ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Nov; 21 (11): 1031 - 1034
http://www.chinaphar.com

G-2964A and C734A genetic polymorphisms of *CYP1A2* in Chinese population[1]

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KEY WORDS cytochrome P-450 CYP1A2; polymerase chain reaction; genotype; restriction fragment length polymorphism; Chinese

ABSTRACT

AIM: To observe the G-2964A and C734A genetic polymorphisms of human *CYP1A2* in Chinese population. **METHODS**: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was employed in genotyping analysis. **RESULTS**: The allele frequencies of A-2964 were 0.25 and 0.22 in Qidong and Changsha populations, respectively. The incidences of A734 were 0.68 in Qidong population and 0.66 in Changsha population. No more than two low-inducibility/activity alleles were presented in one person. **CONCLUSION**: The distribution of the G-2964A and C734A genetic polymorphisms did not show significant difference between Chinese and Japanese populations. The incidence of C734A in Chinese was also similar to that in Caucasian population.

INTRODUCTION

Cytochrome P-450 1A2 (CYP1A2) plays an important role in the metabolism of many drugs and procarcinogens, such as phenacetin[1], acetaminophen[2], 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine (PhIP), 2-amino-3, 8-dimethylimidazo [4, 5-f] quinoxaline (MeIQx)[3] and aflatoxinB1[4]. Thus, it is involved in some drug interactions and the development of several cancers like bladder and colon cancer. Phenotyping analysis with caffeine metabolite ratio shows large individual difference and polymorphism of the enzyme activity[5,6]. Nucleotide sequence analysis further confirms the genetic polymorphisms of *CYP1A2*[7-9]. Nakajima reported that a point mutation from G (wild type) to A (mutated type) at position - 2964 in the 5′-flanking region of *CYP1A2* gave rise to a *Dde*I cleavage site. The homozygous wild (G/G type), the homozygous mutant (A/A type), and the heterozygous mutant (G/A type) were tentatively termed as the w/w, m/m, and w/m type. Allele frequencies in Japanese subjects were 0.77 and 0.23 for the wild G and mutated A types of alleles, respectively. Sachse found that the polymorphism in the intron1 of *CYP1A2*, namely a C to A transversion at position 734, could be identified by PCR-RFLP with *Bsp*120I. The homozygous C/C type, the homozygous A/A type and the heterozygous C/A type were termed as the w/w, m/m and w/m type. Allele frequencies in Germans were 0.68 and 0.32 for A and C at position 734, respectively. Either of the 5′-flanking region and the intron I polymorphisms appears to be linked with the inducible expression of *CYP1A2* in smokers, but not in non-smokers with the index of urinary caffeine metabolite ratio[7,8]. In this work, we studied the G-2964A and C734A genetic polymorphisms in two Chinese populations from different locations.

MATERIALS AND METHODS

Subjects This study was approved by the Ethics Committee of Hunan Medical University. Two hundred and forty one unrelated Chinese healthy Han volunteers (163 living in Qidong, Jiangsu province; 78 in Changsha, Hunan province) aged 20 - 50 a were recruited for the study after giving their written informed consents. All healthy individuals were in good health based on their medical history, physical examination, and laboratory evaluation.

[1] Project supported by the National Natural Science Foundation of China, № F39330230 and by China Medical Board of New York grants 92-568 and 99 - 697.

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Received 2000-04-20 Accepted 2000-09-01

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Nov; 21 (11)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Chemicals All materials for genotyping analysis were bought from Sangon Co. (Shanghai, China). All other supplies were of AR grade unless otherwise indicated.

Genotyping analysis was measured by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) Fresh blood 5 mL was drawn in edetic acid tubes. The peripheral lymphocytes were isolated immediately and stored at −20 ℃ until analysis. Genomic DNA was extracted from peripheral lymphocytes with phenol-chloroform followed by ethanol precipitation[10], and DNA samples were dissolved in Tris 10 mmol/L and edetic acid 1 mmol/L (pH 8.0) and stored at 4 ℃ until used. The PCRs of *CYP1A2* were performed as described previously[7,8] with slight modification to obtain more legible bands in electrophoresis. The 5′-flanking region of *CYP1A2* was amplified by using forward primer P1f (5′-GCTACACATGATCGAGCTATAC-3′) and reverse primer P2r (5′-CAGGTCTCTTCACTGTAAAGTTA-3′). A 25 μL PCR mixture comprised 10 × PCR buffer (Mg^{2+}), 0.4 μmol/L of each primer, 200 μmol/L dNTPs, 1.5U *Taq* polymerase and 1 μg of genomic DNA. PCR was performed with an initial denaturation for 2 min at 94 ℃ followed by 30 cycles of 1.5 min at 94 ℃, 2 min at 56 ℃, 2 min at 72 ℃ and a terminal extension for 7 min at 72 ℃. The intron 1 of *CYP1A2* was amplified by using P3f (5′-CAACCCTGCCAATCTCAAGCAC-3′) and P4r (5′-AGAAGCTCTGTGGCCGAGAAGG-3′). Genomic DNA samples 1 μg was added to a 25 μL PCR mixture consisting of 10 × PCR buffer (Mg^{2+}), 0.2 μmol/L of each primer and dNTPs 200 μmol/L. After an initial denaturation for 5 min, 1.5 U *Taq* polymerase was added and 39 cycles of amplification were performed under the following conditions: denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 15 s, and extension at 72 ℃ for 1 min. Then the amplified DNA fragments including the polymorphic sites in the 5′-flanking region or the intron 1 were separately digested with *Dde*I and *Bsp*120I at 37 ℃ for 24 h, and subjected to electrophoresis in a 2 % agarose gel and visualized by ethidium bromide staining.

Data analysis The incidences of alleles in different populations were compared using Chi-square test in Excel (Microsoft). A *P* value less than 0.05 was considered statistically significant.

RESULTS

G-2964A and C734A polymorphisms The point mutations of G-2964A in the 5′-flanking region and C734A in the intron 1 of *CYP1A2* existed in both Chinese populations. Fig 1 showed that G-2964A point mutation gave rise to a *Dde*I cleavage site while the C734A point mutation resulted in the loss of a *Bsp*120I cleavage site. The amplified 596 bp DNA fragments from the G/G type gene remained uncleaved by *Dde*I, whereas those from the type A/A were cut into two fragments of 464 and 132 bp. On *Bsp*120I digestion, the fragments from the intron 1 of the A/A type gene gave only a single undigested band of 920 bp; the fragments from the type C/C gave two bands of 709 and 211 bp; the type C/A gave three bands of 920, 709 and 211 bp.

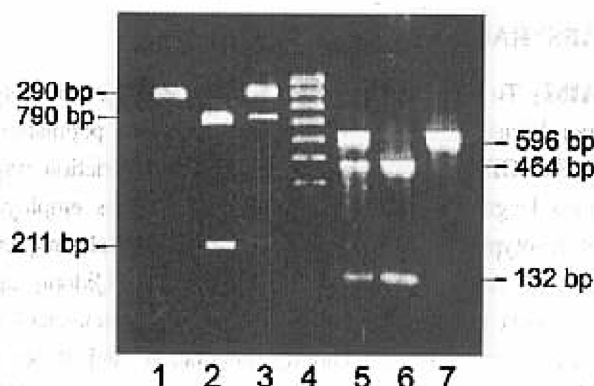

Fig 1. Restriction fragment length polymorphism (RFLP) analysis of *CYP1A2* genotypes. Lane 1, genotype with A/A at 734; lane 2, genotype with C/C at 734; lane 3, genotype with C/A at 734; lane 4, DNA marker; lane 5, genotype with G/A at −2964; lane 6, genotype with A/A at −2964; lane 7, genotype with G/G at −2964. Lane 1, 2, and 3, *Bsp*120I-digested intron 1 products of PCR amplifications; lane 5, 6, and 7, *Dde*I-digested 5′-flanking region products of PCR amplifications.

Allele frequencies The allele frequencies in different populations were similar with respect to either genetic polymorphism (Tab 1).

Tab 1. Allele frequencies for the G-2964A and C734A genetic polymorphisms of human *CYP1A2* in different populations.

Allele		Qidong	Changsha	Japan	Germany
G-2964A	G	0.75	0.78	0.77	–
	A	0.25	0.22	0.23	–
C734A	A	0.68	0.66	0.61	0.68
	C	0.32	0.34	0.39	0.32
Reference				[7,9]	[8]

Genotypes The distribution of the genotypes of either population was in Hardy-Weinberg equilibrium. The incidences of the genotypes did not have significant difference between the two populations (Tab 2, $P > 0.5$). We divided the subjects into nine genotypes according to different nucleotides at positions −2964 and 734: GGAA, GAAA, AAAA, GGCA, GACA, AACA, GGCC, GACC, and AACC. For example, the GGAA genotype had homozygous guanine at position −2964 and homozygous adenine at 734, and the person with both heterozygous mutants was regarded as GACA genotype. None of the 241 subjects under the genotyping test was identified as AACA, GACC, or AACC genotype.

Tab 2. Frequencies of different genotypes with respect to the G-2964A and C734A genetic polymorphisms of human *CYP1A2* in Qidong and Changsha populations.

Genotype C734A	Genotype G-2964A	Qidong (n)	Changsha (n)	χ^2, P
AA	GG	27 (16.6 %)	17 (21.8 %)	
	GA	30 (18.4 %)	13 (16.7 %)	
	AA	11 (6.7 %)	3 (3.85 %)	$\chi^2 = 3.97$
AC	GG	56 (34.4 %)	21 (26.9 %)	($P > 0.5$)
	GA	29 (17.8 %)	16 (20.5 %)	
	AA	–	–	
AA	GG	10 (6.13 %)	8 (10.3 %)	
	GA	–	–	
	AA	–	–	

DISCUSSION

Recently, some positive and negative regulatory cis-elements[11,12] were reported to exist in the human *CYP1A2* gene. And the liver-specific factors regulate the expression of *CYP1A2* through binding to these regions. It is likely that the G-2964A point mutation was to affect the affinities of the liver-specific factors and decrease the inducibility/activity of CYP1A2[7]. And the C734A point mutation might be in linkage with the G-2964A point mutation to affect the inducibility/activity of CYP1A2. Our work indicated that G-2964 and A734 together were associated with the high inducibility/activity of CYP1A2 (unpublished).

An interesting linkage was found between the two genetic polymorphisms in the 241 Chinese subjects studied for the first time. The person with homozygous A at position −2964 had homozygous A at position 734, and homozygous C at position 734 was accompanied by homozygous G at position −2964. No more than two low-inducibility/activity alleles presented in one person. The AACC, GACC or AACA genotype that was thought to have lower inducibility/activity of CYP1A2 than the other genotypes with respect to the two genetic polymorphisms did not exist in the studied subjects. However, whether this phenomenon occurs in other populations remains to be examined.

The incidence of homozygous A at −2964 was slightly higher in Qidong population than in Changsha population, and *vice versa* for homozygous C at 734. Although the allele frequencies did not have significant difference between the two Chinese populations, there seemed to be some linkage between the two mutations to avoid too low expression of *CYP1A2*, which might decrease the effect of the heredity on the enzyme activity.

Qidong is a special region where the annual incidence of hepatocelluar carcinoma is more than 52.18 per 100 000 persons[13]. We found that the activity of CYP1A2 in Qidong population (0.38 ± 0.21, $n = 142$) was significantly higher ($P < 0.01$) as compared to Changsha population (0.32 ± 0.20, $n = 229$) (unpublished). However, the frequencies of different alleles did not show statistical difference either between the two Chinese populations or between Oriental populations and Caucasian populations[7,8], which supported the idea that CYP1A2 was affected by environmental factors more than by heredity[14].

REFERENCES

1 Kobayashi K. Inhibitory effects of antiarrhythmic drugs on phenacetin O-deethylation catalysed by human CYP1A2. Br J Clin Pharmacol 1998; 45: 361–8.

2 Patten CJ, Thomas PE, Guy RL, Lee M, Gonzalez FJ, Guengerich FP, *et al*. Cytochrome P450 enzymes involved in acetaminophen activation by rat and human liver microsomes and their kinetics. Chem Res Toxicol 1993; 6: 511–8.

3 Boobis AR, Lynch AM, Murray S, de la Torre R, Solans A, Farre M, *et al*. CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. Cancer Res 1994; 54: 89–94.

4 Gallagher EP, Kunze KL, Stapleton PL, Eaton DL. The kinetics of aflatoxin B1 oxidation by human cDNA-expressed and human liver microsomal cytochromes P450 1A2 and 3A4. Toxicol Appl Pharmacol 1996; 141: 595–606.

5 Butler MA, Lang NP, Young JF, Caporaso NE, Vineis P, Hayes RB, *et al*. Determination of CYP1A2 and NAT2 phenotypes in human population by analysis of caffeine urinary metabolites. Pharmacogenetics 1992; 2: 116–127.

6 Ouyang DS, Huang SL, Wang W, Xie HG, Xu ZH, Zhou

HH. Phenotypic polymorphism and gender-related differences of CYP1A2 activity in a Chinese population. Br J Clin Pharmacol 1999; 47: in press.

7 Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T. Genetic polymorphism in the 5′-flanking region of human *CYP1A2* gene: effect on the CYP1A2 inducibility in humans. J Biochem 1999; 125: 803 – 808.

8 Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C→A polymorphism in intron 1 of the cytochrome P450 *CYP1A2* gene tested with caffeine. Br J Clin Pharmacol 1999; 47: 445 – 449.

9 Chida M, Yokoi T, Fukui T, Kinoshita M, Yokota J, Kamataki T. Detection of three genetic polymorphisms in the 5′-flanking region and intron 1 of human CYP1A2 in the Japanese population. Jpn J Cancer Res 1999, 90: 899 – 902.

10 Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1989.

11 Chung I, Bresnick E. Regulation of the constitutive expression of human CYP1A2 gene: Cis elements and their interactions with proteins. Mol Pharmacol 1992; 267: 677 – 685.

12 Quattrochi LC, Vu T, Tukey RH. The human CYP1A2 gene and induction by 3-methylcholanthrene — a region of DNA that supports Ah-receptor binding and promoter-specific induction. J Biol Chem 1994; 269: 6964 – 6954.

13 Zhu RY. Review of the epidemiology of hepatocelluar carcinoma. Research on hepatocelluar carcinoma in Qidong 1997; 12: 1 – 3.

14 Tantcheva-Poor I, Zaigler M, Rietbrock S, Fuhr U. Estimation of cytochrome P450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. Pharmacogenetics 1999; 9: 131 – 144.

中国人群中 *CYP1A2* 基因 G-2964A 和 C734A 多态性分布

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关键词 细胞色素 P-450 CYP1A2；聚合酶链反应；基因型；限制片长多态性；中国人

目的：研究中国人群中 *CYP1A2* 基因 G-2964A 和 C734A 多态性分布．**方法**：应用聚合酶链反应-限制片长多态性技术对 163 名启东人和 78 名长沙人进行基因型分析．**结果**：等位基因 A-2964 在启东和长沙人中的发生率分别是 0.25 和 0.22．A734 的发生率在启东人中为 0.68，而在长沙人中为 0.66．一个受试者的两条等位基因中至多含有两个底活性位点．**结论**：G-2964A 和 C734A 基因多态性在中国人和日本人中的分布没有显著性差异，C734A 基因多态性在中国人中的分布也类似于白种人．

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