frequencies of *CYP1A2**2, *CYP1A2**7, *CYP1A2**9, and *CYP1A2**11 were too low to be detected. The frequency of the *CYP1A2**1C mutation in Chinese

patients with *P. multiflorum*-induced acute liver injury differed from that in healthy Chinese people, indicating that *CYP1A2**1C is probably related to metabolism of *P. multiflorum*, which is followed by acute liver injury.

Key words: CYP1A2; Drug-induced liver injury; Gene polymorphism; *Polygonum multiflorum*

INTRODUCTION

Reports of adverse reactions induced by traditional Chinese medicines (TCM) have increased (Ko, 2004). *Polygonum multiflorum* is one form of TCM, and is sold as a food supplement to improve immune function and enhance hair growth through its antioxidant properties (Wong et al., 2006). However, adverse reactions induced by *P. multiflorum* are common, with patients developing drug-induced liver injury (DILI) and even liver failure (Mazzanti et al., 2004; Panis et al., 2005; Cárdenas et al., 2006; Jung et al., 2011). Although the mechanism is unknown, many studies have suggested that an idiosyncratic reaction occurs, which is related to genetic polymorphisms in various enzymes (Empey, 2010).

Genetic variation in cytochrome P450 (*CYP450*) is known to contribute to inter-individual variation in drug metabolism and response to medication (Polimanti et al., 2012). For example, *CYP450 1A2* gene polymorphisms are thought to be involved in the metabolism of theophylline; a study by Uslu et al. (2010) suggested that genetic alterations in *CYP1A2* play a role both in the pharmacogenetics of theophylline and in the development of chronic obstructive pulmonary disorder. Gervasini et al. (2012) found that the *CYP3A4**1B-*CYP3A5**1 haplotype may have a profound impact on tacrolimus pharmacokinetics, which may be related to the occurrence of toxicity or acute rejection in renal transplant recipients treated with tacrolimus.

Among CYP enzymes, CYP1A2 is prominently involved in the biotransformation of emodin, which is the putative component contributing to *P. multiflorum*-induced liver injury (Zhang et al., 2010). Some *CYP1A2* gene variants have been reported to be responsible for altered activity of the *CYP1A2* gene. While *CYP1A2**1F has been associated with increased activity, *CYP1A2**1C, *CYP1A2**1K, *CYP1A2**3, *CYP1A2**4, *CYP1A2**6, *CYP1A2**7, *CYP1A2**8, *CYP1A2**11, *CYP1A2**15, and *CYP1A2**16 variants have been associated with decreased *CYP1A2* gene activity (Soyama et al., 2005; Anonymous, 2013). However, no studies have examined *CYP1A2* gene polymorphisms in acute liver injury induced by *P. multiflorum*. In this study, we examined the clinical causes of acute liver injury induced by *P. multiflorum.*

MATERIAL AND METHODS

Subjects and case recruitment

Data from 355 DILI patients admitted to the First Affiliated Hospital, Zhejiang University between January 2008 and December 2012 were collected. Diagnoses were established using the Maria criteria scale, which is based on drug use history before the symptomatic period, physical examination upon admission, and the presence of acute hepatitis as ascertained

by laboratory investigations or histological findings. Experienced gastroenterologists in the field of toxic hepatitis indicated suspected DILI cases and excluded other possible causes of hepatitis such as hepatitis A, B, C, and E viruses, Epstein-Barr virus, cytomegalovirus, herpes simplex virus, autoimmune hepatitis, Wilson's disease, and alcohol. A total of 43 cases of *P. multiflorum* (herbs and pharmaceuticals)-induced liver injury were included in this study according to the diagnostic criteria for DILI. Another 43 healthy volunteers were chosen as controls. All participants provided written informed consent and the study protocol was approved by the ethical review committee.

CYP1A2 genotyping

For DNA extraction, genomic DNA was isolated from the peripheral blood of each subject using Ezup Column Blood Genomic DNA Isolation Kits (Shanghai, Hanbo Industry Co., Ltd.; Shanghai, China).

For polymerase chain reaction (PCR) amplification and DNA sequencing, the -3860 G>A (*CYP1A2**1C), -163 C>A (*CYP1A2**1F), *CYP1A2**2, *CYP1A2**7, *CYP1A2**9, and *CYP1A2**11 alleles were detected by reverse transcription (RT)-PCR amplification using the primer pairs shown in Table 1 followed by DNA sequencing. The Chromas software (version 2.3) was used to analyze single-nucleotide polymorphisms (SNPs) of *CYP1A2*.

Table 1. Location of the polymorphisms, primers and the length of PCR products.

Polymorphisms in *CYP1A2*	Primers	Length of PCR products (bp)
-3860 G>A *CYP1A2**1C	F: 5'-GCTACACATGATCGAGCTATAC-3' R: 5'-CAGGTCTCTTCACTGTAATGTTA-3'	598
-163 C>A *CYP1A2**1F	F: 5'-CCCAGAAGTGGAAACTGAGA-3' R: 5'-GGGTTGAGATGGAGACATTC-3'	242
63 C>G *CYP1A2**2	F: 5'-ATGAATGAATGAATGTCTC-3' R: 5'-CTCTGGTGGACTTTTCAG-3'	201
3533 G>A *CYP1A2**7	F: 5'-CCTCCTCAGCACAACAAG-3' R: 5'-CTAGCAGGGACAAACAGC-3'	166
248 C>T *CYP1A2**9	F: 5'-GTATTCTGGGTGCTCAAGG-3' R: 5'-GGTGGAGGTGTAGAGGTCA-3'	276
558 C>A *CYP1A2**11	F: 5'-ACCTTCTCCATCGCCTCT3' R: 5'-AGCATCTCATCGCTACTCT-3'	215

Statistical analysis

Genotype and allelic frequencies of polymorphisms at each site were determined. The chi-square test was used to assess differences in the distribution of polymorphisms at each site between patients and controls.

RESULTS

Clinical characteristics of the cases

Forty-three cases of *P. multiflorum*-induced liver injury were recruited, accounting for 12.1% of 355 cases of DILI induced by TCM. These cases were also evaluated using the Maria

criteria scale. The following results were obtained: scores in 8 cases were >17, 26 cases were 14-17, and 9 cases were 10-13. Forty-one cases of patients were recovered after treatment, except for 2 cases, which were automatically discharged (Table 2).

Table 2. Characteristics of patients included in the study.

	Male/female	Age (years)	Length of hospitalization (days)	Maria scale (degree)
Polygonum multiflorum-induced DILI	20/23	46.1 ± 10.2	21.7 ± 10.7	>17:8
14-17:26				
10-13:9				
Control	21/22	48.2 ± 10.5	ND	ND

CYP1A2 genotyping

CYP1A2 alleles were detected by RT-PCR and confirmed by gel electrophoresis (Figure 1). The Chromas software was used to analyze SNPs of *CYP1A2* (a representative result for *CYP1A2**1C is shown in Figure 2).

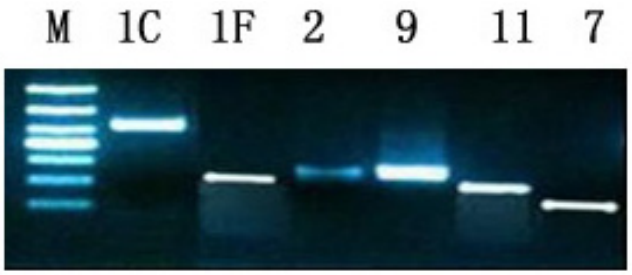

Figure 1. Gel electrophoresis results for *CYP1A2* allele.

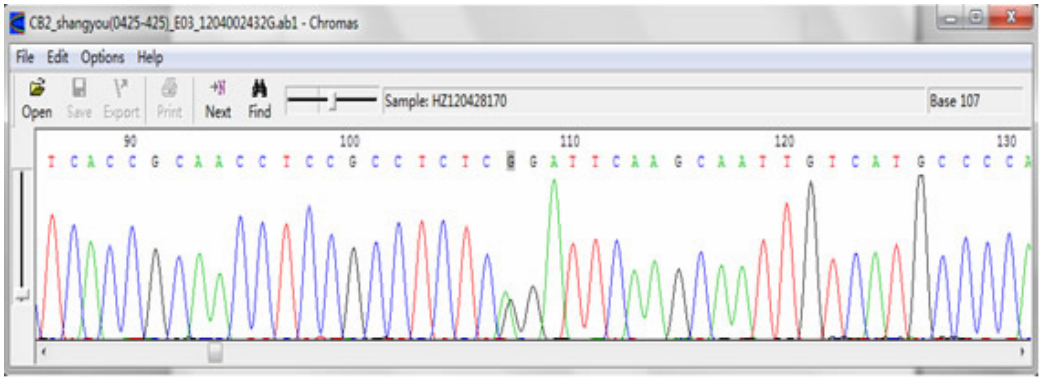

Figure 2. Gene sequence diagram of -3860 G>A *CYP1A2**1C (G/A).

Distribution of each genotype and allele frequency differences in DILI patients and controls

The distribution of genotype and allelic frequencies in DILI patients and controls are summarized in Table 3. The results revealed an association between the G>A allele at position -3860 in *P. multiflorum*-induced DILI patients (46.5 *vs* 27.9%). The frequency of the *CYP1A2**1F allele was 63.9% in *P. multiflorum*-induced DILI patients, compared to 57.0% in healthy controls; this difference was not statistically significant. The allelic frequencies of *CYP1A2**2, *CYP1A2**7, *CYP1A2**9, and *CYP1A2**11 were too low to be detected.

Table 3. Distribution of each genotype and allele in *Polygonum multiflorum* induced DILI patients and healthy controls.

Polymorphism (N = 43)	Genotypes (%)			Allele frequency
*CYP1A2**1C[a]	G/G	G/A	A/A	
DILI patients	11 (25.6%)	24 (55.8%)	8 (18.6%)	A = 46.5%
Healthy controls	23 (51.2%)	16 (37.2%)	4 (9.3%)	A = 27.9%
*CYP1A2**1F	C/C	C/A	A/A	
DILI patients	11.6%	48.8%	39.5%	A = 63.9%
Healthy controls	20.9%	44.2%	34.9%	A = 57.0%
*CYP1A2**2	C/C	C/G	G/G	
DILI patients	100%	0%	0%	G = 0%
Healthy controls	100%	0%	0%	G = 0%
*CYP1A2**7	G/G	G/A	A/A	
DILI patients	100%	0%	0%	A = 0%
Healthy controls	100%	0%	0%	A = 0%
*CYP1A2**9	C/C	C/T	T/T	
DILI patients	100%	0%	0%	T = 0%
Healthy controls	100%	0%	0%	T = 0%
*CYP1A2**11	C/C	C/A	A/A	
DILI patients	100%	0%	0%	A = 0%
Healthy controls	100%	0%	0%	A = 0%

DILI = drug-induced liver injury. [a]$P < 0.05$ (Chi-square test).

DISCUSSION

Complementary medicines including herbal preparations and nutritional supplements are widely used without prescriptions. Thus, there has been an increasing incidence of hepatotoxicity with the use of these agents (Ko, 2004).

Idiosyncratic liver toxicity caused by any drug is typically referred to as DILI. Most DILI involves reactions that appear to be unrelated to drug dose or concentration (Lucena et al., 2011). Although relatively rare, DILI is a serious clinical problem, with up to 10% of cases showing simultaneous severe elevations in alanine transaminase and bilirubin and development of liver failure. Metabolic idiosyncrasy is one of the main mechanisms of DILI, which is related to gene polymorphisms in metabolic enzymes. CYP450s are the main phase I drug metabolizing enzymes; genetic polymorphisms in CYP450 genes may mediate some cases of DILI (Deng et al., 2012).

SNPs have been identified that may result in variant CYP450 enzyme expression and/or activity (McGraw and Waller, 2012). In this study, we chose representative alleles of *CYP1A2*, among which *CYP1A2**1F is reported to be associated with increased activity,

while the *CYP1A2**1C, *CYP1A2**2, *CYP1A2**7, and *CYP1A2**11 variants are associated with decreased activity of the *CYP1A2* gene (Zhou et al., 2009). The allelic frequency in the control group (ethnic Chinese) was similar to that reported in a study of ethnic Japanese subjects (Soyama et al., 2005). *CYP1A2**1C and *CYP1A2**1F showed allelic frequencies of 27.9 and 57.0%, respectively (N = 43) in our study, compared to 23.6 and 62.8%, respectively, in the Japanese population. We did not detect mutations in *CYP1A2**2, *CYP1A2**7, *CYP1A2**9, and *CYP1A2**11 because these allelic frequencies were too low. These alleles are present at frequencies of 0.2-0.4% in the Japanese population (Soyama et al., 2005). Based on our results, there was no significant difference in the allelic frequencies of *CYP1A2**1F, *CYP1A2**2, *CYP1A2**7, and *CYP1A2**11. However, the *CYP1A2**1C frequency in *P. multiflorum*-induced DILI patients was 46.5%, which was significantly higher compared to the frequency of 27.9% in healthy controls. Thus, subjects with the *CYP1A2**1C mutation may have decreased activity of the CYP1A2 protein, thereby inhibiting the metabolism of *P. multiflorum* and causing accumulation of toxic substances.

In conclusion, mutations in *CYP1A2**1C may cause *P. multiflorum*-induced liver injury. The incidence of *P. multiflorum*-induced liver injury is low, and this study included relatively few patients. Thus, further study into the mechanism of *P. multiflorum*-induced liver injury is necessary.

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