CYP1A2 polymorphism -1545 C>T (rs2470890) is associated with side effects of clozapine

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Abstract

Background: *CYP1A2* polymorphisms have been suggested to be associated with the side effects of antipsychotics. Studies on this are scarce and have been conducted with various antipsychotics or in small samples regarding clozapine. The aim of the present study was to test for an association between *CYP1A2* polymorphism -1545 C>T (rs2470890) and side effects in a larger sample during long-term clozapine treatment.

Methods: A total of 237 patients on clozapine treatment completed the Liverpool University Neuroleptic Side-Effect Rating Scale (LUNSERS) assessing clozapine induced side effects. Of these patients, 180 completed the questionnaire satisfactorily, agreed to provide a blood sample and were successfully genotyped for the polymorphism studied.

Results: The TT genotype of *CYP1A2* polymorphism -1545 C>T (rs2470890) was associated with significantly greater severity of side effects in general during clozapine treatment (p=0.011). In subanalysis all seven types of side effects (sympathicotonia-tension; depression-anxiety; sedation; orthostatic hypotension; dermal side effects; urinary side effects; sexual side effects) appeared numerically (but insignificantly) more severe among TT carriers. Only the intensity of urinary side effects reached a trend-like level (p=0.088). The second finding of the present study was that the use of mood stabilizers was more common among patients with TT genotype (OR=2.63, p=0.004).

Conclusions: The finding suggests an association between *CYP1A2* polymorphism -1545 C>T (rs2470890) and the side effects of clozapine. However, it should be regarded as tentative and more studies with larger samples will be required to confirm the result.

Keywords: -1545 C>T, rs2470890, clozapine, side effects, antipsychotic
**Background**

Clozapine is the most effective antipsychotic in treatment refractory schizophrenia [1]. However, due to infrequent, but serious side effects, such as agranulocytosis, its use has been limited. There are also several less serious and more frequent side effects including sedation, weight gain, constipation, hypersalivation, hypotension and urinary incontinence which may lead to impaired quality of life or discontinuation of clozapine treatment.

Clozapine is metabolized mainly by the cytochrome P450 enzyme CYP1A2 [2]. It has been reported that CYP1A2 gains in importance during long-term treatment, i.e. under saturation conditions [3]. The wide interindividual variation in the expression and activity of CYP1A2 suggests a role for genetic factors, e.g. single nucleotide polymorphisms as well as for epigenetic and environmental factors, including smoking, coffee drinking and co-medication [4,5,2]. The genetic polymorphisms of CYP1A2 may affect clozapine clearance and plasma levels. The *1F allele has been reported to be associated with increased enzyme inducibility and *1C with decreased inducibility [6,7]. In addition, poor clozapine response and low plasma drug levels have been found in smokers with the −163A/A genotype [8,9].

*CYP1A2* polymorphisms have been suggested to be associated with the side effects of antipsychotics. Increased severity of tardive dyskinesia in patients with the A allele of the CYP1A2*1C polymorphism and in another study with the (C-->A) of the CYP1A2 has been observed [3,10]. It has also been reported that the CYP1A2 -163C>A polymorphism was associated with clozapine-induced generalized tonic-clonic seizures [11]. Recently, it was reported that patients with adverse drug reactions to clozapine had a higher frequency of CYP1A2 low activity allele and lower CYP1A2-mRNA levels than patients without adverse effects [12]. To the best of our knowledge, there is only one earlier study reporting a possible association between the -1545 C>T (rs2470890) and the side effects of antipsychotics. In that study 1545
C>T was associated with tardive dyskinesia in the initial analysis, but after multiple corrections, the finding was insignificant [13].

Interesting findings on the association of CYP1A2 and the side effects of clozapine are scarce, and these studies have been conducted on fairly small samples. The aim of the present study was to test for an association between CYP1A2 polymorphism -1545 C>T (rs2470890) and side effects in a larger sample during long-term clozapine treatment.

**Methods**

**Patients**

The study included 237 patients with diagnoses of schizophrenia, schizophreniform, schizoaffective or delusional disorder according to the International Classification of Diseases, Tenth Revision (ICD-10). The diagnoses were set by experienced psychiatrists. All patients were ≥18 years of age, Caucasian, of Finnish origin, and on clozapine treatment. Of the 237 patients, four were excluded because of inadequately filled LUNSERS data (at least 20% of responses missing). A total of 190 patients (112 men and 78 women) had commercial laboratory sample for measuring serum clozapine + norclozapine concentration and 187 patients were successfully genotyped for the -1545 C>T (rs2470890).

The study was conducted in three hospital districts in Western Finland (Satakunta, Pirkanmaa and Seinäjoki). The patients were recruited at secondary in- and outpatient clinics and from sheltered accommodation units. All participants (n=237) were asked to complete the Liverpool University Neuroleptic Side-Effect Rating Scale (LUNSERS) [14]. This is a self-reported questionnaire with 51 items assessing the intensity of antipsychotic side effects (0=not at all, 4= very much). Of the items, 41 assess antipsychotic-induced side effects and ten serve as “red herrings” the purpose of which is to detect patients who may be over-reporting symptoms. The reliability and validity of LUNSERS is well-established [15]. As the LUNSERS scale has not been validated in relation
with the use of atypical antipsychotics, a factor analysis was performed in this study to detect the clinically meaningful factors for side effects of different types. This analysis resulted in eight clinical factors, named sympathicotonia-tension, depression-anxiety, sedation, orthostatic hypotension, dermal side effects, urinary side effects, sexual side effects and menstrual side effects, of which the last one was omitted from this study. Information on medical history and duration of clozapine treatment were collected from medical records. The study was approved by the local ethics committee. All participants gave informed consent on entry to the study. This study was carried out in accordance with the code of Ethics of the World Medical Association (Declaration if Helsinki).

**DNA extraction and genotyping**

For DNA extraction, 9.0 ml EDTA-whole blood was drawn from the participants and stored in a freezer at -20 C. Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Minikit and automated biorobot M48 extraction (Qiagen, Hilden, Germany). Genotyping of rs2470890 was performed using Taqman SNP Genotyping Assay (assay C_1642455_10) and ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

**Statistical analyses**

Analysis of variance (ANOVA) and T-tests were used on LUNSERS total scores, factor scores, clozapine dose and concentration comparisons between different CYP1A2 genotype groups. Chi-square statistics was used in the calculations between frequency of use of medications and CYP1A2 genotypes. A general linear univariate model (ANCOVA) was used to calculate the
interactive effect of antipsychotic dose and CYP1A2 genotype on LUNSERS total score. All calculations were performed with SPSS for Windows software (version 19).

Results

A total of 187 patients (110 men [61%] and 70 women [39%]) were successfully genotyped for -1545 C>T (rs2470890), and of these 180 had a valid LUNSERS rating. Mean age was 43.1 (11.0) years (range 20-67 years).

The TT genotype of CYP1A2 polymorphism -1545 C>T (rs2470890) was associated with significantly greater severity of side effects during clozapine treatment. The mean LUNSERS scores were 45.1, 36.8 and 37.9 among TT, TC and CC carriers respectively ($p=0.038$), and 45.1 vs. 37.1 among TT carriers vs. TC or CC carriers ($p=0.011$). In subanalysis all seven types of side effects (sympathicotonia-tension; depression-anxiety; sedation; orthostatic hypotension; dermal side effects; urinary side effects; sexual side effects) were more severe among TT carriers, but the finding was not statistically significant. Only the intensity of urinary side effects reached a trend-like level ($p=0.088$).

The use of mood stabilizers was more common among patients with TT genotype than with TC/CC (OR=2.63, $p=0.004$) (Table 1). The clozapine or norclozapine (or their sum or ratio) did not differ between TT carriers and CC/TC carriers. There was a trend for higher mean clozapine dose among patients with TT genotype (431 mg/day) than among CC/TC genotypes (390 mg) ($p=0.078$) (Table 1). In general linear univariate analysis the interaction between total antipsychotic dose and genotype of the CYP1A2 polymorphism -1545 C>T (rs2470890) together explained 5.0% of the variance in LUNSERS scale total score (complete model: $p=0.037$, power=0.682; antipsychotic dose: $\eta^2=0.018$, $p=0.08$; CYP1A2 genotype: $\eta^2=0.035$, $p=0.051$).
Discussion

In the present study the TT genotype of CYP1A2 polymorphism -1545 C>T (rs2470890) was associated with greater severity of side effects during clozapine treatment. In subanalysis all seven types of side effects (sympathicotonia-tension; depression-anxiety; sedation; orthostatic hypotension; dermal side effects; urinary side effects; sexual side effects) were more severe among TT carriers, but this finding was not statistically significant. Only the intensity of urinary side effects reached a trend-like level (p= 0.088). In earlier studies different CYP1A2 polymorphisms have been reported to be associated with adverse effects of antipsychotics [10-12]. Increased severity of tardive dyskinesia has been reported in patients with the A allele of the CYP1A2*IC polymorphism, CYP1A2 -163C>A polymorphism has been reported to be associated with clozapine-induced generalized tonic-clonic seizures and patients with adverse drug reactions to clozapine have had a higher frequency of CYP1A2 low activity allele [10-12]. It has also been suggested that concomitant absence of CYP1A2*F and the presence of CYP1A2*C could generate CYP1A2 phenotypes which could predispose to adverse effects of clozapine [16].

To the best of our knowledge, there is only one published study assessing an association between the -1545 C>T (rs2470890) and antipsychotic side effects [13]. In that study an excess of the T allele was suggested among patients with tardive dyskinesia [13]. The finding of the present study concurred with that finding.

Higher plasma concentrations of clozapine are associated with increased frequency of some side effects. In earlier studies, higher plasma concentrations of clozapine and its metabolite N-desmethylclozapine have been reported in patients with two CYP1A2 variants associated with reduced enzyme activity (−3860A, −2467del, −163C, −739G, and/or −729T) compared with those with one or none [17]. It could be expected that the higher frequency of side effects during clozapine treatment among TT carriers observed in the present study could be explained by higher plasma concentrations of clozapine in those individuals. However, the clozapine or
norclozadine (or their sum or ratio) did not differ between TT carriers and CC/TC carriers. Nevertheless, there was a trend for higher mean clozapine dose among patients with TT genotype (431 mg/day) than among patients carrying CC/TC genotypes (390 mg). The total dose of all antipsychotics (including clozapine + all other concomitant antipsychotics) did not differ between patients with TT genotype and patients with CC/TC genotypes. Thus the higher frequency of side effects during clozapine treatment among TT carriers was not explained by higher clozapine concentrations.

Smoking is a well-known CYP1A2 inductor, and lower plasma concentrations of clozapine have been measured in smokers with the −163A/A genotype [8,9,18]. The C allele of CYP1A2*1F (intron) is in complete LD with the C allele of CYP1A2 1545 C>T (exon) [13]. The C allele of CYP1A2*1F has been found to lead to decreased inducibility among smokers, but the functional impact of CYP1A2 1545 C>T is not known [6,7]. If smoking was more common among patients with TT genotype than among CC/TC genotypes in the present study, it could theoretically explain why TT carriers needed higher clozapine doses and yet the serum clozapine concentrations were not increased. However, smoking was as common among patients with TT genotype and with CC/TC genotypes. Another lifestyle factor that could explain the difference in CYP1A2 metabolism is coffee drinking. It has been suggested that caffeine in coffee inhibits clozapine metabolism and increases serum clozapine concentrations [19]. Coffee drinking was not assessed in the present study. However, among Finnish patients the effect of drinking instant coffee on serum clozapine concentrations has been reported to be of minor clinical relevance in most cases [20].

The second finding of the present study was that the use of mood stabilizers was more common among TT genotype of CYP1A2 polymorphism -1545 C>T than among CT or CC carriers. The reason for this is unclear. It is possible that the TT carriers who also experienced more adverse effects also complained more about other symptoms, such as anxiety and depression.

Differentiating between adverse subjective experience of antipsychotics, negative symptoms of
schizophrenia and depressive features is challenging. Therefore, it is possible that as a result of these complaints a mood stabilizer was added and this combination could have resulted in increased frequency or severity of adverse effects. It is also possible that the TT genotype is associated with CYP1A2 induction and thus leads to weaker antipsychotic response and due to the addition of a mood stabilizer. The most commonly added mood stabilizer in the present study was valproic acid. Carbamazepine induces CYP1A2 [21]. However, carbamazepine was seldom used in our patients and there was no difference in the frequency of carbamazepine use between TT carriers and CC/TC carriers.

The main limitation of our study is the fairly small sample size, which markedly reduces the power to detect small or moderate effects. However, in this naturalistic study practically all patients with clozapine treatment who were able to give their informed consent were recruited in this geographical area. Another limitation is that LUNSERS is a self-reported questionnaire with the rating for each item ranging from “not at all” to “very much” without any descriptors or criteria. This could have affected inter-rater reliability. However, the reliability and validity of LUNSERS is well-established [15].

Conclusions

The present findings suggest an association between TT genotype of CYP1A2 polymorphism -1545 C>T (rs2470890) and side effects of clozapine. Likewise the use of mood stabilizers was more common among TT genotype of CYP1A2 polymorphism -1545 C>T than among CT or CC carriers. These findings should be regarded as tentative and more studies with larger samples will be required to confirm the results.

Competing Interest

The authors declare that they have no competing interests.
Authors’ Contributions

EL, OK and NS participated in the design of the study. NS and MV participated in patient recruitment. NM and TL carried out the molecular genetic studies and participated in sequence alignment. OK performed the statistical analysis. MV wrote the manuscript. All authors read and approved the final manuscript.

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References


Additional files provided with this submission:

Additional file 1: CYP1A2_Table1.docx, 19K
http://www.biomedcentral.com/imedia/5692182241012080/supp1.docx