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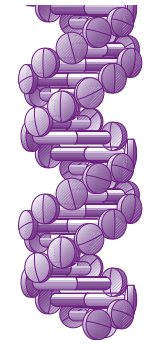

CYP450 genotype and aggressive behavior on selective serotonin reuptake inhibitors

Aim: Genetic variants for selective serotonin reuptake inhibitor (SSRI) metabolizing enzymes have been hypothesized to be a risk factor for aggression as adverse drug effect of SSRIs. Our aim was to assess the possible involvement of these polymorphisms on aggression when using SSRIs. **Materials & methods:** A retrospective noninterventional case-control study was performed on 18 cases. The genetic profile of two main genes involved in the metabolism of SSRIs was determined, and predicted phenotype frequencies were compared with Dutch controls and literature data. **Results:** Predicted CYP2C19 and CYP2D6 phenotypes for all SSRIs analyzed together did not show a significant difference between cases and controls. **Conclusion:** We found no supporting evidence for a significant relationship between *CYP2C19* and *CYP2D6* polymorphisms, and aggression in patients using SSRIs.

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Keywords: adverse drug reactions • CYP450 • psychiatric PGx

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Aggressive behavior following the use of antidepressants, mostly selective serotonin reuptake inhibitors (SSRIs), is a major point of attention [1,2]. For example, fluoxetine has been cited in numerous US criminal cases as a potential cause of violent behavior, also known as 'Prozac (fluoxetine) killings'. Cause and effect have been debated for a long time as the conditions for which these drugs are prescribed are also linked with acts of aggression.

Like other side effects, the occurrence of aggression in patients treated with SSRIs might be correlated with drug-exposure levels. In that case, inherited genetic variants for drug-metabolizing enzymes that are involved in the metabolism of SSRIs may influence the risk of aggression by generating an increased risk of high blood concentrations of the drugs involved. SSRIs are metabolized by CYP450 enzymes. The activity of this enzyme system is, for a substantial part, genetically determined, although it can also be affected by intrinsic factors, such as, age, gender and comorbidity as well as extrinsic factors, such as, nutrition and drug–drug interactions. SSRIs are metabolized by CYP2D6, CYP2C19, CYP3A4 and CYP1A2, the contribution of each enzyme depends on the SSRI involved. Fluoxetine, for instance, is metabolized predominantly by CYP2D6, whereas citalopram is metabolized by CYP2C19 (~40%), CYP2D6 (~30%) and CYP3A4 (~30%) [3]. The general population can be divided by CYP genetic testing into predicted poor (PM), intermediate (IM), extensive/normal metabolizers and ultrarapid metabolizers. For the main enzymes involved in the metabolism of SSRIs, being CYP2D6 and CYP2C19, 5–10 and 2–3% of the population is deficient in enzyme activity, respectively, due to genetic variants. In case that the metabolism of a drug depends for a substantial part on these enzymes, as is the case for many antidepressants, this genetic condition may cause high exposure to these drugs when these patients are treated with standard doses. This could make these patients more susceptible to adverse drug reactions. Indeed, a study by Lucire and Crotty [4] suggested that antidepressant-induced akathisia-related homicides were associated with these genetic variations in metabolizing genes of the CYP450 family.

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Knowledge of risk factors for developing adverse drug reactions (ADRs), among which violent behavior on SSRIs, is of utmost importance for daily practice. This concerns medical aspects, which affect clinical decision process of the psychiatrist and the treatment of the patient, but also has social and juridical aspects. Especially since dosing of antidepressants can be prescribed nowadays at adjusted doses, based on genotype, as evidence-based guidelines are available in The Netherlands [3]. Whether violent behavior can be simply defined as an ADR of SSRIs is, however, not straightforward. Since SSRIs are also prescribed to attenuate aggressive symptoms.

We used the unique information of The Netherlands Pharmacovigilance Centre and they collected in the last 10 years 88 registrations of aggressive behavior on SSRIs. We conducted a study based on the registrations in the period 2010–2014 only, reasoning that individuals before 2010 would be difficult to reach, resulting eventually in 50 registrations. Our objective was to determine the genetic profile of the two main genes involved in the metabolism of the SSRIs in reported aggressive behavior, being *CYP2D6* and *CYP2C19*, and compare the frequency of predicted phenotypes with control groups.

Materials & methods

Study population

A retrospective noninterventional case-control study was conducted at The Netherlands Pharmacovigilance Centre Lareb of reports submitted between 1 January 2010 and 31 December 2014. In these reports, the suspect drug was one of the following SSRIs: citalopram, escitalopram, fluoxetine, fluvoxamine,

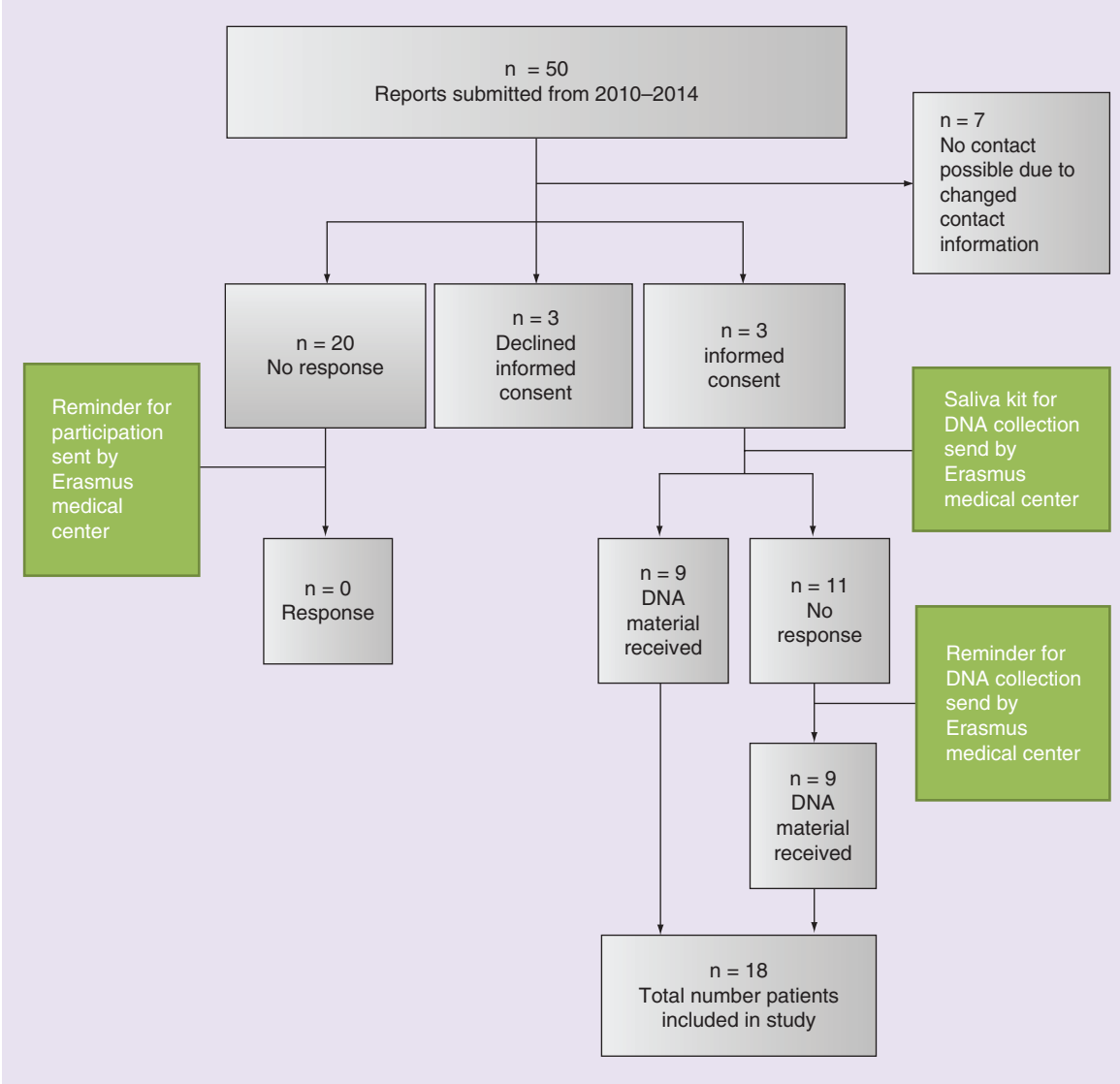

Figure 1. Inclusion flowchart. Overview of the inclusion of cases in the study.

Table 1. Genotyping CYP2D6 and CYP2C19 result cases.

NA number	SSRI	Enzyme relevant in metabolism	*CYP2C19* genotype	CYP2C19 phenotype	*CYP2D6* genotype	CYP2D6 phenotype
M053705	Fluoxetine	CYP2D6	**1/*1*	EM	**1/*9*	EM
M053708	Fluoxetine	CYP2D6	**1/*17*	EM	**1/*2*	EM
M053709	Fluoxetine	CYP2D6	**1/*17*	EM	**1/*2*	EM
M053721	Fluoxetine	CYP2D6	**1/*3*	IM	**2/*41*	EM
M053723	Fluoxetine	CYP2D6	**1/*8*	IM	**1/*1*	EM
M053707	Citalopram	CYP2C19, 2D6, 3A4	**1/*1*	EM	**2/*5*	IM
M053722	Citalopram	CYP2C19, 2D6, 3A4	**1/*1*	EM	**5/*9*	IM
M053706	Citalopram	CYP2C19, 2D6, 3A4	**2/*17*	IM	**1/*2*	EM
M053720	Citalopram	CYP2C19, 2D6, 3A4	**2/*17*	IM	**1/*4*	IM
M053712	Escitalopram	CYP2C19, 2D6, 3A4	**1/*1*	EM	**1/*1*XN	UM
M053718	Escitalopram	CYP2C19, 2D6, 3A4	**1/*2*	IM	**1/*9*	EM
M053717	Fluvoxamine	CYP2D6	**1/*1*	EM	**2/*5*	IM
M053711	Fluvoxamine	CYP2D6	**17/*17*	UM	**4/*41*	IM
M053719	Paroxetine	CYP2D6	**1/*2*	IM	**1/*5*	IM
M053710	Missing	NA	**1/*1*	EM	**1/*2*	EM
M053714	Missing	NA	**1/*1*	EM	**1/*2*	EM
M053716	Missing	NA	**1/*1*	EM	**1/*2*	EM
M053724	Missing	NA	**1/*2*	IM	**1/*2*	EM

EM: Extensive metabolizer; IM: Intermediate metabolizer; SSRI: Selective serotonin reuptake inhibitor; UM: Ultrarapid metabolizer.

paroxetine, sertraline or venlafaxine and the adverse drug reaction was either 'anger' or 'aggression' coded with the medical dictionary for regulatory activities preferred terms. The Netherlands Pharmacovigilance Centre Lareb maintains the spontaneous reporting system in The Netherlands. The study population is based on patients of whom anger or aggression has been reported by either a physician, pharmacist or the patient. The study received a waiver from the local institutional review board at the Erasmus University Medical Center in Rotterdam based on the nature of the research. All patients provided written informed consent for DNA analysis.

Outcome

The *CYP2D6*/*CYP2C19* allelic and predicted phenotype frequencies were compared between cases and a healthy blood donor control group as well as with CYP2D6 and CYP2C19 literature data in Caucasians [5,6]. Additionally, the frequency of CYP2D6-predicted phenotypes in the cases was compared with the outcome of routine diagnostic pharmacogenetic requests from a psychiatric population submitted to the Department of Clinical Chemistry, Erasmus Medical Center, over the period of January 2014 until December 2015.

Genotyping

DNA collection was either performed with the Oragene DNA (OG-500) saliva kit (DNA Genotek®, ON, Canada) or a buccal cell sample with the sterile PurFlock® Ultra swabs (ME, USA) in DNA-stabilizing solution (AutoGenomics, CA, USA). The latter option for DNA collection was chosen for the individuals who did not reply to the initial request providing a saliva sample, to ensure that the method of sampling was not a limiting factor. The MagNA Pure Compact instrument (Roche Diagnostics Nederland B.V.) with the accompanied 'nucleic acid isolation kit I' was used for DNA isolation. The genetic variants for *CYP2D6*

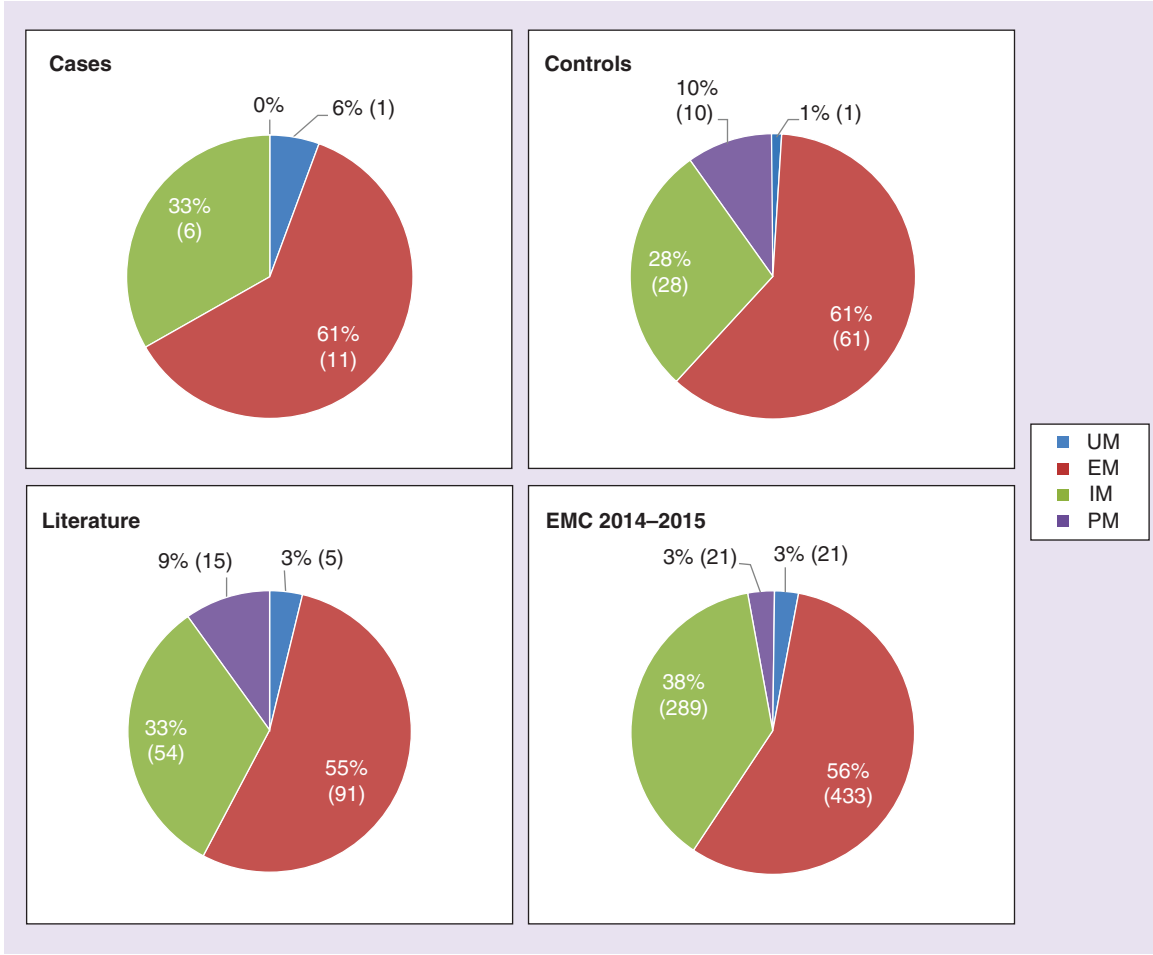

Figure 2. CYP2D6 phenotype frequencies. These pie charts give an overview of the CYP2D6 phenotype frequencies among cases (anger or aggression during selective serotonin reuptake inhibitor treatment), controls (healthy Dutch individuals), literature (Rebsamen *et al.* 2009) and a cohort of *CYP2D6*-genotyped psychiatric patients over a period of 2014–2015 in the Erasmus University Medical Center, Rotterdam, The Netherlands. EM: Extensive metabolizer; IM: Intermediate metabolizer; PM: Poor metabolizer; UM: Ultrarapid metabolizer.

(**2* [active allele]; **3*, **4*, **5*, **6*, **7*, **8*, **14* [inactive alleles]; **9*, **10*, **12*, **17*, **29*, **41* [decreased activity alleles]; XN [increased activity allele]) and *CYP2C19* (**2*, **3*, **4*, **5*, **6*, **7*, **8* [inactive alleles]; **9*, **10* [decreased-activity alleles]); **17* (increased-activity allele) were analyzed on two platforms, that is, INFINITI® CYP450 2D6I/CYP450 2C19+ assay (AutoGenomics) with TaqMan® allelic discrimination (Applied Biosystems, CA 94404) or XL-PCR, as is the standard procedure of the International Reference Centre Pharmacogenetics in Rotterdam, using validated standard operating procedures. The *CYP2D6* and *CYP2C19* control groups existed of 99 healthy Dutch blood donors that were genotyped with the AmpliChip CYP450 test. The predicted phenotypes were derived by taking into account the alleles tested for in the cases. For the *CYP2C19*17* allele, additional TaqMan assays were run in the control group since the *CYP2C19*17* allele is not present on the AmpliChip.

Statistical analysis

Statistical testing was performed using Fisher exact test, in which due to low numbers of individuals, PM and IM were combined as were extensive metabolizers and ultrarapid metabolizers, yielding two groups. The combination of one active and one inactive allele as well as patients with two decreased activity alleles were scored as an IM. All genetic variants were tested for violation ($p < 0.05$) of the Hardy–Weinberg equilibrium with the χ^2 test. The Bonferroni-corrected significance threshold for all analysis was set at $p = 0.01$.

Results

From the 50 approached individuals, 20 agreed on participation and collection of saliva for DNA testing. DNA material was received from 18 individuals (Figure 1), yielding an overall response rate of 36%. From the cases included, five patients used fluoxetine,

four citalopram, two fluvoxamine, two escitalopram, one paroxetine and in four cases this information was not reported. Most patients were treated for depression (n = 13), two patients received the SSRI for borderline personality disorder, two patients for anxiety disorder and one patient was treated for post-traumatic stress disorder. Gender was reported in 88% of the cases, giving a male/female ratio of 40/60. None of the genetic variants violated Hardy–Weinberg equilibrium ($p > 0.05$). A summary of the *CYP2D6* and *CYP2C19* genotype results is shown in Table 1. No *CYP2D6* and no *CYP2C19* PMs were found among our cases. *CYP2D6* and *CYP2C19* genotype based predicted phenotype distribution in cases, healthy blood donor controls, literature and, in the case of *CYP2D6*, the cohort with psychiatric cases is displayed in Figures 2 & 3, respectively. Comparison of CYP2D6-predicted phenotypes for all SSRIs together illustrated neither significant difference in frequency between cases with healthy blood donor controls, nor with literature ($p = 0.80$; $p = 0.62$). Also for CYP2C19-predicted phenotypes, no difference was observed in the distribution between cases and healthy blood donor controls, or with literature ($p = 0.79$; $p = 0.28$). Additional analysis (Table 2) per found *CYP2D6* allelic variant (**2, *4, *5, *9, *41*) in the cases illustrated a difference in the frequency of the *CYP2D6*9* allele ($p = 0.026$), with this allelic variant being more frequent in cases (17%) compared with healthy blood donor controls (2%). However, this association did not pass the Bonferroni significance threshold, and is thus found to be non-significant after correction. With regard to *CYP2C19* allelic variants (**2, *3, *17*), no significant distribution differences were found between cases and healthy blood donor controls (Table 2). When analyzed per drug, the relative most aberrant predicted phenotypes were found with citalopram, where three out of four individuals had the CYP2D6 IM phenotype and two out of four, the CYP2C19 IM phenotype (Table 1). However, these numbers are too small to draw any conclusion.

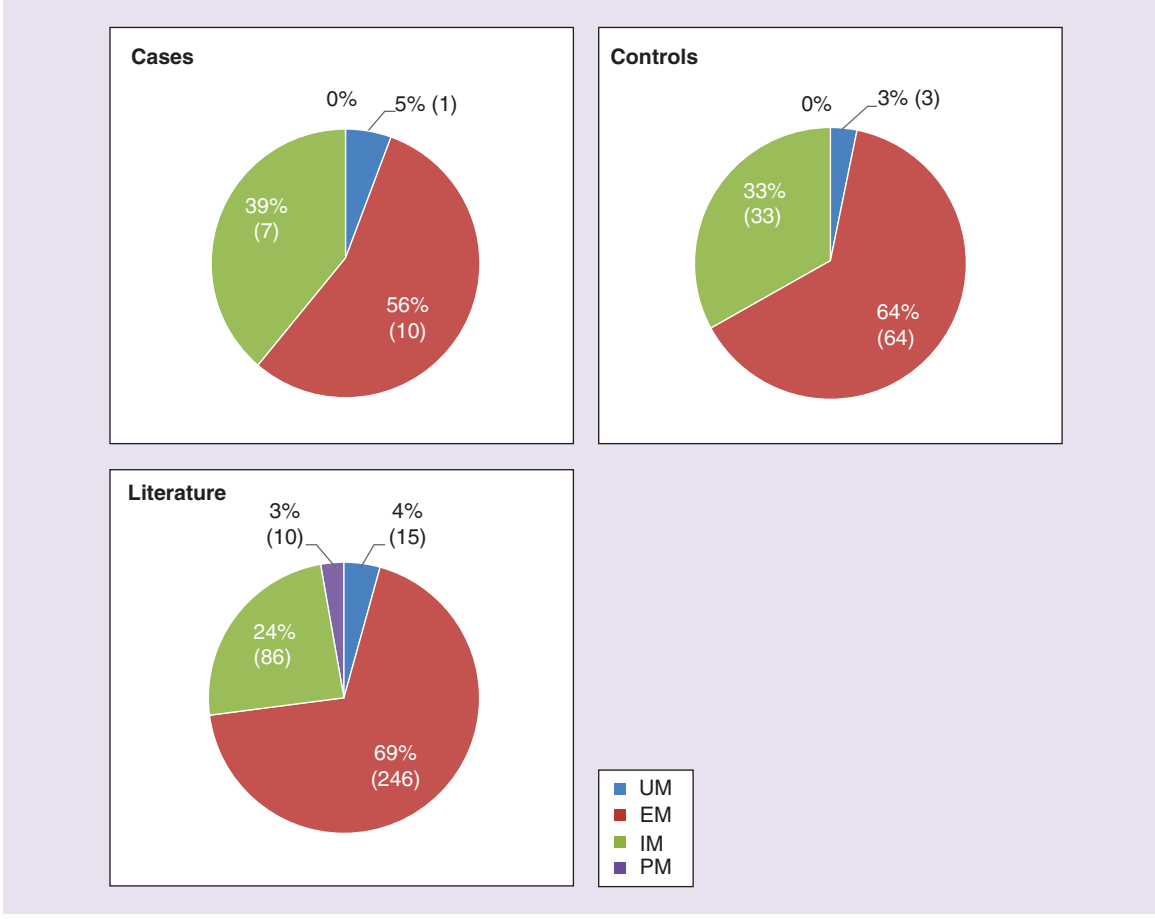

Figure 3. CYP2C19 phenotype frequencies. These pie charts give an overview of the CYP2C19 phenotype frequencies among cases (aggression during selective serotonin reuptake inhibitor treatment), controls (healthy Dutch individuals) and literature.
EM: Extensive metabolizer; IM: Intermediate metabolizer; PM: Poor metabolizer; UM: Ultrarapid metabolizer.
Data taken from [6].

Table 2. CYP2D6 and CYP2C19 allelic variants cases versus controls.

Genotype	Cases; % (n)	Controls; % (n)	p-value
CYP2D6			
**2*:			0.63
– Yes	56 (10)	62 (61)	
– No	44 (8)	38 (38)	
**4*:			0.15
– Yes	11 (2)	29 (29)	
– No	89 (16)	71 (70)	
**5*:			0.11
– Yes	22 (4)	9 (9)	
– No	78 (14)	91 (90)	
**9*:			0.026
– Yes	17 (3)	2 (2)	
– No	83 (15)	98 (97)	
**41*:			0.73
– Yes	11 (2)	18 (18)	
– No	89 (16)	82 (81)	
CYP2C19			
**2*:			0.64
– Yes	28 (5)	23 (33)	
– No	72 (13)	67 (66)	
**3*:			0.16
– Yes	6 (1)	0 (0)	
– No	94 (17)	100 (99)	
**17*:			0.19
– Yes	22 (4)	39 (37)	
– No	78 (14)	61 (57)	

Controls are 99 healthy Dutch blood donors genotyped with the AmpliChip CYP450.

Discussion

The relationship between SSRI-related adverse drug reactions and *CYP2D6* and *CYP2C19* genotype was based on the idea that genetic variants that result in decreased metabolizer status will have an increased blood concentration of SSRIs, thereby increasing their risk on adverse drug reactions. Since aggression is reported to be an ADR on SSRI, it is possible that aberrant genotypes for *CYP2D6* and/or *CY2C19* are a risk factor for aggressive behavior on SSRIs, although it is as yet unclear if indeed an increased drug concentration also is correlated with an increased risk of aggressive behavior. So far, only one Australian study (Lucire and Crotty [4]) has reported in 2011 on this relation between *CYP2D6* and *CYP2C19* genotype with aggressive behavior, and this study is often mentioned nowadays in legal cases involving aggressive behavior on SSRIs.

We had the unique opportunity to investigate in the Dutch population of 18 patients that had been officially registered at our pharmacovigilance center in the period 2010–2014, on having aggressive behavior during the use of SSRIs. In this group, however, we were not able to confirm that patients who experience aggression during use of SSRIs had a significant higher incidence of variant alleles for CYP2D6 and CYP2C19 isoenzymes, predicted decreased metabolism. In fact, we did not detect a single CYP2D6 or CYP2C19 PM. Based on 18 cases and a PM frequency of 5–10%, we would have expected at least 0–2 CYP2D6 PMs in case of an identical frequency between cases and controls, and >2 CYP2D6 PMs if indeed a strong correlation between CYP2D6-predicted phenotype and risk on aggressive behavior would have been present. With an outcome of 0 CYP2D6 PMs, we feel that the hypothesis that CYP2D6 deficiency would play a role in the risk on aggressive behavior was not substantiated. However, we have to bear in mind that these results are based on a small number of patients. Therefore, these results should be interpreted with caution.

Information about the effect of polymorphisms in genes coding for CYP2D6 and CYP2C19 isoenzymes on the occurrence of aggression when using SSRIs in literature is very sparse. As indicated, Lucire and Crotty [4] examined the relation between variants in metabolizing genes of the CYP450 family *CYP2C9*, *2C19* and *2D6* and antidepressant-induced akathisia. Akathisia is known to be associated with suicide and homicide [7,8]. The authors found a significantly increased frequency of variant alleles when analyzing *CYP2D6*, *CYP2C19* and *CYP2C9* in 85 akathisia subjects among their 129 cases compared with 150 randomly selected primary care patients (odds ratio: 19.3; 95% CI: 2.57–144.5; p = 0.00014). However, akathisia is not identical to aggressive behavior. Ten of the subjects who represented the extreme violent end of the spectrum for akathisia subjects were described in detail. Six of them committed homicide and four tried to commit homicide. Eight of these ten individuals were on SSRI drugs for which genotyping would predict impaired metabolism, as they were IM or PM for those drugs. Of the akathisia patients, 98.8% of the 85 patients carried at least one variant allele in either *CYP2D6*, *CYP2C19* or *CYP2C9*. Indeed, we did not test for *CYP2C9* in our population, since the involvement of this particular cytochrome in SSRI metabolism is absent [3]. We feel that including CYP2C9 in this type of investigation is not a valid approach since the effect of having a *CYP2C9* variant allele would then weigh equally as having impaired *CYP2D6* alleles. Mechanistically, this seems a wrong approach in our opinion. When analyzing our cases, 100% of subjects carried a variant allele, yielding no

CYP2D6 and *CYP2C19 * 1*1* individuals. Yet, taking into consideration that the *CYP2D6*2* allele is encoding normal enzymatic activity and the *CYP2C19*17* an increased rather than decreased enzymatic activity, the number of individuals in our study having decreased or null activity encoding genetic variants for either CYP2D6 or CYP2C19 is 12 out of 18, being 66%. A comparison with any control group should in our opinion thus be done with the predicted enzymatic activity rather than variant allele frequencies.

Potentially, the effect of genotype is specific for a particular SSRI: in that respect, citalopram was the drug in our study showing a tendency toward a higher than expected frequency of CYP2D6 IM and CYP2C19 IMs I, although group size is too small to perform any relevant statistical analysis. A future study, focusing on specific drugs, like citalopram, may indicate if the finding in our paper is indeed a significant trend. Aggressive behavior can have many causes and wide range of factors may play a role, including environment, a patient's social and medical history, interpersonal relations, genetics, neurochemistry and endocrine function, and substance abuse. In this study, no information about other possible causes or blood levels of SSRIs was available. Future confirmatory studies should include these other factors and use aggression scales to define this complex behavior in a more specialized manner.

Purpose of our study was to demonstrate whether *CYP2D6* and *CYP2C19* genotypes would have a dominant effect on causing aggressive behavior on SSRIs, not to show if any effect is present. The predictive power should then be visible also in absence of other factors in order for these genotypes to be a useful marker. As genotyping for predicting aggressive behavior on SSRIs was not supported by our study, genotyping for *CYP2D6* and *CYP2C19* still remains a valuable tool in predicting the risk of other side effects of SSRIs.

In summary, in our retrospective noninterventional case-control study of 18 cases from The Netherlands Pharmacovigilance Centre Lareb, we did not find a significant relationship between CYP2D6- and/or CYP2C19-predicted decreased activity based on genetic polymorphisms and the occurrence of aggression in patients using SSRIs.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- Aggressive behavior as an adverse drug effect when using antidepressants, mostly selective serotonin reuptake inhibitors (SSRIs), is a major medical and social problem.
- Inherited genetic variants for drug metabolizing enzymes involved in the metabolism of SSRIs, such as CYP2D6 and 2C19, have been hypothesized to be a risk factor.
- This study aims to investigate the possible involvement of genetic polymorphisms in CYP2D6 and CYP2C19 on aggressive behavior when using SSRIs.

Materials and methods

- A retrospective non-interventional case-control study on aggressive behavior with SSRIs of cases reported to the Netherlands Pharmacovigilance Centre Lareb was performed.
- Cases submitted between January 1 2010, and December 31 2014, where the reported adverse drug reaction was anger or aggression and where the treatment was with one of the following SSRIs: citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline or venlafaxine, were included.
- The genetic profile of the two main genes (CYP2C19 and CYP2D6) involved in the metabolism of SSRIs was determined, and predicted phenotype frequencies were compared with Dutch controls and literature data.

Results

- 18 individuals were willing to provide DNA for genetic analysis.
- No CYP2D6 and no CYP2C19 poor metabolizers were found among our cases.
- Predicted CYP2C19 and CYP2D6 phenotypes for all SSRIs analyzed together did not show a significant difference in incidence between cases and controls.

Conclusion

- We found no evidence for a significant relationship between CYP2D6 and/or CYP2C19 genetic polymorphisms and the occurrence of aggressive behavior in patients using SSRIs.

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