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Effects of *CYP1A2*1C* and *CYP1A2*1F* Genotypes on the Activity and Inducibility of CYP1A2 Determined by Urinary Caffeine Metabolite Ratio in Koreans

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Abstract

The effects of common variants of CYP1A2 gene (CYP1A2*1C and CYP1A2*1F) on the CYP1A2 activity and inducibility were controversial. The aim of the present study is to investigate the effects of CYP1A2*1C and CYP1A2*1F on the activity of CYP1A2 determined by urinary caffeine metabolite ratio in Koreans. As might be expected, there was large inter-individual variation (16-folds) of CYP1A2 activity ranged from 2.41 to 39.58. The mean CYP1A2 activity of smokers was significantly higher than that of non-smokers. The frequencies of CYP1A2*1C (-3858A) and *1F (-164A) alleles were 0.219 and 0.646, respectively. The effect of CYP1A2*1C on the CYP1A2 activity was not significant. However, the CYP1A2 activity of subjects with AA genotype for CYP1A2*1F allele was significantly lower than that of non-AA genotypes (CC, or CA). Interestingly, the significant effect of CYP1A2*1F allele on CYP1A2 activity was not observed in nonsmokers. Our results suggest that CYP1A2*1F allele rather than CYP1A2*1C allele significantly influences on the inducibility of CYP1A2 in Koreans. Owing to small sample size of our study, further studies should be conducted to reveal the inter-ethnic difference or the gene-environmental interaction.

Keywords: CYP1A2, Genotype, Inducibility, Caffeine metabolism, Korean

The cytochrome P450 1A2 (CYP1A2) is one of the major drug-metabolizing enzymes constitutively expressed in the liver, and it is well documented that an individual's CYP1A2 metabolic activity could be assessed by caffeine phenotyping test. It has been previously revealed that the CYP1A2 activities determined by urinary caffeine metabolite ratio had wide inter-individual variations (14 folds) in Koreans[1]. In addition to our previous data, numerous evidences showed that the higher CYP1A2 activity in cigarette smoking population is resulted from the induction of CYP1A2 expression[2,3]. There is wide inter-individual variation of CYP1A2 activities not only in general population but also the sub-groups according to smoking status. Therefore, the inter-individual variations of CYP1A2 activities may be resulted from the differences in the contents of CYP1A2 enzyme, which could be affected by genetic susceptibility to the environmental factors, such as a well-known CYP1A2 inducer, cigarette smoking.

In the past few years, many of CYP1A2 gene mutations influencing on the CYP1A2 inducibility and/or activity have been reported[4-6]. Among these mutations, the effects of *CYP1A2*1C* (G-3858A, same as G-2964A) and *CYP1A2*1F* (C-164A, same as C734A) polymorphism in the 5′-flanking and intron 1 region respectively on the CYP1A2 inducibility/activity were controversial[7-9]. Recently, it was reported by Han *et al*. in Chinese, that *CYP1A2*1C* and *CYP1A2*1F* polymorphism was associated with the CYP1A2 induction by omeprazole or cigarette smoking[10]. However, some investigators have failed to detect the difference of steady state concentration using CYP1A2 substrates according to the *CYP1A2*1C* or *CYP1A2*1F* genotypes[11], nevertheless Sache *et al*. and Nakajima *et al*. had revealed the functional significance of the respective polymorphism in smokers[4,6,9].

The metabolic pathway of caffeine is very complicate and the metabolite ratio determining CYP1A2 activity is depended on the N3-demethylation pathway. The several phenotyping methods using well-

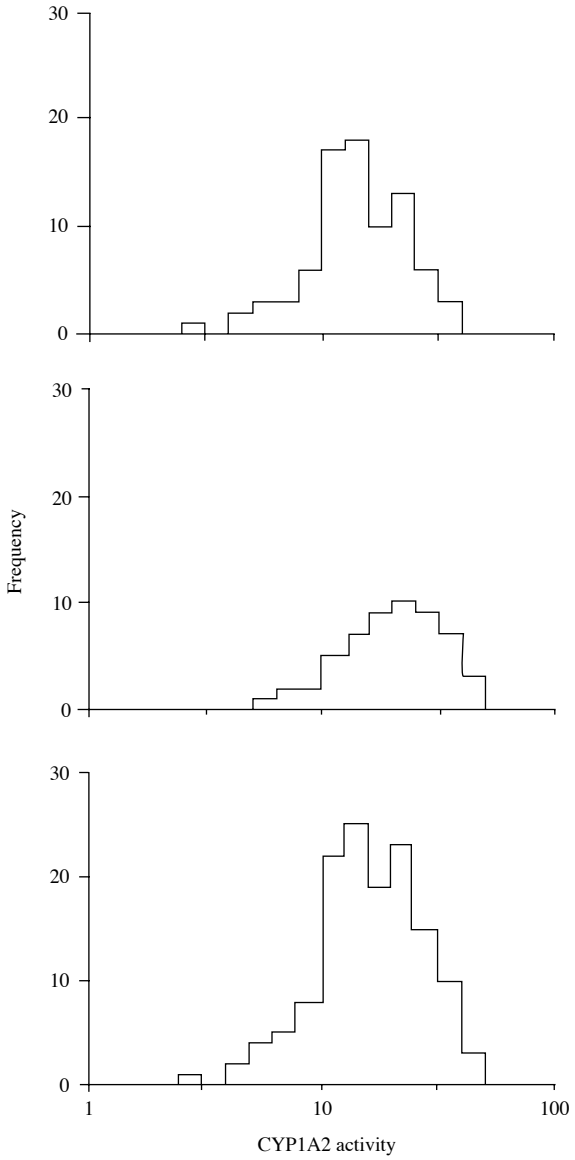

Figure 1. The frequency distribution of CYP1A2 activity in total (n=137, lower panel), smoker (n=55, middle panel) and nonsmokers (n=82, upper panel).

known substrate, caffeine, have been applied for determining CYP1A2 activity. Among these phenotyping methods, the urinary metabolite ratio determined by urinary molar ratio of 1, 7-dimethylxanthine (17X) and 1, 7-dimethyluric acid (17U) to caffeine (1, 3, 7-trimethylxanthine; 137X), [(17U+17X)/137X], was known to be sensitive and better correlated with the rate constant for caffeine N3-demethylation[12].

Recently, Han X. M. *et al*. reported that the *CYP1A2 *1C* and *1F* polymorphism was associated with the inducibility/activity of CYP1A2 determined by plasma caffeine metabolite ratio (17X/137X)[7]. However, the effects of these mutations on the inducibility/activity of CYP1A2 (urinary metabolite ratio) were quite different with our preliminary data in Koreans, especially in *CYP1A2*1F* mutation[13].

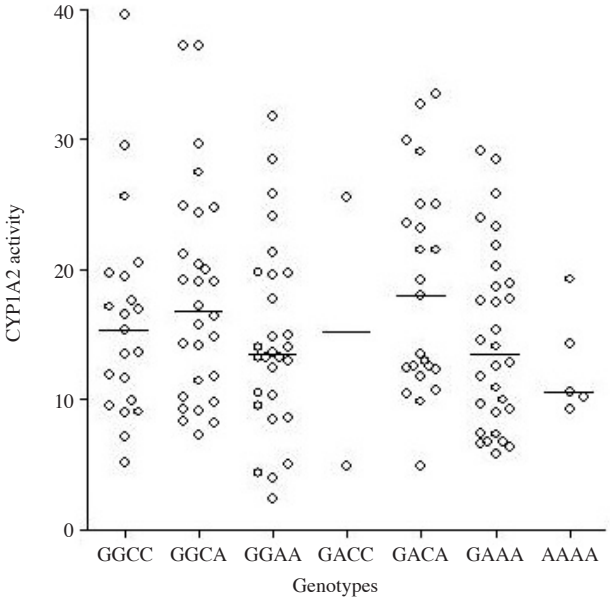

Figure 2. Scatter plot of CYP1A2 activity in subjects with 7 different genotypes for CYP1A2*1C and CYP1A2*1F alleles.

Therefore, in the present study, we analyzed the relationship between the two genetic polymorphisms (*CYP1A2*1C* and *CYP1A2*1F*) and the inducibility/activity of CYP1A2 determined by urinary caffeine metabolite ratio [(17U+17X)/137X] in 137 Korean volunteers.

Inter-individual Variation of CYP1A2 Activity

16-folds variation of CYP1A2 activity was observed at the range from 2.41 to 39.58 (16.14±7.74) in total subjects. As shown in Figure 1, the logarithmic transformed CYP1A2 activity was not normally distributed as analyzed by the Shapiro-Wilk test of normality in nonsmokers (W=0.9691, P=0.0453). In total subjects and smoker subgroup, the log activity was normally distributed (W=0.9818, P=0.0639 and W=0.9634, P=0.0926). The coefficient of variation (CV%), skewness and kurtosis of the log CYP1A2 activity in total subjects, nonsmokers and smokers were 19.39%, -0.4097 and 0.2784, 19.33%, -0.6182 and 0.7935, and 17.3%, -0.5907 and -0.1728 respectively. The mean CYP1A2 activity of smokers (n=55, 19.45±8.57, range from 5.0 to 39.58) was significantly higher than that of nonsmokers (n=82, 13.93

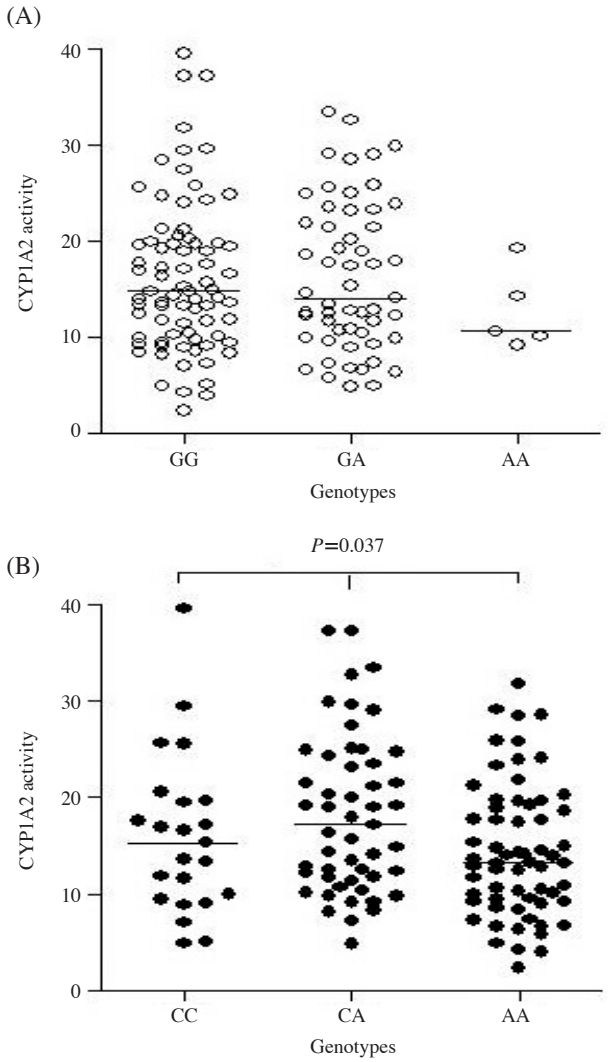

Figure 3. The effects of CYP1A2*1C or CYP1A2*1F allele on the CYP1A2 activity in 137 Koreans. (A) The CYP1A2*1C allele did not influence on the CYP1A2 activity, while (B) CYP1A2*1F allele decreased the CYP1A2 activity.

±6.26, range from 2.41 to 29.53) (1.4 folds, $P<0.0001$). There was no significant difference ($P=0.2051$) in the ratios between men (16.53±7.88, n=113) and women (14.32±6.89, n=24).

Frequencies of CYP1A2*1C and CYP1A2*1F

CYP1A2 genotyping revealed that the frequencies of the *CYP1A2*1C* (-3858A) and **1F* (-164A) allele were 0.219 and 0.646, respectively. The genotypes frequencies of G/G, G/A and A/A for the *CYP1A2*1C* mutation were 0.562, 0.401 and 0.037, respectively, and those of C/C, C/A and A/A for the *CYP1A2*1F* mutation were 0.168, 0.372, 0.460, respectively. As shown in Figure 2, there were 7 genotypes according to different nucleotide at positions -3858 (G>A) and -164 (C>A): GG/CC, GG/CA, GG/AA, GA/CC, GA/CA, GA/AA and AA/AA. The genotypes of AA/CC and AA/CA were not identified in the 137 included Koreans.

Table 1. Comparisons of CYP1A2 activity among the genotypes in nonsmokers.

Genotype		N	CYP1A2 activity	*P* value*	*P* value#
C-164A	G-3858A				
C/C	G/G	16	14.91±6.74		0.580
	G/A	1	4.95	–	
	A/A	–			
C/A	G/G	15	14.93±5.69		
	G/A	12	14.54±6.74	N.S.	
	A/A	–	–		
A/A	G/G	17	11.34±4.77		
	G/A	17	14.89±7.24	N.S.	
	A/A	4	13.60±4.22	N.S.	
Genotype		N	CYP1A2 activity	*P* value*	*P* value#
G-3858A	C-164A				
G/G	C/C	16	14.91±6.74		0.2366
	C/A	15	14.93±5.69	N.S.	
	A/A	17	11.34±4.77	0.042	
G/A	C/C	1	4.95		
	C/A	12	14.54±6.99	N.S.	
	A/A	17	14.89±7.24		
A/A	C/C	–			
	C/A	–			
	A/A	4	13.60±4.22		

*P values were calculated when respective genotype was compared with their wild genotypes (e.g., G/G, or C/C).

#P values were calculated when 3 genotypes for G-3858A or C-164A were compared (e.g., C/C, C/A and A/A; G/G, G/A, A/A).

Effects of CYP1A2 Genotypes on the Activity

As shown in Figure 3A, the mean CYP1A2 activities of the G/G, G/A and A/A genotypes in total subjects were 16.24±7.90 (n=77), 16.33±7.78 (n=55) and 12.73±4.14 (n=5), respectively and there was no significant difference among them. As summarized in Table 1 and 2, in nonsmokers, there was also no significant difference according to the genotypes with G/G, G/A and A/A (13.65±5.91 (n=48), 14.42±7.12 (n=30) and 13.60±4.22 (n=4), respectively). The mean CYP1A2 activities of these genotypes in smokers were 20.52±8.97 (n=29), 18.62±8.07 (n=25) and 9.25 (n=1), respectively. There was also no significant difference among them. Comparing the activities of respective genotype in smokers with nonsmokers, the activity of G/G and G/A genotype in smokers was significantly higher than in nonsmokers

Table 2. Comparisons of CYP1A2 activity among the genotypes in smokers.

Genotype		N	CYP1A2 activity	*P* value*	*P* value#
C-164A	G-3858A				
C/C	G/G	5	20.05±11.4		0.0633
	G/A	1	25.61	–	
	A/A	–	–	–	
C/A	G/G	13	21.46±9.50		
	G/A	11	22.90±7.16	N.S.	
	A/A	–	–	–	
A/A	G/G	11	19.61±7.91		
	G/A	13	14.46±6.91	N.S.	
	A/A	1	9.25	–	
Genotype		N	CYP1A2 activity	*P* value*	*P* value#
G-3858A	C-164A				
G/G	C/C	5	20.05±11.4		0.4201
	C/A	13	21.46±9.50	N.S.	
	A/A	11	19.61±7.91	N.S.	
G/A	C/C	1	25.61		
	C/A	11	22.90±7.16	–	
	A/A	13	14.46±6.91	0.0108$	
A/A	C/C	–			
	C/A	–		–	
	A/A	1	9.25	–	

*P values were calculated when respective genotype was compared with their wild genotypes (e.g., G/G, or C/C).

#P values were calculated when 3 genotypes for G-3858A or C-164A were compared (e.g., C/C, C/A and A/A; G/G, G/A, A/A).

$A/A vs. C/A.

($P<0.0001$ and $P=0.0453$, respectively). However, the activity of A/A genotype in smokers (9.25, n=1) was not higher, even lower than in nonsmokers (13.60 ±4.22, n=4) although the sample size was small.

The mean CYP1A2 activities of C/C, C/A and A/A genotypes for the *CYP1A2*1F* allele in total subjects were 16.09±8.30 (n=23), 18.22±8.11 (n=51) and 14.50±6.89 (n=63) respectively, and there were significant difference among them ($P=0.037$, one-way ANOVA) (Figure 3B). As summarized in Table 1 and 2, those of C/C, C/A and A/A genotypes in smokers were 20.97±10.45 (n=6), 22.12±8.36 (n=24) and 16.52±7.67 (n=25) respectively and, there was a trend of decreased activity in subgroup with A/A genotype ($P=0.0633$, one-way ANOVA). However, in nonsmokers, the mean activities of respective genotypes were not significantly different (C/C=14.33± 6.96, n=17; C/A=14.76±6.18, n=27; A/A=13.17 ±6.07, n=38, $P=0.58$ by one-way ANOVA) comparing the activity between nonsmokers and smokers, the activities of C/A genotype in smokers were significantly higher than those in nonsmokers ($P=0.0007$). However, the activities of C/C ($P=0.0922$) and A/A genotypes ($P=0.0583$) in smokers showed only tendency of higher activity. Interestingly, the activities of A/A genotype were significantly lower than those of non-A/A genotypes (C/C and C/A genotypes) in smokers ($P=0.0192$) and total subjects ($P=0.0208$), whereas there was no significant difference between A/A genotype and non-A/A genotype in nonsmoker ($P=0.307$). BThese results indicate that CYP1A2*1F might influence on the CYP1A2 inducibility.

Discussion

To investigate the two different point mutations on the activity of CYP1A2, we failed to detect the significant difference of the CYP1A2 activities among 7 genotypes determined by combinations of two point mutations in total (n=137, $P=0.4335$), nonsmokers (n=82, $P=0.4753$) and smokers (n=55, $P=0.9175$) as analyzed by Kruskal-Wallis test. As mentioned above, the activity between G/G and non-G/G genotype in G-3858A site was not different, however, the activity between A/A and non-A/A genotype in C-164A site was different in total subjects ($P=0.0208$) and in smokers ($P=0.0192$) but in nonsmokers ($P=0.3071$). The mean activity with A/A genotypes was 14.50±6.89 and with non-A/A genotypes was 17.55 ±8.17 in total subjects, which was significant difference ($P=0.0208$). These results suggested that the CYP1A2 activity may be regulated by CYP1A2*1F genotype, particularly in smokers. It is well known that CYP1A2 activity is modulated by many environmental factors including diet, smoking and environmental pollutants. Therefore, our phenotype data might be influenced by unknown environmental factors. We suggest that CYP1A2*1F rather than CYP1A2*1C is an important genetic factor for regulating the CYP1A2 activity in Koreans, particularly in smokers. Contrary to previous results obtained from Caucasian and Chinese population[6,8-10], our current study demonstrates that the AA genotype of the *CYP1A2*/A*1F polymorphism is associated with decreased CYP1A2 inducibility. This discrepancy may be attributable to several causes. First, the frequencies of other SNP, such as *CYP1A2*/T-2467delT and *CYP1A2**1C, in Caucasians were quite different from those in Koreans or other Asians[5,8-10]. Furthermore, the linkage disequilibrium of these SNPs might differ among these ethnic populations. Second, there are many methods to measure in vivo CYP1A2 activity using caffeine as a substrate. Some investigators measured CYP1A2 activity using the plasma metabolite ratio (17X: caffeine), whereas others measured

it using urinary metabolite ratios (e.g. [17X+17 U]: caffeine or 5′-acetylamino-6-formylamino-3-methyluracil (AFMU) +1X+1 U):17 U)[1,3,7,14]. These issues should be resolved by an additional study that uses identical methods for the measurement of CYP1A2 activity in different ethnic groups. Therefore, further studies including different ethnic groups with large populations should be conducted to reveal the effects of other SNPs in CYP1A2 gene and to investigate the inter-ethnic difference of pharmacogenetics of CYP1A2.

Methods

Subjects

137 Korean unrelated healthy volunteers aged 21-33 years (23.1±1.9; 82 nonsmokers and 55 regular smokers) were recruited after giving their written informed consent for both phenotyping and genotyping analysis of CYP1A2. All subjects were in good health on the basis of medical history and physical examination. The ethics committee in Inha University Hospital approved the study.

Phenotyping of CYP1A2

Subjects were asked to refrain from taking the caffeine-containing food and beverage for at least 48 hours before the study. After overnight fasting, a cup of instant coffee (containing 110 mg caffeine) was drunk after emptying the bladder and urine was voided 4 hours after coffee intake. Subsequently, one hour spot urine was collected and was analyzed by high-performance liquid chromatography as described previously[1]. The urinary caffeine metabolite ratio [(17U +17X)/137X] on a molar basis was used as an index of CYP1A2 activity[1,12].

Genotyping of CYP1A2*1C and CYP1A2*1F

Genomic DNA was extracted from peripheral lymphocytes with a standard phenol-chloroform extraction method. For the detection of a point mutation from G to A in 5′-flanking region (*CYP1A2*1C*) which give rise to a *Dde*I cleavage site, PCR was performed as described previously[4]. Briefly, 5′-flanking region of CYP1A2 was amplified by PCR using sense primer 1A2-3993S (5′-GCT ACA CAT GAT CGA GCT ATA C-3′) and antisense primer 1A2-3458A (5′-CAG GTC TCT TCA CTG TAA AGT TA-3′). A 50 μL PCR mix comprised 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM dNTPs, 0.2 μM of each of the primers, 1.5 U Taq polymerase (TaKaRa TaqTM, TaKaRa Biomedicals) and 1 μL of genomic DNA. PCR was performed with an initial denaturation for 5 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at 53°C, 3 min at 72°C, and a terminal extension for 10 min at 72°C. The G/A polymorphism were identified using the amplified PCR product by followed digestion with *Dde*I restriction enzyme. For the detection of a point mutation from C to A (*CYP1A2*1F*) in intron 1 of CYP1A2, PCR amplification was performed with sense primer 1A2-221 (5′-TGA GGC TCC TTT CCA GCT CTC A-3′) and antisense primer 1A2+23 (5′-AGA AGC TCT GTG GCC GAG AAG G-3′). The condition for the PCR reaction was same as described above except the annealing temperature (60°C) and the C/A polymorphism was identified by routine PCR-RFLP analyses (Bsp120I restriction enzyme) according to the method described by Sachse, C. *et al*.[6]. Cleavage products were analyzed by 2% agarose gel electrophoresis.

Statistical Analysis

The CYP1A2 activities among the genetically defined groups were statistically compared by one-way analysis of variance, Kruskal-Wallis test, or unpaired t-test using GraphPad Prism program (Ver. 4.0, GraphPad Software Inc., CA, USA). The normality test was performed using the Shapiro Wilk test after logarithmic transformation of data from all groups.

Acknowledgements

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