1. Introduction

As reviewed by Eichelbaum et al. [1], most patient populations show large inter-individual variability with respect to drug response and toxicity. Even when administered at standard doses, a substantial proportion of patients fail to respond, respond only partially, or experience adverse drug reactions (ADRs) for all major classes of drugs (e.g., opioids, tricyclic antidepressants, serotonin reuptake inhibitors, ACE inhibitors, β-adrenoreceptor agonists, statins, etc.). For two individuals of the same weight on the same dose, drug concentrations in plasma can vary substantially. This variation can be of physiological, pathophysiological, environmental or genetic origin. In general, potential risk factors for ADRs include a patient's age, sex, co-morbidities, co-medication, organ dysfunction (especially of liver and kidneys), diet, as well as some lifestyle variables, such as smoking habits and alcohol intake (Fig. 1). However, a drug's absorption, distribution, metabolism and interactions with its target often are determined by genetic differences. Pharmacokinetic and pharmacodynamic variations can appear at the level of drug metabolizing enzymes (e.g., the cytochrome P450 system), drug transporters, drug targets or other biomarker genes. Pharmacogenetics or toxicogenetics can therefore be relevant in forensic toxicology. This review presents relevant aspects together with some examples from daily routines.

2. Reasons for pharmacokinetic variations

2.1. Drug metabolism

The hepatic cytochrome P450 system is responsible for the first phase of the metabolism and elimination of numerous endogenous and exogenous molecules and ingested chemicals. The P450 enzymes convert these substances into electrophilic intermediates, which are then conjugated by phase II enzymes (e.g., UDP glucuronosyltransferases; N-acetyltransferases) to form hydrophilic derivatives that can be excreted [8]. The P450 superfamily comprises several subfamilies that are designated by letter according to the system of nomenclature recommended by an international committee [9–11] and data are continuously upgraded [12]. Although humans probably have at least 60 unique P450 genes within several subfamilies [10], only a subset (e.g., CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) is responsible for metabolism of the vast majority of prescribed and over-the-counter drugs [13–27]. Table 1 presents a list of selected drugs that are metabolized by specific cytochrome P450 isoforms.
Because of genetic polymorphism of the CYP P450 enzymes, populations could be classified into three major phenotypes [28]:

- the ultra-rapid metabolizers (UM), with more than two active genes encoding a certain P450;
- the extensive metabolizers (EM), carrying two functional genes;
- the poor metabolizers (PM), lacking functional enzymes due to defective or deleted genes;
- heterozygous individuals, carrying one functional and one defective PM associated allele and intermediate metabolizers (IM) carrying alleles partially decreasing enzyme activity. These genotypes have reduced but not absent CYP activity.

Subjects with PM associated variants are at increased risk of suffering from adverse side effects due to drug overdose or of experiencing therapeutic failure due to poor metabolism of a prodrug to the active metabolite (Fig. 2). In contrast, UMs have significantly increased enzyme activity, which can result in

![Diagram](image)

**Fig. 1.** Variables determining response to a drug. Modified from [2].

<table>
<thead>
<tr>
<th>Enzyme Substrates</th>
<th>Inhibitors</th>
<th>Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Antidepressants: amitriptyline, clomipramine, duloxetine, fluvoxamine, imipramine, maprotiline, mirtazapine</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>Others: aminopyrine, amiodarone, caffeine, melatonin, paracetamol, phenacetin, propafenone, propranolol, tacrine, theophylline, verapamil, R-warfarin, zolpidem</td>
<td>Enoxacin</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td></td>
<td>Antipsychotics: clozapine, haloperidol, olanzapine, promazine, thioridazine, zotepine</td>
<td>Fluvoxamine</td>
</tr>
<tr>
<td></td>
<td>Others: bupropion, cyclophosphamide, desmethylseledeline, methadone</td>
<td>Sulphaphenazole</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Others: bupropion, cyclophosphamide, desmethylseledeline, methadone</td>
<td>Fluconazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluoxetine</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>NSAIDs: diclofenac, ibuprofen, naproxen, piroxicam</td>
<td>Fluvoxamine</td>
</tr>
<tr>
<td>Others: losartan, phenobarbital, phenytoin, tolvaptamide, valproic acid, valsartan, S-warfarin</td>
<td>Sulphaphenazole</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Antidepressants: amitriptyline, citalopram, clomipramine, escitalopram, imipramine</td>
<td>Fluvoxamine</td>
</tr>
<tr>
<td>Others: diazepam, lansoprazole, omeprazole, phenytoin, proguanil, propranolol</td>
<td>Ticlopidine</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Antiarhythms: encainide, flecainide, propafenone</td>
<td>Bupropion</td>
</tr>
<tr>
<td>Antidepressants: amitriptyline, citalopram, clomipramine, desipramine, escitalopram, fluoxetine, fluvoxamine, imipramine, mirtazapine, nortriptyline, paroxetine, venlafaxine</td>
<td>Duloxetine</td>
<td>Barbiturates</td>
</tr>
<tr>
<td>Antipsychotics: aripiprazole, clozapine, haloperidol, olanzapine, perphenazine, risperidone, sertindole, thioridazine</td>
<td>Fluoxetine</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>β-Blockers: alpenolol, bufuralol, metoprolol, propranolol, timolol</td>
<td>Paroxetine</td>
<td>Phenytion</td>
</tr>
<tr>
<td>Others: atomezetine, codeine, donepezil, galantamine, phenformin, tramadol</td>
<td>Quinidine</td>
<td>Rifampicin</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Antidepressants: amitriptyline, citalopram, clomipramine, escitalopram, imipramine, mirtazapine, nefazodone, sertraline, venlafaxine</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>Antipsychotics: aripiprazole, clozapine, haloperidol, pimozide, quetiapine, risperidone, sertindole, ziprasidone</td>
<td>Grapefruit juice</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>Benzodiazepines: alprazolam, clonazepam, diazepam, midazolam, triazolam</td>
<td>Iracnoazole</td>
<td>Phenytion</td>
</tr>
<tr>
<td>Calcium channel antagonists: diltiazem, felodipine, nifedipine, verapamil</td>
<td>Nefazodone</td>
<td>Rifampicin</td>
</tr>
<tr>
<td>Statins: atorvastatin, lovastatin, pravastatin, simvastatin</td>
<td>Troleandomycin</td>
<td></td>
</tr>
</tbody>
</table>
subtherapeutic blood levels of the enzyme substrate. In case of a prodrug the metabolism to the active metabolite is increased.

Functional CYP polymorphisms consist of single nucleotide polymorphisms (SNPs) accounting for the majority of genetic variants, gene deletions, gene duplications. Deleterious genetic variants that create inactive gene products might be; e.g., small insertions and deletions causing frame shift mutations. [28]. Mutations or polymorphisms in introns can create altered splice sites, while base exchanges in exons can result in amino acid changes with possible effects on substrate specificity. The copy number variation, where multiple functional gene copies of one allele can result in increased drug metabolism, can also be an important aspect in genetically determined absence of drug response or in case of a prodrug, drug-related adverse events and fatalities.

2.2. Drug transporters

Transporters and transporter-related proteins play a major role in drug absorption, disposition, toxicity and efficacy and significant transporter-mediated drug interactions can occur [29]. These types of interactions can be inhibitory, inductive, or both, and may involve influx or efflux transporters. They are of interest if: (1) the elimination of the affected drug or the distribution into a target tissue is mediated primarily by the transporter and (2) the interaction results in the concentration of the affected drug at the site of action [30,31].

For example, the P-glycoprotein (P-gp) efflux pump belongs to the ATP-binding cassette (ABC) family transporters and is encoded by the MDR1 gene (or ABCB1). In humans, P-gp is present in several tissues that are important for drug absorption, distribution and elimination (Fig. 3). Due to the broad substrate specificity of P-gp, inhibitors or inducers of P-gp may produce significant drug–drug interactions. Another important group are the organic anion (protein) transporters (OATPs or OATs) encoded by the solute carrier gene family (SLC). These Na+-independent transporters partly rely on co-transport and are responsible for the transport of a wide range of endogenous compounds as well as drugs.

Organic cation transporter (OCT) interactions, as well as peptide transporter (PEPT) and nucleoside transporter (NT) interactions, have also been described.

The TransportDB website currently lists as many as 1,022 transporters for *Homo sapiens* alone [32]. For example, the so-called Pharmacogenomics of Membrane Transporters (PMT) project is at the crossroads of pharmacogenomics and transporter biology. Recently, allele frequencies and findings regarding functional

![Fig. 2. Pharmacological effects depending on metabolism of an active drug (A) or prodrug (B). Genotypes determine enzyme activity and metabolism of a drug, thus causing increased (overdose/side effects), normal, or lacking pharmacological effects of a drug. Modified from [2].](image1)

![Fig. 3. In humans, P-gp is present in several tissues important for drug absorption, distribution and elimination, such as the apical membrane of intestinal epithelial cells, the canalicular membrane of the hepatocytes, the capillary endothelial cells of the brain, the apical membrane of the placental syncytiotrophoblasts and the apical membrane of the renal proximal tubular cells. In these tissues P-gp functions as an efflux pump are involved in the entry of substances into these tissues. Reprinted from [30] with permission from Elsevier.](image2)
variants in drug transporter systems were summarized by Kroetz et al. [33].

2.3. Drug targets

Pharmaceutical agents generally exert their effects by binding to and subsequently modulating the activity of specific protein, nucleic acid or other molecular (such as membrane) targets, such as the β2-adrenoceptors, insulin receptors, angiotensin-converting enzyme and others. Factors such as drug interactions and especially polymorphisms in genes encoding these targets may influence the sensitivity to selected drugs. These polymorphisms are considered to be significant in cases where inter-individual variations in drug plasma concentrations are minimal, but where major pharmacodynamic differences can be observed [6]. Information about therapeutic targets are given by several databases such as Drugbank [34], Potential Drug Target Database (PDTD) [35], or Therapeutic Target Database (TTD) [36].

Pathway databases are also helpful for understanding drug interactions; e.g., the well-known drug interaction table of Flockhart [37], the Therapeutically Relevant Multiple Pathways (TRMP) [38] or the Small Molecule Pathway Database (SMPDB) [39] with information on the relevant organs, organelles, subcellular compartments, protein cofactors, protein locations, metabolite locations, etc. According to the authors, the concept of metabolic pathway mapping has actually proven to be so effective that it has been extended to describe protein signalling, protein–DNA interactions and many other molecular biological phenomena.

2.4. Other biomarkers

According to the National Institutes of Health working group, a biomarker is a characteristic that is objectively measured as an indicator of normal biological processes, pathogenic processes, or a physiological response to a therapeutic intervention [40]. In the past, physiological parameters such as body temperature, blood pressure, colour of urine, heart beat rate, respiratory sounds or a lot of parameters deriving from clinical chemistry such as serum creatinine, urinary glucose or enzymes in blood or urine have been considered to be potential biomarkers. Recently, Marrer and Dieterle [41] reviewed biomarkers for kidney safety, including serum cystatin C, expression markers KIM-1 (kidney injury molecule-1) and lipocalin-2 (NGAL), urinary β2-microglobulin for the monitoring of tubular re-absorption or total protein for the glomerular filtration membrane. Biomarkers for liver safety (α-gluthathione- S-transferase (GST-α), γ-glutamyl-transpeptidase (GGT), paraoxynase-1 (PON-1), purine nucleotide phosphorylase (PNP), malate dehydrogenase (MDH)), cardiac safety (cardiac troponin 1 and T (cTn-1 and cTn-T)), natriuretic peptides (B-type natriuretic peptide (BNP)), and vascular safety (Willebrand factor (vWF), vWF propeptide (vWFPp), caveolin-1 (Cav-1)) have also been presented. However, all of these markers are more useful in drug safety assessment during or in development.

Today, the term biomarker increasingly refers to molecular and cellular markers joined with the development of modern scientific fields like proteomics, metabolomics, pharmacogenomics or better toxicogenomics. Reactive molecules produce DNA adducts, protein adducts and metabolites and represent biomarkers of exposure [42]. Many DNA adducts can result in mutations, while irreversible changes in the DNA structure can alter the genetic information content. Mutations represent biomarkers of effect. Microarray technologies can move biomarker discovery forward and result in genomic/genetic tests, protein assays or other analytical tests [43,44]. The application of mass spectrometric procedures is useful for such purposes [45].

3. Ethnic variations in drug dispositions

A drug metabolism phenotype is partly determined by the inheritance of various alleles, which exhibit variations among different ethnic groups. For example, the frequency of the CYP2D6 PM phenotype is much higher in population groups of Western Caucasian origin (5–10%) than in populations from the Far East or in Asian ethnic groups (0–2%) [46–50]. Major human polymorphic variant CYP2D6 alleles and their global distribution are described in Table 2. The frequency of PMs with the CYP2C19 allele is lower in the Western Caucasians (2–4%) compared to the frequencies observed among Orientals (15–25%), where it reaches as high as 60–70% in the Pacific islands [50–52]. Recently Sistonen et al. [20] described altered activity variants of CYP2C9, CYP2C19, and CYP2D6 occurring globally in all geographic regions and reported these to reach extremely high frequencies in some populations. Each of the CYP genes studied showed a distinct geographic pattern of variation and population substructure that can strongly affect variations seen in pharmacogenetic loci. However, according to this study, several geographic regions are still poorly characterized. Global heterogeneity and ethnic differences have also been reported with respect to the frequency of variant alleles of the genes expressing many other drug metabolizing enzymes [50,53–57] as well as the gene expressing P-gp [58,59]. Other ethnic differences also exist in the mutations of a number of drug targets, for example, those concerning the β-adrenoreceptor [60] or the serotonin receptor [61]. Consequently, ethnic variations in drug metabolism phenotypes as well as differences in drug transporters or drug targets should be taken into consideration in both clinical as well as forensic cases.

4. Applications in forensic toxicology

Interpretation of forensic-toxicological results is a task of great importance that demands knowledge of many different aspects of analytical toxicology, as well as pharmacokinetics and pharmaco-dynamics [62]. Especially in post-mortem toxicology, further aspects such as site- and time-related differences in post-mortem drug concentrations have to be considered, which complicates extrapolation from post-mortem to ante-mortem concentrations.

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Table 2

<table>
<thead>
<tr>
<th>Major variant allelea</th>
<th>Mutation</th>
<th>Consequence</th>
<th>Allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caucasians</td>
</tr>
</tbody>
</table>

CYP2D6*2xm  Gene duplication/multiduplication  Increased enzyme activity  1–5  0–2  2  10–16
CYP2D6*4    Defective splicing  Inactive enzyme  12–21  1  2  1–4
CYP2D6*5    Gene deletion  No enzyme  2–7  6  4  1–3
CYP2D6*10   P345, S486T  Unstable enzyme  1–2  51  6  3–9
CYP2D6*17   T107I, R296C, S486T  Altered affinity for substrates  0  0  20–35  3–9

a All variant alleles are listed by the Human CYP Allele Nomenclature Committee at http://www.imm.ki.se/cypalleles/cyp2d6.
In daily routine, concentrations of drugs have to be compared with the same kind of reference values for both therapeutic and toxic levels. Ratios of parent drug to metabolite concentrations (P/M ratio) can be helpful to decide whether an intake was acute (high ratio) or the result of a chronic use. However, other variables also influence the P/M ratio, including interactions between different drugs and genetic variations described above. For these reasons, genotyping has become more common in forensic toxicology. Holmgren and Ahlner [62] developed a scheme for determining the appropriateness of genotyping in several clinical or forensic settings (Table 3). Some case studies and case reports relevant to forensic toxicology are presented here. Although investigations and reports on genetically determined fatalities are still sparse up to date, modern biomarkers are expected to become a useful tool for forensic medicine in the future.

### 4.1. Opioids

The CYP2D6 system, in particular, is responsible for the metabolism of a number of opioid drugs such as codeine, tramadol, dihydrocodeine, oxycodone, hydrocodone, ethylmorphine, and at least part of methadone. The CYP2D6 PM phenotype affects opioid analgesics in different ways [68]: it reduces analgesic effects of codeine, decreases the clearance of methadone and reduces the efficacy of tramadol.

#### 4.1.1. Tramadol

The weak racemic opioid tramadol is metabolized by hepatic CYP2D6 to O-demethyltramadol (ODT) (Fig. 4). (+)-ODT has been shown to have an affinity for μ-opioid receptors that is approximately 200 times greater than that of the parent drug. Thus, (+)-ODT is largely responsible for opioid receptor mediated

---

### Table 3

Situations in which genotyping could give additional information. Modified according to Holmgren and Ahlner [62].

<table>
<thead>
<tr>
<th>Analytical findings or other information</th>
<th>Factors to determine</th>
<th>Is a polymorphic enzyme involved?</th>
<th>Interactions with other drugs?</th>
<th>Perform genotyping?</th>
</tr>
</thead>
<tbody>
<tr>
<td>High ratio between parent drug and metabolite: accidental death or suicide?</td>
<td>Yes</td>
<td>Yes</td>
<td>Consider</td>
<td>Yes</td>
</tr>
<tr>
<td>Abnormal high or low ratios between parent drug and metabolite</td>
<td>No</td>
<td>–</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Adverse drug reactions in anamnesis</td>
<td>Yes</td>
<td>Yes</td>
<td>Consider</td>
<td>No</td>
</tr>
<tr>
<td>No – No</td>
<td>Yes</td>
<td>Yes</td>
<td>Consider</td>
<td>No</td>
</tr>
<tr>
<td>High ratio between parent drug and metabolite: intake according to prescription was claimed</td>
<td>Yes</td>
<td>Yes</td>
<td>Consider</td>
<td>No</td>
</tr>
</tbody>
</table>

![Fig. 4. Metabolic pathway of tramadol involving polymorphic CYP enzymes.](image)
analgesia, whereas (+)- and (-)-tramadol contribute to analgesia by inhibition of serotonin and noradrenaline reuptake.

Recently, the influence of the CYP2D6 genotype and CYP2D6 inhibitors was investigated with respect to enantiomeric plasma levels of tramadol and ODT as well as to tramadol [69]. Concentrations of (+)-ODT differed in the four genotype groups (PMs < IMs < EMs < UM) and co-medication with CYP2D6 inhibitors decreased (+)-ODT concentrations. In PMs, non-response rates to tramadol treatment were increased 4-fold compared with the other genotypes, allowing the conclusion that the CYP2D6 genotype determines ODT concentrations and influences efficacy of tramadol treatment. Pharmacokinetic differences between EMs and UMs were also measured by Kirchheiner et al. [70]; UMs were more sensitive to tramadol than were EMs. According to the authors, tramadol may frequently cause adverse effects in southern European and Northern African populations that have a high proportion of UMs.

An opioid-related respiratory depression in a patient receiving tramadol was reported by Stamer et al. [71]. Complete recovery occurred after naloxone administration, thus confirming opioid intoxication. Analysis of the patient’s genotype revealed a CYP2D6 gene duplication resulting in ultra-rapid metabolism of tramadol to its active metabolite (+)-ODT. A concomitant renal impairment resulting in decreased metabolite clearance further enhanced opioid toxicity.

In 33 autopsy cases, Levo et al. [72] analysed both, the CYP2D6 genotype and the concentrations of tramadol and its metabolites O- and N-demethyltramadol. They found a correlation between the number of functional CYP2D6 alleles and the ratios of tramadol to ODT as well of tramadol to N-demethyltramadol. The number of UMs was overrepresented in these fatalities. There were two important findings of this study: (1) genetic variation in drug metabolizing enzymes can be analysed in post-mortem blood, and (2) genetic variation correlates well with the parent drug to metabolite ratios.

4.1.2. Codeine

Comparable to tramadol, O-demethylation of the prodrug codeine to its metabolite morphine is essential for its opioid activity and, thus, the CYP2D6 genotype specifically influences the efficacy and side effects (Fig. 5). The analgesic effect of codeine has been reported to be substantially reduced in subjects found to be PMs [68,73–75]. In the following some case reports concerning codeine are presented.

A 72-year-old man developed life-threatening respiratory depression after a 3-day treatment with codeine (3×25 mg/day). The adverse events were mediated by: (1) high concentrations of morphine owing to an UM genotype; (2) transient reduction in renal function with reduced clearance; and (3) co-medication with clarithromycin and voriconazole that blocked CYP3A4 as alternative pathway for codeine metabolism [76].

A breast-fed neonate whose mother received codeine 30 mg/day died on day 13 of morphine poisoning. The mother had an UM genotype and, thus, high amounts of morphine were formed from codeine, which then were transferred to the baby [77].

A 29-month-old previously healthy child experienced apnea resulting in brain injury following a dose of acetaminophen and codeine 2 days after an uneventful anaesthetic for tonsillectomy. A genetic polymorphism leading to ultra-rapid metabolism of codeine into morphine resulted in narcosis and apnea [78]. However, CYP2D6 genotyping is not only performed to prove the responsibility of genetic polymorphism for side effects. It is also important to prove an (accidental) intoxication; e.g., by dosage error when alternatively a UM genotype could be seen as responsible for a fatality [79,80].

4.1.3. Dihydrocodeine

Similarly to codeine, dihydrocodeine is converted by CYP2D6 to dihydromorphine, which has activity comparable to that of morphine. EMs have been demonstrated to have 7-fold higher dihydromorphine concentrations compared with PMs [81]. Quinidine-induced poor metabolism produced a 3- to 4-fold decrease in dihydromorphine plasma concentrations [82].

4.1.4. Oxycodone

Oxycodone, a semi-synthetic opioid with μ-receptor agonist-mediated effects, is metabolized to its active metabolite oxymor-
phone by O-demethylation via the polymorphic CYP2D6. In a recent clinical study, the mean oxymorphone/oxycodone ratios were reported as 0.0031 and 0.00081 in EMs and PMs, respectively. According to these results, the oxymorphone formation depends on CYP2D6, but no differences were found in the post-operative analgesic effect of intravenous oxycodone between the two CYP2D6 genotypes [83]. By assessing the prevalence of CYP2D6 polymorphisms and covariables, oxycodone fatalities were also hypothesised to be partially due to poor drug metabolism caused by CYP2D6 variant alleles. A retrospective analysis of 15 oxycodone cases was followed by genotyping and chemical-toxicological analysis. Genotyping provided a more definitive interpretation of oxycodone toxicity in four cases. Therefore, pharmacogenomics was proposed to serve as an adjunct in the determination of the cause and manner of death in forensic toxicology and a pharmacogenomic algorithm for genotyping has been proposed [84]. However, as also described by others [85], the PM genotype was rare and drug–drug interactions in EMs constituted a more frequent and important finding.

4.1.5. Ethylmorphine
In a fatality of a 10-month-old boy ethylmorphine which is a common ingredient of antitussive preparations, was administered. Ethylmorphine is partially metabolized to morphine by CYP2D6. Genotyping revealed an EM genotype, signifying a normal capacity for metabolizing ethylmorphine to morphine [86]. Autopsy report concluded that death was associated with opioid-induced sedation and respiratory depression, and a respiratory infection combined with sleeping position that could have impeded breathing.

4.1.6. Methadone
Methadone is partially metabolized by CYP2D6 and methadone toxicity was assumed to be partially due to CYP2D6*3, *4, and *5 variant alleles, resulting in poor drug metabolism. However, in a retrospective analysis performed on covariables and risk factors of 21 methadone cases, CYP2D6 mutations were not shown as yet to be directly associated with methadone toxicity [87].

Otherwise, methadone is mostly given as a racemic mixture, but CYP2B6 metabolism is stereo-selective toward the (S)-enantiomer. (R)-methadone mediates its narcotic effect by activating the µ-opioid receptor, whereas (S)-methadone inhibits the cardiac potassium channel, contributing to the cardiac side effects of methadone treatment. Decreased CYP2B6 activity was found to be associated with high plasma concentrations of (S)- but not (R)-methadone and with a greater risk of cardiac side effects and death [88].

4.1.7. Fentanyl
The opioid fentanyl is metabolized via CYP3A4 to norfentanyl, however, CYP3A5 is also a catalyst of fentanyl oxidation [89]. The majority of the Caucasian population is carrier of the *3 allele, thus resulting in lowered CYP3A5 activity. In an examination of fentanyl-related deaths, homozygous CYP3A5*3 individuals showed impaired metabolism of fentanyl if they additionally carried the CYP3A4*1B, especially the homozygous genotype [90]. The authors concluded that genotyping of CYP3A4*1B and CYP3A5*3 may serve as molecular autopsy.

4.2. Ecstasy
3,4-Methylendioxymethamphetamine (MDMA), the main ingredient of ecstasy, is metabolized via CYP2D6 and PMs have been suggested to possibly bear a greater risk of becoming intoxicated by this drug. However, in two small published studies, no PM was found among individuals who died after taking MDMA [91,92]. Nonetheless, caution has to be taken, as misclassification of the genotype might have been possible due to the number of SNPs investigated. In addition to CYP2D6, several other P450 isoenzymes have the capacity to contribute to the oxidative metabolism of MDMAs [93,94].

4.3. Benzodiazepines

In some cases, subjects who have taken prescribed drugs according to a physician’s directions cannot be prosecuted unless it is proven that they had overdosed the medication. For example, benzodiazepines, especially diazepam, commonly found in drugged drivers, are metabolized via CYP2C19. A high P/M ratio between diazepam and desmethyl Diazepam indicates an acute intake. Genotyping may be necessary in specific cases, because a PM who has not deliberately overdosed will also show a high P/M ratio [62].

4.4. Antidepressants and antiepileptics

A 9-year-old boy was treated with methylphenidate, clonidine, and fluoxetine, and over a 10-month period signs and symptoms suggestive for metabolic toxicity were observed. Finally, he suffered a status epilepticus and cardiac arrest. At autopsy, fluoxetine and norfluoxetine were several fold higher than expected and drug intoxication was stated as cause of death. Genetic testing revealed a gene defect at the CYP2D6 locus, which resulted in poor metabolism of fluoxetine and exonerated the suspected adoptive parents [95].

The tricyclic antidepressant amitriptyline is metabolized by the polymorphic CYP2D6 and CYP2C19 [Fig. 6]. In a series of forensic autopsies, Koski et al. [96] found positive correlations between the proportion of hydroxylated metabolites and the number of functional copies of CYP2D6 and between the proportion of demethylated metabolites and the functional copies of CYP2C19. Especially when a suicidal intent is being proven, CYP genotyping was proposed as a useful tool to add valuable information to the interpretation of forensic-toxicological results.

Hair analysis also provided inter-individual variations of amitriptyline metabolism and correlated with CYP2C19 and CYP2D6 polymorphisms [97].

Major pathways of doxepin metabolism involving polymorphic CYP enzymes are described in Fig. 7. In a case of fatal doxepin poisoning with an undetermined manner of death, a completely non-functional CYP2D6 genotype (*3/*4) indicated a total absence of CYP2D6 enzyme and suggested a PM phenotype [98]. The doxepin concentration in blood was 2.4 mg/L (16–80 times higher than considered therapeutic in a living patient), the concentration of nordoxepin 2.9 mg/L, and the doxepin/nordoxepin ratio 0.83, the lowest found among 35 other post-mortem cases analysed during the same year. In 20 doxepin poisonings, a ratio of at least 3.8 (up to 75) was found, with only one case having a ratio as low as 2.0. No alcohol or other drugs were detected in the presented case. The CYP2C19 genotype was determined to be that of an EM. The high concentration of nordoxepin was not consistent with an acute intoxication and it was assumed that the defective genotype contributed to the death, possibly involving repeated high dosage of doxepin. The manner of death was denoted accidental, not suicidal.

A patient developed severe adverse effects and did not experience any therapeutic effect of venlafaxine. Further investigations indicated that the patient was a PM for CYP2D6, the most important phase I enzyme to metabolize venlafaxine [99]. This corroborated that polymorphisms in the CYP450 gene influence the metabolic activity of the corresponding enzymes, thus affecting the subsequent serum drug levels and their metabolites. In another study, fatal venlafaxine poisonings were demonstrated
to be associated with a high prevalence of drug interactions, but the relative CYP2D6 activity did not predispose to high venlafaxine concentrations [100]. Kobylecki et al. [101] also recently found no significant association between substance abuse disorder and suicidal behaviour with the CYP2D6 and CYP2C19 genotypes among patients with schizophrenia genotypes.

Of all the new antiepileptic drugs, only phenytoin undergoes significant metabolism by cytochrome P450 isozymes with significant genetic polymorphisms (CYP2C9, CYP2C19). According to Anderson [102], studies are still needed to identify genetic and biomarkers to identify patients at risk for serious idiosyncratic reactions. Identification of experimental and clinical evidence linking functional changes associated with gene mutations to epilepsy syndromes would help to provide new molecular targets for future antiepileptic drugs.

Clinically relevant pharmacokinetic drug interactions with antidepressants are the object of recent research [103–105] as are pharmacogenetics in analgesia [2,106–108] as well as in other areas of medication. Relevant findings should be taken into consideration in forensic toxicology, where many of these substances are of interest in daily routines.

4.5. Carisoprodol

The centrally-acting skeletal muscle relaxant carisoprodol is metabolized to meprobamate by CYP2C19. In cases of driving under the influence of drugs the carisoprodol-to-meprobamate ratio reflects the number of active CYP2C19 alleles, indicating a gene-dosage effect [109].

5. Conclusion

Pharmacogenetic analysis in forensic settings and especially in post-mortem toxicology may reveal new aspects in daily routines.
Therefore, an approach of molecular analysis including DNA genotyping, together with forensic-toxicological analyses, can be viewed as progress. In post-mortem cases in particular, these approaches, together with macroscopic and microscopic scrutiny, can be of relevance in modern medicolegal investigations. Because other so-called “invisible diseases” like heart and liver abnormalities can also be ascribed to genetic defects, post-mortem genetic testing and the term “molecular autopsy” could find their way into forensic practice [7,110,111].

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