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***ABCB1* gene polymorphisms are associated with fatal intoxications involving venlafaxine but not citalopram**

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Abstract

P-glycoprotein (P-gp), encoded by the *ABCB1/MDR1* gene, is a drug transporter at the blood-brain barrier. Several polymorphisms in the *ABCB1* gene are known to affect the activity and/or expression of P-gp, thereby influencing the treatment response and toxicity of P-gp substrates like citalopram and venlafaxine. In this study we aimed to investigate the frequency of *ABCB1* genotypes in forensic autopsy cases involving these two antidepressants. Further, the distribution of *ABCB1* genotypes in deaths related to intoxication was compared to cases not associated to drug intoxication. The study included 228 forensic autopsy cases with different causes and manners of deaths. The *ABCB1* single nucleotide polymorphisms (SNPs) G1199A, C1236T, C3435T and G2677T/A for these individuals were determined. The SNPs C1236T and C3435T in venlafaxine positive cases were significantly different between the intoxication cases and non-intoxications. This was not seen for cases involving citalopram, indicating that the effect of genetic variants might be substrate specific. This novel finding should, however, be confirmed in future studies with larger number of cases.

Keywords

ABCB1 – Citalopram – Forensic material – Genotype – Postmortem – Venlafaxine

Introduction

Postmortem genetic testing (molecular autopsy) has been suggested to provide insights for the interpretation of forensic toxicological results [1-4]. So far, much research has focused on cytochrome P450 polymorphisms [5-11]. There is now a need for studies on the possible impact of other genetic polymorphisms in forensic autopsy cases.

P-glycoprotein (P-gp), encoded by the *ABCB1* gene (also known as *MDR1*), is expressed in the endothelium of blood capillaries of the brain (blood-brain barrier; BBB) as well as in the kidneys and the intestine [12]. In order for drugs to exert its effects on the central nervous system (CNS), the drug has to pass the BBB. In the BBB P-gp acts as an efflux pump, which removes its substrates from the brain back into the blood-stream and is capable of extruding drugs with different chemical structures and mechanisms of action, such as antidepressants and antipsychotics [13-20]. Venlafaxine and citalopram are antidepressant drugs and substrates of P-gp. While citalopram belongs to the pharmacodynamic class of selective serotonin reuptake inhibitors (SSRIs), venlafaxine belongs to the class of dual serotonin and noradrenaline reuptake inhibitors (SNRIs) [21].

The *ABCB1* gene is located in the human chromosome 7 band p21-21.1 [22]. Three of the identified single nucleotide polymorphisms (SNPs) in the *ABCB1* gene, C3435T, G2677T/A and C1236T are believed to affect the activity and/or expression level of the protein [23-25]. The SNPs have also been associated with altered pharmacokinetics and treatment response [26-28]. Clinical efficiency of antidepressant as well as antipsychotic treatment has been correlated with *ABCB1* genetic polymorphisms and substrate properties of several drugs [28-31]. To our knowledge, only two studies focusing on the influences of P-gp genotype in postmortem samples have been published [32, 33], and so far none on antidepressant drugs.

Interpretation of postmortem results is not always an easy task [34], and studies focused on the use of genotyping in the forensic case work are warranted. The overall aim of the present study was to investigate the frequency of *ABCB1* genotypes in forensic autopsy cases positive for citalopram or venlafaxine. Further, the distribution of *ABCB1* genotypes in deaths related to intoxication was compared to cases not associated to drug intoxication.

Methods

Case selection

From the Department of Forensic Genetics and Forensic Toxicology in Linköping, Sweden, 228 cases positive for venlafaxine and citalopram (venlafaxine n=116, citalopram n= 112) during the toxicological screening were identified and genotyped for *ABCB1* SNPs. At the time of the study, the cases were routinely screened for medical substances and ethanol (screening for illegal drugs was only performed upon request). All included cases were classified using International Classification of Disease-9 (ICD-9) codes by the responsible forensic pathologist and divided into intoxications (n=73: suicides, accidents and undetermined; ICD codes E950, E980, E859, E866) and cases not related to drug intoxication (n=155: non-intoxications; comprising all other causes of death not related to intoxications). Information related to each of the cases (age, gender and other circumstances surrounding death) was provided from the forensic pathology and toxicology databases at the Swedish National Board of Forensic Medicine. Demographic characteristics of the individuals are presented in Table 1. The study was approved by the Regional Ethical Review Board in Linköping, Sweden.

Toxicological analysis

Routine screening of a wide range of prescription drugs was determined in femoral blood by capillary gas chromatography (GC) with nitrogen-phosphorus detector. In brief, two different extraction methods were used; an alkaline and a neutral extraction. 1.0 g of femoral blood was used in both extraction procedures and standard curves, made by adding known amounts of each analyte to drug-free blood, were used for the quantification of drugs [35]. The concentrations of venlafaxine (plus the main metabolite O-desmethylvenlafaxin) and citalopram (plus the main metabolite desmethylcitalopram) were quantified by using this method. Ethanol was determined in blood according to a previously described method [36].

Genotyping for ABCB1

The following *ABCB1* SNPs, G1199A (exon 11, rs2229109), C1236T (exon 12, rs1128503), G2677T/A (exon 21, rs2032582) and C3435T (exon 26, rs1045642) were identified by PCR and pyrosequencing. In brief, genomic DNA was extracted using King Fischer ML (Thermo Scientific, Vantaa, Finland). Extracted DNA was stored frozen in -20° C until analyzed. PCR and sequencing primers were designed using the PSQAssay Design program (Qiagen, Uppsala, Sweden). PCR primers, sequencing primers and dispensation order for the four SNPs are shown in Table 2. For PCR amplification, the reactions were optimized for MgCl₂ concentration (1.5 mM) and annealing temperature (58°C), and each primer was used at a final concentration of 0.4 µM. HotStarTaq master mixture (VWR International, Stockholm, Sweden) was used and all reactions were performed on a Mastercycler gradient instrument (Eppendorf, Hamburg, Germany) in a total volume of 12.5 µl. The four SNPs were analyzed using a PyroMark Q96MD instrument (Qiagen, Uppsala, Sweden) according to the manufactures protocol.

Statistics

Statistical differences between groups were calculated using the Fischer's exact test. The distributions of individuals homozygous variant were compared to individuals with the wild type or heterozygous genotype. A P-value < 0.05 was considered statistically significant. All statistical analysis was performed using StatView® (SAS® Institute, Cary; NC, version 5.0).

Results

General results

The study includes 116 cases positive for venlafaxine and 112 cases positive for citalopram. The general characteristics of the cases, analytical results, including concentrations of parent drug and main metabolite, age, sex, cause and manner of death are presented in Table 1. The material comprised 85 women (37%) and 143 men (63%) in total with ages ranging between 18 and 90 years (median 56 years). 91 different substances were detected (median number of drugs were 4; range 1-11). In 39 cases (17%), venlafaxine or citalopram were the only drug identified and none of the cases were classified as intoxications. The median concentration of venlafaxine was 0.50 µg/g and for the main metabolite O-desmethylvenlafaxine 0.30 µg/g. For the citalopram positive cases, the median citalopram concentration was 0.40 µg/g and for the main metabolite desmethylcitalopram 0.10 µg/g. Most frequently found drugs in addition to venlafaxine and citalopram are displayed in Table 3. According to the forensic pathologist, the death was related to intoxication (suicide, accident or undetermined) in 73 cases (32% of all cases). 103 cases were classified as suicides (45% of all cases) and 35 of these cases were associated with intoxication (34% of all suicides). The remaining 68 suicide cases were not associated with drug intoxication and hanging was the most frequently cause of death (n=45) followed by drowning (n=11). The manner of death was considered natural in 51 cases and the 57 cases were classified as accidents. The age, sex, drug and metabolite concentrations in

relation to other found drugs and cause and manner of death, in all individual cases, are presented in Tables S1 and S2 of the Electronic Supplementary Material.

The genotype frequencies of the *ABCB1* gene are given in Table 4. All the 228 cases, with exception of 3 cases for C1236T, were successfully genotyped for the SNPs (G1199A, C1236T, C2677T/A and C3435T) and did not significantly deviate from the Hardy-Weinberg (HW) equilibrium.

ABCB1 genotypes in intoxication and non-intoxication cases

The frequencies of the *ABCB1* genotypes in intoxications (n=73; comprising suicides, accidents and undetermined intoxication cases) and non-intoxications (n=155; comprising all other causes of deaths not related to intoxication) are presented in Table 5. The SNPs C1236T and C3435T in venlafaxine positive cases were significantly different between the intoxication cases and the non-intoxication cases (C1236T p=0.0173; C3435T p=0.0074). The same trend was observed for G2677T in venlafaxine positive cases, but the comparison did not reach statistical significance (p=0.0597). Very few individuals positive for venlafaxine were homozygous variants for the SNPs (i.e. individuals 1236TT, 2677TT or 3435TT) in the intoxication cases as compared to the other cases. The genetic variant 1236 and 3435 was significantly different between intoxications and non-intoxications in the whole (citalopram+venlafaxine) material. For the SNP G1199A, a low frequency of the A variant was found and the genotype distribution was not significantly different between intoxications and non-intoxications (no A/A homozygous individuals was found). Very few cases were found to carry the 2677A variant so they were excluded from the analysis. No significant difference in *ABCB1* genotype distribution was seen for the citalopram positive cases.

Discussion

Accidental deaths caused by drugs may rely on various causes, including variations in accumulation of drug and therapeutic response. Transport proteins may play an essential role in drug toxicity and efficacy. The knowledge about the distribution of relevant polymorphisms in the gene of e.g. *ABCB1* in a population might be valuable information when understanding the cause and manner of deaths in forensic cases. Therefore we investigated the frequencies of four SNPs in the *ABCB1* gene to evaluate whether the distribution differed between intoxication and non-intoxication deaths. In the present population we found that the genotype distribution of C1236T, G2677T and C3435T differed in intoxication cases as compared to non-intoxication cases involving venlafaxine.

In humans, P-gp is present in several tissues that are important for drug absorption, distribution and elimination [37]. With regard to the effect of the SNPs on antidepressants few studies are available. Uhr and colleagues published a clinical study of *ABCB1* polymorphisms in depressed patients and provided the first evidence that genetic variants account for differences in the clinical efficacy of antidepressants [28]. They found an association between SNPs in *ABCB1* and remission in patients taking P-gp substrates (including venlafaxine and citalopram). This is in accordance with our findings that the *ABCB1* genotype might influence antidepressant response.

To our knowledge, only two studies with postmortem samples and genotyping for SNPs in *ABCB1* have so far been published. The effect of three SNPs (C1236T, G2677T and C3435T) on postmortem digoxin concentration in 112 subjects was studied and they found that the T-T-T haplotype allele frequency was higher in the group with high digoxin concentration (≥ 7 nmol/l) and that the females might have a higher risk of digoxin intoxication [33]. In a study

with methadone, no association between the SNPs and methadone concentrations or metabolite concentration was found [32]. In our study we found an association between *ABCB1* genotype and drug intoxication in cases involving venlafaxine. This was not seen for cases positive for citalopram, indicating that the effect of genetic variants might be substrate specific.

In the venlafaxine positive cases in the present study, the intoxication group contained no homozygous variant individuals (1236 TT n=0; 2677 TT n=1 and 3435TT n=4). In this forensic material, a carrier homozygous variant for 1236, 2677 and/or 3435 is more likely to have died by suicide not associated with drug intoxication (1236 TT, 73% suicides; 2677TT, 67% suicides and 3435TT 57% suicides) and hanging being the most common cause of death. Of the individuals with the TT-TT-TT (1236-2677-3435) haplotype, 70% (7/10 cases) were suicides (six hangings and one drowning) and none of them were associated with intoxication (suicide, accident or undetermined). If these cases are associated with low therapeutic effects (assuming that they are on medication for depression), severe side effects or a behavioral phenotype leading to the individuals being more prone to suicide, remains to be elucidated. Of the individuals with homozygous variant for one of the SNPs (2677 TT n=1 and 3435TT n=4) in the intoxication group, two were suicides by intoxication, one accident and one undetermined (all cases had venlafaxine concentration at the higher end of the therapeutic range).

The published studies on the effect of *ABCB1* polymorphisms on the effects of antidepressants are inconclusive [38-41]. The studies have not been consistently performed and the role of *ABCB1* polymorphisms in pharmacokinetics and pharmacodynamics needs to be further studied. Recently, a study reported that polymorphisms in the *ABCB1* gene are

associated with the severity of depression and the effectiveness of the antidepressant escitalopram [42]. The influence of *ABCB1* polymorphism on the disposition of the S- and R-enantiomers of citalopram in plasma and cerebrospinal fluid (CSF) was examined under steady-state conditions in 15 patients with major depression treated with citalopram. The 2677TT genotype was associated with lower plasma and CSF concentrations than the 2677GT or 2677GG. The 2677 GG/GT genotype was associated with a better treatment response ($p = 0.001$) compared with the 2677TT genotype [43]. Others have not found any associations between SNPs and treatment or drug concentrations. Menu *et al.* studied whether the response to antidepressants in 117 depressed patients differed in patients with the 3435CC genotype as compared to patients with the 3435CT and 3435TT genotypes. They found no association between the SNP and antidepressant efficacy or tolerance [44]. In another paper where the associations between *ABCB1* polymorphisms and citalopram response and tolerance were studied, no association was found [45].

When dealing with intoxication cases, it is important to consider not only the measured drug concentrations but also possible co-factors that can influence the interpretation of the toxicological results. According to several studies, venlafaxine has a relatively higher fatal toxicity compared with SSRIs and other newer antidepressants [46-49]. Different explanations regarding this finding have been suggested. Launiainen *et al.* recently reported that fatal venlafaxine poisonings were associated with a high prevalence of drug interactions [50]. This is in line with results previously reported by us [7] and also evident from the present data. The most frequently found prescription drugs, in addition to venlafaxine and citalopram, were zopiclone, paracetamol, propiomazine/dihydropropiomazine, diazepam, alimemazine, nordiazepam and mirtazapine. The present results support the importance of considering adverse drug combinations as one of the major risk factors leading to fatal drug intoxications

[51,52]. In this context, it should also be noted that among the newer antidepressants, like venlafaxine and citalopram, differences are present both in toxicity and in interaction potential with alcohol [53].

Studies of genetic factors that might influence the risk of intoxication are of value in forensic toxicology. In our postmortem study with 228 cases we found that the genotype distribution of SNPs C1236T and C3435T significantly differed between intoxications and non-intoxications indicating that these polymorphisms might influence the function of P-gp leading to toxicity risk (i.e. high drug concentration in the brain) or unsatisfied therapeutic effect due to low drug concentration in the brain. However, our novel findings should be replicated in future studies with larger groups of cases and with other antidepressants.

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Table 1 General information related to the included postmortem cases

	Venlafaxine positive cases		Citalopram positive cases	
Sex, n (%)				
Male	75	(65%)	68	(61%)
Female	41	(35%)	44	(39%)
Age, years				
Median (range)	50	(18 - 86)	59	(19 - 90)
Manner of death ^a , n (%)				
Suicide	58	(50%)	45	(40%)
Accident	27	(23%)	30	(27%)
Natural	23	(20%)	28	(25%)
Other	8	(7%)	9	(8%)
Intoxications ^a , n (%)				
Suicides	17	(47%)	18	(49%)
Accidents	16	(44%)	13	(35%)
Undetermined	3	(8%)	6	(16%)
Suicides ^a , n (%)				
Hanging	28	(48%)	17	(38%)
Drowning	6	(10%)	5	(11%)
Intoxication	17	(29%)	18	(40%)
Other	7	(12%)	5	(11%)
Drug concentration, µg/g				
Parent: median (range)	0.50	(0.05 - 6.6)	0.40	(0.05 - 10.5)
Metabolite: median (range)	0.30	(0 - 1.6) ^b	0.10	(0 - 0.8) ^c

^aAll included cases were classified by the responsible pathologist using ICD-9 codes.

^bO-desmethylvenlafaxine

^cDesmethylocitalopram

Table 2 Primers, sequencing primers and dispensation order for the *ABCB1* SNPs

<i>PCR primers</i>		
SNP	Forward primer	Reverse primer
G1199A	bio* ATTGACAGCTATTCGAAGAGTG	CCTTAACTTCTTTTCGAGATGG
C1236T	bio* GTCTGTGAATTGCCTTGAAGT	CAGCCACTGTTTCCAACC
G2677T/A	bio* GGACAAGCACTGAAAGATAAG	AGGGAGTAACAAAATAACACTGAT
C3435T	bio* ACATTGCCTATGGAGACAAC	TAGGCAGTGACTCGATGAAG
<i>Sequencing primers</i>		
SNP	Sequencing primers	Dispensation order
G1199A	CTTTTCGAGATGGGTAA	GA CTCGAGTGAC
C1236T	TGCACCTTCAGGTTCA	TGAGCTCAGAT
G2677T/A	TTAGTTTGACTCACCTTCC	GCCAGTCAGCTC
C3435T	CTTTGCTGCCCTCAC	CAGAGTCTCT

*bio: biotinylated

Table 3 Most frequently found drugs (n) in addition to venlafaxine and citalopram in the postmortem cases divided into intoxications (comprising suicide, accidents and undetermined intoxication cases) and non-intoxications (comprising all other causes of deaths not related to intoxication)

Venlafaxine positive cases		Citalopram positive cases	
Intoxications	Non-intoxications	Intoxications	Non-intoxications
Propiomazine ^a (18)	Ethanol (31)	Ethanol (18)	Paracetamol (19)
Zopiclone (15)	Mirtazapin (13)	Propiomazine ^a (17)	Ethanol (16)
Ethanol (13)	Zopiclone (12)	Zopiclone (11)	Mirtazapine (7)
Diazepam (12)	Alimemazine (10)	Nordiazepam (6)	Alimemazine (6)
Nordiazepam (8)	Paracetamol (10)	Paracetamol (5)	Diazepam (5)

^aPropiomazine and/or dihydropropiomazine

Table 4 Genotype frequencies found in the venlafaxine positive cases (n=116), the citalopram positive cases (n=112) and in all cases (i.e. venlafaxine + citalopram cases, n=228)

SNP	Genotype	Venlafaxine positive cases		Citalopram positive cases		All cases	
G1199A	G/G	109	(94%)	108	(96%)	217	(95%)
	G/A	7	(6%)	4	(4%)	11	(5%)
C1236T ^a	C/C	45	(39%)	40	(36%)	85	(38%)
	C/T	58	(51%)	47	(42%)	105	(47%)
	T/T	11	(10%)	24	(22%)	35	(16%)
G2677T/A	G/G	44	(38%)	39	(35%)	83	(36%)
	G/T	49	(42%)	40	(36%)	89	(39%)
	T/T	13	(11%)	23	(21%)	36	(16%)
	G/A	3	(3%)	4	(4%)	7	(3%)
	T/A	7	(6%)	6	(5%)	13	(6%)
C3435T	C/C	26	(22%)	23	(21%)	49	(21%)
	C/T	58	(50%)	56	(50%)	114	(50%)
	T/T	32	(28%)	33	(29%)	65	(29%)

^aGenotyping for C1236T failed for two venlafaxine positive cases and one citalopram positive case.

The number (and percentage) of cases with different genotypes are displayed.

All genotypes are in Hardy-Weinberg equilibrium.

Table 5 Genotype frequencies found in the venlafaxine and citalopram positive cases divided into intoxications (comprising suicides, accidents and undetermined intoxication cases) and non-intoxications (comprising all other causes of deaths not related to intoxication)

SNP	Genotype	Venlafaxine positive cases				Citalopram positive cases				All cases			
		Intoxications		Non-intoxications		Intoxications		Non-intoxications		Intoxications		Non-intoxications	
G1199A	G/G	34	(94%)	75	(94%)	36	(97%)	72	(96%)	70	(96%)	147	(95%)
	G/A	2	(6%)	5	(6%)	1	(3%)	3	(4%)	3	(4%)	8	(5%)
	Total	36		80		37		75		73		155	
	p-value	> 0.9999				> 0.9999				> 0.9999			
C1236T	C/C + C/T	35	(100%)	68	(86%)	31	(84%)	56	(76%)	66	(92%)	124	(81%)
	T/T	0	-	11	(14%)	6	(16%)	18	(24%)	6	(8%)	29	(19%)
	Total	35		79		37		74		72		153	
	p-value	0.0173				0.4638				0.0483			
G2677T/A	G/G + G/T	32	(97%)	61	(84%)	28	(80%)	51	(76%)	60	(88%)	112	(80%)
	T/T	1	(3%)	12	(16%)	7	(20%)	16	(24%)	8	(12%)	28	(20%)
	Total	33		73		35		67		68		140	
	p-value	0.0597				0.8042				0.1729			
C3435T	C/C + C/T	32	(89%)	52	(65%)	28	(76%)	51	(68%)	60	(82%)	103	(66%)
	T/T	4	(11%)	28	(35%)	9	(24%)	24	(32%)	13	(18%)	52	(34%)
	Total	36		80		37		75		73		155	
	p-value	0.0074				0.5100				0.0179			

The number (and percentage) of cases with different genotypes are displayed.

P < 0.05 Fishers exact test; intoxications vs. non-intoxications

Table S1 Age, sex, venlafaxine (VEN) and O-desmethylenlafaxine (ODV) femoral blood concentrations in relation to other found drugs and cause and manner of death.

Case	Age (sex)	Concentration		Other drugs in femoral blood	Cause of death	Manner of death
		VEN µg/g	ODV µg/g			
1	71 (M)	0.1	ND	Tramadol	Hanging	Suicide
2	24 (M)	0.2	0.2	Ethanol	Gunshot	Suicide
3	37 (F)	0.3	0.5	Ethanol Tramadol Propiomazine Dihydropropiomazine Zolpidem Zopiclone	Intoxication	Suicide
4	49 (M)	0.4	0.2	Ethanol	Acute myocardial infarction	Natural
5	41 (F)	0.6	0.4	Ethanol Mirtazapine	Hanging	Suicide
6	28 (M)	0.1	0.1	Ethanol Diazepam Nordazepam Mirtazapine Propiomazine Dihydropropiomazine Zopiclone	Intoxication	Suicide
7	51 (F)	2.4	1.6	7-amino-clonazepam Diazepam Nordazepam Mirtazapine Zopiclone	Hanging	Suicide
8	19 (F)	0.3	0.3	None	Hanging	Suicide
9	31 (M)	0.7	0.2	Ethanol	Hanging	Suicide
10	43 (M)	2.1	0.2	Ethanol Alimemazine Diazepam GHB	Intoxication	Undetermined
11	59 (M)	0.7	0.1	Promethazine Zopiclone	Coronary artery atherosclerosis	Natural
12	84 (M)	2	0.2	None	Hanging	Suicide
13	61 (M)	1.2	0.5	Ethanol Lamotrigine Metoprolol Mirtazapine Diazepam Nordazepam Oxycodone Propiomazine Tramadol Zopiclone	Hanging	Suicide
14	34 (M)	0.4	0.2	Ethanol Alimemazine Desmethylalimemazine Mirtazapine	Intoxication	Accident
15	39 (M)	0.7	0.5	Ethanol Alimemazine Desmethylalimemazine	Intoxication	Accident
16	56 (M)	0.5	0.4	Mirtazapine	Hanging	Suicide
17	44 (M)	0.07	ND	Ethanol Mirtazapine Desmethylmirtazapine Topiramate Zopiclone	Intoxication	Suicide
18	49 (F)	0.9	0.4	Bupropione	Intoxication	Suicide
19	41 (M)	0.07	ND	None	Hanging	Suicide
20	35 (M)	0.1	0.2	Ethanol Alimemazine	Hanging	Suicide
21	38 (F)	0.5	0.2	None	Jump from hight	Suicide
22	25 (M)	0.2	ND	Ethanol Oxazepam	Drowning	Suicide
23	33 (F)	0.7	0.5	Mirtazapine	Hanging	Suicide
24	44 (M)	0.05	0.2	Ethanol THC	Fall from hight	Accident
25	57 (M)	0.09	0.4	Ethanol Alprazolam Dextropropoxyphene	Intoxication	Suicide
26	56 (F)	1.5	0.3	Ethanol	Hanging	Suicide

27	62 (M)	0.9	0.5	Alimemazine Paracetamol Tramadol Ethanol	Coronary artery atherosclerosis	Natural
28	50 (F)	0.1	0.2	None	Drowning	Accident
29	86 (F)	0.2	0.4	None	Drowning	Suicide
30	63 (F)	0.08	0.2	None	Gastrointestinal hemorrhage	Natural
31	48 (F)	0.8	ND	Carbamazepine Alimemazine Desmethylalimemazine	Gastrointestinal hemorrhage	Undetermined
32	28 (M)	0.3	0.4	Ethanol	Hanging	Suicide
33	37 (M)	1.1	0.1	Ethanol Olanzapine Zopiclone	Hanging	Suicide
34	58 (M)	1.6	ND	Alprazolam Moclobemide Oxomoclobemide	Intoxication	Suicide
35	59 (M)	0.1	0.2	Ethanol	Coronary artery atherosclerosis	Natural
36	66 (F)	0.2	0.6	7-amino-nitrazepam Promethazine	Causa mortis ignota	Undetermined
37	50 (M)	1.9	0.5	Alimemazine Desmethylalimemazine Quetiapine	Acute myocardial infarction	Natural
38	36 (F)	2.5	0.4	Codeine Morphine Promethazine Desmethylprometazine	Intoxication	Undetermined
39	51 (M)	2.4	0.9	Ibuprophen Diazepam Nordiazepam Lamotrigine Mirtazapine Propiomazine Dihydropropiomazine Quetiapine Zopiclone	Intoxication	Suicide
40	47 (M)	0.09	0.2	Ethanol Carbamazepine THC	Road traffic fatality	Accident
41	33 (M)	0.5	ND	Ethanol 7-amino-clonazepam Amphetamine	Intoxication	Accident
42	28 (M)	2	0.4	Methadone	Intoxication	Accident
43	63 (F)	2.2	0.7	Ethanol Lamotrigine Mirtazapine Desmethyilmirtazapine Theophylline Zopiclone	Aspiration caused by drug intoxication	Accident
44	43 (M)	0.4	0.8	Pregabalin	Pneumonia	Natural
45	56 (M)	0.1	ND	Alprazolam Dihydropropiomazine Flupentixol Nordazepam Zopiclone	Coronary artery atherosclerosis	Natural
46	54 (F)	1.4	0.2	Amitriptyline Nortriptyline Levomepromazine Desmethyllumpromazine Mirtazapine Desmethyilmirtazapine Propiomazine Dihydropropiomazine Tramadol O-desmethyiltramadol	Intoxication	Suicide
47	54 (M)	1.4	0.6	Diazepam Nordazepam Dihydropropiomazine Hydroxyzine Sertraline Desmethylsertraline Zolpidem Zopiclone	Intoxication	Suicide

48	69 (M)	0.3	0.3	Diazepam	Jump from height	Suicide
49	57 (M)	0.6	ND	Nordazepam Alimemazine Methadone Zopiclone	Intoxication	Accident
50	45 (M)	0.2	0.2	Ethanol	Stair fall	Accident
51	64 (F)	1.2	0.7	Ethanol	Causa mortis ignota	Undetermined
52	65 (M)	0.2	0.4	None	Hanging	Suicide
53	47 (M)	1.1	ND	Ethanol	Hanging	Suicide
54	59 (F)	0.4	0.7	None	Road traffic fatality	Accident
55	50 (M)	0.1	0.2	None	Hanging	Suicide
56	49 (M)	0.4	0.2	Ethanol	Gastrointestinal hemorrhage	Natural
57	52 (F)	0.2	0.3	None	Unspecified means	Suicide
58	33 (F)	0.7	0.5	Mirtazapine	Hanging	Suicide
59	63 (F)	5.1	0.6	Cyanide Fentanyl Metoprolol Carbon monoxide Zopiclone	Intoxication	Accident
60	32 (F)	0.9	ND	Biperiden Diazepam Nordazepam Ethylmorphine Haloperidol Levomepromazine 7-amino-nitrazepam Dihydropropiomazine Zopiclone Olanzapine	Intoxication	Accident
61	57 (M)	0.3	0.5	Amitriptyline Diazepam Nordazepam Codeine Morphine Paracetamol	Coronary arteriosclerosis	Natural
62	24 (M)	1.2	0.4	Alimemazine Lamotrigine Mirtazapine Desmethyilmirtazapine	Hanging	Suicide
63	62 (M)	0.9	0.5	Ethanol	Coronary arteriosclerosis	Natural
64	64 (M)	0.8	0.4	Biperiden Carbon monoxide Zopiclone	Fire	Accident
65	50 (F)	0.1	0.2	None	Drowning	Accident
66	86 (F)	0.2	0.4	None	Drowning	Suicide
67	38 (F)	0.2	0.5	Carbon monoxide Zopiclone	Gas	Suicide
68	28 (M)	0.3	0.4	Ethanol	Hanging	Suicide
69	37 (M)	1.1	0.1	Zopiclone Olanzapine Ethanol	Hanging	Suicide
70	26 (M)	0.4	ND	Alimemazine THC Buprenorphine Mirtazapine Norbuprenorphine Phenazepam	Intoxication	Accident
71	58 (M)	1.6	ND	Alprazolam Moclobemide Oxomoclobemide Ethanol	Intoxication	Suicide
72	59 (M)	0.1	0.2	Ethanol	Coronary arteriosclerosis	Natural
73	31 (M)	0.6	0.3	7-amino-clonazepam Codeine Methadone Morphine	Hanging	Suicide
74	66 (F)	0.2	0.6	7-amino-nitrazepam Promethazine	Unknown	Natural
75	66 (M)	0.2	ND	Alimemazine Dihydropropiomazine Ethanol	Drowning	Suicide
76	50 (M)	1.9	0.5	Alimemazine	Acute ischemic	Natural

				Desmethyldiazepam	myocardial injury	
77	36 (F)	2.5	0.4	Quetiapine		
				Ibuprofen	Intoxication	Undetermined
				Codeine		
				Morphine		
				Promethazine		
78	51 (M)	2.4	0.9	Desmethyldiazepam		
				Diazepam	Intoxication	Suicide
				Nordazepam		
				Propiomazine		
				Dihydropropiomazine		
				Zopiclone		
				Quetiapine		
				Lamotrigine		
79	25 (M)	0.5	ND	Mirtazapine		
				Dextropropoxyphene	Intoxication	Suicide
				Diazepam		
				Nordazepam		
				Buprenorphine		
80	33 (M)	0.5	ND	Norbuprenorphine		
				Amphetamine	Intoxication	Accident
				7-amino-clonazepam		
81	28 (M)	2	0.4	Ethanol		
				Methadone	Intoxication	Accident
82	63 (F)	2.2	0.7	Theophylline		
				Zopiclone	Obstruction of respiratory tract	Accident
				Lamotrigine		
				Mirtazapine		
				Desmethyldiazepam		
83	49 (M)	0.1	ND	Ethanol		
				Diazepam	Intoxication	Accident
				Nordazepam		
				Levomopromazine		
				Desmethyldiazepam		
				Mirtazapine		
				Desmethyldiazepam		
				Ethanol		
84	43 (M)	0.4	0.8	Pregabalin	Bronchopneumonia	Natural
85	85 (M)	0.2	0.2	Mirtazapine	Hanging	Suicide
86	56 (M)	0.1	ND	Alprazolam	Coronary arteriosclerosis	Natural
				Nordazepam		
				Flupentixol		
				Dihydropropiomazine		
				Zopiclone		
87	54 (M)	1.4	0.6	Diazepam	Intoxication	Suicide
				Nordazepam		
				Hydroxyzine		
				Dihydropropiomazine		
				Zopiclone		
				Zolpidem		
				Sertraline		
				Desmethyldiazepam		
88	69 (M)	0.3	0.3	Diazepam	Jump from height	Suicide
				Nordazepam		
89	57 (M)	0.6	ND	Alimemazine	Unknown	Natural
				Methadone		
				Zopiclone		
90	45 (M)	0.2	0.2	None	Fall	Accident
91	64 (M)	0.3	0.5	Paracetamol	Gun shot	Undetermined
				Mirtazapine		
				Olanzapine		
92	30 (M)	0.9	0.3	Alprazolam	Intoxication	Accident
				Amphetamine		
				Methadone		
				Methamphetamine		
				Metoprolol		
93	27 (M)	1.1	0.2	Alimemazine	Intoxication	Suicide
				Diazepam		
				Oxazepam		
				Propiomazine		
				Dihydropropiomazine		
				Zolpidem		
94	43 (M)	0.8	0.4	Zopiclone	Hanging	Suicide
				Lamotrigine		
95	79 (M)	0.3	ND	Alprazolam	Fall	Undetermined
				Cocaine		

				Bensoylecgonine		
				Paracetamol		
				Ethanol		
96	69 (F)	0.8	0.4	Diazepam	Old myocardial infarction	Natural
				Nordazepam		
				Paracetamol		
				Mirtazapine		
				DesmethyImirtazapine		
97	49 (M)	0.6	ND	Alimemazine	Hanging	Suicide
				Nordazepam		
				Hydroxizine		
				Promethazine		
				Desmethylpromethazine		
				Ethanol		
98	53 (M)	0.4	0.3	Ethanol	Intracerebral hemorrhage	Natural
99	47 (F)	1.1	0.6	Cyanide	Intoxication	Accident
				Carbon monoxide		
				Mianserine		
				DesmethyImianserine		
				Ethanol		
100	38 (M)	0.6	0.2	Alimemazine	Intoxication	Accident
				Zopiclone		
				Pregabalin		
101	62 (F)	0.2	0.3	Ethanol		Suicide
102	43 (M)	0.2	0.8	Diazepam	Bronchopneumonia	Natural
				Nordazepam		
				Ephedrine		
				Ethylmorphine		
				Morphine		
				Paracetamol		
103	18 (F)	0.2	0.4	Methadone	Intoxication	Accident
				Zopiclone		
				Ethanol		
104	50 (M)	0.3	0.2	Diazepam	Intoxication	Suicide
				THC		
				Ethanol		
105	56 (F)	0.5	0.6	Amitriptyline	Excessive heat	Accident
				Carbon monoxide		
				Paracetamol		
				Pregabalin		
				Ethanol		
106	84 (F)	1.8	0.9	Paracetamol	Coronary arteriosclerosis	Natural
				Tamoxiphen		
				Ethanol		
107	59 (F)	1.1	0.4	None	Drowning	Suicide
108	40 (F)	0.5	1	Alprazolam	Intoxication	Accident
				Codeine		
				Morphine		
				7-amino-nitrazepam		
				Paracetamol		
				Zopiclone		
				Quetiaphine		
				Ethanol		
109	66 (F)	0.3	0.4	None	Hanging	Suicide
110	25 (M)	0.6	0.3	Alimemazine	Intoxication	Suicide
				Desmethylalimemazine		
				Propiomazine		
				Dihydropropiomazine		
				Zopiclone		
				Desmethylsertraline		
111	73 (F)	0.5	0.3	Alimemazine	Drowning	Suicide
				Desmethylalimemazine		
				Olanzapine		
				Reboxetine		
112	66 (M)	0.1	0.2	Paracetamol	Intracerebral hemorrhage	Natural
113	44 (F)	6.6	ND	Amitriptyline	Intoxication	Suicide
				Dextropropoxyphene		
				Ethylmorphine		
				Carisoprodol		
				Codeine		
				Morphine		
				Paracetamol		
114	58 (F)	0.2	0.2	Paracetamol	Hanging	Suicide
115	44 (F)	0.1	0.1	Mirtazapine	Intracerebral	Natural

116	20 (F)	1.7	0.6	Quetiapine	hemorrhage Hanging	Suicide
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M, male; F, female; VEN, venlafaxine; ODV, O-desmethylenlafaxine; GHB, gammahydroxybutyrate; THC, tetrahydrocannabinol; ND, not detected.

Table S2 Age, sex, citalopram (CIT) and desmethylcitalopram (DCIT) femoral blood concentrations in relation to other found drugs and cause and manner of death.

Case	Age (sex)	Concentration		Other drugs in femoral blood	Cause of death	Manner of death
		CIT µg/g	DCIT µg/g			
1	66 (M)	8.4	0.4	Carbon monoxide	Intoxication	Suicide
2	65 (M)	0.6	0.1	Nortriptyline Diazepam Nordazepam Tramadol O-desmethyltramadol Caffeine Paracetamol	Cardiosclerosis	Natural
3	71 (F)	0.3	0.1	Zopiclone	Hanging	Suicide
4	86 (F)	0.3	0.2	Nortriptyline 7-amino-nitrazepam	Intoxication	Suicide
5	63 (M)	0.6	0.1	Ethanol	Vitium cordis	Undetermined
6	59 (F)	0.5	0.1	Ethanol Oxazepam 7-amino-flunitrazepam Alimemazine Desmethylalimemazine Codeine Carisoprodol Meprobamate Paracetamol	Corpus alienum	Accident
7	57 (M)	0.2	0.1	None	Acute ischemic myocardial lesion	Natural
8	22 (F)	0.2	0.1	None	Hanging	Suicide
9	36 (F)	0.2	0.1	Topiramate Levetiracetam	Epilepsy	Natural
10	47 (M)	1	0.1	Nitrazepam 7-amino-nitrazepam Alprazolam	Intoxication	Suicide
11	84 (M)	0.7	0.3	Dextropropoxyphene Clomethiazole Paracetamol	Burns	Accident
12	46 (M)	0.5	ND	Nortriptyline	Intoxication	Suicide
13	84 (M)	0.3	0.1	O-desmethyltramadol Paracetamol	Skull fracture	Accident
14	71 (M)	0.1	ND	None	Peritonitis	Natural
15	54 (M)	0.4	0.2	Ethanol Carbon monoxide	Intoxication	Accident
16	54 (F)	0.05	ND	Ethanol Propiomazine Dihydropropiomazine 7-amino-flunitrazepam	Intoxication	Suicide
17	59 (F)	0.3	0.2	None	Bleeding gastric ulcer	Natural
18	55 (M)	0.6	0.1	Ethanol	Cardiac disease	Natural
19	65 (F)	1	0.2	None	Hanging	Suicide
20	37 (M)	1.2	0.4	Ethanol Mianserine Desmethylmianserine Diazepam Nordazepam Caffeine Paracetamol	Hanging	Suicide
21	61 (M)	0.3	0.1	None	Hanging	Suicide
22	62 (F)	0.08	ND	Ethanol	Intoxication	Accident
23	74 (M)	0.3	0.1	None	Cardiosclerosis and extreme cold	Accident
24	77 (F)	0.9	0.1	Carbon monoxide Zopiclone	Intoxication	Accident
25	29 (M)	0.4	0.1	Ethanol Alimemazine Desmethylalimemazine Carbamazepine Hydroxyzine	Intoxication	Suicide
26	82 (M)	0.7	0.2	None	Fat embolism	Accident
27	63 (M)	0.4	0.2	None	Acute myocardial	Natural

28	77 (M)	0.4	0.1	Phenazone	infarction	Natural
				Hydroxyzine	Bleeding oesophageal	
				7-amino-nitrazepam	varices	
29	76 (F)	0.05	0.1	None	Bronchial pneumonia	Accident
30	30 (F)	0.2	ND	None	Hanging	Suicide
31	56 (F)	0.2	0.1	Nortriptyline	Drowning	Suicide
				Mirtazapine		
32	86 (F)	0.2	0.1	Desmethylmirtazapine	Drowning	Suicide
				Paracetamol		
33	56 (M)	0.2	ND	Ethanol	Hanging	Suicide
34	87 (M)	1.9	0.2	None	Brainstem injury	Accident
35	58 (F)	1.1	0.8	Codeine	Intoxication	Accident
				Morphine		
				Promethazine		
				Desmethylpromethazine		
				Nitrazepam		
				7-amino-nitrazepam		
				Salicylic acid		
36	65 (M)	0.9	ND	Dextropropoxyphene	Intoxication	Accident
				Diazepam		
				Nordazepam		
				Zopiclone		
37	46 (F)	0.5	0.1	None	Epilepsy	Natural
38	67 (M)	0.5	0.1	None	Hanging	Suicide
39	60 (F)	0.6	0.1	Ethanol	Intoxication	
				Diazepam		
				Nordazepam		
40	73 (F)	0.3	0.1	Zopiclone	Drowning	Suicide
				Dihydropropiomazine		
41	19 (F)	0.3	ND	Promethazine	Intoxication	Suicide
				Desmethylpromethazine		
				Diazepam		
				Nordazepam		
				Hydroxyzine		
42	36 (M)	8.5	0.3	Ethanol	Intoxication	Suicide
43	89 (M)	0.4	ND	Nordazepam	Hanging	Suicide
44	72 (F)	0.1	ND	Zopiclone	Intoxication	Suicide
45	87 (F)	0.1	0.1	None	Corpus alienum	Accident
46	69 (M)	0.6	0.1	None	Hanging	Suicide
47	49 (M)	0.7	0.1	Ethanol	Intoxication	Accident
				Propiomazine		
				Dihydropropiomazine		
				Zopiclone		
48	20 (M)	0.2	ND	None	Hanging	Suicide
49	48 (M)	0.08	ND	None	Gunshot	Suicide
50	85 (F)	0.5	0.1	Paracetamol	Cardiosclerosis	Accident
51	90 (M)	0.3	ND	Paracetamol	Jump from high	Suicide
52	61 (M)	0.2	ND	Zolpidem	Drowning	Suicide
53	40 (F)	0.6	0.2	Aceton	Diffuse myocardial scarring	Natural
					Intoxication	
54	48 (M)	0.4	ND	Nordazepam		Accident
				Promethazine		
				Zopiclone		
				Ethanol		
55	76 (M)	0.3	0.1	Metroprolol	Intoxication	Suicide
				Oxazepam		
				Propiomazine		
				Dihydropropiomazine		
				Zolpidem		
				Mirtazapine		
56	26 (F)	10.5	0.7	Hydroxyzine	Intoxication	Suicide
				Propiomazine		
				Dihydropropiomazine		
				Tramadol		
				O-desmethyltramadol		
57	50 (F)	1	0.1	Alimemazine	Chronic alcoholism	Natural
				Promethazine	Fire	
58	56 (M)	0.4	0.1	Cyanide		Accident
				Carbon monoxide		
				Mirtazapine		
59	50 (M)	5	0.2	Dextropropoxyphene	Intoxication	Suicide
				Ethanol		
60	49 (F)	0.4	0.1	Alimemazine	Unknown	Natural

61	62 (F)	0.6	0.2	Hydroxyzine Zolpidem Ethanol Buspiron Mirtazapine Desmethyilmirtazapine	Lung emphysema	Natural
62	84 (F)	0.2	0.1	None	Thrombosis/embo- lus in cerebral arteries	Natural
63	37 (F)	0.3	ND	Methadone Zopiclone Olanzapine	Intoxication	Suicide
64	57 (M)	0.4	ND	Paracetamol Propiomazine Dihydropropiomazine Zopiclone Tramadol	Intoxication	Accident
65	54 (M)	1	0.2	Methadone Paracetamol Mirtazapine Desmethyilmirtazapine	Coronary arteriosclerosis	Natural
66	87 (M)	0.4	0.1	Paracetamol	Hanging	Suicide
67	74 (F)	1.4	0.2	Dihydropropiomazine Ethanol	Suffocation	Accident
68	55 (M)	0.3	ND	Ethanol	Drowning	Accident
69	74 (M)	0.4	0.3	Diazepam Nordazepam Ethylmorphine Morphine Mianserine	Intoxication	Accident
70	27 (M)	0.7	0.2	Buprenorphine Norbuprenorphine Ethanol	Rectal cancer	Natural
71	59 (M)	0.3	0.1	Diazepam Nordazepam	Drowning	Accident
72	51 (M)	0.3	ND	Alimemazine	Intoxication	Undetermined
73	49 (F)	0.9	0.1	Prometazine Ethanol	Obstruction of respiratory tract	Accident
74	74 (M)	0.5	0.1	Diazepam Nordazepam Metoprolol Ethanol	Thrombosis/embo- lus in cerebral arteries	Natural
75	72 (F)	1.3	0.2	Paracetamol	Gas	Suicide
76	76 (M)	0.6	0.1	Carbon monoxide Zopiclone Ethanol	Traffic	Accident
77	82 (M)	0.3	0.1	None	Intoxication	Undetermined
78	60 (F)	0.7	0.3	Paracetamol Zopiclone Ethanol	Intoxication	Suicide
79	38 (M)	4.7	0.2	Alimemazine Desmethyialimemazine Alprazolam Ketamine Metoprolol Zopiclone	Sepsis	Natural
80	51 (F)	0.3	ND	Metoclopramide Lidocaine Morphine Paracetamol	Hanging	Suicide
81	74 (M)	0.2	ND	Phenytoin	Old myocardial infarction	Natural
82	57 (M)	0.8	0.2	None	Hanging	Suicide
83	56 (M)	0.6	ND	Ethanol	Intoxication	Undetermined
84	65 (F)	0.3	ND	Flecainide Ethanol	Unknown	Natural
85	49 (M)	0.5	0.1	THC	Cardiomegaly	Natural
86	42 (M)	1.1	0.1	Alimemazine Desmethyialimemazine Metoprolol Zolpidem Ethanol Promethazine Desmethylpromethazine		

87	64 (F)	0.2	ND	Acetone Hydroxyzine Dihydropropiomazine	Chronic myocardial ischemia	Natural
88	72 (M)	0.5	0.1	Dihydropropiomazine	Intoxication	Suicide
89	78 (F)	0.3	0.1	Diazepam Paracetamol Zopiclone Ethanol	Drowning	Suicide
90	46 (M)	0.2	ND	Propiomazine Dihydropropiomazine Ethanol	Intoxication	Undetermined
91	47 (M)	0.3	0.1	Carbon monoxide	Gas	Suicide
92	84 (F)	0.2	ND	Paracetamol Tramadol	Coronary arteriosclerosis	Natural
93	88 (M)	0.4	0.1	Norverapamil Verapamil	Hanging	Suicide
94	51 (M)	0.3	ND	Morphine Duloxetine Metphormine Ethanol	Intoxication	Undetermined
95	46 (M)	0.8	0.1	Dextropropoxyphene Paracetamol Zopiclone	Cardiomyopathy	Natural
96	66 (M)	0.5	0.1	None	Injury by unspecified means	Undetermined
97	40 (M)	2.4	0.2	Cocaine Benzoylecgonine	Falling from high	Undetermined
98	46 (M)	0.5	0.1	Alimemazine Desmethyalmemazine Dextropropoxyphene Morphine Paracetamol Promethazine Desmethylpromethazine Zopiclone	Intoxication	Accident
99	26 (M)	0.2	0.1	Lamotrigine	Hanging	Suicide
100	81 (F)	0.7	0.1	Phenytoin Mianserine Desmethylmianserine Paracetamol Propiomazine Dihydropropiomazine	Intoxication	Suicide
101	61 (M)	0.2	ND	Ethanol	Intoxication	Accident
102	43 (M)	0.2	0.1	Ethanol	Drowning	Accident
103	64 (M)	0.5	0.2	None	Intracerebral hemorrhage	Natural
104	59 (F)	0.2	0.1	Paracetamol Mirtazapine	Acute ischemic myocardial injury	Natural
105	58 (M)	0.3	0.1	Mirtazapine Ethanol	Pulmonary embolism	Natural
106	53 (F)	0.3	0.1	Paracetamol Zolpidem Ethanol	Intoxication	Suicide
107	77 (F)	0.5	0.1	Paracetamol	Hanging	Suicide
108	58 (M)	0.9	ND	Alprazolam Moclobemide Oxomoclobemide	Intoxication	Suicide
109	56 (F)	1	0.2	Metoclopramide Paracetamol Tramadol Ethanol	Obstruction of respiratory tract	Accident
110	71 (M)	0.2	ND	Paracetamol	Gunshot	Suicide
111	47 (M)	0.6	0.1	Acetone Alimemazine Metoclopramide Mirtazapine	Diabetes mellitus with hyperosmolar coma	Natural
112	55 (F)	0.3	0.1	Nordazepam Propiomazine Dihydropropiomazine Zopiclone Mirtazapine Desmethyalmirtazapine Ethanol	Intoxication	Accident

M, male; F, female; CIT, citalopram; DCIT, desmethylocitalopram; THC, tetrahydrocannabinol; ND, not detected.