Research Article

เภสัชพันธุศาสตร์ของยาคลอซาปีนในผู้ป่วยจิตเภท และความสัมพันธ์กับประสิทธิภาพในการรักษา และอาการไม่พึงประสงค์จากการใช้ยา

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บทคัดย่อ

เภสัชพันธุศาสตร์ของยาคลอซาปีนในผู้ป่วยจิตเภท และความสัมพันธ์กับประสิทธิภาพในการรักษา และอาการไม่พึงประสงค์จากการใช้ยา

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ยาคลอซาปีน เป็นยาจิตเวชกลุ่มใหม่ (atypical antipsychotic) ที่ได้รับการรับรองให้ใช้สำหรับรักษาโรคจิตเภทที่ดื้อต่อการรักษา หรือผู้ป่วยที่มีความคิดหรือพยายามฆ่าตัวตาย โดยพบว่ามีหลายปัจจัยที่ส่งผลต่อการตอบสนองทางคลินิกต่อการรักษาด้วยยาคลอซาปีนซึ่งรวมไปถึงปัจจัยการแปรผันทางพันธุกรรม การศึกษานี้จึงมีวัตถุประสงค์เพื่อประเมินความสัมพันธ์ระหว่างปัจจัยทางพันธุกรรมของคน และการตอบสนองทางคลินิกต่อการรักษา รวมไปถึงการเกิดอาการไม่พึงประสงค์จากยาในผู้ป่วยจิตเภทที่ได้รับการรักษาด้วยยาคลอซาปีน **วิธีดำเนินการวิจัย:** อาสาสมัครผู้ใหญ่ที่ใช้ยาคลอซาปีนมานานมากกว่า 1 เดือน และยินยอมเข้าร่วมโครงการจะถูกเจาะเลือดหลังจากได้รับประทานยาคลอซาปีนไปแล้วประมาณ 12 ชั่วโมง ข้อมูลทางคลินิก และผลตรวจทางห้องปฏิบัติการจะถูกเก็บจากแฟ้มข้อมูลผู้ป่วย ลักษณะทางพันธุกรรมของยีน *CYP1A2 ABCB1 HTR2A*; T102C และ *DRD2*; Taq IA จะถูกวิเคราะห์โดยวิธี TaqMan® real-time PCR ระดับยาคลอซาปีน และเมตาบอไลท์ (N-Desmethyl clozapine) ในกระแสเลือดถูกวัดโดยวิธี liquid chromatography-tandem mass spectrometry (LC-MS/MS) สำหรับการประเมินการตอบสนองทางคลินิกต่อการรักษานั้นจะประเมินโดยใช้ CGI-S และ คะแนน Thai HoNOS **ผลการศึกษา:** ลักษณะอาการทางคลินิกของคนไข้ส่วนใหญ่ (ร้อยละ 88, n=37/42) จะอยู่ในระดับปกติถึงเจ็บป่วยเล็กน้อย (CGI-S = 1-3) โดยมีผู้ป่วย 5 คน หากประเมินโดยทั้ง CGI-S และ คะแนน Thai HoNOS แล้วจะพบว่ามีอาการปกติ หรือมีอาการที่คงที่แล้ว (CGI-S = 1 และ Thai HoNOS = 0) ขนาดของการใช้ยาคลอซาปีนโดยเฉลี่ย+ค่าเบี่ยงเบนมาตรฐาน (ต่ำสุด-สูงสุด) คือ 75.63±68 (6.25-400.00) มิลลิกรัมต่อวัน ส่วนระดับยาที่ปรับฐานโดยขนาดยาแล้วพบว่ามีระดับต่ำกว่าในกลุ่มสูบบุหรี่เมื่อเทียบกับคนที่ไม่สูบบุหรี่อย่างมีนัยสำคัญทางสถิติ(1.11±0.87 และ 2.12±1.60 นาโนกรัมต่อมิลลิลิตรของขนาดยาต่อวัน ตามลำดับ) และยังพบว่ามีขนาดสูงในกลุ่มที่ใช้ยาฟลูออกซีทีนอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับกลุ่มที่ไม่ได้ใช้ยาฟลูออกซีทีน (2.69±1.68 และ 1.48±1.24นาโนกรัมต่อมิลลิลิตรของขนาดยาต่อวัน ตามลำดับ) การแปรผันทางพันธุกรรมของยีน *ABCB1* (rs1045642) มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับอัตราส่วนของระดับยาคลอซาปีนต่อเมตาบอไลท์ และยังพบว่ามีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับการที่มีระดับ neutrophile ต่ำกว่าร้อยละ 50 ส่วนการตอบสนองทางคลินิกต่อการรักษาโดยการประเมิน CGI-S นั้นพบว่ามีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับการแปรผันทางพันธุกรรมของยีน *DRD2* Taq IA และการใช้ยาฟลูเฟนาซีนร่วม **สรุปผลการวิจัย:** จากการวิจัยพบถึงความสัมพันธ์ของการแปรผันทางพันธุกรรมของยีน *DRD2* (Taq IA) และยีน *ABCB1* (rs1045642) กับทั้งประสิทธิภาพของการรักษา และแนวโน้มการเกิดอาการไม่พึงประสงค์จากยาคลอซาปีน โดยความสัมพันธ์นี้อาจเกิดจากทั้งการทำงานของยีนหลายๆยีนร่วมกัน และการแปรผันของระดับยา ซึ่งจากผลการศึกษาสามารถสนับสนุนแนวคิดเกี่ยวกับการแปรผันของยีนกับผลการรักษา ซึ่งจะนำมาซึ่งความเข้าใจในเภสัชพันธุศาสตร์ของยาคลอซาปีนที่มากขึ้น และนำไปปรับใช้ในการใช้ยาในการรักษาผู้ป่วยจิตเภท

คำสำคัญ: เภสัชพันธุศาสตร์, ประสิทธิภาพ, อาการไม่พึงประสงค์จากการใช้ยา, คลอซาปีน

Pharmacogenomics of clozapine in schizophrenia patients and associations with efficacy and adverse drug reactions

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Abstract

Pharmacogenomics of clozapine in schizophrenia patients and associations with efficacy and adverse drug reactions

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Clozapine, an atypical antipsychotic drug, is approved for use in treatment-resistant schizophrenia or accompanied by persistent suicidal or self-injurious behavior. Multiple factors could play a role affecting the clinical response of clozapine in schizophrenic patients, including genetic variations. Our objectives were to evaluate the associations between host genetic factors and clinical response, as well as adverse drug reactions, in schizophrenia patients receiving clozapine. **Methods:** Consenting adults receiving clozapine for at least 1 month had a blood sample collected 12 hours after their last dose of clozapine. Clinical data and laboratory data were collected from patient chart. Genotyping candidate genes (*CYP1A2, ABCB1, HTR2A*; T102C, and *DRD2*; Taq IA) was performed TaqMan® real-time PCR. Plasma clozapine and its active metabolite (N-Desmethyl clozapine) concentrations were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Clinical responses were evaluated by CGI-S and Thai HoNOS scores. **Results:** The clinical symptoms of the majority of patients (88%, n=37/42) were normal to mildly ill in severity (CGI-S = 1-3); 5 patients were in the categories of normal or stable illness by CGI-S and Thai HoNOS (CGI-S = 1 and Thai HoNOS = 0). The average + standard deviation (min-max) dose of clozapine was 75.63±68 (6.25-400.00) mg/day. Dose-normalized clozapine concentrations were statistically significant lower in smokers than non-smokers (1.11+0.87 and 2.12+1.60 ng/mL/mg of dose per day, respectively). Moreover, dose-normalized clozapine concentrations were statistically significant higher in fluoxetine users than non-users (2.69±1.68 and 1.48±1.24 ng/mL/mg of dose per day, respectively). A genetic polymorphism of *ABCB1* (rs1045642) was statistically significant associated with the ratio of clozapine to N-Desmethyl clozapine, as well as neutrophil profiles (% neutrophile of less than 50). The non-genetic factors, fluphenazine used and a genetic polymorphism of *DRD2* Taq IA were statistically significant associated with the clinical response evaluated by CGI-S. **Conclusion:** We found associations between genetic polymorphisms of *DRD2* (Taq IA) and *ABCB1* gene (rs1045642) with clinical efficacy and potential adverse drug reaction of clozapine. These associations are likely complicated by multiple genes and their interactions and may also be related to clozapine dose or plasma concentrations. These preliminary findings support exploring other potential candidate genes that could provide a better understanding of the pharmacogenomics of clozapine and help optimize treatment for schizophrenia patients.

Keywords: Pharmacogenomics, Efficacy, Adverse drug reactions, Clozapine

Introduction

Schizophrenia is a chronic psychiatric disorder that has a prevalence of approximately 1% globally. Antipsychotic drugs are the first line treatment for schizophrenia but about 30% of patients respond poorly to these treatments, termed “treatment-resistant schizophrenia; TRS” (Siskind *et al.*, 2018). Clozapine, an atypical antipsychotic drug, is approved for use in TRS and has favorable efficacy for many symptomatic conditions, including aggression, suicide, and self-injury (Chakos, Lieberman, Hoffman, Bradford, & Sheitman, 2001; Tiihonen *et al.*, 2017). Clozapine is an antagonist to serotonin (5-HT2A, 5-HT2C, 5-HT6, 5-HT7), dopamine (D4), muscarinic 1, and alpha 1-adrenergic with high binding affinity (<10 nM affinity) (Meltzer, 1994). Clozapine is metabolized to an active metabolite, N-desmethylclozapine, and then further oxidized to clozapine N-oxide (Pirmohamed, Williams, Madden, Templeton, & Park, 1995). The major metabolizing enzymes *in vivo* are CYP1A2 and CYP3A4; while *in vitro* CYP3A4 (70%), CYP1A2 (15%), and CYP2C19/CYP2C8/FMO3 (5%) enzymes has been reported (Wagmann, Meyer, & Maurer, 2016).

The clinical response rate of clozapine treatment in schizophrenic patients is between 30 to 60%; leaving a high percentage of patients who do not respond to treatment (Lewis *et al.*, 2006; McEvoy *et al.*, 2006). Multiple factors could play a role affecting the clinical response of clozapine in schizophrenic patients, including genetic variations. Previous studies have shown that some variations in genes relating to pharmacodynamics (*DRD1, DRD2, DRD3, DRD4, COMT, HTR2C, HTR6, HTR1A, HTR3A, SLC6A3*, and *SLC6A4*) and pharmacokinetics (*CYP1A2, CYP2C19*, and *ABCB1*) were associated with clinical response of clozapine (Numata, Umehara, Ohmori, & Hashimoto, 2018), especially polymorphism in *CYP1A2* *1F which had high enzymes inducibility. Genetic variations of CYP1A2 are associated with lower plasma clozapine concentrations and unsuccessful treatment. (Eap *et al.*, 2004). Conversely, a gene-gene interaction study revealed a desirable clinical response with variations in *HTR2A T102C, HTR2A his452tyr, HTR2C−330-GT/-244-CT, HTR2C Cys23Ser, SLC6A4 5-HTTLPR*, and *H2−1018-G/A* (Sriretnakumar, Huang, & Müller, 2015).

Clozapine-induced agranulocytosis (CIA) (Absolute neutrophil count (ANC) ≤ 500/mm3) is a factor limiting its widespread use (Nielsen, Dahm, Lublin, & Taylor, 2010). The reported incidence of CIA within the first year of clozapine treatment is 0.8 (Alvir, Lieberman, Safferman, Schwimmer, & Schaaf, 1993) but frequently occurs within the first 6–18 weeks of treatment (Atkin *et al.*, 1996). Even though the incidence of CIA is low it can be life-threatening. Moreover, clozapine associated with single drop or cumulative drop within 3 weeks of white blood cell (WBC) < 3500/mm3 (leukopenia) and/or ANC < 2000/mm3 (granulocytopenia) ("CLOZARIL [package insert]," 2017).

The mechanism of CIA is not well understood but studies have shown associations between genetic variations and clozapine adverse drug reactions (ADR). A genome-wide association study (GWAS) and an exome-sequencing study revealed an association between *HLA* gene variations and CIA, specifically *HLA-DQB1* (126Q) (P=4.7x1014, odds ratio (OR)=0.19, 95% confidence interval (CI)=0.12–0.29) (Goldstein *et al.*, 2014). A case-control study has also demonstrated that patients who carry *HLA-DQB1* had a higher risk of experiencing CIA (16.9 times higher) (Athanasiou *et al.*, 2011). A study in Japan reported an increased risk of having clozapine-induced agranulocytosis/granulocytopenia (CIAG) in patients with *HLA-B*5901* (p=3.81x10-8, OR=10.7) (Saito *et al.*, 2016). Other studies have shown an association between *ABCB1* polymorphisms and clozapine ADR (van der Weide *et al.*, 2017).

Clozapine is widely used as first line treatment for unresponsiveness schizophrenia or accompanied by persistent suicidal or self-injurious behavior in Thailand. However, the pharmacogenomics of clozapine in Thai population is unknown. Our study aimed to evaluate the association of genetic variations of genes involved in clozapine pharmacokinetics, *CYP1A2* and *ABCB1*, and pharmacodynamics, *HTR2A*; T102C, His452Tyr, *DRD2*; Taq IA, with efficacy and safety of clozapine in schizophrenia patients.

Methodology

Subjects

Schizophrenic patients diagnosed by psychiatrist as International Statistical Classification of Disease-10 (ICD-10) F20-29 by DSM-IV and DSM-V who had received clozapine treatment for more than 1 month were considered eligible for enrollment. Patients who had congenital neutropenia or immune mediated-neutropenia or received any cancer treatments were excluded. All participants were attending out-patient clinics at Suan-Prung hospital, Chiang Mai, Thailand and the study was approved by the local ethics committee. Eligible participants received a complete description of the study before being asked for written informed consent.

Sample collection

Blood samples were collected in 2 EDTA tubes each 6 mL for genetics and plasma drug concentration analyses. Samples were collected in the morning, about 12 hours after the last dose or prior to receiving their scheduled dose of clozapine. Samples were stored at -20ºC until analysis.

Evaluation of Treatment Outcome

Treatment efficacy was evaluated using The Clinical Global Impression – Severity (CGI-S) score and Thai Health of the Nation Outcome Scales (Thai HoNOS). The CGI-S response was defined as having a score of 1 and 2 (not at all ill and borderline mentally ill) for CGI-S while the full response had only a score 1 (normal). The HoNOS stable group was defined as having a score of 0 for Thai HoNOS. The CGI-S and HoNOS stable group was defined as having a score of 1 for CGI-S and 0 scores for Thai HoNOS. Safety was evaluated using white blood cell (WBC) and absolute neutrophil counts (ANC) (Llanchezhian, Joseph, & Rabinarayan). Patient safety profiles were classified as leukopenia: WBC<3,500/mm3; granulocytopenia: ANC <2,000/mm3; and agranulocytosis: ANC<500/mm3.

Genetic Analysis

Sample preparation and DNA extraction: DNA was extracted from whole blood samples (EDTA tubes) by PureLink® Genomic DNA Kits (Life Technologies, CA, USA). DNA concentrations and the purity were measured by NanoDrop spectrophotometer at wavelength 260 nm (Thermo Fisher Scientific, Wilmington, DE, USA). All DNA samples were stored at Suandok Repository Unit, Omics Center for Health Sciences: OCHS, Faculty of Medicine, Chiang Mai University

Genotyping: The TaqMan® SNP Genotyping assay (Life Technologies, Camarillo, CA, USA) was used to genotype *DRD2* Taq IA (rs1800497), *HTR2A* 102C>T (rs6313), and *ABCB1* (rs7787082), and the TaqMan® Drug Metabolism Genotyping assay was used for *ABCB1* 3435C>T (rs1045642) and *CYP1A2* -163C>A (*1F) (rs762551).

Measurement of plasma clozapine and N-desmethylclozapine concentrations

Plasma samples were separated by centrifuging at 800 x g for 10 minutes and stored at -70ºC until analysis. Clozapine and N-desmethylclozapine were quantified using a validated liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) Assay (Couchman *et al.*, 2018; Wohlfarth, Toepfner, Hermanns-Clausen, & Auwärter, 2011). Deuterated analytes (d4-clozapine and N-desmethylclozapine-d8) were used as internal standards. Analytes were extracted from plasma using a liquid-liquid extraction under alkaline condition using ethyl acetate as organic solvent. Chromatographic separation was performed on a Synergi Polar RP column using a gradient elution with 1.0 mM ammonium formate and methanol. Both analytes were quantified over the concentration range of 20 to 1,000 ng/mL, with a lower limit of quantification (LLOQ) of 20 ng/mL. Accuracy ranged from 101% to 104% for clozapine and 97% to 100% for N-desmethylclozapine, with precision <4.0% for both analytes.

Statistical analysis

Hardy–Weinberg equilibrium, allele, and genotype frequencies of all candidate SNPs were analyzed using the Haploview program v4.2 (Broad Institute, Cambridge, MA, USA). The distribution of data among groups was analyzed using a Chi-square test. After testing for the normality, associations were further analyzed by chi-square, ANOVA, Mann–Whitney U-test, or Kruskal–Wallis test. The multivariate multiple regression analysis was used to analyze multiple factors and outcomes. Statistically significant were considered as p values <0.05. Statistical analyses were performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Forty-two participants (male 64%, female 36%) were enrolled and the clinical characteristics are shown in Table 1. The majority of patients (64.3%) were diagnosed with continuous paranoid schizophrenia, and most were non-smokers (59.5%) and non-alcohol drinkers (88.1%). Of note, 60% of patients were caffeine users. Almost all participants had co-medication (41/42, 97.6%). The frequently co-medication used were trihexyphenidyl (73.81%), fluphenazine decanoate (30.95%), trifluoperazine (21.43%), valproate (21.43%), fluoxetine (21.43%), lithium (19.05%), risperidone (19.05%), and perphenazine (19.05%). The mean+SD (min-max) duration of clozapine used from initial dose was 42.27±29.48 months (1.00-82.10) and duration of clozapine current dose was 23.44±25.75 months (1.00-81.80).

Table 1 Demographic and clinical characteristics of patients *(N = 42)*

Characteristics		N	(%)
Gender	Male	27	(64.29)
	Female	15	(35.71)
Age, years (mean ±SD) (min-max)	45.05±11.10 (21-64)		
Weight, kg (mean ±SD) (min-max)	70.38±13.07 (51-100.7)		
Height, cm (mean ±SD) (min-max)	162.47±8.85 (146-180)		
Marital status	Single	27	(64.29)
	Widowed	1	(2.38)
	Married	14	(33.33)
Nationality	Thai	39	(92.86)
	Tai Yai	1	(2.38)
	Burmese	2	(4.76)
Ethnicity	Thai	39	(92.86)
	Tai Yai	1	(2.38)
	Myanmar	2	(4.76)
Education level	Illiterate	5	(11.90)
	Primary school	13	(30.95)
	High school	19	(45.24)
	Bachelor's degree or higher	5	(11.90)
Primary diagnosis	Paranoid schizophrenia, Continuous	27	(64.29)
	Schizoaffective disorder, manic type	4	(9.52)
	Paranoid schizophrenia, Incomplete remission	2	(4.76)
	Schizophrenia, unspecified, Continuous	2	(4.76)
	Paranoid schizophrenia, Episodic with progressive deficit	1	(2.38)
	Paranoid schizophrenia, Complete remission	1	(2.38)
	Undifferentiated schizophrenia, Continuous	1	(2.38)
	Paranoid schizophrenia, Episodic remittent	1	(2.38)
	Undifferentiated schizophrenia, Episodic with progressive deficit	1	(2.38)
	Schizoaffective disorder, depressive type	1	(2.38)
	Residual schizophrenia, Continuous	1	(2.38)
Clozapine dose ,mg/day (mean ±SD) (min-max)	75.63±68 (6.25-400)		
Clozapine duration from initial dose, month (mean ±SD) (min-max)	42.27±29.48 (1.00-82.10)		
Clozapine duration of current dose, month (mean ±SD) (min-max)	23.44±25.75 (1.00-81.80)		

Table 1 Demographic and clinical characteristics of patients *(N = 42)* (*Continued*)

Characteristics		N	(%)
Co-morbidities	Hypertension	11	(26.19)
	Diabetes	4	(9.52)
	Dyslipidemia	2	(4.76)
	Asthma	1	(2.38)
	Allergy	1	(2.38)
	Osteoarthritis	1	(2.38)
	None	28	(66.67)
Smoking	Yes	16	(38.10)
	No	21	(50.00)
	Used to	4	(9.52)
	Not known	1	(2.38)
Alcohol use	Yes	4	(9.52)
	No	30	(71.43)
	Used to	7	(16.67)
	Not known	1	(2.38)
Caffeine use	Yes	25	(59.52)
	No	16	(38.10)
	Not known	1	(2.38)
Narcotics history	Yes	4	(9.52)
	No	36	(85.71)
	Not known	2	(4.76)
Suicidal history	Yes	2	(4.76)
	No	38	(90.48)
	Not known	2	(4.76)

Efficacy

All patients had good drug compliance. Forty-seven percent of patients were categorized as mild illness (CGI-S = 3) and the median Thai HoNOS total score was 1 (IQR = 0 - 20). The highest Thai HoNOS score of 4 was reported for some participants with hallucination/ delusion and other mental and behavioral problems (Table 2). Forty percent of patients were classified as CGI-S response and 55% were stable as evaluated by HoNOS. However, only five patients were stable in both CGI-S and HoNOS.

Table 2 Clinical evaluation *(N = 42)*

Clinical data		
Compliance score, Mean ± SD (min-max)		9.36±1.01 (6-10)
CGI-S, Mean ± SD (min-max)		2.57±0.97 (1-5)
CGI-S category, N (%)	1 (Normal, not at all ill)	7 (16.67)
	2 (Borderline mentally ill)	10 (23.81)
	3 (Mildly ill)	20 (47.62)
	4 (Moderately ill)	4 (9.52)
	5 (Markedly ill)	1 (2.38)
	6 (Severely ill)	0

Table 2 Clinical evaluation *(N = 42)* (*Continued*)

Clinical data		
Thai HoNOS score category, Median ± IQR (min-max)	1.Overactive, aggressive, disruptive, or agitated	0±0 (0-3)
	2.non-accidental self-injury	0±0 (0-2)
	3.Problem drinking or drug-taking	0±0 (0-3)
	4.Cognitive problems	0±0 (0-3)
	5.Physical illness or disability problems	0±0 (0-2)
	6.Problems with hallucinations and delusions	0±0.5 (0-4)
	7.Problems with depressed mood	0±0 (0-1)
	8.Other mental and behavioral problems	0±0.5 (0-4)
	9.Problems with relationships	0±0 (0-2)
	10.Problems with activities of daily living	0±0 (0-2)
Thai HoNOS total score (n=111), Median ± IQR (min-max)		1±4 (0-20)
CGI-S response, N (%)		17 (40.48)
CGI-S full response, N (%)		7 (16.67)
HoNOS stable, N (%)		22 (55.00)
CGI-S and HoNOS stable, N (%)		5 (12.20)

Plasma Clozapine and Metabolites Concentrations

Clozapine plasma and metabolite concentrations are shown in Table 3. The mean+ standard deviation, SD (min-max) clozapine dose was 75.63 ± 68 mg/day (6.25 – 400 mg/day). The mean concentrations of clozapine and N-desmethyl clozapine (N-Des) were 114.38±130.99 ng/ml and 60.14±72.69 ng/ml, respectively. When dose normalized, the clozapine concentrations were significantly lower among smokers than the non-smokes (1.11±0.87 and 2.12±1.6 ng/mL/mg of dose per day, respectively; p<0.05). Moreover, dose normalized clozapine concentrations were significantly higher among fluoxetine users than non-users (2.69±1.68 and 1.48±1.24 ng/mL/mg of dose per day, respectively; p<0.05). The multivariate multiple regression analysis of dose normalized clozapine concentrations with non-genetic factors (compliance, smoking, caffeine use, and fluoxetine) and genetic factors (*ABCB1*; 3435C>T, rs7787082, and *CYP1A2* -163C>A) found the significantly association with only fluoxetine use and smoking (p<0.05) (Table 4).

Table 3 Clozapine dosage regimen and concentrations in smokers and non-smokers, Mean ± SD (min-max)

Clozapine data	Total *(N = 42)*	Smoking *(N = 16)*	Non-smoking *(N = 25)*
Clozapine dose (mg/day)	75.63±68 (6.25-400)	104.69±112.90 (12.50-400.00)	57.75±65.93 (6.25-300.00)
Dosing Interval (hours)	16.02±4.06 (12.40-38.30)	16.85±6.16 (13.00-38.30)	15.51±1.91 (12.40-19.10)
Clozapine concentration (ng/mL)	114.38±130.99 (10-573.00)	86.88±82.81 (10.00-308.00)	118.00±139.34 (10.00-573.00)
N-Desmethyl Clozapine concentration (ng/mL)	60.14±72.69 (5-301.00)	57.38±74.62 (5.00-301.00)	56.36±68.73 (5.00-291.00)
Clozapine concentration/Dose (ng/mL x day/mg)	1.74±1.42 (0.13-6.72)	*1.11±0.87 (0.13-3.64)	*2.12±1.60 (0.20-6.72)

Table 3 Clozapine dosage regimen and concentrations in smokers and non-smokers, Mean ± SD (min-max) (*Continued*)

Clozapine data	Total (N = 42)	Smoking (N = 16)	Non-smoking (N = 25)
Clozapine concentration/ N-Des concentration	2.61±1.92 (0.63-10.80)	2.54±1.96 (0.63-8.40)	2.66±1.97 (1.02-10.80)
N-Desmethyl Clozapine concentration/Dose (ng/mL x day/mg)	0.79±0.65 (0.10-2.91)	0.58±0.50 (0.10-1.98)	0.92±0.73 (0.10-2.91)
Clozapine concentration + N-Des concentration/Dose (ng/mL x day/mg)	2.53±2.02 (0.27-9.24)	1.69±1.32 (0.27-5.62)	3.04±2.26 (0.30-9.24)

*p<0.05

Table 4 Multivariate Multiple Regression Results for factors associated with dose normalized clozapine concentrations

Variable	Beta	t	*p-value*
Fluoxetine	-0.307	-2.113	0.041*
Smoking	0.302	2.076	0.045*

*p<0.05

Associations between genetic polymorphisms and treatment outcome

The genotypic frequencies and allelic frequencies of *ABCB1*; 3435C>T, rs7787082, *CYP1A2* -163C>A, *DRD2* Taq IA, *HTR2A* T102C are shown in Table 5. For *CYP1A2* only *1F was tested and defined as homo 1F (*1F/*1F) (59.52%) and non-homo 1F (other) (40.48%). The *DRD2*; Taq IA was defined as A1 allele (A) and A2 allele (G).

Table 5 Genotypic frequency and allelic frequency of candidate genes

Gene	Polymorphism	Genotype	N	Frequency	Allele	Allele frequency
ABCB1	rs7787082	GG	13	30.95%	G	0.548
		AG	20	47.62%	A	0.452
		AA	9	21.43%		
ABCB1	rs1045642	CC	13	30.95%	C	0.607
		CT	25	59.52%	T	0.393
		TT	4	9.52%		
CYP1A2	rs762551	CC	1	2.38%	C	0.214
		CA	16	38.10%	A	0.786
		AA	25	59.52%		
CYP1A2	*1F	Other	17	40.48%		
		*1F/*1F	25	59.52%		
HTR2A	102C>T	CC	5	11.90%	C	0.321
		CT	17	40.48%	T	0.679
		TT	20	47.62%		
DRD2	Taq IA	GG	15	35.71%	G (A2)	0.548
		AG	16	38.10%	A (A1)	0.452
		AA	11	26.19%		

The association between genetic polymorphisms and clozapine/metabolite concentrations are shown in Table 6. A statistically significant association was observed between *ABCB1* rs1045642 and a higher clozapine/N-Des concentration ratio in patients carrying CC and CT than the ones carrying TT. This result indicates lower metabolic activity in adults with CC and CT genotypes, consistent with a high clozapine concentration/Dose ratio and low N - Des/Dose ratio.

Table 6 Association between genetic polymorphisms, clozapine concentration and metabolism

Genes	Polymorphisms	Genotypes	Clozapine concentration /Dose		Clozapine concentration /N-Des† concentration		N-Des concentration /Dose		Clozapine concentration + N-Des concentration/Dose	
			Mean ± SD	(min-max)	Mean ± SD	(min-max)	Mean ± SD	(min-max)	Mean ± SD	(min-max)
ABCB1	rs7787082	GG	2.15 ± 1.50	(0.40 - 5.73)	2.23 ± 1.01	(0.63 - 4.20)	1.07 ± 0.78	(0.20 - 2.91)	3.22 ± 2.25	(0.60 - 8.64)
		GA and AA	1.55 ± 1.37	(0.13 - 6.72)	2.78 ± 2.20	(0.89 - 10.80)	0.67 ± 0.56	(0.10 - 2.52)	2.22 ± 1.87	(0.27 - 9.24)
ABCB1	rs1045642	CC and CT	1.76 ± 1.43	(0.13 - 6.72)	*2.72 ± 1.98	(0.89 - 10.80)	0.78 ± 0.65	(0.10 - 2.91)	2.53 ± 2.03	(0.27 - 9.24)
		TT	1.53 ± 1.51	(0.40 - 3.64)	*1.58 ± 0.63	(0.63 - 2.00)	0.95 ± 0.75	(0.20 - 1.98)	2.49 ± 2.23	(0.60 - 5.62)
CYP1A2	*1F	Other	1.54 ± 0.88	(0.40 - 2.98)	2.63 ± 1.68	(1.05 - 8.40)	0.70 ± 0.45	(0.10 - 1.80)	2.25 ± 1.27	(0.60 - 4.78)
		*1F/*1F	1.87 ± 1.70	(0.13 - 6.72)	2.59 ± 2.10	(0.63 - 10.80)	0.85 ± 0.77	(0.10 - 2.91)	2.72 ± 2.40	(0.27 - 9.24)
HTR2A	102C>T	CC	1.13 ± 1.18	(0.20 - 3.20)	3.63 ± 2.82	(1.75 - 8.40)	0.35 ± 0.29	(0.10 - 0.80)	1.48 ± 1.45	(0.30 - 4.00)
		CT and TT	1.82 ± 1.45	(0.13 - 6.72)	2.47 ± 1.77	(0.63 - 10.80)	0.85 ± 0.67	(0.14 - 2.91)	2.67 ± 2.06	(0.27 - 9.24)
DRD2	Taq IA	A1A1	1.08 ± 0.82	(0.40 - 3.04)	2.87 ± 2.15	(1.02 - 8.40)	0.50 ± 0.35	(0.10 - 1.04)	1.58 ± 1.09	(0.60 - 4.08)
		A1A2/A2A2	1.97 ± 1.52	(0.13 - 6.72)	2.51 ± 1.86	(0.63 - 10.80)	0.90 ± 0.71	(0.10 - 2.91)	2.87 ± 2.17	(0.27 - 9.24)

* $p < 0.05$
†N – Des; N – Desmethyl Clozapine

The association between clinical response and host genetics is shown in Table 7. Only *DRD2* Taq IA was shown to be statistically significantly associated with clinical response. Genetic polymorphisms of the *DRD2* Taq IA gene were associated with the Fully clinical response evaluated by CGI-S criteria. The multivariate multiple regression analysis showed the statistically significant association of CGI-S fully clinical response with fluphenazine decanoate used and *DRD2* Taq IA ($p<0.05$) as shown in Table 8. No associations between co-morbidities, sex, ethnicity, and smoking status and clinical outcomes were found. There was also no association between allele and haplotype and clinical outcome.

Table 7 Association between genetics polymorphism and clinical response, n (%)

Gene	Polymorphism	Genotype	CGI-S response		CGI-S fully response		HoNOS stable		CGI-S and HoNOS stable	
			Yes	No	Yes	No	Yes	No	Yes	No
ABCB1	rs7787082	GG	4 (23.53)	9 (36.00)	2 (28.57)	11 (31.43)	8 (36.36)	4 (22.22)	1 (20.00)	11 (30.56)
		GA and AA	13 (76.47)	16 (64.00)	5 (71.43)	24 (68.57)	14 (63.64)	14 (77.78)	4 (80.00)	25 (69.44)
ABCB1	rs1045642	CC and CT	15 (88.24)	23 (92.00)	6 (85.71)	32 (91.43)	20 (90.91)	17 (94.44)	5 (100.00)	33 (91.67)
		TT	2 (11.76)	2 (8.00)	1 (14.29)	3 (8.57)	2 (9.09)	1 (5.56)	0 (0.00)	3 (8.33)
CYP1A2	*1F	Other	7 (41.18)	10 (40.00)	1 (14.29)	16 (45.71)	8 (36.36)	8 (44.44)	0 (0.00)	17 (47.22)
		*1F/*1F	10 (58.82)	15 (60.00)	6 (85.71)	19 (54.29)	14 (63.64)	10 (55.56)	5 (100.00)	19 (52.78)
HTR2A	102C>T	CC	1 (5.88)	4 (16.00)	0 (0.00)	5 (14.29)	2 (9.09)	2 (11.11)	0 (0.00)	5 (13.89)
		CT and TT	16 (94.12)	21 (84.00)	7 (100.00)	30 (85.71)	20 (90.91)	16 (88.89)	5 (100.00)	31 (86.11)
DRD2	Taq IA	A1A1	5 (29.41)	6 (24.00)	*4 (57.14)	*7 (20.00)	5 (22.73)	4 (22.22)	2 (40.00)	8 (22.22)
		A2A2/A1A2	12 (70.59)	19 (76.00)	*3 (42.86)	*28 (80.00)	17 (77.27)	14 (77.78)	3 (60.00)	28 (77.78)

*$p<0.05$

Table 9 Association between genetic polymorphisms and neutrophil profiles

Gene	Polymorphism	Genotype	Neutrophil, n (%)	
			< 50 %	> 50 %
ABCB1	rs7787082	GG	2 (15.38)	11 (84.62)
		GA and AA	4 (13.79)	25 (86.21)
ABCB1	rs1045642	CC and CT	4 (10.53) *	34 (89.47) *
		TT	2 (50.00) *	2 (50.00) *
CYP1A2	*1F	Other	3 (17.65)	14 (82.35)
		*1F/*1F	3 (12.00)	22 (88.00)
HTR2A	102C>T	CC	0 (0.00)	5 (100.00)
		CT and TT	6 (16.22)	31 (83.78)
DRD2	Taq IA	A1A1	1 (9.09)	10 (90.91)
		A2A2/A1A2	5 (16.13)	26 (83.87)

*$p<0.05$

Table 8 Multivariate Multiple Regression Results for factors associated with CGI-S fully response

Variable	Beta	t	*p-value*
Fluphenazine decanoate	0.538	4.209	<0.001*
DRD2 Taq IA	-0.260	-2.029	0.049*

*$p<0.05$

No adverse reactions were observed in patients treated with clozapine in this cross-sectional study. The laboratory data showed only abnormal neutrophil levels in 6 patients which were lower than 50%. The genetic association analysis found that *ABCB1* rs1045642 TT genotype was significantly associated with a lower neutrophil level (neutrophil count < 50%) when compared to CC and CT genotypes, Table 9 ($p<0.05$).

Discussion

The average maintenance doses of clozapine in our study were 75 mg/day (range 6.25 to 400 mg/day), which is lower than the recommended dose. The recommended target doses of clozapine for schizophrenia is 300-450 mg/day ("CLOZARIL [package insert]," 2017) with the maximum total daily dose of 900 mg/day. The majority of participants were clinically stable and mildly ill using CGI-S category and HoNOS (CGI-S<3 and HoNOS=0), except 5 patients (12%) for whom their clinical status was moderately to markedly ill (n=4 and 1, respectively). The clozapine dose in our study was comparable to another study in Thailand in which reported an average dose of 100 mg (range 6.25 mg to 800 mg) (Sudthanaphan, 2014). In other Asian populations the average clozapine dose was below 300 mg/day, different from Caucasian patients (range 300 to 600 mg/day). Average clozapine doses per day for non-smoking females have been reported to be 150 mg versus 300 mg for smoking males (de Leon *et al.*, 2020). In our study, the clozapine dose was 57.8 mg/day in non-smokers and 104.7 mg/day in smokers. We observed an impact of smoking on clozapine pharmacokinetics, with the clozapine/metabolite ratio almost two-fold higher in non-smokers compared to smokers. Dose normalized clozapine concentrations were also significantly lower among smokers than non-smokers.

The minor allele frequencies of candidate genes in our study were similar to those observed in Han Chinese. For example, the G allele frequency of *ABCB1*; rs7787082

was 0.548 and the A allele was 0.452 [Han Chinese (G: A = 0.5500:0.4499)]. (National Library of Medicine, 2021) The minor allele frequency of *ABCB1*; rs1045642 (T) previously reported in the Thai population was 0.3600 to 0.3902. (Chuwongwattana *et al.*, 2020; Nuntamool *et al.*, 2017), which was similar to our study (T = 0.3930). A study in Thai autistic children and adolescence showed a *CYP1A2*; rs762551 minor allele frequency of 0.239 (Medhasi *et al.*, 2016), comparable to 0.214 in our study. The allele frequency of *DRD2*; Taq IA observed was also concordant with other studies in Thai (A2:A1 = 0.5488:0.4512) (Nuntamool *et al.*, 2017) and Han Chinese populations from PubMed database (G=0.5936, A=0.4064).

The metabolic activity was calculated as clozapine concentration/dose ratio. The average clozapine concentration/dose was 1.74 ng/mL x day/mg, which was lower than the ratio in a study of Chinese patients (Ruan *et al.*, 2019). Previously, the average clozapine concentration/dose ratio was 1.96 ng/mL x day/mg in male smokes and 2.07 ng/mL x day/mg in female smokers but in non-smokers was higher, 2.47 ng/mL x day/mg in males and 2.95 ng/mL x day/mg in females (Ruan *et al.*, 2019). This study showed the association of fluoxetine used with clozapine concentration which increased significantly by 45% of dose-normalized clozapine concentrations and increased by 38% of its active metabolite. The result was similar to the study of fluoxetine effect on the plasma concentrations of clozapine and its major metabolites in patients with schizophrenia which increased by 58% and 36% of clozapine and norclozapine plasma concentrations respectively (Spina *et al.*, 1998). The strong CYP2D6 inhibitor, fluoxetine, appears to inhibit the metabolism of clozapine which was metabolized by CYP2D6. In addition, the FDA approved clozapine dose reduction in individuals who have CYP2D6 poor metabolizers ("CLOZARIL [package insert]," 2017). However, the majority participants in this study did not use fluoxetine (33/42) leading to low average plasma concentrations of clozapine.

In addition, the *CYP1A2* *1C was not shown to be associated with this ratio while the *CYP1A2* *1F showed a minimal effect, which is consistent with our study. This may have been because the majority of patients in our study were homo- and hetero-*CYP1A2**1F, and only 1 patient had the *CYP1A2* rs762551 CC genotype. Furthermore, several studies indicate that non-genetic factors may also affect *CYP1A2* activity (Gunes & Dahl, 2008). It is important to highlight that although the clozapine concentration/dose ratio in our study was low in some patients they still respond to this treatment. The effect of co-medication (fluphenazine) and long-term used of clozapine resulting to stable disease. Thus, clozapine dose was low and showed a low plasma clozapine concentration. In contrast, another study showed a clozapine concentration/dose ratio of 0.34 ng/mL x day/mg, which was associated with a poor response (Eap *et al.*, 2004). Thus, it remains important to further evaluate other genetic and non-genetic factors and response to treatment.

Several studies have shown that clozapine is a substrate for the efflux transporter p-glycoprotein (P-gp) encoded by the *ABCB1* gene. (Thorn, Müller, Altman, & Klein, 2018). We found a low metabolic ratio of clozapine to its metabolite in *ABCB1*; rs1045642 CC and CT genotypes. Previous studies demonstrated greater *ABCB1* expression levels and P-gp activity in patients with C allele, and lower activity in patients with T allele. (Hoffmeyer *et al.*, 2000) Thus, individuals with a TT genotype may have higher metabolic activity of clozapine compared to other genotypes. However, several studies showed low binding affinity of clozapine to P-gp. (Boulton, DeVane, Liston, & Markowitz, 2002; Moons, de Roo, Claes, & Dom, 2011)

The current study recruited patients who had received clozapine treatment for schizophrenia for more than one month. When the clinical symptoms were classified as stable and non-stable, the majority of the patients were observed to be clinically stable. We observed an association between the *DRD2* TaqIA A2A2 and A1A2 genotype and clinical outcome. Previous studies reported that the *DRD2* TaqIA A1 allele affects dopamine D2 receptor expression by decreasing the density of the receptor. (Noble, 2003; Pohjalainen *et al.*, 1998) In addition, studies have shown

smaller caudate in the elderly who carried the A1 allele as compared to those without. (Li, Papenberg, Kalpouzos, Bäckman, & Persson, 2018) The decrease in dopamine receptors may allow clozapine to completely block the D2 receptor; thereby exerting a positive clinical response. Similarly, several studies have shown the effect of *DRD2* Taq IA on antipsychotics treatment response. A pharmacogenomics study in African Americans using clozapine showed that the A1 allele of *DRD2* Taq IA could predict a good clinical response. (Hwang *et al.*, 2005) Moreover, studies of aripiprazole also showed a tendency to a good response for A1 allele carriers. (Kwon *et al.*, 2008; Miura *et al.*, 2012) Thus, decreasing the receptor density may lead to higher efficacy and allow lower therapeutic doses of clozapine. We observed a statistically significantly lower clozapine dose per day in patients with A1A1 genotype than A1A2 and A2A2 genotypes (25 mg/day vs 62.5 mg/day, respectively, $p<0.05$).

No adverse reactions were observed in patients treated with clozapine in this cross-sectional study, which may have been attributed to the low doses used. However, we did observe six patients whose neutrophil percentage was less than 50%. The low percentage of neutrophil was associated with *ABCB1* rs1045642, which is consistent with a Dutch study which reported an association between *ABCB1* rs1045642 TT genotype and agranulocytosis and neutropenia. (van der Weide *et al.*, 2017)

This cross-sectional, retrospective study lacks baseline values of CGI-S, HoNOS score, and co-medication used profile for all participants, likely leading to bias. Moreover, the one-point blood collection may lead to an unclarified association of clozapine level and clinical response. The incidence of leukopenia, granulocytopenia, and agranulocytosis was very low compared to prior studies (Alvir *et al.*, 1993; Sudthanaphan, 2014), which may have been driven by our relatively small sample size. Multiple genes play a role in clozapine treatment response; (Numata *et al.*, 2018) and it is important to continue to evaluate host genetics in large cohorts of patients to help optimize treatments for schizophrenia patients.

Conclusions

Forty-two schizophrenia patients on clozapine for more than one month were recruited. Most of them were normal to mild illness. The average clozapine dose per day was lower than the usual therapeutic dose. Non-genetic factors, smoking and fluoxetine used, were significantly associated with dose-normalized clozapine concentrations. In addition, using fluphenazine as co-medication was significantly associated with the patients' clinical response. A genetic polymorphism of *ABCB1* (rs1045642) was associated with the ratios of clozapine to its active metabolite, as well as neutrophil profiles (% neutrophile of less than 50). Furthermore, a genetic polymorphism of *DRD2* Taq IA was significantly associated with the patients' clinical response evaluated by CGI-S. Genetic factors may influence the clinical response and adverse reactions from clozapine in schizophrenia patients.

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Conflict of Interest

The authors declare no conflict of interest.

Ethics approval statement

This study was approved by the ethical committees of Suan-Prung hospital, Chiang Mai, Thailand (number 13/2563).

References

Alvir, J. M., Lieberman, J. A., Safferman, A. Z., Schwimmer, J. L., & Schaaf, J. A. (1993). Clozapine-induced agranulocytosis. Incidence and risk factors in the United States. *N Engl J Med, 329*(3), 162-167. doi:10.1056/nejm199307153290303

Athanasiou, M. C., Dettling, M., Cascorbi, I., Mosyagin, I., Salisbury, B. A., Pierz, K. A., *et al.* (2011). Candidate gene analysis identifies a polymorphism in HLA-DQB1 associated with clozapine-induced agranulocytosis. *J Clin Psychiatry, 72*(4), 458-463. doi:10.4088/JCP.09m05527yel

Atkin, K., Kendall, F., Gould, D., Freeman, H., Liberman, J., & O'Sullivan, D. (1996). Neutropenia and agranulocytosis in patients receiving clozapine in the UK and Ireland. *Br J Psychiatry, 169*(4), 483-488. doi:10.1192/bjp.169.4.483

Boulton, D. W., DeVane, C. L., Liston, H. L., & Markowitz, J. S. (2002). In vitro P-glycoprotein affinity for atypical and conventional antipsychotics. *Life Sci, 71*(2), 163-169. doi:10.1016/s0024-3205(02)01680-6

Chakos, M., Lieberman, J., Hoffman, E., Bradford, D., & Sheitman, B. (2001). Effectiveness of second-generation antipsychotics in patients with treatment-resistant schizophrenia: a review and meta-analysis of randomized trials. *Am J Psychiatry, 158*(4), 518-526. doi:10.1176/appi.ajp.158.4.518

Chuwongwattana, S., Jantararoungtong, T., Prommas, S., Medhasi, S., Puangpetch, A., & Sukasem, C. (2020). Impact of CYP2C19, CYP3A4, ABCB1, and FMO3 genotypes on plasma voriconazole in Thai patients with invasive fungal infections. *Pharmacol Res Perspect, 8*(6), e00665. doi:10.1002/prp2.665

CLOZARIL [package insert]. (2017). In Novartis Pharmaceuticals Corporation (Ed.).

Couchman, L., Fisher, D. S., Subramaniam, K., Handley, S. A., Boughtflower, R. J., Benton, C. M., & Flanagan, R. J. (2018). Ultra-fast LC-MS/MS in therapeutic drug monitoring: Quantification of clozapine and norclozapine in human plasma. *Drug Test Anal, 10*(2), 323-329. doi:10.1002/dta.2223

de Leon, J., Rajkumar, A. P., Kaithi, A. R., Schoretsanitis, G., Kane, J. M., Wang, C.-Y., *et al.* (2020). Do Asian Patients Require Only Half of the Clozapine Dose Prescribed for Caucasians? A Critical Overview. *Indian journal of psychological medicine, 42*(1), 4-10. doi:10.4103/IJPSYM.IJPSYM_379_19

Eap, C. B., Bender, S., Jaquenoud Sirot, E., Cucchia, G., Jonzier-Perey, M., Baumann, P., *et al.* (2004). Nonresponse to clozapine and ultrarapid CYP1A2 activity: clinical data and analysis of CYP1A2 gene. *J Clin Psychopharmacol, 24*(2), 214-219. doi:10.1097/01.jcp.0000116646.91923.2f

Goldstein, J. I., Jarskog, L. F., Hilliard, C., Alfirevic, A., Duncan, L., Fourches, D., *et al.* (2014). Clozapine-induced agranulocytosis is associated with rare HLA-DQB1 and HLA-B alleles. *Nat Commun, 5*, 4757. doi:10.1038/ncomms5757

Gunes, A., & Dahl, M. L. (2008). Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. *Pharmacogenomics, 9*(5), 625-637. doi:10.2217/14622416.9.5.625

Hoffmeyer, S., Burk, O., von Richter, O., Arnold, H. P., Brockmöller, J., Johne, A., *et al.* (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A, 97*(7), 3473-3478. doi:10.1073/pnas.050585397

Hwang, R., Shinkai, T., De Luca, V., Müller, D. J., Ni, X., Macciardi, F., *et al.* (2005). Association study of 12 polymorphisms spanning the dopamine D(2) receptor gene and clozapine treatment response in two treatment refractory/intolerant populations. *Psychopharmacology (Berl), 181*(1), 179-187. doi:10.1007/s00213-005-2223-5

Kwon, J. S., Kim, E., Kang, D. H., Choi, J. S., Yu, K. S., Jang, I. J., & Shin, S. G. (2008). Taq1A polymorphism in the dopamine D2 receptor gene as a predictor of clinical response to aripiprazole. *Eur Neuropsychopharmacol, 18*(12), 897-907. doi:10.1016/j.euroneuro.2008.07.010

Lewis, S. W., Barnes, T. R. E., Davies, L., Murray, R. M., Dunn, G., Hayhurst, K. P., *et al.* (2006). Randomized controlled trial of effect of prescription of clozapine versus other second-generation antipsychotic drugs in resistant schizophrenia. *Schizophrenia bulletin, 32*(4), 715-723. doi:10.1093/schbul/sbj067

Li, X., Papenberg, G., Kalpouzos, G., Bäckman, L., & Persson, J. (2018). Influence of the DRD2/ANKK1 Taq1A polymorphism on caudate volume in older adults without dementia. *Brain Struct Funct, 223*(6), 2653-2662. doi:10.1007/s00429-018-1650-0

Llanchezhian, R., Joseph, C. R., & Rabinarayan, A. (2012). Urushiol-induced contact dermatitis caused during Shodhana (purificatory measures) of Bhallataka (Semecarpus anacardium Linn.) fruit. *Ayu, 33*(2), 270-273. doi:10.4103/0974-8520.105250

McEvoy, J. P., Lieberman, J. A., Stroup, T. S., Davis, S. M., Meltzer, H. Y., Rosenheck, R. A., *et al.* (2006). Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. *Am J Psychiatry, 163*(4), 600-610. doi:10.1176/appi.ajp.163.4.600

Medhasi, S., Pasomsub, E., Vanwong, N., Ngamsamut, N., Puangpetch, A., Chamnanphon, M., *et al.* (2016). Clinically relevant genetic variants of drug-metabolizing enzyme and transporter genes detected in Thai children and adolescents with autism spectrum disorder. *Neuropsychiatr Dis Treat, 12*, 843-851. doi:10.2147/ndt.S101580

Meltzer, H. Y. (1994). An overview of the mechanism of action of clozapine. *J Clin Psychiatry, 55 Suppl B*, 47-52.

Miura, I., Takeuchi, S., Katsumi, A., Mori, A., Kanno, K., Yang, Q., *et al.* (2012). Effects of aripiprazole and the Taq1A polymorphism in the dopamine D2 receptor gene on the clinical response and plasma monoamine metabolites level during the acute phase of schizophrenia. *J Clin Psychopharmacol, 32*(1), 106-109. doi:10.1097/JCP.0b013e31823f87ac

Moons, T., de Roo, M., Claes, S., & Dom, G. (2011). Relationship between P-glycoprotein and second-generation antipsychotics. *Pharmacogenomics, 12*(8), 1193-1211. doi:10.2217/pgs.11.55

National Library of Medicine. (2021). Reference SNP (rs) Report. Retrieved from https://www.ncbi.nlm.nih.gov/snp/rs7787082?vertical_tab=true

Nielsen, J., Dahm, M., Lublin, H., & Taylor, D. (2010). Psychiatrists' attitude towards and knowledge of clozapine treatment. *J Psychopharmacol, 24*(7), 965-971. doi:10.1177/0269881108100320

Noble, E. P. (2003). D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *Am J Med Genet B Neuropsychiatr Genet, 116b*(1), 103-125. doi:10.1002/ajmg.b.10005

Numata, S., Umehara, H., Ohmori, T., & Hashimoto, R. (2018). Clozapine Pharmacogenetic Studies in Schizophrenia: Efficacy and Agranulocytosis. *Front Pharmacol, 9*, 1049. doi:10.3389/fphar.2018.01049

Nuntamool, N., Ngamsamut, N., Vanwong, N., Puangpetch, A., Chamnanphon, M., Hongkaew, Y., *et al.* (2017). Pharmacogenomics and Efficacy of Risperidone Long-Term Treatment in Thai Autistic Children and Adolescents. *Basic Clin Pharmacol Toxicol, 121*(4), 316-324. doi:10.1111/bcpt.12803

Pirmohamed, M., Williams, D., Madden, S., Templeton, E., & Park, B. K. (1995). Metabolism and bioactivation of clozapine by human liver in vitro. *J Pharmacol Exp Ther, 272*(3), 984-990.

Pohjalainen, T., Rinne, J. O., Någren, K., Lehikoinen, P., Anttila, K., Syvälahti, E. K., & Hietala, J. (1998). The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Mol Psychiatry, 3*(3), 256-260. doi:10.1038/sj.mp.4000350

Ruan, C. J., Zang, Y. N., Wang, C. Y., Cheng, Y. H., Sun, C., Spina, E., & de Leon, J. (2019). Clozapine Metabolism in East Asians and Caucasians: A Pilot Exploration of the Prevalence of Poor Metabolizers and a Systematic Review. *J Clin Psychopharmacol, 39*(2), 135-144. doi:10.1097/jcp.0000000000001018

Saito, T., Ikeda, M., Mushiroda, T., Ozeki, T., Kondo, K., Shimasaki, A., *et al.* (2016). Pharmacogenomic Study of Clozapine-Induced Agranulocytosis/Granulocytopenia in a Japanese Population. *Biol Psychiatry, 80*(8), 636-642. doi:10.1016/j.biopsych.2015.12.006

Siskind, D. J., Lee, M., Ravindran, A., Zhang, Q., Ma, E., Motamarri, B., & Kisely, S. (2018). Augmentation strategies for clozapine refractory schizophrenia: A systematic review and meta-analysis. *Aust N Z J Psychiatry, 52*(8), 751-767. doi:10.1177/0004867418772351

Spina, E., Avenoso, A., Facciolà, G., Fabrazzo, M., Monteleone, P., Maj, M., *et al.* (1998). Effect of fluoxetine on the plasma concentrations of clozapine and its major metabolites in patients with schizophrenia. *Int Clin Psychopharmacol, 13*(3), 141-145. doi:10.1097/00004850-199805000-00009

Sriretnakumar, V., Huang, E., & Müller, D. J. (2015). Pharmacogenetics of clozapine treatment response and side-effects in schizophrenia: an update. *Expert Opin Drug Metab Toxicol, 11*(11), 1709-1731. doi:10.1517/17425255.2015.1075003

Sudthanaphan, K. (2014). Prevalence and characteristic of clozapine induced neutropenia in patients with schizophrinia. *Journal of Somdet Chaopraya Institute of Psychiatry, 8*(1), 11-21.

Thorn, C. F., Müller, D. J., Altman, R. B., & Klein, T. E. (2018). PharmGKB summary: clozapine pathway, pharmacokinetics. *Pharmacogenet Genomics, 28*(9), 214-222. doi:10.1097/FPC.0000000000000347

Tiihonen, J., Mittendorfer-Rutz, E., Majak, M., Mehtälä, J., Hoti, F., Jedenius, E., *et al.* (2017). Real-World Effectiveness of Antipsychotic Treatments in a Nationwide Cohort of 29 823 Patients With Schizophrenia. *JAMA Psychiatry, 74*(7), 686-693. doi:10.1001/jamapsychiatry.2017.1322

van der Weide, K., Loovers, H., Pondman, K., Bogers, J., van der Straaten, T., Langemeijer, E., *et al.* (2017). Genetic risk factors for clozapine-induced neutropenia and agranulocytosis in a Dutch psychiatric population. *Pharmacogenomics J, 17*(5), 471-478. doi:10.1038/tpj.2016.32

Wagmann, L., Meyer, M. R., & Maurer, H. H. (2016). What is the contribution of human FMO3 in the N-oxygenation of selected therapeutic drugs and drugs of abuse? *Toxicol Lett, 258*, 55-70. doi:10.1016/j.toxlet.2016.06.013

Wohlfarth, A., Toepfner, N., Hermanns-Clausen, M., & Auwärter, V. (2011). Sensitive quantification of clozapine and its main metabolites norclozapine and clozapine-N-oxide in serum and urine using LC-MS/MS after simple liquid-liquid extraction work-up. *Anal Bioanal Chem, 400*(3), 737-746. doi:10.1007/s00216-011-4831-8