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Influence of *CYP2D6* genotype on the disposition of the enantiomers of venlafaxine and its major metabolites in postmortem femoral blood

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ABSTRACT

Venlafaxine (VEN) is an antidepressant drug mainly metabolized by the cytochrome P450 (CYP) enzyme CYP2D6 to the active metabolite O-desmethylvenlafaxine (ODV). VEN is also metabolized to N-desmethylvenlafaxine (NDV) via CYP3A4. ODV and NDV are further metabolized to N,O-didesmethylvenlafaxine (DDV). VEN is a racemic mixture of the S- and R-enantiomers and these have in vitro displayed different degrees of serotonin and noradrenaline reuptake inhibition. The aim of the study was to investigate if an enantioselective analysis of VEN and its metabolites, in combination with genotyping for *CYP2D6*, could assist in the interpretation of forensic toxicological results in cases with different causes of deaths. Concentrations of the enantiomers of VEN and metabolites were determined in femoral blood obtained from 56 autopsy cases with different causes of death. The drug analysis was done by liquid chromatography tandem mass spectrometry (LC/MS/MS) and the *CYP2D6* genotyping by PCR and pyrosequencing. The mean (median) enantiomeric S/R ratios of VEN, ODV, NDV and DDV were 0.99 (0.91), 2.17 (0.93), 0.92 (0.86) and 1.08 (1.03), respectively. However, a substantial variation in the relationship between the S- and R-enantiomers of VEN and metabolites was evident (S/R ratios ranging from 0.23-17.6). In six cases, a low S/R VEN ratio (mean 0.5) was associated with a high S/R ODV ratio (mean 11.9). Genotyping showed that these individuals carried two inactive *CYP2D6* genes indicating a poor metabolizer phenotype. From these data we conclude that enantioselective analysis of VEN and ODV can predict if a person is a poor metabolizer genotype/phenotype for CYP2D6. Knowledge of the relationship between the S- and R-enantiomers of this antidepressant drug and its active metabolite is also important since the enantiomers display different pharmacodynamic profiles.

Keywords: CYP2D6, Enantiomers, Forensic toxicology, Postmortem toxicology, Venlafaxine

1. Introduction

Venlafaxine (VEN) is an antidepressant drug belonging to the pharmacodynamic class of dual serotonin and noradrenaline reuptake inhibitors (SNRIs) [1]. VEN is administered as a racemic mixture (50:50) of the S- and R-enantiomers. Both enantiomers are pharmacologically active but the S-enantiomer inhibits only serotonin reuptake, while the R-enantiomer inhibits both serotonin and noradrenaline reuptake [1]. In vitro studies have shown that VEN is extensively metabolized via the cytochrome P450 (CYP) enzymes. CYP2D6 catalyzes the metabolism of VEN to its main metabolite O-desmethylvenlafaxine (ODV) and CYP3A4 is the major enzyme responsible for formation of the minor metabolite N-desmethylvenlafaxine (NDV). Both metabolites are then further metabolized to N,O-didesmethylvenlafaxine (DDV), possibly by CYP2D6 [2,3]. ODV contributes to the overall pharmacological effect of VEN since it exhibits a pharmacological profile similar to that of VEN. It has been shown that CYP2D6 displays stereoselectivity towards the R-enantiomer [4].

Inter-individual variability in the activity of the CYP enzymes has clinical and toxicological implications for drug response. CYP2D6 is one of the most important enzymes involved in the metabolism of centrally acting drugs including antidepressants. The *CYP2D6* gene is highly polymorphic with more than 70 different alleles reported (<http://www.cypalleles.ki.se>). Most of them are rare and associated with a decrease or absence of CYP2D6 activity. An individual carrying a genotype with no active allele is in concordance with the poor metabolizer (PM) phenotype. The lack of functional activity of CYP2D6 may lead to an impaired metabolism of many drugs, such as VEN, resulting in high plasma drug concentrations, lack of therapeutic response and/or an increased risk for side-effects. Another risk group is individuals who have a substantially increased metabolism, e.g. due to more than two functional copies of the *CYP2D6* gene, which can lead to therapeutic failure. These so called ultra-rapid metabolizers (UMs) may require higher doses than usual to reach therapeutic drug levels [5].

Many of the newer generation of antidepressants, including SNRIs and selective serotonin reuptake inhibitors (SSRIs), have been shown to be safer in overdose as compared to the older tricyclic antidepressants [6]. Overdoses with VEN have been associated with several adverse effects such as sedation, tachycardia, seizures, hypotension, hypertension and serotonin syndrome [7-13] and

fatal overdoses have been reported for VEN alone or in combination with other compounds [14,15]. VEN has also been shown to have a relatively higher toxicity compared to the SSRIs [10,13,16-18]. Further, it has been suggested that VEN may be more toxic in PMs of CYP2D6 [19-21]. Consequently, therapeutic doses to such individuals may result in drug concentrations similar to those found in overdoses, especially if also concomitant drugs with CYP enzyme inhibition properties are taken.

Interpretation of postmortem toxicological results is not always an easy task due to a number of pitfalls [22-24]. For many substances there is no sharp dividing line between toxic and lethal drug concentrations [25]. When a drug exists as a racemic mixture the picture is even more complicated [26-28]. One strategy to attack this problem is to perform a drug analysis that can separate between the different enantiomers. We have recently developed such a method for the determination of VEN and its three major demethylated metabolites in plasma and whole blood [29]. In addition, postmortem genotyping and the analysis of metabolite/parent drug ratios have been suggested to provide insights for the interpretation of forensic toxicological results [30-33]. Thus, the aim of the present study was to scrutinize possible relations between the *CYP2D6* genotype and the disposition of the enantiomers of VEN and its metabolites in femoral blood from forensic autopsy cases.

2. Materials and methods

2.1. Experimental design

The included cases were selected from all forensic autopsies that were performed during 2008 in Sweden. Specimens of blood, urine and other biological material were collected in 5,103 cases and sent to one central laboratory for forensic toxicology. In a broad drug screening analysis, 92 of all cases were found to be positive for VEN. Femoral blood was available in 86 cases. Blood was collected in tubes containing potassium fluoride as preservative (1-2%, v/v). After exclusion of badly decomposed samples and cases with too little blood available for drug analysis 66 cases were remaining. Enantioselective analysis of VEN and its metabolites, along with genotyping for *CYP2D6*, were performed in femoral blood from the selected 66 cases (for details, see below). Seven of the cases were positive for citalopram in the previous drug screening and these were re-analyzed with an

enantioselective HPLC method as described previously [34]. Information related to each of the cases (age, gender, other found drugs, cause and manner of death) was provided from the forensic pathology and toxicology databases at the Swedish National Board of Forensic Medicine. The study was approved by the Regional Ethical Review Board in Linköping, Sweden (No. M87-08).

2.2. Toxicological analysis

A wide range of basic, neutral and acidic prescription drugs were determined in femoral blood by using capillary gas chromatography (GC) fitted with a nitrogen-phosphorus detector [35]. Ethanol was determined in blood by head-space GC according to a previously described method [36]. Urine and/or blood samples were also analyzed for different classes of illicit drugs (opiates, cannabis, amphetamines, cocaine and gamma-hydroxybutyrate) after a direct request by the responsible forensic pathologist.

2.3. Enantioselective determination of venlafaxine and its metabolites

The concentrations of the S- and R-enantiomers of VEN and its metabolites ODV, NDV and DDV in postmortem femoral blood were determined by using a liquid chromatography tandem mass spectrometry (LC/MS/MS) method as described previously [29]. The blood samples were pre-treated by solid-phase extraction using Isolute C8 columns 100 mg (International Sorbent Technology Ltd). Briefly, the columns were activated with 1 ml methanol and 1 ml ultrapure water. A mixture of 0.2 ml blood sample, 3 ml ultrapure water and 20 µl internal standard (mexiletine, 0.5 mM) was added to the columns. The columns were washed with 1 ml ultrapure water, followed by 2 ml methanol:ultrapure water (50:50; v/v) and 2 ml acetonitrile. After elution with 1.5 ml acetonitrile with 10 mM trifluoric acid and evaporation with nitrogen at 50°C, the analytes were reconstituted in 100 µl mobile phase (tetrahydrofuran:ammonium acetate, 10 mM, pH 6; 10:90 v/v) and 2 µl was injected.

The LC/MS/MS system consisted of an Acquity liquid chromatography system (Waters) and an API 4000 tandem quadrupole instrument equipped with an electrospray interface (TURBO V™ source, TurbolonSpray® probe) operating in positive ion mode (Applied Biosystem/MSD Sciex). Chromatographic separation was performed on a Chirobiotic-V column (5 µm particle size, 250 x 2.1

mm; Astec/Supelco) protected with a 5 µm in-line filter (VICI AB Int.) at a mobile phase flow rate of 0.2 mL/min. The column was kept at 10°C using a Jones Chromatography Model 7955 column chiller/heater. Multiple reaction monitoring (MRM) of the most abundant transitions originating from product ions of the protonated molecular ions was used for quantification. The data were processed using Analyst 1.4.2 software (Applied Biosystem/MSD Sciex). Calibration curves were in the range of 0.003-1.2 µg/g (for each enantiomer of VEN and ODV; 10-4000 nM) and 0.001-0.55 µg/g (for each enantiomer of NDV and DDV; 5-2000 nM). The lower limit of quantification was 0.14 ng/g (0.5 nM) for the enantiomers of VEN, 0.13 ng/g (0.5 nM) for the enantiomers of ODV, 0.07 ng/g (0.25 nM) for the enantiomers of NDV and 0.06 ng/g (0.25 nM) for the enantiomers of DDV.

2.4. Genotyping for *CYP2D6*

Genomic DNA was extracted using King Fischer ML (Thermo Scientific, Vantaa, Finland). Extracted DNA was stored frozen in -20° C until analyzed. Three single nucleotide polymorphisms (SNPs), *3, *4 and *6, were included in the genotyping of *CYP2D6* (Table 1). These alleles were identified by PCR and pyrosequencing as earlier described [37] with some minor modifications [38]. The copy number variation (CNV) of *CYP2D6* was also determined to identify carriers of whole gene deletion (*CYP2D6**5) or multiple gene copies (*CYP2D6**N). For determination of the gene copy number, the pseudogene *CYP2D8* was co-amplified with the *CYP2D6* gene in the PCR as described previously [39] with some minor modifications [38]. Identification of the genes that was multiplied in samples with more than two gene copies was determined using peak pattern recognition of programs from the SNP analysis [38].

The *CYP2D6* genotypes were assigned based on the alleles identified. Alleles not carrying any of the determined polymorphisms were classified as *1 (*wild-type*). The outcomes of the genotype analysis were categorized into three groups: individuals carrying no active gene (i.e. carrier of only the *3, *4, *5 or *6 alleles, also known as poor metabolizers, PMs), individuals carrying one active gene (i.e carrier of *1 in combination with one of the alleles *3, *4, *5 or *6, also known as intermediate metabolizers, IMs) and individuals with two active genes (i.e. carrier of two *1 alleles, also known as extensive metabolizers, EMs).

2.5. Statistical analysis

Descriptive statistics of mean, median and range were used to describe the data. Mean and standard deviation (SD) was calculated for the enantiomeric (S/R) concentration ratios and the metabolite/parent drug (M/P) concentration ratios. Statistical comparisons between the groups were analyzed by the Mann-Whitney test. A *P*-value less than 0.05 were considered statistically significant. All statistical analyses were performed using StatView® (SAS® Institute, Cary, NC, version 5.0).

3. Results

3.1. General results

Ten out of the selected 66 cases were excluded due to problems associated with the drug analysis and genotyping. The reason for this exclusion was either badly decomposed samples or extremely high VEN levels resulting in uncertain drug quantifications. The final study material of 56 cases comprised 18 women (32%) and 38 men (68%) with ages ranging between 19 and 86 years (mean age 48.7 ± 14.3 years). Analytical results, including total (S+R) concentrations of VEN and main metabolites as well as S/R-ratios, together with age, sex, cause and manner of death, other analytical findings and genotype results are presented in Table 2. The 56 cases were rank ordered after the highest concentration of VEN in blood. Fifty-two different drugs were detected (mean number of drugs 3.6 ± 2.5 ; range 1-11). In 10 cases (18%), VEN was the only drug identified and none of these cases was classified as intoxication. Most frequently found prescription drugs in addition to VEN were zopiclone ($n=12$), mirtazapine ($n=10$), alimemazine ($n=7$), diazepam ($n=7$), propiomazine/dihydropropiomazine ($n=7$) and citalopram ($n=5$). Ethanol was found in 26 cases (46%). According to the forensic pathologist, the death was related to intoxication (suicide, accident or undetermined) in 17 cases (30%). Twenty-two cases (39%) were classified as suicides not associated with drug intoxication and 17 of these were hangings (30%). The manner of death was natural in 9 cases (16%) and the remainder ($n=8$, 14%) were classified as accidents or undetermined.

3.2. Enantioselective analysis of venlafaxine and CYP2D6 genotyping

The results from the enantioselective analysis of VEN and the *CYP2D6* genotyping are presented in Table 2. For all 56 cases the mean (median) S/R ratios of VEN, ODV, NDV and DDV were 0.99 (0.91), 2.17 (0.93), 0.92 (0.86) and 1.08 (1.03), respectively. In Fig. 1 and 2 the included cases were divided into three groups related to *CYP2D6* genotype: cases with 2 active genes (EM, $n=26$), cases with 1 active allele (IM, $n=24$) and cases without any active alleles (PM, $n=6$). No cases with more than 2 active genes (UM) were found. When the results were divided into these groups (0, 1, and 2 active *CYP2D6* genes, respectively), the mean S/R ratios for VEN were 0.5 ± 0.17 , 1.07 ± 0.67 and 1.01 ± 0.34 (0 vs. 1 $p<0.01$; 0 vs. 2 $p<0.01$), the mean S/R ratios for ODV were 11.9 ± 4.37 , 1.27 ± 1.12 and 0.99 ± 0.36 (0 vs. 1 $p<0.001$; 0 vs. 2 $p<0.001$), the mean S/R ratios for NDV were 0.51 ± 0.03 , 0.77 ± 0.26 and 1.12 ± 0.55 (0 vs. 1 $p<0.05$; 0 vs. 2 $p<0.01$; 1 vs. 2 $p<0.05$), and the mean S/R ratios for DDV 1.78 ± 0.43 , 1.0 ± 0.33 and 1.02 ± 0.27 (0 vs. 1 $p<0.01$; 0 vs. 2 $p<0.01$) (Fig. 1). The mean M/P concentration ratios for the cases with 0 active genes were 0.23 ± 0.13 for ODV/VEN, 0.74 ± 0.46 for NDV/VEN and 0.14 ± 0.14 for DDV/VEN (Fig. 2). The mean M/P ratios for the groups with 1 and 2 active genes were 1.26 ± 1.35 and 2.01 ± 1.36 for ODV/VEN, 0.21 ± 0.20 and 0.22 ± 0.17 for NDV/VEN, and 0.25 ± 0.29 and 0.42 ± 0.44 for DDV/VEN, respectively.

The mean (median) concentrations of VEN, ODV, NDV and DDV in all 56 cases were 0.93 (0.60), 0.65 (0.52), 0.24 (0.08) and 0.14 (0.11) $\mu\text{g/g}$, respectively. In five cases (9, 13, 31, 32 and 44) ODV could not be analyzed due to interference of tramadol in the chromatogram. In case 47 NDV could not be detected. When dividing the cases according to the strategy originally presented by Druid and Holmgren [35] 16 cases were classified as Group B cases (certified death by intoxication with more than one drug) and 26 cases were classified as Group C cases (certified other cause of death, in which the circumstances excluded the possibility of incapacitation by drugs). The mean (median) concentration of VEN in B and C cases were 1.31 (1.0) and 0.78 (0.59) $\mu\text{g/g}$. For the main metabolite ODV, the mean (median) concentrations were 0.61 (0.68) and 0.56 (0.52) $\mu\text{g/g}$, respectively. None of the cases were considered as single drug intoxications with VEN (i.e. Group A cases [35]).

3.3. Enantioselective analysis of citalopram

The seven cases containing citalopram were re-analyzed with an enantioselective HPLC method. Three cases were excluded due to reasons explained in Section 3.1. The total (S+R) mean concentrations of citalopram in femoral blood in the remaining four cases were $1.2 \pm 0.84 \mu\text{g/g}$ (range 0.11-3.7 $\mu\text{g/g}$). The mean concentrations of S- and R-citalopram were $0.66 \pm 0.41 \mu\text{g/g}$ (range 0.06-1.9 $\mu\text{g/g}$) and $0.72 \pm 0.56 \mu\text{g/g}$ (range 0.05-1.8 $\mu\text{g/g}$), respectively. In case 11 no detectable levels of R-citalopram was found indicating intake of the pure S-enantiomer of citalopram (i.e. escitalopram).

4. Discussion

The present novel study reports how the *CYP2D6* genotype influences the enantiomeric disposition of VEN, and its three main metabolites, in femoral blood from forensic autopsy cases. A substantial variation in the relationship between the S-and R-enantiomers of parent drug and metabolites was evident with S/R ratios ranging from 0.23 to 17.6. In six cases, a low S/R VEN ratio (mean 0.5) was associated with a high S/R ODV ratio (mean 11.9). Genotyping showed that these individuals carried two inactive *CYP2D6* genes. From these data we conclude that enantioselective analysis of VEN and ODV can predict if a person is a PM genotype/phenotype for *CYP2D6*.

The presented mean S/R ratios of 0.99, 2.17 and 0.92 for VEN, ODV and NDV, respectively, are in accordance with previously published results from clinical studies. Five patients receiving individually adapted VEN doses between 75-225 mg/day displayed after three weeks treatment mean plasma S/R ratios of 1.16, 0.94 and 1.01 for VEN, ODV and NDV, respectively, and all included patients were EMs for *CYP2D6* [40]. Wang and co-workers [41] reported a mean plasma S/R ratio of 1.3 following administration of 50 mg of VEN to nine human subjects. Eap and co-workers [42] have published a case report with a *CYP2D6* EM patient displaying a serum S/R VEN ratio of 1.9 and a S/R ODV ratio of 0.81. Gex-Fabry et al. [43,44] investigated 35 patients on chronic medication with VEN and they reported S/R ratios between 0.4-2.5 (median 1.1) for VEN and 0.7-16.4 (median 0.97) for ODV. In that study, the patients were not genotyped but with our data in mind it is plausible that the patient with a high S/R ODV ratio of 16.4 had a low S/R VEN ratio and was a PM genotype/phenotype for *CYP2D6*. To compare forensic data with clinical data is difficult; information

about ingested dose and the time of sampling in relation to the time when the drug was taken are rarely known. Further, the possibility that VEN undergoes postmortem drug redistribution has to be considered [45]. However, since this process mainly depends on passive drug release from drug reservoirs (e.g. lungs and liver) it seems reasonable to assume that no major differences exist in the redistribution of the VEN enantiomers. Moreover, studies in an animal model have evidenced an equal postmortem redistribution of the enantiomers of citalopram, also a basic lipophilic antidepressant, and its metabolites [46,47].

According to several studies VEN has a relatively higher fatal toxicity compared to SSRIs and other newer antidepressants [10,13,16,17,48-50]. Different explanations to this finding have been suggested. Launiainen et al. [50] recently reported that fatal VEN poisonings were associated with a high prevalence of drug interactions. Another explanation might be possible differences in prescribing practice of VEN compared with SSRIs. VEN may be more often prescribed to patients with prior prescriptions of another antidepressant drug [51,52]. With regard to VEN toxicity, possible physiological drug effects also have to be considered. Decreased CYP2D6 activity has been associated with cardiovascular toxicity following VEN treatment [20]. One cannot exclude a possible link between this finding and the observed differences in enantiomeric disposition of VEN and metabolites in EMs and PMs. As mentioned above, the VEN enantiomers display somewhat different activities on serotonin and noradrenalin reuptake [1]. For ODV, NDV and DDV, only few data have been published regarding the pharmacological profiles. To our knowledge, no data are available describing the effects of the separate enantiomers of the metabolites. Such information would be of great interest to further elucidate potentially toxic effects after VEN ingestion.

The high number of fatalities associated with VEN underscores the importance of correct interpretation of toxicological results in forensic cases. Postmortem concentrations of VEN have been relatively well characterized previously and the VEN levels in our study (mean 0.93 µg/g, median 0.6 µg/g) are within the range reported by others [45,49,50,53]. A median VEN concentration of 1.05 mg/L (range 0.1-5.5 mg/L) was found in six deaths associated with serotonin toxicity [54]. In a compilation of reference concentrations of different antidepressants [49] the cases were divided into three groups (A, single drug intoxication; B, multiple drug intoxication; C, postmortem control)

according to the strategy originally presented by Druid and Holmgren [35] (for further details, see Section 3.2). In the study by Reis et al. [49], the median VEN concentrations were 31 ($n=16$), 4.1 ($n=54$) and 0.3 ($n=142$) $\mu\text{g/g}$ in Groups A, B and C, respectively. When dividing the present study material into Group B and C cases median VEN levels of 1.0 and 0.59 $\mu\text{g/g}$, respectively, were obtained. To this end, Goeringer et al. [53] concluded that postmortem blood concentrations of VEN may be considered therapeutic between 0.07 and 0.3 mg/L. Reis et al. [49] presented a somewhat wider range between 0.1 and 1.0 $\mu\text{g/g}$ (10th and 90th percentile) for their postmortem control cases (Group C), which also is supported by the present data.

Low metabolite/parent drug (M/P) ratios are typically observed in acute drug intoxications, in which the death occurred before any substantial concentration of metabolite has been formed. In a therapeutic drug monitoring (TDM) material the median M/P ratio of ODV/VEN was 3 (range 0.6-10) and the corresponding ratios in postmortem cases were 0.2 and 1.0 in Groups B and C, respectively [49]. In the present study, the median M/P ratio was 0.4 in B cases and 1.27 in C cases. In a study with 100 patients treated with VEN, the median ODV/VEN ratio was 1.8 (range 0.07-13.7) [21]. Twenty-five of the patients were genotyped for CYP2D6 and the mean ODV/VEN ratios were 0.25, 1.16, 3.3 and 10.3 for PMs (6*/4*, *5/*4 or *6/*6), IMs (*1/*4), EMs (*1/*1) and UMs (2x*1/*1), respectively [21]. Our results correspond well with those obtained by Shams et al. [21] as the present mean ODV/VEN ratios were 0.23 and 2.01 for PMs and EMs, respectively. From the available data it is obvious that it is difficult to distinguish between an acute drug intake and a PM of CYP2D6 when only looking at the M/P ratio between ODV and VEN.

In 46 of the cases (82%) other drugs were found with an average of 3.6 drugs/case. Many of the drugs found are metabolized by the same enzymes as VEN, and some are also known to be inhibitors of the enzymes. These interactions may affect the pharmacokinetics of the drugs. Several drugs detected during the routine drug screening are metabolized by CYP2D6. Patients who are CYP2D6 PMs, or are taking drugs that may interact, may receive concentrations in the range of those found in overdose when taking therapeutic doses [20,55]. Thus, drug interactions involving VEN may have occurred in several of the cases included in the present study. The combination of tramadol and VEN was found in five cases and the risk of serotonin syndrome, which can be fatal, cannot be negligible

[54]. Other drugs found in our cases are inducers or inhibitors of CYP enzymes, e.g. carbamazepine [56] and dextropropoxyphene [57].

The *CYP2D6* genotyping results from this study can at a first sight look different from earlier studies in postmortem materials (Table 3). Of our 56 cases, six cases (10.7%) were identified with a genotype corresponding to a poor metabolism and none with ultra-rapid metabolism was identified. In a Finnish study with 123 VEN cases, a low prevalence of PMs (1.6%) and a relative high prevalence of UMs (9.8%) were found [50]. In another Finnish study, 33 cases positive for tramadol were genotyped and 12% PMs and 12% UMs were found [58]. Zackrisson et al. [38] have in an earlier Swedish study genotyped 239 intoxication suicide cases, 254 violent suicide cases and 205 natural death cases. The mean number of PMs found in these three materials was 6.8% and the corresponding figure for UMs was 2.6% [38]. If comparing these figures with the present 56 cases we had to expect to find 3.8 PMs (we observed six), and 1.4 UMs (we observed none). The small number of cases included in our study can explain the deviant genotype frequencies. In the studies by Druid et al. [30], Holmgren et al. [59] and Carlsson et al. [34] a lower prevalence of *CYP2D6* PM genotypes occurred compared to the general Caucasian population [60]. These differences in frequencies are most probably due to differences in number of included cases. The high prevalence of UMs in the two Finnish studies is supported by another Finnish study by Sistonen et al. [61]. They reported a higher *CYP2D6*-related metabolic rate in Finns than in other Scandinavian populations (1-2%) [5,62].

The problem of drug identification in postmortem cases when using LC/MS/MS can be seen in cases where drugs produce a product ion of similar mass/charge ratio at the same retention time in the chromatography. This problem occurs in the present method when the opioid tramadol is detected together with ODV, the major VEN metabolite. These substances are structurally similar with identical molecular mass (263.37 g/mol) and molecular formula ($C_{16}H_{25}NO_2$). The problem with production of identical product ions has been identified by others [63] and highlights the need to consider both parent drug and drug metabolites in interferences in LC/MS/MS methods.

A comprehensive toxicological analysis is essential when fatal drug intoxications are investigated [64]. The present study shows that information about the disposition of the enantiomers of VEN and its metabolites could assist in the interpretation of forensic toxicological results. A low S/R VEN ratio

was strongly related to a high S/R ODV ratio in individuals lacking two functional *CYP2D6* alleles. We therefore conclude that enantioselective analysis of VEN and ODV can predict if a person is a PM genotype/phenotype for *CYP2D6*. Knowledge of the relationship between the S- and R-enantiomers of this antidepressant drug and its active metabolite is also important since the enantiomers display different pharmacodynamic profiles.

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Table 1
CYP2D6 genetic variant alleles.

Allele	Nucleotide change, cDNA	RefSNP ID	Effect on protein	Enzyme activity
<i>CYP2D6*1</i>	wild-type			normal
<i>CYP2D6*1xN</i>	wild-type and gene duplication		xN active genes	increased
<i>CYP2D6*3</i>	2549delA	rs35742686	frameshift	none
<i>CYP2D6*4</i>	1846G>A	rs3892097	splicing defect	none
<i>CYP2D6*4xN</i>	1846G>A and gene duplication		xN inactive genes	none
<i>CYP2D6*5</i>	gene deletion		CYP2D6 deleted	none
<i>CYP2D6*6</i>	1707delT	rs5030655	frameshift	none

Table 2

Age, sex and blood drug concentrations in relation to cause and manner of death and the results of the genotyping.

Case (sex/age)	Total (S+R) concentration				S/R ratio				Other drugs in blood (concentration)	Cause of death (Manner of death)	CYP2D6 genotype
	VEN	ODV	NDV	DDV	VEN	ODV	NDV	DDV			
1 (F/51)	2.96	3.06	0.51	0.62	0.87	0.94	1.13	1.22	7-amino-clonazepam (0.05) Diazepam (0.2) Nordazepam (0.2) Mirtazapine (0.1) Zopiclone (0.02)	Hanging (Suicide)	*1/*1
2 (M/51)	2.92	1.63	0.21	0.16	1.03	0.85	1.10	1.97	Citalopram (0.2) Demethylcitalopram (0.1) Diazepam (0.3) Nordiazepam (0.3) Lamotrigine (4.3) Mirtazapine (0.1) Propiomazine (0.04) Dihydropropiomazine (0.08) Quetiapine (1.0) Zopiclone (0.4)	Intoxication (Suicide)	*1/*4
3 (F/63)	2.81	1.74	0.92	0.43	0.71	1.18	0.48	1.06	Ethanol (1.7) Lamotrigine (3.3) Mirtazapine (0.2) Desmethyilmirtazapine (0.1) Theophylline (3.0) Zopiclone (0.04)	Aspiration caused by drug intoxication (Accident)	*1/*1
4 (F/36)	2.80	0.79	0.28	0.11	1.25	0.68	0.54	0.70	Codeine (2.1) Morphine (0.02) Promethazine (0.4) Desmethylpromethazine (0.2) Ibuprophen (3.0)	Intoxication (Undetermined)	*1/*4
5 (M/43)	2.77	0.40	0.68	0.09	1.07	1.66	0.51	0.74	Ethanol (1.2) Alimemazine (0.2) Diazepam (0.2) GHB (82)	Intoxication (Undetermined)	*1/*5
6 (M/84)	2.42	0.33	1.15	0.12	0.72	11.1	0.50	1.77	None	Hanging (Suicide)	*4/*4
7 (M/28)	2.40	0.85	0.60	0.30	0.80	2.50	0.55	1.34	Methadone (0.5)	Intoxication (Accident)	*1/*1
8 (M/50)	2.36	0.98	1.10	0.31	0.63	2.54	0.42	1.11	Alimemazine (0.2) Desmethyialimemazine (0.1) Quetiapine (0.2)	Acute myocardial infarction (Natural)	*1/*4xN
9 (F/56)	2.05	NA	0.62	0.28	0.74	-	0.70	1.24	Ethanol (0.5) Alimemazine (0.07) Paracetamol (6) Tramadol (1.2)	Hanging (Suicide)	*1/*1
10 (M/54)	1.80	1.18	0.12	0.17	1.07	0.84	0.81	1.35	Diazepam (1.3) Nordazepam (0.2) Dihydropropiomazine (0.06) Hydroxyzine (0.8) Sertraline (0.3) Desmethylsertraline (0.1) Zolpidem (0.17) Zopiclone (0.12)	Intoxication (Suicide)	*1/*4
11 (M/58)	1.76	0.10	0.03	0.004	1.00	0.93	0.32	0.42	Alprazolam (0.07) Citalopram (0.9) Moclobemide (103) Oxomoclobemide (11.2)	Intoxication (Suicide)	*1/*1
12 (61/M)	1.64	1.02	0.44	0.31	0.72	1.26	0.86	1.04	Ethanol (1.2) Lamotrigine (3.4) Metoprolol (0.2) Mirtazapine (0.1)	Hanging (Suicide)	*1/*4
13 (F/54)	1.53	NA	1.36	0.27	0.47	-	NC	1.99	Amitriptyline (1.5) Nortriptyline (0.9) Levomepromazine (1.0) Desmethylevomepromazine (0.6) Mirtazapine (2.2) Desmethyilmirtazapine (0.6) Propiomazine (0.03)	Intoxication (Suicide)	*4/*5

									Dihydropropiomazine (0.1) Tramadol (34) O-desmethyltramadol (1.3)		
14 (F/64)	1.42	0.98	0.16	0.15	1.08	0.78	1.06	1.02	Ethanol (1.9)	Causa mortis ignota (Undetermined)	*1/*4
15 (M/47)	1.36	0.11	0.49	0.04	0.59	12.3	0.46	1.99	Ethanol (1.6)	Hanging (Suicide)	*4/*4xN
16 (M/37)	1.34	0.25	0.19	0.05	0.90	1.57	0.55	1.30	Ethanol (1.2) Olanzapine (0.1) Zopiclone (0.17)	Hanging (Suicide)	*1/*3
17 (F/48)	1.19	0.42	1.01	0.17	0.28	5.92	0.42	0.95	Carbamazepine (3.2) Alimemazine (0.8) Desmethylalimemazine (0.7)	Gastrointestinal hemorrhage (Undetermined)	*1/*4
18 (M/62)	1.12	1.04	0.08	0.05	1.84	0.79	0.41	0.31	Ethanol (1.6)	Coronary artery atherosclerosis (Natural)	*1/*5
19 (F/49)	1.12	0.84	0.11	0.16	0.91	0.89	0.87	1.09	Bupropione (8.5)	Intoxication (Suicide)	*1/*1
20 (F/33)	0.99	0.89	0.15	0.19	0.85	0.89	1.08	1.28	Mirtazapine (0.1)	Hanging (Suicide)	*1/*4
21 (M/31)	0.97	0.39	0.04	0.05	1.06	0.75	0.90	1.01	Ethanol (2.3)	Hanging (Suicide)	*1/*3
22 (M/57)	0.88	0.12	0.06	0.02	1.20	0.92	0.45	0.50	Alimemazine (0.06) Methadone (2.2) Zopiclone (0.15)	Intoxication (Accident)	*1/*4
23 (M/39)	0.87	0.85	0.08	0.08	1.29	0.80	0.97	1.08	Ethanol (1.3) Alimemazine (2.5) Desmethylalimemazine (0.8)	Intoxication (Accident)	*1/*4
24 (M/59)	0.82	0.19	0.43	0.06	0.43	13.2	0.54	1.85	Promethazine (0.1) Zopiclone (0.02)	Coronary artery atherosclerosis (Natural)	*3/*4
25 (F/41)	0.74	0.72	0.03	0.08	1.14	0.79	1.75	1.28	Ethanol (0.2) Mirtazapine (0.1)	Hanging (Suicide)	*1/*1
26 (M/33)	0.69	0.19	0.39	0.06	0.56	17.6	0.53	2.14	Ethanol (3.6) 7-amino-clonazepam (0.3) Amphetamine (0.2)	Intoxication (Accident)	*4/*4
27 (F/38)	0.65	0.46	0.06	0.15	0.80	1.18	0.77	1.01	None	Jump from hight (Suicide)	*1/*4
28 (M/56)	0.60	1.00	0.16	0.34	0.80	0.93	0.62	0.97	Mirtazapine (0.1)	Hanging (Suicide)	*1/*3
29 (M/49)	0.59	0.64	0.06	0.15	1.04	0.94	0.71	1.03	Ethanol (0.65)	Acute myocardial infarction (Natural)	*1/*1
30 (M/49)	0.54	0.40	0.05	0.11	1.02	0.96	1.01	1.03	Ethanol (0.7)	Gastrointestinal hemorrhage (Natural)	*1/*1
31 (F/37)	0.50	NA	0.07	0.20	1.02	-	2.03	1.17	Ethanol (1.4) Tramadol (1.3) Propiomazine (0.2) Dihydropropiomazine (0.4) Zolpidem (0.4) Zopiclone (0.5)	Intoxication (Suicide)	*1/*1
32 (M/34)	0.48	NA	0.28	0.16	0.41	-	0.59	0.85	Diazepam (0.1) Nordazepam (0.06) Oxycodone (0.9) Propiomazine (0.05) Tramadol (0.1) Zopiclone (0.1)	Intoxication (Accident)	*1/*4
33 (M/69)	0.46	0.84	0.15	0.31	0.59	1.27	0.60	0.98	Diazepam (0.1) Nordazepam (0.1)	Jump from hight (Suicide)	*1/*1
34 (M/43)	0.42	1.33	0.10	0.33	0.82	0.89	1.16	1.25	Pregabalin (4.9)	Pneumonia (Natural)	*1/*4xN
35 (M/28)	0.41	0.52	0.01	0.02	2.04	0.77	NC	0.44	Ethanol (1.6)	Hanging (Suicide)	*1/*1
36 (F/59)	0.37	0.72	0.05	0.13	1.11	0.70	1.53	1.12	None	Road traffic fatality (Accident)	*1/*1
37 (F/19)	0.33	0.54	0.06	0.12	0.63	0.98	0.86	1.18	None	Hanging	*1/*4

38 (M/24)	0.32	0.55	0.04	0.10	1.16	0.84	1.54	1.21	Ethanol (1.4) Citalopram (0.1)	(Suicide) Gunshot (Suicide)	*1/*1
39 (M/65)	0.28	0.57	0.09	0.15	0.81	0.84	1.25	1.00	None	Hanging (Suicide)	*1/*1
40 (F/86)	0.21	0.79	0.06	0.15	0.75	0.83	0.97	0.98	None	Drowning (Suicide)	*1/*4
41 (F/66)	0.20	1.10	0.05	0.23	1.37	0.83	0.85	0.97	7-amino-nitrazepam (0.12) Promethazine (0.1)	Causa mortis ignota (Undertemined)	*1/*4
42 (F/50)	0.19	0.41	0.06	0.08	0.86	0.86	1.12	0.94	None	Drowning (Accident)	*1xN/*4
43 (M/56)	0.19	0.41	0.02	0.02	1.68	0.90	1.02	0.54	Alprazolam (0.04) Dihydropropiomazine (0.1) Flupentixol (0.04) Nordazepam (0.1) Zopiclone (0.07)	Coronary artery atherosclerosis (Natural)	*1/*1
44 (M/71)	0.19	NA	0.02	0.02	1.94	-	0.81	0.45	Citalopram (0.06) Tramadol (0.1)	Hanging (Suicide)	*1/*1
45 (M/45)	0.18	0.36	0.05	0.08	0.93	0.87	1.12	0.96	Ethanol (0.4)	Stair fall (Accident)	*1/*4
46 (M/50)	0.16	0.31	0.03	0.08	0.84	0.74	1.00	0.99	None	Hanging (Suicide)	*1/*1
47 (M/59)	0.15	0.29	ND	0.02	1.16	0.97	-	1.11	Ethanol (0.2)	Coronary artery atherosclerosis (Natural)	*1/*1
48 (M/28)	0.15	0.27	0.01	0.03	1.47	0.95	2.39	1.59	Ethanol (2.3) Citalopram (2.5) Diazepam (0.1) Nordazepam (0.05) Mirtazapine (0.2) Propiomazine (0.09) Dihydropropiomazine (0.4) Zopiclone (0.3)	Intoxication (Suicide)	*1/*1
49 (M/57)	0.13	0.68	0.02	0.10	0.99	0.77	1.08	0.90	Ethanol (1.9) Alprazolam (0.05) Dextropropoxyphene (1.0)	Intoxication (Suicide)	*1/*1
50 (M/35)	0.12	0.44	0.04	0.05	0.71	0.99	1.14	1.03	Ethanol (1.5) Alimemazine (0.09)	Hanging (Suicide)	*1/*1
51 (F/63)	0.10	0.43	0.02	0.09	1.27	0.73	1.46	0.88	None	Gastrointestinal hemorrhage (Natural)	*1/*1
52 (M/47)	0.10	0.38	0.06	0.19	0.63	0.96	0.94	0.81	Ethanol (1.9) Carbamazepine (2.3) THC (0.003)	Road traffic fatality (Accident)	*1/*1
53 (M/44)	0.10	0.04	0.16	0.04	0.23	5.47	0.52	0.93	Ethanol (1.7) Mirtazapine (0.4) Desmethyilmirtazapine (0.1) Topiramate (3.1) Zopiclone (0.5)	Intoxication (Suicide)	*4/*4xN
54 (M/25)	0.09	0.18	0.01	0.03	0.92	0.93	2.13	1.29	Ethanol (1.2) Oxazepam (0.5)	Drowning (Suicide)	*1/*1
55 (M/41)	0.09	0.17	0.001	0.01	3.74	1.14	NC	0.55	None	Hanging (Suicide)	*1/*5
56 (M/44)	0.07	0.29	0.05	0.10	0.74	0.86	0.60	0.94	Ethanol (0.3) THC (0.003)	Fall from hight (Accident)	*1/*1
Mean	0.93	0.65	0.24	0.14	0.99	2.17	0.92	1.08			
<i>SD</i>	<i>0.88</i>	<i>0.52</i>	<i>0.33</i>	<i>0.12</i>	<i>0.53</i>	<i>3.57</i>	<i>0.46</i>	<i>0.40</i>			
Median	0.60	0.52	0.08	0.11	0.91	0.93	0.86	1.03			
<i>Range</i>	<i>0.07-2.96</i>	<i>0.04-3.06</i>	<i>0.001-1.36</i>	<i>0.004-0.62</i>	<i>0.23-3.74</i>	<i>0.68-17.6</i>	<i>0.32-2.39</i>	<i>0.31-2.14</i>			

M, male; F, female; VEN, venlafaxine; ODV, O-desmethylvenlafaxine; NDV, N-desmethylvenlafaxine; DDV, N,O-didesmethylvenlafaxine; GHB, gamma-hydroxybutyrate; THC, tetrahydrocannabinol; NA, not analyzed; ND, not detected; NC, not calculated; N, gene copy number; SD, standard deviation. All femoral blood concentrations are µg/g, except ethanol which is g/L.

Table 3

A compilation of studies with reported frequencies of forensic autopsy cases with a genotype corresponding to either a poor or ultrarapid metabolism for CYP2D6.

Description of postmortem study material	<i>n</i>	% PM	% UM	Reference
Cases positive for venlafaxine ^a	56	10.7	0	Present data
Cases positive for venlafaxine ^a	123	1.6	9.8	Launiainen et al. [50]
Intoxication suicide cases	239	4.6	2.5	Zackrisson et al. [38]
Violent suicide cases	254	7.9	4.7	Zackrisson et al. [38]
Natural death cases	205	7.8	0.5	Zackrisson et al. [38]
Cases positive for citalopram and/or escitalopram ^a	50	6.0	4.0	Carlsson et al. [34]
Cases positive for citalopram ^a	53	3.8	NA	Holmgren et al. [59]
Cases positive for tramadol ^a	33	12	12	Levo et al. [58]
Supposedly overdose death cases ^b	22	0	NA	Druid et al. [30]
Consecutive cases ^a	24	4.2	NA	Druid et al. [30]

n, number of individuals; PM, poor metabolizer; UM, ultrarapid metabolizer; NA, not analyzed.

^a Included cases represented all causes of deaths.

^b Included cases had high parent drug to metabolite ratios of drugs metabolized by CYP2D6.

Figure legends

Fig. 1. Enantiomeric (S/R) concentration ratios for venlafaxine (VEN) and its metabolites O-desmethylvenlafaxine (ODV), N-desmethylvenlafaxine (NDV) and N,O-didesmethylvenlafaxine (DDV) in femoral blood after dividing the cases into *CYP2D6* genotypes (0 active genes $n=6$, 1 active gene $n=24$ and 2 active genes $n=23$). Values are means $\pm$ SD.

Fig. 2. Metabolite/parent drug (M/P) concentration ratios and total concentration for venlafaxine (VEN) and its metabolites O-desmethylvenlafaxine (ODV), N-desmethylvenlafaxine (NDV) and N,O-didesmethylvenlafaxine (DDV) in femoral blood after dividing the cases into *CYP2D6* genotypes (0 active genes $n=6$, 1 active gene $n=24$ and 2 active genes $n=23$). Values are means $\pm$ SD.

Figure 1

Figure 2