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Cytochrome *P*450 2D6 genotyping of fatal intoxications using Pyrosequencing

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Abstract. Many commonly used pharmaceuticals such as antidepressants and neuroleptics as well as some illegal drugs are metabolised by the Cytochrome *P*450 enzyme debrisoquine 4-hydroxylase (CYP2D6). Seven to ten percent of Caucasians lack this enzyme which can lead to adverse reactions and in some cases to unexpected intoxication even with fatal outcome, upon administration of drugs in normal therapeutic doses. 236 individuals who had died due to intoxication of pharmaceuticals were genotyped for CYP2D6 and compared to a reference group of 281 blood donors. A single nucleotide polymorphism (SNP) method was used to identify five CYP2D6 alleles: *1 (wt), *2, *3, *4 and *6. Allele *5, a complete gene deletion, was identified by a multiplex amplification of long DNA fragments. The prevalence of the CYP2D6 PM genotype in the fatal intoxications was lower (4.7%) compared to the blood donors (8.5%). A significant decrease ($p < 0.005$) was found in the CYP2D6*4 allele frequency among the fatal intoxications.

Keywords: Genotyping; CYP2D6; SNP; Pyrosequencing; Post-mortem

1. Introduction

The Cytochrome *P*450 (CYP) debrisoquine 4-hydroxylase is a liver enzyme coded from the CYP2D6 gene in the Cytochrome *P*450 gene cluster, CYP2D6-D8P, on chromosome 22 in humans. The CYP2D6 enzyme metabolises a variety of toxic plant substances and many drugs, such as antidepressants, neuroleptics, opiates, antiarrythmics and antihypertensive agents [1]. Seven to ten percent of Caucasians lack this enzyme and will have an increased risk for adverse reactions sometimes with fatal intoxication. More than 80 different alleles have been reported for CYP2D6 [2]. The majority of these alleles are rare and associated with a decreased or none CYP2D6 activity known as a poor metabolism phenotype (PM). The most frequent PM alleles are *4, *5, *3 and *6, in decreasing order. These four alleles can predict 93–98% of the PM phenotypes in Caucasians [3–5]. In contrast, 1–7% of the Caucasian population have increased CYP2D6 activity (ultrarapid metabolisers) [3,6]. In previous studies on CYP2D6 in forensic materials [7,8], we found a lower frequency of PMs.

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Table 1
Number of individuals with 0, 1 or 2 functional alleles in intoxications and blood donors

	Number of functional alleles			Total number of individuals
	0	1	2	
	q/q	p/q	p/p	
Intoxications	11 (4.7%)	87 (37%)	138 (58%)	236
Blood donors	24 (8.5%)	127 (45%)	130 (46%)	281

Our aim in this work was to study different Cytochrome *P*450 systems such as CYP2D6, CYP2C9 and CYP2C19 in a larger group of deaths caused by intoxication of pharmaceuticals or illegal drugs. This is a report from a part of the work that comprises CYP2D6.

2. Materials and methods

Samples were taken from 242 consecutive cases when the causes of death were considered by the forensic pathologist to be fatal intoxication with a pharmaceutical. Samples from 281 blood donors were also collected.

At the autopsy, femoral blood was collected and potassium fluoride was added as a preservative. Genomic DNA was extracted with GenoM48™ robotic workstation (GenoVision, Vienna, Austria). Genomic DNA from the blood donors was extracted according to the DTAB/CTAB method [9]. The Regional Ethics Committee (Faculty of Health Sciences, Linköping University, Sweden) gave permission for analysis.

Four alleles associated with the PM phenotype were studied, CYP2D6*3 (2549A>del), *4 (1846G>A), *5 (deleted sequence) and CYP2D6*6 (1707T>del). Additionally the polymorphism 2850C>T in allele *2 was identified. That polymorphism is also present in the alleles *4K, *8, *11–12, *14, *17, *20, *28–32 and *34–35 some of which are associated with a normal or increased enzyme activity and others with low or no activity [4]. The allele *1 (wt) also can include other polymorphisms. The allele CYP2D6*5 was identified using a multiplex long PCR method [10]. The other alleles were identified by single nucleotide polymorphism (SNP) analysis using Pyrosequencing [11–13].

3. Results

The CYP2D6 genotype was established in 236 of the 242 samples. In 11 of the 236 fatal intoxications (4.7%), a PM genotype was found compared to 8.5% in the blood donors. Considering the number of functional alleles (0, 1 and 2) a significant difference

Table 2
Allele frequencies

Alleles	Frequencies	
	Intoxications	Blood donors
*1	0.428	0.367
*2	0.341	0.324
*3	0.015	0.014
*4	0.169	0.244
*5	0.038	0.043
*6	0.008	0.009

($p < 0.0005$) was found (Table 1). The total number of nonfunctional alleles (*3, *4, *5 and *6) was also found significantly decreased ($p < 0.005$) among the fatal intoxications. The allele *4 was found less frequent ($p \approx 0.001$) among the intoxications (Table 2).

4. Discussion

Earlier we have shown a lower frequency of poor metabolisers [7,8] in forensic intoxication cases. However, the number of cases in those studies was small. We wanted to investigate a larger material of forensic cases of intoxications to confirm or discharge those results. The result presented here shows that there is a lower frequency of PM alleles in the forensic cases compared to the blood donors. The decrease of the CYP2D6*4 allele in the fatal intoxications is statistically significant. To our knowledge this has not been shown earlier. The reasons for these findings are so far not known, but further studies are in progress.

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