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IMPORTANCE OF PHARMACOGENETIC AND ENVIRONMENTAL FACTORS FOR VARIATION IN CAFFEINE DISPOSITION: WITH SPECIAL EMPHASIS ON CYP1A2, CYP2A6, NAT2 AND XO

Academic Thesis

The public defence for the degree of Doctor of Philosophy at Karolinska Institutet will be held at Karolinska Institutet, Alfred Nobel Allé 8, Floor 6, Lecture room 6F, Karolinska University Hospital, Huddinge

13th April, 2012: 9:30

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Stockholm 2012

Abstract

Inter-individual variation in response to drugs conveys considerable risk of either therapy failure or drug toxicity. Caffeine, as one of the most frequently used psychoactive substances in the world, is not an exception, and both lack of effects and adverse reactions have been observed after usual doses. In addition to inter-individual differences at the drug-receptor level and drug-drug interactions, variability in caffeine metabolism has been proposed as a possible explanation. The objective of this study was to investigate the influence of pharmacogenetic and environmental factors, which are known to be of importance for inter-individual variation in drug disposition, on the activity of enzymes involved in caffeine metabolism, namely cytochrome P450 1A2 (CYP1A2) and 2A6 (CYP2A6), *N*-acetyltransferase-2 (NAT2) and xanthine oxidase (XO). The study involved unrelated healthy volunteers from three ethnically distinct populations: Serbian (n=140), Swedish (n=190) and Korean (n=150). Phenotyping of CYP1A2, CYP2A6, NAT2 and XO was performed using caffeine as a probe drug and measuring concentration of parent compound and its metabolites in plasma or urine samples. CYP1A2 activity was estimated by 17X/137X plasma ratio, and CYP2A6, NAT2 and XO activities were determined by 17U/17X, AFMU/(AFMU+1X+1U) and 1U/(1U+1X) urinary ratios, respectively. In addition, subjects were genotyped for the most important *CYP1A2*, *CYP2A6* and *NAT2* polymorphisms.

Daily consumption of at least three cups of coffee in non-OC (oral contraceptive) users significantly increased CYP1A2 enzyme activity in both Swedes ($P<0.0001$) and Serbs ($P=0.0002$). When additionally controlling for smoking, the observed difference remained significant in both populations ($P\leq0.02$). Significant association of heavy coffee consumption with high CYP1A2 enzyme activity was observed only in carriers of −163 A/A, and increasing effect of −163C>A on CYP1A2 inducibility was found in both Serbian ($P=0.022$) and Swedish ($P=0.016$) nonsmoking heavy coffee consumers. There was no significant difference in CYP1A2 enzyme activity among genotypes in non–heavy coffee consumers. Controlling for the effect of smoking, heavy coffee consumption habit and OC use, significantly lower 17X/137X ratio was observed in Serbs than in Swedes ($P=0.0003$). Comparison between Swedes and Koreans revealed that functional *CYP2A6* alleles were more frequent in former, whereas the defective were more frequent in latter ($P\leq0.002$). *CYP2A6* genotype significantly affected enzyme activity in both populations ($P=0.004$), while no effect of sex, age, cigarette smoking or OC use was observed. CYP2A6 activity was higher in Swedes compared to Koreans, with 3.16% of Swedes and 18.75% of Koreans being slow metabolizers ($P=0.0001$). The observed differences between the two populations remained significant when controlling for the genotype effect, i.e. within rapid ($P=0.0007$) and intermediate ($P=0.04$) genotype groups. Rapid acetylator is the predominant NAT2 phenotype in Serbs, present in 55% of the population. Significant NAT2 genotype-phenotype association was detected in Serbs ($P<0.0001$), Swedes ($P=0.03$) and Koreans ($P=0.008$). Partial NAT2 genotype-phenotype discordance in Serbian rapid acetylators could not be explained by *NAT1* gene polymorphism. Koreans display significantly higher NAT2 activity compared to Swedes, and the difference remained significant when controlling for the influence of cigarette smoking, sex, or OC use ($P <0.0001$), as well as *NAT2* genotype ($P=0.016$). Swedes and Koreans significantly differed in terms of *NAT2* genotype groups distribution ($P<0.0001$). Stratified by genotype, among carriers of at least one wild type *NAT2*4* allele Koreans display higher enzyme activity compared to Swedes ($P=0.004$). No significant influence of smoking or sex on NAT2 activity was observed in Serbs, Swedes or Koreans. OC use significantly increased NAT2 activity in Swedish women ($P=0.007$). In terms of XO activity, no significant difference between Swedes and Koreans was detected. In Swedes, higher XO activity was observed in women compared to men ($P=0.003$), and the effect remained significant after controlling for OC use ($P=0.01$). OC use and cigarette smoking did not affect XO activity in either Swedes or Koreans.

In conclusion, habitual heavy coffee consumption induces CYP1A2 enzyme activity in carriers of *CYP1A2* −163 A/A genotype. *CYP2A6* genotype, but not sex, age, cigarette smoking and OC use, significantly affect CYP2A6 enzyme activity. *NAT2* genotype, but not sex and cigarette smoking, significantly affect NAT2 enzyme activity. Cigarette smoking and OC use do not affect XO enzyme activity. In Swedes, female sex and oral contraceptive use are associated with higher XO and NAT2 enzyme activities, respectively. Swedes display significantly higher CYP1A2 and CYP2A6 activities compared to Serbs and Koreans, respectively. Koreans display significantly higher NAT2 enzyme activity compared to Swedes. Serbs differ from other Caucasians in terms of *N*-acetylation capacity, due to unprecedented high prevalence of rapid acetylator phenotype. There is no inter-ethnic difference between Swedes and Koreans in terms of XO enzyme activity.