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Association between *CYP1A2* gene single nucleotide polymorphisms and clinical responses to clozapine in patients with treatment-resistant schizophrenia

Rajkumar AP, Poonkuzhali B, Kuruvilla A, Srivastava A, Jacob M, Jacob KS. Association between *CYP1A2* gene single nucleotide polymorphisms and clinical responses to clozapine in patients with treatment-resistant schizophrenia.

Objectives: Despite clozapine's superior clinical efficacy in treatment-resistant schizophrenia (TRS), its adverse effects, need for periodic leukocyte monitoring, cost and variable clinical outcomes mandate a clinical need to predict its treatment response. Although cytochrome P450 1A2 (CYP1A2) is the principal determinant of metabolism of clozapine, the role of *CYP1A2* gene in the clinical response to clozapine is uncertain. Hence, we investigated its association with treatment responses and adverse events of clozapine in TRS.
Methods: We evaluated four single nucleotide polymorphisms (SNP) in the *CYP1A2* gene, clinical responses and serum clozapine levels in 101 consecutive patients with TRS on stable doses of clozapine. We defined clozapine response *a priori* and investigated allelic and genotypic associations. We assessed the socio-demographic and clinical profiles, premorbid adjustment, traumatic life events, cognition and disability of the participants, using standard assessment schedules for appropriate multivariate analyses.
Results: Our results revealed that *CYP1A2* gene SNP (**1C, *1D, *1E* and **1F*) were not associated with clozapine treatment response, adverse effects, serum clozapine levels or with disability (p values > 0.10).
Conclusions: As *CYP1A2* gene SNP do not help to predict the clinical response to clozapine, routine screening for them prior to start clozapine is currently unwarranted. We suggest future longitudinal genome-wide association studies investigating clinical and pharmacogenetic variables together.

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Keywords: clozapine; cytochrome P-450 CYP1A2; pharmacogenetics; single nucleotide polymorphism

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Significant outcomes

- *CYP1A2* gene SNP (**1C, *1D, *1E* and **1F*) were not associated with treatment response and adverse effects of clozapine in patients with TRS.
- *CYP1A2* gene SNP (**1C, *1D, *1E* and **1F*) were not associated with serum clozapine levels in patients with TRS.

- Routine screening for *CYP1A2* gene SNP prior to start clozapine is currently unwarranted.

Limitations

- Cross-sectional study design.
- Dichotomous categorisation of clinical response to clozapine is debatable.
- Outcome measures to assess the treatment responses in schizophrenia are diverse.

Introduction

Clozapine is the drug of choice for the management of treatment-resistant schizophrenia (TRS) (1). It is a serotonin-(5-HT1A, 5-HT2A, 5-HT2C, 5-HT3A, 5-HT3B, 5-HT6 and 5-HT7) dopamine (D1–D4) antagonist (SDA), which also acts on histaminergic, adrenergic and cholinergic receptors (2). The advantages of clozapine include its superior clinical efficacy, its ability to reduce negative symptoms as well as the risk for suicide (3) and its low propensity to produce movement disorders (4). Its disadvantages are sub-optimal response in 40–70% patients with TRS (5), adverse events such as seizures, agranulocytosis, weight gain and dyslipidaemia (6), high cost and the need for periodic leucocyte monitoring. There is a clinical need to determine factors associated with good response to clozapine in order to predict its clinical outcomes and to prevent unnecessary use in patients who are unlikely to improve with clozapine (7).

Cytochrome P-450 1A2 (CYP1A2), a member of the cytochrome P-450 mixed-function oxidase system, is the principal determinant of clozapine metabolism by N-demethylation and N-oxidation (8). CYP1A2 enzyme activity influences the serum clozapine levels (9–11). Inadequate serum clozapine levels are associated with sub-optimal clinical response to clozapine in many patients (12–14). Hence, we get the impetus to evaluate the association between *CYP1A2* gene polymorphisms and clinical response to clozapine. The *CYP1A2* gene (gene ID: 1544) is located in 15q24.1 and spans 7.8 kilo bases, comprising seven exons, six introns and an enhancer region (15,16). CYP1A2 enzyme activity has been reported to show marked inter-individual variations (up to 60-fold), because of various genetic and environmental factors (17). Genetic factors alone may explain 35–75% of the variations seen in CYP1A2 enzyme activity (16). In addition, marked inter-individual variations (15- to 40-fold) have been also documented in the expression levels of CYP1A2 mRNA and protein (16). At least 33 single nucleotide polymorphisms (SNP) and 17 haplotypes in the *CYP1A2* gene have been identified so far (18). Among these SNP, *CYP1A2*1C* causes decreased enzyme activity *in vivo* and *CYP1A2*1F* leads to higher inducibility of the enzyme (18). *CYP1A2*1D* and *CYP1A2*1E* are relatively more frequent in Asian populations and have been previously investigated for their influence of CYP1A2 enzyme activity (16,18,19). However, the association between these four SNP and clinical responses to clozapine in TRS remains uncertain.

Although evidence for association between response to clozapine and *5HT2A* (20) as well as *5HT3A* (21) gene SNP exists, results of other studies investigating the clinical (22–25), genetic (26–28) and other biological predictors (29,30) are mostly inconclusive, with many of them being contradictory or pending replication. The Royal Dutch Association for the advancement of pharmacy has recently evaluated therapeutic dose recommendations for clozapine based on CYP2D6 genotypes and concluded against any specific recommendations (31). Although non-synonymous coding SNP have not been found to be associated with clozapine treatment response (16,17), case studies have claimed an association between *CYP1A2*1F* SNP and treatment resistance to clozapine (9). Ultra-rapid CYP1A2 activity, because of *CYP1A2*1F* polymorphism, has been hypothesised to yield low serum clozapine levels and poor treatment response (10). *CYP1A2*1F* has also been associated with higher induction of the enzyme by smoking (10) and heavy caffeine consumption (11). However, subsequent studies were negative for the association between *CYP1A2* SNP and serum clozapine levels, as well as clozapine treatment response (32–34). These reports did not exclusively study patients with established TRS and investigated only less than 80 participants (9,32–34). They did not use structured assessment of clinical variables such as premorbid adjustment, traumatic life events, cognition and disability nor did they adjust for these variables. Hence, investigating the association between the *CYP1A2* gene and clinical response to clozapine in a relatively larger sample of patients with TRS, accompanied by structured assessment of clinical variables, is desired.

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Aims of the study

Our principal aim is to evaluate the association between four SNP in the *CYP1A2* gene (*CYP1A2*1C, CYP1A2*1D, CYP1A2*1E* and *CYP1A2*1F*) and the clinical responses as well as adverse effects to clozapine in patients with TRS, while adjusting for the effects of confounding clinical variables. Our secondary aims include investigating the association between these four SNP and the serum clozapine levels, disability and cognition of patients with TRS.

Materials and methods

Study design

We used a pharmacogenetic association study to investigate the association between these functionally relevant four SNP in the *CYP1A2* gene and clinical responses to clozapine.

Setting

We conducted this study in the Department of Psychiatry, Christian Medical College (CMC), Vellore, India, a tertiary referral centre for the management of psychiatric disorders. The hospital has short-term inpatient services, daily outpatient and regular follow-up clinics. Patients with schizophrenia are initially treated with either dopamine antagonists or serotonin dopamine antagonists (SDA). Clozapine is never used as the first line antipsychotic medication and is reserved for patients with TRS. Standard international guidelines (35,36) are followed to monitor total and differential leukocyte counts of all patients receiving clozapine. Their metabolic parameters are also periodically monitored. Detailed medical records of treatment are maintained for all patients. Most of our outpatients with schizophrenia live in the community with their families. Their medications are directly provided by their first-degree relatives or spouses, who report any degree of non-adherence to the treating psychiatrists during periodically scheduled follow-up visits.

Recruitment of participants

We invited all consecutive patients, who satisfied the following eligibility criteria, into the study: (a) Diagnostic and Statistical Manual of Mental Disorders-IV TR diagnosis of schizophrenia (37), (b) established treatment resistance in the past after failure to respond at least two adequate antipsychotic trials, as documented by treating psychiatrists. An adequate antipsychotic trial was defined by 600 mg chlorpromazine equivalents for a duration of at least 6 weeks with good drug compliance. The two adequate antipsychotic trials included at least one adequate trial with a SDA, (c) on stable dose regimens of clozapine for at least 12 weeks with good drug compliance during that period, (d) origin of South Indian ethnicity. Written informed consent was obtained from the patients and from their first-degree relatives. Patients with severe neurological illnesses, intellectual disability and sensory impairment, precluding the assessment, were excluded.

Clinical assessment

We used the following instruments: (a) Brief Psychiatric Rating Scale (BPRS) to assess treatment response to clozapine (38), (b) Abnormal Involuntary Movements Scale (AIMS) to measure neuroleptic-induced dyskinesia (39), if present, (c) Addenbrooke's Cognitive Examination (ACE-R), a brief cognitive test battery to evaluate cognitive status (40), (d) World Health Organisation Disability Assessment Scale II to quantify the disability (41), (e) Childhood Traumatic Events Scale (CTES) to assess early traumatic experiences before the age of 17 (42), (f) Recent Traumatic Event Scale (RTES) to assess traumatic experiences within the past 3 years (42), (g) Premorbid Adjustment Scale (PAS) to assess premorbid functioning retrospectively (43) and (h) a structured questionnaire to collect sociodemographic, clinical and treatment data. We also recorded data about developmental delays, obstetric complications, urbanisation, recent migrations, smoking, caffeine as well as grape juice consumption and anthropometric measures. ACE-R, CTES, RTES and PAS were translated into the local language, Tamil, and then back translated to English by bilingual health professionals. The final versions were obtained by consensus among the translators who emphasised on content and on conceptual, semantic and technical equivalence.

Serum clozapine assay

Peripheral venous blood samples were collected from all participants by venipuncture, 12 h after their last clozapine dose. Serum clozapine levels were measured by deproteinisation with diethyl ether and subsequent high-performance liquid chromatography with ultra-violet detection (44). The serum clozapine levels were expressed as ng/ml.

CYP1A2 genotyping

Genomic DNA was isolated from whole blood using QIAamp DNA mini Kit (Qiagen GmBH, Hilden, Germany). *CYP1A2*1C, CYP1A2*1D, CYP1A2*1E*

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and *CYP1A2*1F* were genotyped using previously published PCR-restriction fragment length polymorphisms method (19). Briefly, 200 ng of genomic DNA was subjected to PCR amplification using appropriate primers (19) and Genei™ Red Dye PCR master mix (Genei, Bangalore, India). Amplified PCR products were subjected to restriction digestion with appropriate enzymes, *Bse*L I, *Nde* I, *Bsu*R I and *Bsp* 120I (Fermentas-Genetix biotech Asia, New Delhi, India) respectively for *CYP1A2*1C, CYP1A2*1D, CYP1A2*1E* and *CYP1A2*1F*. The digested products were separated by gel electrophoresis in a 3% agarose gel. Then, they were identified by the unique patterns, characteristic to their specific genotypes.

Data collection

The protocol of the study was approved by the Institutional Review Board of CMC, Vellore, India. We provided a fact sheet about the details of this study to all participants. We discussed those details and obtained written informed consent from the participants and from their first-degree relatives or spouses. Every participant was individually assessed for psychopathology using BPRS. The participants were examined for tardive dyskinesia, when present, its severity was recorded using the AIMS. Another independent investigator, who was blind to the clozapine response status, used other instruments and assessed various clinical variables by detailed personal interviews with the participants and their primary care givers. She accessed the medical records of all participants with their consent. The principal investigator (A. P. Rajkumar), who was blind to clozapine response status and to the clinical data, carried out *CYP1A2* genotyping of all samples. Hence, separate investigators collected data on outcome variable of clozapine treatment response (S. Bhuvaneshwari), exposure variables of *CYP1A2* genotype (A. P. Rajkumar) and clinical variables (C. Chitra). They ensured that they were blind to each others' findings till the completion of the study. We followed standard quality control procedures to ensure the accuracy of our data collection, data entry and of the *CYP1A2* genotyping.

Statistical analyses

We initially analysed the study variables using descriptive statistics. Many researchers prefer to define the response to clozapine by greater than 20% reduction in the total score of BPRS (45). However, most clinical psychiatrists do not refer to non-response based on a change on any rating scale, but rather on the presence of persistent positive or negative symptoms (46). Moreover, due to our cross-sectional study design, we defined the response to clozapine, with the widely used cross-sectional threshold of having BPRS total score of 35 or less (45,46). We dichotomously categorised the participants, who had BPRS total scores equal to or less than 35, as clozapine responders. We calculated the *CYP1A2* allele frequencies in our sample and checked whether they were in Hardy-Weinberg Equilibrium (HWE). We calculated the allelic odds ratios (ORs) with 95% confidence intervals (CIs). We used the Cochran-Armitage Test for Trend (CATT) to assess statistical significance of the association between *CYP1A2* genotypes and clinical response to clozapine. We used one sample Kolmogorov-Smirnov test to check for the normal distribution of all continuous variables. We compared the means of psychopathology and disability scores and serum clozapine levels between *CYP1A2* genotypes, using the Kruskal-Wallis test. We used appropriate multivariate statistics to adjust for the effects of clinical variables. We estimated the prerequisite sample size and post hoc power using Quanto 1.2.4 software (47). We performed other analyses using the statistical software packages, STATA 12.0 and PLINK v1.07 (48).

Sample size estimation

A previous study using BPRS for the assessment of clinical outcome has reported that 44.3% patients with TRS were clozapine non-responders (49). The minor allele (C) frequency of *CYP1A2*1F* (rs762551) in the Asian population is 0.386 (50). We estimated that the prerequisite sample size to be 34 cases of clozapine non-responders for an unmatched case control study two-sided test, with 5% alpha error, 80% power and with odds ratio (OR) of 2.5. The variant allele (del-T) frequency of *CYP1A2*1D* (rs35694136) is 0.414 (51). We estimated the prerequisite sample size to be 33 cases of clozapine non-responders for an unmatched case control study two-sided test with 5% alpha error, 80% power and with OR of 2.5.

Results

Sample characteristics

We assessed 113 consecutive patients. We excluded six patients, who were not completely compliant with clozapine, within the past 12 weeks. One patient with severe Parkinson's disease and another with moderate intellectual disability were also excluded. Among the 105 patients, confirmed to be eligible, 101 consented to participate, making the response

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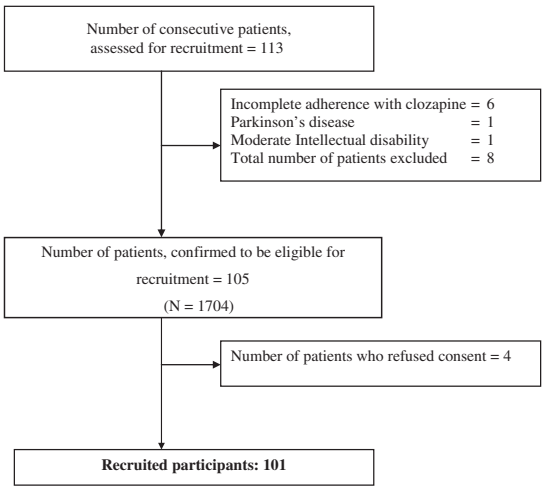

Fig. 1. Flowchart for the recruitment of participants.

rate as 96.2%. Common reasons for refusing consent were lack of interest in study objectives and reluctance to provide blood samples. We present the flowchart for recruitment of the patients as Fig. 1. Participants ($N = 101$) and those who were excluded ($n = 12$) did not differ significantly on gender ($\chi^2 = 0.04$; d.f. $= 1$; $p = 0.84$), age ($t = -1.41$; d.f. $= 111$; $p = 0.16$) and on their duration of illness ($t = -1.27$; d.f. $= 111$; $p = 0.21$). There were 65 (64.4%) clozapine responders and 36 (35.6%) non-responders, who had BPRS total scores, 36 or more.

Table 1 presents the socio-demographic, clinical and treatment profiles of all participants and of the clozapine responders as well as non-responders. The majority of the participants were single or separated men ($n = 66$; 65.3%) who were unemployed ($n = 60$; 59.4%) and living in urban areas ($n = 58$; 57.4%). The duration of clozapine treatment ranged between 4 and 174 months, with a median value of 28 months. More than half of the participants (53.5%) had received clozapine for longer than 2 years. Thirty seven participants (36.6%) had received electroconvulsive therapy in the past. We did not have any participants who were concurrently on carbamazepine or on oral contraceptive pills. The oral doses of clozapine ranged from 100 to 650 mg/day, with a median value of 350 mg/day. Serum clozapine levels of the participants ranged from 104 to 2547 ng/ml, with a median value of 428 ng/ml.

CYP1A2 allele frequencies

Table 2 shows the *CYP1A2* allele frequencies of the four SNP. The allele frequencies of *CYP1A2*1C* (rs2069514), *CYP1A2*1D* (rs35694136), *CYP1A2*1E* (rs2069526) and *CYP1A2*1F* (rs762551) were consistent with the HWE.

Association between *CYP1A2* SNP and clinical responses to clozapine

Table 3 shows the association between the four SNP in the *CYP1A2* gene and response to treatment with clozapine in patients with TRS. None of the *CYP1A2* alleles and genotypes was significantly associated with clozapine treatment response. The *CYP1A2*1F* CC genotype did not significantly increase the risk of clozapine non-response (OR 2.17; 95% CI 0.74–6.38; $p = 0.16$), when compared to the AA genotype. Multiple logistic regression analyses, adjusting for the effects of various clinical variables, including the serum clozapine levels, confirmed these findings. Table 4 presents the association between these four SNP in the *CYP1A2* gene and psychopathology, serum clozapine levels, cognition and disability. The differences among the median values of these variables between the *CYP1A2* genotypes were not statistically significant. Appropriate multiple quantile regression analyses, adjusting for the effects of other clinical variables, including the serum clozapine levels, also confirmed these findings.

Association between *CYP1A2* SNP and adverse effects to clozapine

Our participants had the following adverse effects, related to clozapine: hypersomnolence (n = 77; 76.2%), sialorrhoea (n = 47; 46.5%), nausea or vomiting ($n = 21$; 20.8%), constipation ($n = 21$; 20.8%), erectile dysfunction (13 men; 27.7%), dyslipidaemia ($n = 12$; 11.9%), clozapine-related seizures ($n = 9$; 8.9%), nocturnal enuresis ($n = 6$; 5.9%) and obesity ($n = 15$; 14.9%). As we never re-challenge patients, who have developed neutropenia with clozapine, none of our participants had past history of neutropenia or agranulocytosis. Association between the four CYP1A2 SNP and all adverse effects were not statistically significant (p values > 0.10). Multiple logistic regression analyses, adjusting for the effects of other clinical variables, including the serum clozapine levels, confirmed these findings.

Secondary analyses

We repeated similar analyses using the following three more definitions for non-response to treatment with clozapine: (a) Total score of BPRS 38 and above (worst quartile); (b) At least one the five selected BPRS items for suspiciousness, hallucinatory behaviours, grandiosity, conceptual disorganisation and unusual thought content was scored moderate and above; (c) At least two of

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Table 1. Socio-demographic, clinical and treatment profiles of the clozapine responders ($n = 65$) and non-responders ($n = 36$)

Characteristics	Sample ($N = 101$) *N* (%) /mean (SD)	Clozapine responders ($N = 65$) *N* (%)/mean (SD)	Clozapine non-responders ($N = 36$) *N* (%)/mean (SD)	$\chi^2/t/U$*	*p* value
Male gender	73 (72.3)	50 (76.9)	23 (63.9)	0.88	0.35
Age (years)	35.43 (9.43)	35.46 (9.07)	35.36 (10.18)	0.20	0.84
Number of years of education	11.86 (3.89)	11.91 (3.84)	11.78 (4.02)	1112.00	0.68
Monthly family income (INR)	4733 (6062)	5065 (6833)	4135 (4363)	986.50	0.19
Body mass index (kg/m^2)	24.54 (4.64)	24.85 (4.50)	23.99 (4.90)	1.20	0.24
Family history of schizophrenia	17 (16.8)	11 (16.9)	6 (16.7)	0.00	0.97
Age of onset of illness (years)	23.07 (7.22)	22.32 (6.10)	24.42 (8.82)	1076.50	0.51
Duration of illness (years)	12.40 (6.77)	13.14 (7.31)	11.06 (5.51)	1.33	0.19
DUP (months)	11.21 (13.38)	11.37 (14.65)	10.92 (10.92)	1065.00	0.45
Presence of AIMS	11 (10.9)	5 (7.7)	6 (16.7)	1.92	0.17
Paranoid sub-type	85 (84.2)	56 (86.2)	29 (80.6)	0.55	0.46
Past history of catatonia	**5 (4.9)**	**0 (0)**	**5 (13.9)**	—	**0.005**†
Total duration of treatment (months)	113.64 (78.46)	117.62 (85.30)	106.47 (64.82)	0.40	0.69
Total duration of clozapine (months)	41.61 (39.58)	45.83 (42.24)	34.00 (33.47)	991.00	0.20
Oral dose of Clozapine (mg)	**340.84 (119.04)**	**321.92 (101.98)**	**375.00 (140.03)**	**−2.42**	**0.02**
Serum clozapine level (ng/ml)	550.53 (378.46)	503.23 (260.37)	635.93 (523.07)	−1.85	0.07
High caffeine intake‡	24 (23.8)	15 (23.1)	9 (25.0)	0.05	0.83
Smoking ≥ one pack/day	**17 (16.8)**	**7 (10.8)**	**10 (27.8)**	**4.79**	**0.03**
BPRS total score	**34.73 (12.45)**	**27.94 (3.79)**	**47.00 (13.27)**	**0.00**	**<0.001**
ACE-R total score	**63.11 (20.78)**	**67.65 (18.64)**	**54.61 (22.77)**	**3.21**	**0.002**
WHODAS-II total score	17.49 (12.98)	16.49 (12.71)	19.28 (13.45)	−1.72	0.09
CTES total score	8.32 (10.48)	8.62 (10.56)	7.78 (10.46)	1101.50	0.62
RTES total score	5.94 (8.71)	5.55 (7.84)	6.64 (10.17)	1156.50	0.92
PAS total score	54.83 (21.29)	53.46 (17.31)	57.31 (27.15)	−0.97	0.34

DUP, duration of untreated psychosis; AIMS, abnormal involuntary movements; BPRS, Brief Psychiatric Rating Scale; ACE-R, Addenbrooke's cognitive examination-revised; WHODAS-II, World Health Organisation Disability Assessment Scale; CTES, Childhood Traumatic Event Scale; RTES, Recent Traumatic Event Scale; PAS, Premorbid Adjustment Scale; INR, Indian rupees. Clozapine non-responders: participants with BPRS total scores 36 and above.

Statistically significant associations with *p* values < 0.05 are presented in bold.

*Chi square or independent samples *t*-test or Mann-Whitney *U* test between responders and non-responders.

†Fisher exact test *p* value (two tailed).

‡Three or more cups of coffee or tea intake/day.

Table 2. CYP1A2 allele frequencies among the participants ($N = 101$)

SNP	Allele		Allele frequencies		Genotype frequencies (n)			HWE* χ^2, d.f.	*p*
	1	2	1	2	11	12	22		
*CYP1A2*1C* rs2069514	G	A	0.891	0.109	81	18	2	0.68, 1	0.41
*CYP1A2*1D* rs35694136	T	—	0.693	0.307	48	44	9	0.06, 1	0.81
*CYP1A2*1E* rs2069526	T	G	0.911	0.089	84	16	1	0.06, 1	0.81
*CYP1A2*1F* rs762551 rs762551	A	C	0.569	0.431	37	41	23	2.29, 1	0.08

*Goodness of fit with HWE.

these five selected BPRS items was scored moderate and above. Absence of statistically significant associations between *CYP1A2* SNP and clozapine treatment response were replicated using these differing outcome definitions for non-response to clozapine. Participants who smoked more than 20 cigarettes a day ($n = 17$) did not differ in their serum clozapine levels (Kruskal-Wallis $\chi^2 = 0.39$; d.f. = 2; $p = 0.82$) and on their clinical responses to clozapine (CATT $\chi^2 = 2.00$; d.f. = 1; $p = 0.16$) depending on their *CYP1A2*1F* genotypes. Multivariate analyses adjusting for the effects of age, oral dose and of body mass index confirmed these findings.

Discussion

This study examined the association between four SNP in the *CYP1A2* gene and clinical responses to clozapine among patients with TRS, accompanied by structured assessment of clinical variables. Our sample size is relatively larger than most of the available studies on this topic (9–11,32,33) and

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Table 3. Association between *CYP1A2* gene SNP and treatment response to clozapine among the clozapine responders ($n = 65$) and non-responders ($n = 36$)

	Allele		Responder		Non-responder				Responder			Non-responder				
SNP	1	2	1	2	1	2	Allelic OR* (95% CI)	Allelic *p*	11	12	22	11	12	22	CATT† χ^2, d.f.	Genotype *p*
*CYP1A2*1C* rs2069514	G	A	115	15	65	7	0.83 (0.27–2.29)	0.69	52	11	2	29	7	0	0.15, 1	0.70
*CYP1A2*1D* rs35694136	T	–	89	41	51	21	0.89 (0.45–1.75)	0.73	31	27	7	17	17	2	0.13, 1	0.72
*CYP1A2*1E* rs2069526	T	G	118	12	66	6	0.89 (0.26–2.72)	0.83	54	10	1	30	6	0	0.05, 1	0.83
*CYP1A2*1F* rs762551	A	C	79	51	36	36	1.54 (0.83–2.88)	0.14	26	27	12	11	14	11	1.87, 1	0.17

*Calculated with variant allele (2) as the exposure variable and clozapine non-response as the outcome variable.
†CATT.

Table 4. Association between *CYP1A2* gene SNP and serum clozapine levels, psychopathology, disability as well as cognitive status among the participants with TRS ($N = 101$)

SNP and variables		Wild type mean (SD)	Heterozygous variant mean (SD)	Homozygous variant mean (SD)	χ^{2*}	*p*
*CYP1A2*1C*	Serum level†	543.67 (389.96)	580.67 (336.91)	557.00 (455.38)	0.38	0.83
rs2069514	Psychopathology‡	34.49 (12.18)	36.67 (14.23)	27.00 (2.83)	0.85	0.65
	Disability§	17.81 (12.96)	14.50 (12.93)	31.00 (7.07)	3.74	0.15
	Cognitive status¶	62.41 (21.19)	68.00 (17.68)	47.50 (31.82)	1.71	0.43
*CYP1A2*1D*	Serum level†	543.56 (322.01)	559.35 (440.77)	544.56 (369.01)	0.22	0.89
rs35694136	Psychopathology‡	36.17 (14.35)	34.43 (10.96)	28.56 (5.15)	2.58	0.28
	Disability§	19.54 (14.47)	15.43 (11.23)	16.56 (12.19)	1.65	0.44
	Cognitive status¶	61.54 (19.70)	64.00 (21.63)	67.11(23.83)	1.15	0.56
*CYP1A2*1E*	Serum level†	569.55 (401.33)	471.50 (217.98)	217.00	1.81	0.40
rs2069526	Psychopathology‡	34.17 (11.31)	38.31 (17.44)	25.00	2.34	0.31
	Disability§	17.18 (12.89)	20.00 (13.53)	3.00	2.11	0.35
	Cognitive status¶	62.80 (21.08)	63.38 (19.72)	85.00	1.70	0.43
*CYP1A2*1F*	Serum level†	475.22 (260.99)	589.84 (383.27)	601.61(507.29)	1.49	0.48
rs762551	Psychopathology‡	35.59 (15.45)	32.93 (9.27)	36.57 (12.11)	1.69	0.43
	Disability§	17.65 (14.27)	17.37 (12.29)	17.43 (12.59)	0.04	0.98
	Cognitive status¶	60.46 (22.05)	66.63 (20.06)	61.09 (19.94)	2.15	0.34

*Kruskal-Wallis test with two degrees of freedom.
†Serum clozapine level in ng/ml.
‡BPRS total score.
§World Health Organisation Disability Assessment-II Scale total score.
¶Addenbrooke's cognitive examination-revised total score.

we have exclusively recruited only patients with established treatment resistance. The strengths of this study include minimal refusal rate, access to well-documented medical records, estimations of serum clozapine levels, testing multiple outcome definitions and structured assessments of clinical variables such as premorbid adjustment, traumatic life events, cognition as well as disability. Consecutive sampling strategy reduced the possibility of selection bias. The independent assessments of *CYP1A2* genotypes, clozapine treatment response and clinical variables minimised the possibility of observer bias. We attempted to minimise the recall bias on the reported clinical variables by interviewing one or more first-degree relatives of the participants and by verifying their follow-up medical records.

The potential limitations of this study include the cross-sectional clinical assessment of response to clozapine and its dichotomous categorisation. We recruited only the participants, who were maintained on stable dosage of clozapine for a minimum duration 12 weeks, when their treating psychiatrists did not need to change their prescription. Hence, their cross-sectional BPRS scores were more indicative of their persistent psychopathology than of any acute fluctuations in their illnesses. Although many researchers define the response to clozapine by the reduction in the total scores of BPRS, most clinical psychiatrists prefer to use the discrete clinical category of non-response based on the presence of persistent positive or negative symptoms (46). Clinical significance of many statistically significant reductions in the total scores of psychiatric rating scales remains uncertain (52). Hence, we analysed multiple BPRS-derived categorical outcome definitions, as dependent variables, by appropriate multivariate models to confirm our findings. Despite the extensive use of BPRS, we should acknowledge that there are more diverse outcome measures to assess the treatment responses in schizophrenia (53).

Our findings suggest that the presence of two SNP (*CYP1A2*1C* and *CYP1A2*1D*) in the 5′ flanking region and two others (*CYP1A2*1E* and *CYP1A2*1F*) in intron 1 of the *CYP1A2* gene are

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not associated with clinical response to clozapine and with serum clozapine levels. *CYP1A2*1F* has been associated with higher induction of CYP1A2 activity by smoking (10) and by heavy caffeine consumption (11). Earlier case studies have reported possible association between *CYP1A2*1F* and clozapine non-response, especially in smokers (9,10). Small uncontrolled samples and lack of multivariate analyses may explain these reports, because subsequent larger studies did not find any significant association between *CYP1A2* SNP and serum clozapine levels or with clinical response to clozapine (32–34). Associations between *CYP1A2* gene and schizophrenia as well as tardive dyskinesia were also not significant, after corrections for multiple comparisons (54). Our results corroborate the available literature and confirm the lack of association between these four SNP in the *CYP1A2* gene and clinical response to clozapine. Our results do not support the association of *CYP1A2*1F* with low serum clozapine levels in smokers (10). Smoking has been reported to have a major influence over serum clozapine levels (55). Its relationship with clozapine treatment response is, however, controversial (56,57). We may consider that poor clozapine response in smokers may be secondary to smoking rather than to *CYP1A2*1F* (56).

Although early studies have claimed that CYP1A2 genotyping could have high clinical utility for the patients on clozapine (10) and CYP450 pharmacogenetic test chips are being currently marketed (58), our results have proved the contrary. On the basis of our study findings, we conclude that these four *CYP1A2* gene SNP do not help to predict the clinical responses to clozapine. Hence, routine screening for them prior to start clozapine is unwarranted at present. Our study also provides new data on the *CYP1A2* allele frequencies in a population of south Indian ethnicity. These allele frequencies are similar to other Asian and sub-Saharan African populations (50,51,59,60).

Schizophrenia is not a single disease, but a heterogeneous polygenic multi-factorial disorder, caused by multiple common genetic variants (61) as well as environmental factors (62). Searches for rare genetic variants, which exert significant effects on the pathogenesis and on clinical responses of schizophrenia, have not been fruitful (63). The pharmacokinetics and pharmacodynamics of clozapine are also complex (64). Hence, searching for a single gene to explain major variances of the clinical response to clozapine in TRS usually yields negative results. We suggest that future studies to predict treatment responses to clozapine in TRS should spread their nets wide to study multiple candidate genes that may be involved in major pharmacodynamic and pharmacokinetic processes of clozapine. Studies which have moved beyond the traditional focus on neurotransmitters and polymorphisms in genes for associated receptors and transporters have, so far, been more successful in elucidating common genetic variants associated with schizophrenia (61). Hence, we may need genome-wide association studies that use longitudinal assessments of clinical outcomes to better understand the intricacies of clozapine pharmacogenetics. Pharmacogenetic association studies should not underestimate the importance of environmental factors, gene-environment interactions and the utility of clinical variables to predict clinical responses to clozapine (7,22–24). Such studies, investigating both clinical and pharmacogenetic factors together, are called for to make progress towards the goal of identifying patients who are most likely to benefit from clozapine and to prevent unnecessary exposure of non-responders to serious adverse effects of the drug.

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