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PITFALLS AND CHALLENGES ASSOCIATED WITH PHENOCONVERSION IN FORENSIC TOXICOLOGY

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Running title: Phenoconversion in forensic toxicology

Abstract

Purpose In recent years, several publications have demonstrated the interest and the usefulness of pharmacogenetics in forensic toxicology. However, this approach remains namely focused on DNA-based phenotype, which may potentially lead to misinterpretation. Other determinants such as co-medication or physiological parameters may also impact the phenotype. This article aims to highlight the importance of considering such determinants in forensic toxicology, through the original case of a heroin-related fatality.

Method Ethanol concentration determination and toxicological screening were performed using gas chromatography with flame ionization detection, liquid chromatography with diode array detection and gas chromatography with mass spectrometry detection. *CYP2C19* and *CYP2D6* genotypes were determined by Taqman® real-time PCR analyses.

Results Femoral blood analyses revealed the presence of ethanol, morphine, codeine, venlafaxine (VEN), O-desmethylvenlafaxine (ODV) and N-desmethylvenlafaxine (NDV), paroxetine, and risperidone. 6-acetylmorphine was also identified in urine. VEN, paroxetine and risperidone were quantified at supra-therapeutic or toxic blood concentrations. NDV was not quantified. The metabolic ratio of VEN (ODV to VEN) was exceptionally low (about 0.7). Pharmacogenetics testing showed that the patient was heterozygous for the *CYP2C19**2 loss-of-function allele, which predict an intermediate metabolism for *CYP2C19*. None of the deficient *CYP2D6* alleles investigated were identified. Those results suggest an extensive *CYP2D6*-metabolism phenotype.

Conclusion A discrepancy was seen between the results of the genomic evaluation and the observed metabolic ratio of VEN. This tends to exclude a genetic origin and lead us to formulate other hypotheses, such as phenoconversion that may have been induced by drug interaction involving patients' regular medications. Phenoconversion is as a complex phenomenon that leads to genotype-phenotype mismatch without any genetic abnormality particularly described for cytochromes P450 2D6 and 2C19. Although transient, phenoconversion can have a significant impact on the analysis and

interpretation of genotype-focused clinical outcomes correlation and in forensic toxicology conclusions.

Keywords: phenoconversion, pharmacogenetics, forensic, personalized medicine

Introduction

Drug metabolism may be influenced by various parameters including enzyme or transporter genetic variants, drug-drug interactions (DDIs), and physiological parameters. Taking into account these different factors allows a better understanding of the variability of the observed clinical response and/or of the measured plasma concentration. This approach is the basis of the development of precision medicine programs [1,2]. Personalized medicine provides more precise tools to physicians to select treatment protocol based on patient, or disease molecular profile which may minimize adverse drug reaction (ADR) and ensure a more successful outcome. This evolution has been driven by the result of several projects including, among other, the Human Genome Project, the HapMap project, 1000genomes, and major technical developments in the field of genomics. It is now routinely applied in therapeutics through pharmacogenetics or pharmacogenomics [3].

Such an integrative approach should also be considered in forensic toxicology in order to avoid any misconception regarding the cause of death (CoD) and/or the manner of death (MoD). In recent years, several publications have demonstrated the interest and the usefulness of pharmacogenetics in order to understand or even to solve several complex lethal intoxication cases [4–6]. Currently, this approach remains namely focused on DNA-based phenotype, which may potentially lead to misinterpretation. Indeed, other determinants such as co-medication or physiological parameters may impact the phenotype and, therein, toxicological conclusions. In this context, this article aims to illustrate the interest of an integrative approach in forensic toxicology through the original case of a heroin-related fatality.

Case presentation

A 35 year-old European Caucasian man was discovered in his home, sitting on his sofa, without any vital signs. He had last been seen alive the day before by his caregiver, a home care nurse. According to anamnestic data collected from the authorities, his medical background consisted of depression and alcohol- and drug-abuse history. His usual treatment included paroxetine 20 mg twice daily, venlafaxine (VEN) extended release 75 mg twice daily, risperidone 2 mg once daily, zopiclone 7.5 mg once daily and acamprosate 363 mg four times daily. This treatment had been initiated several months previously. An autopsy took place the day after the discovery of the corpse. The decedent's height and weight were 188 cm and 77 kg, respectively. The heart weighed 392 g and no macroscopic abnormalities were found. Right and left lungs weighed 843 and 846 g, respectively, and were both edematous and mild congestive. The liver, which presented a slightly fatty aspect, weighed 2377 g. The pancreas weighed 98 g and was congestive. Right and left kidney weighed 176 g and 104 g respectively and were mild congestive. The brain weighed 1395 g with no macroscopic abnormalities. Gastric content was quantified at 0.15 L and no drug residue was found. The rest of the examination was unremarkable and highlighted neither relevant existing diseases nor evidence for ante-mortem violence likely to explain the death. During the autopsy, in order to allow further analysis, several postmortem specimens (including cardiac and femoral blood, bile, gastric content, vitreous humor) were collected in order to establish the cause of death.

Material and method

Toxicological analysis

Chemicals, solvents and reagents

O-desmethylvenlafaxine (ODV) drug standard was purchased from Cerilliant Corporation (Round Rock, TX, USA). VEN and paroxetine drug standards were purchased from Lipomed AG (Arlesheim, Switzerland). Risperidone drug standard was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Prazepam internal standards were bought from LGC (Molsheim, France). N-propanol was bought from Fischer Scientific SA (Illkirch-Graffenstaden, France) whereas Methylmilnacipran (a

non-marketed molecule) was obtained as a gift from the pharmaceutical laboratory Pierre Fabre (Labège, France). All solvents and reagents were of HPLC grade or analytical grade. Milli-Q water (Millipak® Express 40 filter, Merck Millipore [Molsheim, France]) was used throughout the analysis. Drug-free human plasma was obtained from the hospital blood bank (Angers Hospital, France).

Systematic toxicological investigations

Ethanol (EtOH) concentration was determined using gas chromatography with flame ionization detection (GC-FID) (6850, Agilent Technologies, Santa Clara, CA, USA) after precipitation using sodium tungstate and sulfuric acid 1N. The internal standard was n-propanol. The calibration range was 0.1 to 4 g/L.

The determination and quantitation of drugs of abuse (DOA) was yielded using LC-MS/MS (API4000, AB Sciex, Framingham, MA, USA) after protein precipitation using a methanolic solution containing ZnSO₄ and deuterated internal standards at 100 µg/L for opioids, amphetamines and cocaine. After centrifugation, the supernatant was injected. A SPE column (Strata-X, 25 µm, 20×2mm, Phenomenex, Le Pecq, France) was connected to the Kinetex® phenyl-hexyl column (2.6 µm, 100 *3 mm, Phenomenex, Le Pecq, France). The mobile phase consisted in ammonium formate 2 mM, 0.2% formic acid, water (A), ammonium formate 2 mM, 0.2% formic acid, acetonitrile (B) and ammonium formate 2 mM, water (C). For the determination of Δ⁹-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (THC-COOH) concentrations, deuterated standards at 50 µg/L in methanol and acetonitrile+ 0,1% formic acid were added to samples. After centrifugation, the supernatant was half diluted with water-formic acid 1%. A SPE column Kinetex PFP (50 x 2.1 mm, 5 µm, Phenomenex, Le Pecq, France) was connected to the Kinetex C18 column (50 x 2.1 mm - 2.6 µm, Phenomenex, Le Pecq, France). The mobile phase including: water+0.1% formic acid (A1), methanol +0,1% formic acid (B1), water (C1) and acetonitrile-0.2% formic acid (C2).

Comprehensive screenings of general drugs and toxic compounds were performed using two different methods: liquid chromatography with diode array detection (LC-DAD) and gas chromatography with mass spectrometry detection (GC-MS). For LC-DAD analysis, sample preparation was performed using two methods. In the first method, 25 μL of internal standard (prazepam, 20 mg/L in methanol) was added to 500 μL of sample, followed by a liquid/liquid extraction and the addition of 30 μL of sodium hydroxide (1 M) and 5 mL of dichloromethane. After mixing the preparation for 1 min and centrifuging briefly, the non-aqueous (organic) supernatant was evaporated to dryness at 50° C under nitrogen gas. The residue was dissolved in 50 μL of methanol then completed to a total volume of 70 μL with water. In the second method, 25 μL of internal standard (methylmipranpran 20 mg/L in methanol) was added to 500 μL of sample and 100 μL of sodium hydroxide (4 M). Then an extraction using 4 mL of hexane/isoamyl alcohol (80/20, v/v) was performed. After mixing and centrifugation, the organic layer was isolated and mixed with 100 μL of hydrochloric acid (0.02 M). Following vortex mixing and centrifugation, the aqueous layer was kept for injection into the chromatographic system (1100 HPLC system, Agilent Technologies, Les Ulis, France). For both preparation procedures, the injection volume was 10 μL . The chromatographic separation was achieved over 20 minutes run time on an Uptisphere C18 ODB column (5 μm , 100x 2.1 mm) (Agilent Technologies, Les Ulis, France). A spectra UV scan between 210 nm and 400 nm was performed throughout the analytical run. Data acquisition was performed at three wavelengths (210 nm, 230 nm and 254 nm). Products were identified by comparing UV spectra and retention times to a in-house spectrum library (established using authentic standards).

For GC-MS, 50 μL of a 1 mg/mL internal standard (3-(5, 11-dihydro-10, 10-dioxo-11-methyl-dibenzo[c,f]-thiazepinyloxy) tropane maleate or RN927, a non-commercialized antihistaminic molecule) was added to 1 mL of urine sample then extracted using 5 mL of dichloromethane under alkaline conditions (500 μL of carbonate 1 M buffer pH 9.2 and 50 μL of sodium hydroxide 1 M). The organic phase was evaporated at 50°C under nitrogen gas and the residue dissolved in 100 μL of hexane/isopropanol mixture (85/15, v/v). Finally, 2 μL of the dissolved sample was injected (splitless mode) in the GC-MS system (Agilent GC system model *HP* 6890N, Agilent Technologies, Santa

Clara, CA) with a HP5 MS 5% phenylmethyl column (30 m, internal diameter 0.25 mm, 0.25 μ m film) (Agilent Technologies, Les Ulis, France). The injector temperature was set to 275°C. The initial column temperature (70°C) was increased to 210°C at a rate of 30°C/min, and held at 210°C, then ramped up to 300°C at a rate of 20°C/min and finally held at 300°C for 15 min. Helium was used as a carrier gas and delivered at a flow rate of 1.6 mL/min. The mass spectrometer source and quad temperature were set at 230°C, and 150°C, respectively. Acquisition was carried out in a scan mode, for m/z ranging from 35 to 500. Peaks spectrum research was realized in comparison with different spectrum libraries (home-made library, Cayman spectral library, Nist 98 library) and peaks identification was confirmed after authentic standard analysis.

For VEN, and ODV quantitation, 25 μ L of internal standard (prazepam 20 mg/L in methanol) were added to 500 μ L of sample, followed by a liquid/liquid extraction and the addition of 30 μ L of sodium hydroxide (1 M) and 5 mL of dichloromethane. After mixing the preparation for 1 min and centrifuging briefly, the non-aqueous (organic) supernatant was evaporated to dryness at 50° C under nitrogen gas. The residue was dissolved in 50 μ L of methanol then completed to a total volume of 70 μ L with water. The injection volume was 10 μ L and the analysis time was 20 minutes using a HP1100 HPLC system (Agilent Technologies, Les Ulis, France). The column was an Uptisphere C18 ODB column (5 μ m, 100x 2.1 mm) (Agilent Technologies, Les Ulis, France). Quantification was performed at 230 nm. Retention time of VEN and ODV were 7.1 and 4.3 min, respectively.

For risperidone and paroxetine quantitation, 25 μ L of internal standard (methylnilnacipran 20 mg/L in methanol) were added to 500 μ L of sample then 100 μ L of sodium hydroxide (4 M). Then an extraction using 4 mL of hexane/isoamylic alcohol (80/20, v/v) was performed. After mixing the preparation, 100 μ L of hydrochloric acid (0.02 M) was added to the organic phase. After centrifugation and elimination of the supernatant, the preparation was injected. The injection volume was 10 μ L and the analysis time was 20 minutes using a HP1100 HPLC system (Agilent Technologies, Les Ulis, France). The column was an Uptisphere C18 ODB column (5 μ m, 100x 2.1 mm) (Agilent Technologies, Les Ulis, France). Quantification was performed at 230 nm. Retention time of risperidone and paroxetine were 8.2 and 9.5 min, respectively.

Validation of quantification methods was based on the Food and Drug Administration (FDA) guidelines for bioanalytical method validation [7] (**Table 1**). Toxicological screening methods were validated according to SFBC-SFTA recommendations [8].

Pharmacogenetics testing

Genomic DNA was isolated from femoral blood (collected in EDTA using an automated procedure performed on a Maxwell® 16 Instrument using dedicated Blood DNA Purification Kit (Promega, Charbonnières-les-Bains, France). DNA quantity and purity were estimated using spectrophotometry on a nanodrop 2000c® (Thermo Scientific™, Saint-Herblain, France). The list of variants genotyped is presented in **Table 2**. Taqman® real-time PCR discrimination assays (Life Technologies, Villebon sur Yvette, France) were performed using 10 ng of DNA and the Type-it Fast SNP Probe PCR Kit (Qiagen, Courtaboeuf, France) using the ABI 7500 real-time PCR system for *CYP2C19* (Life Technologies, Villebon sur Yvette, France) or Rotorgene Q instrument for *CYP2D6* (Qiagen, Courtaboeuf, France).

Results

Toxicological analysis

Toxicological analyses were performed on femoral blood, urine and gastric content. Femoral blood analyses revealed the presence of ethanol, morphine, codeine, VEN, ODV and NDV, paroxetine, and risperidone. Blood drug and toxic concentrations are reported in **Table 3**. Urine analyses highlighted the presence of ethanol, morphine, 6-acetylmorphine (a heroin metabolite), codeine, VEN, ODV and NDV, paroxetine and risperidone. VEN and paroxetine were also detected and quantified in gastric content (volume 0.15 L) at concentrations of: 35 mg/L (equivalent quantity 5.25 mg) and 0.8 mg/L (equivalent quantity 0.12 mg), respectively. NDV was not quantified. Risperidone metabolite (9-hydroxyrisperidone) was not detected (LOD of the analytical method was 10 µg/L). Otherwise,

paroxetine metabolites were not screened as paroxetine is extensively metabolized in several polar and conjugated products that do not contribute to the pharmacological response [9].

Pharmacogenetics testing

The analysis showed that the patient was heterozygous for the *CYP2C19**2 loss-of-function allele, which predict an intermediate metabolism for *CYP2C19*. None of the deficient *CYP2D6* alleles investigated were identified. The patient was heterozygous for *CYP2D6**2, a common allele considered to be in the same normal metabolizer category as *CYP2D6**1 reference allele [10]. Those results suggest an extensive *CYP2D6*-metabolism phenotype.

Discussion

In forensic practice, considering analysis results without taking into account other findings such as medical history or autopsy findings appears to carry a degree of risk. This paradigm is perfectly illustrated by this case. In fact, in addition to the detection of morphine, 6-acetylmorphine and ethanol, several other substances including VEN, paroxetine, and risperidone, have been quantified at supra-therapeutic or toxic blood concentrations, and were also considered as potential contributing factor to death [11-12].

According to literature, expected therapeutic plasma concentrations range from 60 µg/L to 400 µg/L for VEN, from 100 µg/L to 400 µg/L for ODV, from 20 µg/L to 60 µg/L for risperidone, and from 2 µg/L to 65 µg/L for paroxetine respectively [11-12]. In this case, considering the dose regimen, parent's drugs and metabolites concentrations can be considered to have reached their steady-state. Hence, the fluctuations of drug concentrations are minimized. Thus, at first glance, it may appear that these findings are in agreement with massive drug intoxication and so, support the conclusion of an overdose. However, several arguments were not in accordance with this hypothesis. Indeed, the psychiatrist indicated clearly that the decedent did not have any further access to his medication

beyond the daily dosages prescribed which were delivered each day by a caregiver. The last patient's regular medication administration was about 24 hours before the corpse's discovery. In addition, and despite the limits related to this matrix [13], the drug concentrations measured in the gastric content appeared too low to confirm the hypothesis of a massive ingestion of his regular medication. Otherwise, no drug residue was found into the gastric content. Finally, and in our opinion the most relevant element, the ratio of ODV to VEN, which correspond to the metabolic ratio of VEN, was exceptionally low (~ 0.7) compared to the expected ratio in an individual with a normal metabolic profile [14,15]. The metabolic ratio corresponds to the ratio of unchanged drug to metabolite [16] and reflects the enzyme activity. In this case, this abnormality may be related to a genetic variant of the enzyme that may affect the function [14,15].

VEN is a serotonin and noradrenaline reuptake inhibitor (SNRI) mainly prescribed for the treatment of depression [17]. This drug is associated with a number of adverse effects that are generally mild, including tachycardia or increased blood pressure. However, severe adverse effects such as arrhythmia or seizure have been observed in the context of high doses of VEN. Several VEN related fatalities have been reported [18]. VEN is mainly eliminated through hepatic metabolism mediated by the CYP enzyme system. The major elimination pathway for VEN appears to be O-demethylation to ODV via CYP2D6. VEN is also O-demethylated into ODV by CYP2C9 and CYP2C19 and N-demethylated into NDV by CYP3A4, CYP2C19 and CYP2C9. ODV and NDV are then further N-demethylated and O-demethylated into N,O-didesmethylvenlafaxine (DDV) by CYP2C19 and CYP2D6, respectively [15,17,19]. VEN metabolism is characterized by huge inter-individual variability, mainly of genetic origin [15,19]. In fact, CYP2D6 and CYP2C19 exhibit an important polymorphic genetic variability which appear correlated to the metabolic capacities [20]. Such a correlation has been described for several drugs including tramadol and VEN in postmortem blood [14,15,21]. In addition, Jornil *et al*, by using a preliminary model for simulating VEN and ODV elimination, reported simulated ratio values between ODV and VEN for virtual populations with different CYP2C19 and 2CYP2D6 genetically determined metabolic profiles. Thus, for both CYP2C19 and CYP2D6, extensive (EM) and poor metabolizer (PM) combination were simulated [15]. The expected median ODV/VEN ratio were

3.1 (0.53-23), 2.7 (0.46-23), 0.3 (0.06-3.0) and 0.03 (0.005-0.13) for CYP2C19 EM/CYP2D6 EM, CYP2C19 PM/CYP2D6 EM, CYP2C19 EM/CYP2D6 PM, and CYP2C19 PM/CYP2D6 PM, respectively [15]. In this case, the metabolic ratio was strongly suggestive of a CYP2C19 EM/CYP2D6 PM or CYP2C19 PM/CYP2D6 PM individual. All these elements, despite the very likely hypothesis that the death was related to heroin and alcohol abuse, led us to perform pharmacogenetics testing in order to clarify the causes of such a ratio. In this case, the decedent had a CYP2C19 genotype corresponding to an intermediate metabolizer (*CYP2C19**2 heterozygous). None of the CYP2D6 loss-of-function alleles investigated was found, suggesting a normal CYP2D6 activity. In particular, *CYP2D6**4 was absent. Of the non-functional CYP2D6 allele, *CYP2D6**4 is the most frequent (among CYP2D6 PM, approximately 75% carry this allele) [7]. The other alleles investigated are less frequently reported. A major limitation is that CYP2D6 copy number variations (CNV), especially gene deletion, were not investigated here, due to the lack of genetic material. Indeed, most analytical methods designed to determine CYP2D6 CNV require relatively large amount of high quality DNA, which may be difficult to obtain from postmortem blood, as in the reported case. The time between death and blood collection as well as the storage conditions of the deceased body may influence the quality and the quantity of DNA available in whole blood collected during autopsy. This issue also concerns single nucleotide polymorphism genotyping but is particularly marked for CNV determination [22]. However, considering the ethnicity of the decedent (European Caucasian), it is unlikely that a deletion of CYP2D6 would have been found here. Indeed, according to the Pharmacogenomic Knowledge Base (PharmGKB), the *CYP2D6**5 allele frequency and the *CYP2D6**5/*5 diplotype frequency are estimated to be 2.95% and 0.087% in this population, respectively [23]. Thus, although *CYP2D6**4 is the most frequent allele associated with CYP2D6 PM Status, we cannot formally exclude the presence of *CYP2D6**5. Overall, here, pharmacogenetics testing strongly suggests a CYP2C19IM/CYP2D6 EM metabolic status, instead of a CYP2C19 EM/CYP2D6 PM or CYP2C19 PM/CYP2D6 PM metabolic status, as expected.

A discrepancy was seen between the results of the genomic evaluation and the observed metabolic ratio of VEN. This tends to exclude a genetic origin and leads us to formulate other hypotheses such as hepatic disease or DDIs [1,2]. DDIs represent a common clinical problem during the management of patients treated with several drugs and are one of the commonest causes of ADRs [24]. In the case reported here, pharmacokinetic DDIs appear as the most probable hypothesis. Paroxetine and risperidone are both metabolized by CYP2D6 and act as potent -paroxetine- or moderate -risperidone- CYP2D6 inhibitors [25,26]. According to the literature, paroxetine has a high inhibitory interaction profile with CYP2D6 substrates and is frequently associated with serious DDIs and ADRs [27]. Furthermore, according to DDI prediction models [28], paroxetine has a significant impact on VEN pharmacokinetics (prediction of 3.88-fold change in the VEN area under the curve (AUC) (i.e. the definite integral of a curve that describes the variation of a drug concentration in blood as a function of time). Likewise, paroxetine has also a significant impact on risperidone pharmacokinetics (prediction of 3.88-fold change in risperidone AUC also). This non-genetic variability refers to phenoconversion which can lead to a genotype-phenotype mismatch without any genetic abnormality [1,2]. Although transient, this phenomenon has a significant impact on the analysis and interpretation of genotype-focused clinical outcomes correlation study and can also disrupt personalizing therapy in clinical practice. This has been particularly described for cytochromes P450 2D6 and 2C19 [1,2]. Co-medication appears to be one major cause of phenoconversion and the concomitant use of multiple medications significantly increase the likelihood of DDI [29,30]. In fact, administration of therapeutically used drugs can inhibit a DME, mimicking the genetic defect and producing an acquired form of impaired drug metabolism, or conversely induce the DME and give rise to an EM or UM phenocopy normally associated with heritable traits. Most of the time, this phenomenon converts genotypic EMs of a DME to PMs of that DME. DDI do not appear as the only cause of phenoconversion. In fact, phenoconversion has been also documented in inflammatory conditions associated with elevated cytokine, such as human immunodeficiency virus infection, cancer, and liver disease [1,2,31].

Conclusion

This observation emphasizes the importance of a complete phenotype/genotype evaluation in forensic toxicology. Despite the limitation due to the absence of CYP2D6*5 analysis, the discrepancy between the pharmacogenetics testing (suggesting a CYP2C19IM/CYP2D6 EM metabolic status) and the phenotype evaluation (suggesting a CYP2C19 EM/CYP2D6 PM or CYP2C19 PM/CYP2D6 PM metabolic status) led us to conclude to a phenoconversion phenomenon. In order to correctly understand and better interpret toxicological analytical results and so, avoid misconception, such phenomenon has to be considered in forensic toxicology. This is of particularly great importance considering the fact that in our current practice we are increasingly confronted by problematic poisoning cases involving many different regular drugs. Most of the time, these cases concern patients with a long-term psychotropic use history and involve substances with common toxic patterns and/or using the same metabolic pathways, such as antidepressant and/or antipsychotic drugs. Beyond the postmortem toxicological issues, this raises questions about the place given to precision medicine in psychiatric medicine nowadays. In fact, although the importance of the concept of precision medicine is perfectly accepted in other medical areas such as oncology where its use has become commonplace, its place in psychiatric medicine remains unclear and has to be better defined in the best interests of the patient.

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Tables captions:

Table.1: within- and between-run precision (CV%) and accuracy (%) for the analytes in spiked human plasma samples.

Table.2: List of genotyped variants

Table.3: Femoral blood concentrations of identified compounds

Table 1: within- and between-run precision (CV%) and accuracy (%) for the analytes in spiked human plasma samples.

Analyte	Within-run (n=6)				Between-run (n=6)		
	Nominal concentration	Measured concentration	Accuracy%	CV%	Measured concentration (ng/ml)	Accuracy%	CV%
Venlafaxine	250 µg/L	240 µg/L	96	6.3	245 µg/L	98	4.9
	500 µg/L	486 µg/L	97	2.1	495 µg/L	99	5.1
	800 µg/L	778 µg/L	97	3.8	773 µg/L	97	3.5
O-desmethylvenlafaxine	200 µg/L	194 µg/L	97	3.5	202 µg/L	101	2.5
	400 µg/L	428 µg/L	107	3.3	431 µg/L	108	3.9
	800 µg/L	857 µg/L	107	1.8	834 µg/L	104	3.6
Risperidone	250 µg/L	250 µg/L	100	10	250 µg/L	100	8.4
	500 µg/L	520 µg/L	104	8.4	510 µg/L	102	4.7
	1000 µg/L	1060 µg/L	106	4.7	1070 µg/L	107	2.8
Paroxetine	250 µg/L	247 µg/L	99	9.7	260 µg/L	104	8.4
	500 µg/L	517 µg/L	103	10.4	508 µg/L	102	8.1
	800 µg/L	813 µg/L	102	4.9	810 µg/L	101	5.4
Morphine	10 µg/L	9.6 µg/L	96	7.9	10.2 µg/L	102	10.9
	70 µg/L	71.4 µg/L	102	6.4	70.6 µg/L	101	3.7
	180 µg/L	181.8 µg/L	101	3.3	181.0 µg/L	101	2.4
Codeine	10 µg/L	9.9 µg/L	99	8.8	10.5 µg/L	105	4.8
	70 µg/L	70.8 µg/L	101	6.5	69.4 µg/L	99	5.1
	180 µg/L	181.2 µg/L	101	5.6	179.5 µg/L	100	5.9
Ethanol	0.5 g/L	0.5 g/L	100	1.7	0.5 g/L	100	3.7
	1.0 g/L	1.1 g/L	110	5.2	1.1 g/L	110	2.1
	3.0 g/L	3.1 g/L	103	0.9	3.2 g/L	107	2.9

Table 1 List of genotyped variants

Gene symbol	dbSNP reference* (variant allele)	Consequence**
<i>CYP2C19</i>	rs4244285 (<i>CYP2C19</i> *2)	No function
	rs4986893 (<i>CYP2C19</i> *3)	No function
	rs12248560 (<i>CYP2C19</i> *17)	Increased function
<i>CYP2D6</i>	rs16947 (<i>CYP2D6</i> *2)	Normal function
	rs35742686 (<i>CYP2D6</i> *3)	No function
	rs3892097 (<i>CYP2D6</i> *4)	No function
	rs5030655 (<i>CYP2D6</i> *6)	No function
	rs1065852 (<i>CYP2D6</i> *10)	Decreased function

*: <https://www.ncbi.nlm.nih.gov/snp/>; **: <https://www.pharmvar.org/>

Table 1 Femoral blood concentrations of identified compounds

Medication/metabolite	concentration
Venlafaxine (VEN)	2900 µg/L
O-desmethylvenlafaxine (ODV)	200 µg/L
N-desmethylvenlafaxine (NDV)	<i>Detected but not quantified</i>
Risperidone	100 µg/L
Paroxetine	1150 µg/L
Morphine	111 µg/L
Codeine	12 µg/L
Ethanol	2.75 g/L