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Pharmacogenomic screening for agranulocytosis and efficacy with clozapine

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Abstract

Aim: To review genetic biomarkers of agranulocytosis and efficacy with clozapine as a screening tool for the safe and effective use of clozapine.

Methods: A PubMed search was performed using PRISMA guidelines for English articles. Separate searches were conducted using "clozapine" AND "agranulocytosis," and "clozapine" AND ("response" OR efficacy "outcome") AND "schizophrenia". Eligible studies reported positive findings with genetic polymorphism(s) associated with clozapine-induced agranulocytosis (CIA) and clozapine's efficacy. Case reports/series, abstracts, systematic reviews, and meta-analyses were excluded. Negative and genome-wide studies were not formally reviewed but included in the discussion.

Results: Twelve out of 572 CIA studies and 32 out of 126 efficacy studies met the eligibility criteria for this review. Most reviewed studies were conducted in small samples of Jewish, Caucasian, and Asian populations using a candidate gene approach.

Conclusion: Future research needs to address the limitations of the findings from the reviewed studies to enable a combined genetic screening for CIA and clozapine response to optimize the safe and effective use of clozapine without unnecessarily exposing potential clozapine nonresponders to CIA or neutropenia.

Keywords: Pharmacogenomic, screening, clozapine, efficacy, agranulocytosis

INTRODUCTION

Schizophrenia is one of the most complex brain disorders that affects 24 million people worldwide[1]. One out of every three patients is diagnosed with treatment-resistant schizophrenia (TRS), which is an inadequate response to two different antipsychotic medications (APMs) despite adequate dose and duration[2]. Clozapine is the only APM with evidence-based efficacy in managing patients with TRS[3]. Clozapine treatment is cost-effective by reducing rehospitalizations[4], suicidality[5,6], substance use relapse, and improving life quality in the treatment-refractory population[7]. Despite these benefits, clozapine is still underprescribed[8-11], with an average delay of 5 years in initiating clozapine in treatment-refractory patients[8]. There is also a myth that patients do not like using clozapine, resulting in less emphasis on inquiring about patients' experiences with clozapine[12]. However, when asked, patients report more positive experiences[13-15] and fewer concerns with clozapine than perceived by clinicians[16].

Although underutilization and delayed clozapine use may be due to its serious adverse effects (AEs) of myocarditis[17] and metabolic syndrome[18], the most limiting AE has been the clozapine-induced agranulocytosis (CIA)[19]. Agranulocytosis is an absolute neutrophil count (ANC) that is < 500 cells/mm with severe and potentially fatal neutropenia with a fatality rate of 0.05%[20] and can be a common cause of a lethal outcome, particularly after pneumonia[21]. Historically, clozapine was available in Europe for about 30 years before its approval in the United States due to several reports of CIA from Europe. However, clozapine remained available for European psychiatrists if they requested it due to its unique efficacy. Other barriers to prescribing clozapine include a relative lack of training for the mental healthcare providers to safely and effectively use clozapine[22,23] and mandatory blood testing[24] to monitor CIA requiring registration with the Risk Evaluation and Mitigation Strategy (REMS)[25,26]. However, regular blood testing in clozapine patients has been challenged for not being cost-effective in the longer run due to the majority of clozapine patients having CIA between four to five months of clozapine initiation, with the incidence of CIA decreasing from 1.3% to 0.07% after one year[27] with less than one day increase in quality-adjusted survival per patient[28]. This finding was replicated in another study during the first year of clozapine treatment[29]. Additionally, this level of mandatory monitoring may delay the clozapine treatment when some evidence suggests that the earlier the treatment with clozapine, the better the response[30-32]. Therefore, researchers have investigated predictive genetic biomarkers for CIA to identify patients less susceptible to developing CIA, requiring less frequent or no blood monitoring over time. At the same time, those at high risk for CIA may still undergo the current level of white blood cell monitoring (WBC) or be provided alternate treatments[33].

Although the main current focus is on finding genetic predictors for CIA, it is equally, if not more, important to discuss the genetic biomarkers for clozapine response. Similar to the AEs, the clozapine response is also variable and unpredictable, with a short-term response of 32% and a long-term response of 39%[34] in a large number of patients with TRS (40%-70%) remaining treatment-resistant[35,36]. Therefore, a patient with a genetic risk for agranulocytosis and clozapine nonresponse may be unnecessarily exposed to a clozapine trial with potential toxicity. Determination of clozapine response is also warranted as clozapine is the only APM, as opposed to other APMs, that is selected for its superior efficacy in treatment-refractory patients. While the research to determine clinical predictors of CIA is underway, many studies have also investigated genetic biomarkers for clozapine's efficacy. Once genetic predictors with high sensitivity and specificity are developed, genetic testing for clozapine response may be utilized as a screening test to assess if it is worth exposing a high-risk patient to CIA. This review assesses currently available data on genetic

biomarkers for CIA and clozapine's response to propose a genetic screening for both a safe and efficacious use of clozapine. However, the discussion of other clinically serious AEs is beyond the scope of this paper, primarily due to inadequate genetic data and/or lower clinical significance than CIA. Similarly, the reviewed studies investigating clozapine response include those with significant results. However, negative studies are presented in the "DISCUSSION" Section.

METHODS

Literature search

A comprehensive literature search was performed for all currently available eligible studies until December 2022 using PRISMA guidelines[37]. Separate searches were conducted on 18th January 2023 for CIA and clozapine response using the key words: "clozapine" AND "agranulocytosis," and "clozapine" AND ("response" OR efficacy "outcome") AND "schizophrenia". Only peer-reviewed articles on human subjects published in English were eligible. These searches were limited to the titles and abstracts of the papers, which were reviewed against study eligibility criteria, which included studies that compared the frequency of genetic polymorphism(s) associated with CIA from those that did not. Genetic studies investigating the polymorphisms associated with the antipsychotic response to clozapine were also included. Lack of information on clozapine dosing and treatment duration was not an exclusion criterion for efficacy studies since some studies did not report this information. Case reports, case series, conference proceedings, narratives, abstracts, systematic reviews, and meta-analyses were not included. Only studies with significant positive findings were reviewed with or without Bonferroni corrections. Replicated findings were discussed in more detail. The negative and the genome-wide association studies (GWAS) were not included in the study tables for brevity and clarity but were reviewed under the "DISCUSSION" Section.

Data extraction process

Data items collected included the subjects' ethnicity, the genetic variant(s) investigated, study subjects with at least one genetic variant, and the cases and controls with no genetic variant(s). For CIA, these data included ethnicity, haplotype, CIA with haplotype, CIA without haplotype, controls with haplotype, and controls without haplotype, specificity, and sensitivity of the findings, odd ratio, and *P*-values. For the clozapine response, the data included the genetic predictors, sample size, ethnicity, the clozapine dose and treatment duration, assessment measures, and the significance of the results.

RESULTS

Clozapine-induced agranulocytosis

Out of 572 studies of CIA, 494 were excluded following the studies screened for titles and abstracts; out of the remaining 57 studies, 21 met the selection criteria after removing duplicates, and studies with insignificant findings left 12 studies for inclusion in the final review. The results of the literature search are shown in the PRISMA flowchart [Figure 1].

All 12 studies reviewed were completed in various ethnic groups, including the Jewish population, non-Jewish Europeans, and other populations[38], which can be seen in Table 1 below.

The first study investigating the CIA primarily focused onthe Jewish population[39]. However, four studies had Jewish as well as non-Jewish populations[40-43]. One study included participants with non-schizophrenia diagnoses[41]. Three GWAS studies were not included in the 12 formally reviewed studies but have been discussed in the "DISCUSSION" Section[51-53]. However, these studies are briefly discussed in the "DISCUSSION" Section.

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Table 1. Significant genetic predictors of clozapine-induced agranulocytosis

Reference	Ethnicity	Haplotype	CIA cases with haplotype	CIA cases without haplotype	Controls with haplotype	Controls without haplotype	Specificity	Sensitivity	OR	*P*-value
Lieberman *et al.*, 1990[39]	Mainly Jewish (22/31)	HLA-B38	5	0	4	13	100%	55%	16.25	0.005
		HLAB38-DR4-DQw3	5	0	2	15	100%	71%	37.5	0.008
Corzo *et al.*, 1995[40]	Jewish	HSP-70 *9.0-A*	16	8	10	44	81%	62%	8.8	0.0001
	Non-Jewish	HSP-70 *9.0-A*	19	21	6	26	81%	47%	3.92	0.01
Yunis *et al.*, 1995[41]	Ashkenazi Jewish	HLA-B38	14	1	7	25	96%	67%	50.0.	<0.0001
		HLA-DR4	14	1	12	20	95%	54%	23.3	
		HLA-B38, DR4, DQw3	13	2	6	26	93%	68%	28.2	0.0004 <0.0001
		HLA-DRB1*0402	11	13	6	48	79%	46%	5.7	0.002
		HLA-DQB1*0302	11	13	8	46	78%	58%	4.9	0.008
		HLA-DQA1*0301	0	24	18	36	60%	0.0%	12.0	0.008
		HLA-DRB1*11	0	24	13	41	63%	0.0%	7.6	0.007
		HLA-DRB4*0101 HLA_DQB1*0302 HLA-DQA1*0301	10	14	6	48	11%	42%	5.7	0.005
	Non-Jewish	-HLA-DR2	17	8	5	24	75%	62%	4.5	0.01
		-HLA-DR2, DQw1	14	11	4	15	58%	78%	4.8	0.03
		-HLA-B7, DR2, DQw1	6	19	0	19	50%	100%	6.0	0.03
Turbay *et al.*, 1997[42]	Non-Jewish	HSP-70 *9.0-A*	19	21	6	26	81%	47%	3.92	0.01
	Non-Jewish European	HLA-DQA1*0102	14	26	4	28	52%	78%	3.77	0.03
		TNFb4	29	13	6	24	65%	83%	8.92	0.0005
		TNFb5	8	34	22	8	19%	27%	0.08	0.0005
Amar *et al.*, 1998[43]	Jewish & Non-Jewish	HLA-DQB1*0201	5	5	7	6	55%	42%	0.86	0.03
Valevski *et al.*, 1998[44]	Israeli Jewish	HLA-B38	5	5	7	50	91%	42%	10.01	*0.04*
Ostrousky *et al.*, 2003[45]	Jewish	NQ02 372 T>c	14	4	14	40	50	78	3.5	0.04
		NQ 02 202 G>A	17	1	54	26	33	94	8.2	0.05
Lahdelma *et al.*, 2001[46]	Finnish	HLA-A1, HLA-A28, HLA-B16	3	23	11	8	26%	21%	0.95	0.0013
			8	18	1	18	50%	89%	8	0.027
			6	16	2	17	51%	75%	3.2	0.027
Dettling *et al.*, 2001[47]	German Caucasians - non-Jewish	HLA-Cw*7	20	11	30	47	81%	40%	2.85	0.02
		HLA-DPB*0401	23	8	40	33	80%	36%	2.37	0.03
		HLA-DQB*0502	5	26	1	76	74%	83%	14.6	0.04
		HLA-DRB1*0101	3	28	20	57	74%	9%	0.30	0.03
		HLA-DRB3*0202	14	17	21	56	77%	40%	0.45	0.01
Dettling *et al.*,	German Caucasians - non-Jewish	HLA-DRB5*0201	5	37	0	0	0%	100%	0.13	0.005
		HLA-Cw7	29	13	33	42	76%	47%	2.84	0.035

2007[48]	Dutch	-HLA-DRB5*0201-HLA-DRB4*000 -HLA-Cw7-B18 -HLA-DRB5*000 -HLA-Cw7-B39-DRB5*000 -HLA-Cw7-B44-DRB5*000	NA	NA	NA	NA	NA	NA	NA	0.004 0.004 0.005 0.018
Van der Weide *et al.*, 2017[49]		-ABCB C3435T (rs1045642) -NQO2 G1541A	18 14	13 17	101 64	140 177	91% 96%	15% 18%	3.13 5.53	0.05 0.004
Athanasiou *et al.*, 2011[50]	Cohort 1: US, Russia, and South Africa	Cohort1 I:	NA	NA	NA	NA	NA	NA	NA	
		DRD1								< 0.05
		CSF2RB								< 0.05
		NTSR1								< 0.05
	Cohort II: German Caucasians, non-Jewish	-HLA-DQB1 6672G>C Cohort II: -HLA-DQB1 6672G>C	8 9	1 1	24 38	52 71	98% 99%	25.0% 19%	17.33 16.82	0.0015 0.00097

Although some of the findings from the reviewed studies have been replicated with some combinatorial assays providing high genetic predictability for CIA, more research is warranted to confirm the clinical utility of genetic testing to predict CIA reliably. The biggest limitations of the reviewed studies include inadequate sample sizes, failing to assess relatively rare genetic predictors for CIA, and significant variability in findings across different ethnic groups. Only genome-wide assays in significantly large sample studies can address the mentioned limitations.

Antipsychotic response with clozapine

In terms of clozapine efficacy, 126 studies were potentially eligible initially. Subsequent screening of 126 studies for title and abstract excluded 83 studies leaving 69, of which 31 were ineligible, six were duplicates, and the remaining 32 had statistically significant results. The PRISMA flowchart with search details is shown in Figure 2.

As can be seen from Table 2 below, reviewed studies examined the PK and PD genetic markers for clozapine efficacy.

Significant changes in clozapine response were reported in four studies with cytochrome P-450 (CYP) enzyme polymorphisms affecting clozapine's metabolism targeting CYP1A2[54-56], and CYP2C19[57], and one study with ABCB1, the gene for p-glycoprotein[58]. At the same time, 13 studies investigated the effects of various genetic polymorphisms in the dopamine system on the clozapine response. Of these, three studies examined Ser9Gly polymorphism of rs6280 in the DRD3 gene with significant positive effects on clozapine response[59-61]. Four studies reported significant findings between clozapine response and DRD2 polymorphisms[62-65]. The remaining six studies examined clozapine response in polymorphisms of DRD4[66,67], DRD1[68,69], COMT[70], and SCL6A3[85]. Among the serotonergic genes, the HTR2A gene for 5HT2A receptors produced the most replicated positive results in five studies[72-74,81,86]. Two of the five studies

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Table 2. Genetic predictors of clozapine response

Reference	Genetic variant	Sample size	Ethnicity	Clozapine dose	Treatment duration	Assessment measure	Significance
Pharmacokinetic Genetic Markers - CYP P450 Enzymes							
Eap *et al.*, 2004[54]	CYP1A2*1F	33 (4/29)	Four smoking schizophrenia patients were nonresponders to clozapine	Dose range 450-800 mg/day	NA	CGI	2 patients with marked and 2 with moderate improvement on CGI after increasing clozapine to 1,400 mg/day
Balibey *et al.*, 2011[55]	CYP1A2*1F	97	Turkish	Mean dose: 308 ± 92 mg/day	18 weeks	BPRS	2.4-fold higher lack of response in patients with homozygosity for CYP1A2*1F (P = 0.02)
De Brito *et al.*, 2015[56]	CYP1A2*1F	Clozapine responders = 54; Clozapine non-responders = 27	Brazilian	Mean dose in CLZ non-responders = 593 ± 114 mg/day Mean dose in CLZ responders: 535 ± 116 mg/day	2 years	BPRS	74% CLZ nonresponders homozygous *vs.* 33% CLZ responders homozygous for CYP1A2*1F (P = 0.0002)
Piatkov *et al.*, 2017[57]	CYP2C19*17 rs12248560	45	Australian (Caucasian, Asian, Pacific Islander)	NA	3 and 12 months	Not assessed formally	Homozygosity for CYP2C19*17 was associated with a lower CLZ/NCLZ ratio and improved clinical response (P = 0.049)
Lee *et al.*, 2012[58]	ABCB1 rs7787082, rs10248420	15	Korean	Mean dose: 319.0 ± 133.1 mg/day	> 6 months	CGI-I	19/20 patients were responders with A/A for ABCB1 rs7787082 (corrected P = 035) *vs.* 20/31 responders with A/A for ABCB1 rs10248420 (corrected P = 0.046)
Dopamine-Associated Genes							
Shaikh *et al.*, 1996[59]	DRD3 Ser9Gly	133	Caucasians	NA		BPRS	53% non-responders *vs.* 36% responders with DRD3 Ser9Gly 1-1 genotype (P = 0.04)
Scharfetter *et al.*, 1999[60]	DRD3 Ser9Gly	32	Pakistani	Maximum dose = 600 mg/day	6 months	BPRS	Better clozapine response in patients with Gly-9 allele than Ser-9 allele (P = 0058)
Hwang *et al.*, 2010[61]	DRD3 rs2134655 in Caucasian samples, rs1394016 in African American samples	232	Caucasians and African Americans	NA	At least 6 months	BPRS, BPOS, BNEG	-A allele (allele 1) of rs2134655 associated with a better response on the BPOS (P = 0.007) in Caucasians. -A dose-dependent relationship between the T allele of rs1394016 and better response on the BNEG scale (P = 0.018) in African Americans
Huang *et al.*, 2016[62]	DRD2 rs2514218	208	Caucasian and African American	NA	6 months	BPRS	DRD2 rs2514218 significant association b/w G/G genotype and BPRS change (adjusted P = 0.033)
Hwang *et al.*, 2006[63]	DRD2, Taq1B, rs1125394, and rs2242593	232	Caucasian and African American	NA	At least 6 months	BPRS, BPOS	-African Americans with the TaqIB B1 allele were associated with a lesser response than those without (P =0.025) -African Americans with allele 1 of rs1125394 with improved response (P = 0.001) -1-1 genotype of rs2242593 with a better

							response than the 1-2 genotype group in African American (P = 0.016)
Hwang *et al.*, 2005[64]	DRD2 Taq1A, Taq1B, rs1125394	132	Caucasian and African American	NA	BPRS	At least 6 months	-TaqIB frequencies differed b/w responders and nonresponders in African Americans (P = 0.033) -Allele 2 (B2) associated with response African Americans. (P = 0.036). -Allele 1 of rs1125394 (P = 0.029) and TaqIA, allele A2 more frequent in African American responders (P = 0.010) - TaqIA A1 (-) associated with response in African Americans (P = 0.032)
Malhotra 1999[65]	DRD2 -141C Ins/Del	72	NA	NA	10 weeks	BPRS	NA
Hwang *et al.*, 2012[66]	-DRD4 120 bp tandem repeat and 142 bp/140 bp -DRD4 48 bp repeat in Caucasian samples	232	Caucasians and African Americans	NA	6 months	BPRS, BPOS, BNEG	-Association b/w 1-copy allele of 120-bp tandem repeat polymorphism and nonresponse in African Americans (P = 0.004). -Short alleles (≤ 6R) with a better response on the BPOS scale (P = 0.031) in the Caucasians
Zhao *et al.*, 2005[67]	DRD4 48 bp variant number tandem repeat	81	Chinese	200-450 mg/day	2 months	PANSS	Frequencies of DRD4 5 allele (P < 0.05) and 5/5 (P < 0.05) genotypes were significantly higher in nonresponders than that in responders
Hwang *et al.*, 2007[68]	-DRD1 rs265976 -Haplotype SNPs in rs265981, rs4532, rs686	232	Caucasians and African Americans	NA	At least 6 months	BPRS, BPOS, BNEG	-DRD1 rs265976, genotype occurred more frequently in responders in African Americans (P = 0.03) -T-G-A haplotype with poor response in Caucasians (P = 0.027)
Potkin *et al.*, 2003[69]	DRD1 A-48G	15	Caucasian and African American	Average Dose = 460 ± 11 mg/day	5 weeks	BPRS	DRD1 2,2 genotype significantly improved with clozapine treatment, demonstrating a 30% decrease in the BPRS-positive symptoms compared to a 7% worsening for the 1,2 genotypes (P < 0.05)
Woodward *et al.*, 2007[70]	COMT Val108/158Met	86	Caucasian and African American	NA	6 months	COWAT, CIGT, DSST ACTT	Better performance on global cognition (P = 0.01), attention and verbal fluency (P < 0.032), and working memory (P < 0.001) in patients with Met/Met than Val/Val genotype after clozapine treatment
Xu *et al.*, 2010[71]	SLC6A3 rs2975226	160	Chinese	Average dose = 415 ± 97 mg/day	At least 8 weeks	BPRS	SNP rs2975226 (T-71A) in the 5′-regulatory region frequencies were statistically different between CLZ responders and nonresponders allele-wise (adjusted P = 0.000674) and genotype-wise (adjusted P = 0.024)
Serotonin-Associated Genes							
Arranz *et al.*, 1996[72]	HTR2A His452Tyr (rs6314)	153	Caucasian	Dose range 125-600 mg/day	NA	GAS	Tyr452 was associated with poorer CLZ response than those without this allele (11% *vs.* 6%, respectively

Arranz *et al.*, 1998[73]	HTR2A G-1438A (rs6311)	274	Caucasian	Dose range 125-600 mg/day	At least 3 months	GAS	Positive association between CLZ response and G-1438/A-1438 genotype (0.002). and G-1438/A allele (P = 0.004) in sample1 and combined sample.1 and 2. Negative correlation was reported with G/G genotype
Masellis *et al.*, 1998[74]	HTR2A His452Tyr (rs6314)	185	Caucasian, African American, and Asians	NA	At least 6 months	GAS	*HTR2A* his452tyr allele and genotype negatively associated with CLZ response (corrected P = 0.04 and P = 0.04, respectively
Arranz *et al.*, 1995[75]	HTR2A T102C (rs6313)	149	European Caucasian	Dose range - 125-600 mg/day	3 months	GAS	-C102/T102 associated with better CLZ response (59%) than T102/T102 (16%) and C102/C102 (25%) in Schizophrenia patients
Souza *et al.*, 2010[76]	HRT3A rs1062613	140	Caucasian and African American	NA	At least 6 months	BPRS	-HRT3A rs1062613 associated with CLZ response (adjusted P = 0.041). -A-C-C haplotype of rs2276302-rs1062613-rs1150226 was associated with better CLZ response (adjusted P = 0.04)
Rajkumar *et al.*, 2012[77]	HTR3A rs1062613, rs2276302	101	Indian	Mean dose: 304.84 ± 119.04 mg/day	At least 12-weeks	BPRS	-T allele of rs1062613 and G allele of rs2276302 associated with CLZ response (P = 0.02) -Combined clinical predictors and HTR3A markers explained 38 % variability in CLZ response
Kohlrausch *et al.*, 2010[78]	SLC6A4 HTTLPR/rs25531	116	Brazilian (European background)	Average dose = 540.91	At least 3 months	BPRS	HTTLPR/rs25531 with SO/SO or SO/LO genotypes associated with CLZ nonresponse (P = 0.01) and LO/LO with CLZ response (P = 0.04)
Yu *et al.*, 1999[79]	HTR6 267T	99	Chinese	Mean dose: 271.6 mg/day for 267C/C, 287.5 mg/day for 267C/T, and 241.7 mg/day for 267T/T	At least 8 weeks	BPRS	HTR6 267T/T genotype had a better CLZ response than T/C or C/C groups
Sodhi *et al.*, 1995[80]	HTR2C Cys23Ser (rs6318)	162	Caucasian	Dose range = 125-600 mg/day	At least 3 months	GAS	Patients with one or more 5HT2C ser alleles were clozapine responders (P = 0.005)
Combinatorial Genetic Assays to Predict Clozapine Response							
Arranz *et al.*, 2000[81]	HTR2A -102-T/C -1438-G/A -His452Tyr HTR2C -330-GT/-244-CT -Cys23Ser 5HTLLPR -H2 -1018-G/A	200	Caucasian	NA	NA	GAS	-Six polymorphisms 5-HT2A 102-T/C, His452Tyr, -1438-G/A, 5-HT2C _330-GT/244-CT, Cys23Ser, 5-HTTLPR, and H2 1018-G/A) showed the strongest association with CLZ response (P < 0·09) and PPV of 76·86% (P = 0·0001). -Strongest correlations with 5-HT2A 102-T/C, and -1438-G/A,
Hwang *et al.*, 2011[82]	In Caucasians -DRD1 rs686 and DRD3 Ser9Gly *-DRD2 Taq*IB and *DRD3* rs2134655	232	Caucasians and African Americans	NA	At least 6 months	BPRS	In Caucasians, strongest gene interactions observed between *DRD1 rs686* and *DRD3* Ser9Gly (P = 0.048), followed by *DRD2 Taq*IB and *DRD3* rs2134655 (P = 0.037), and *DRD2 Taq*IA & *DRD3* rs2134655 (P = 0.040) for BPRS change; & *DRD1*

	-*DRD1* rs4532 and *DRD3* rs1394016 In African Americans - *DRD2* C957T and *DRD3* Ser9Gly -*DRD1* rs686 and *DRD2 Taq* IA -*DRD1* rs265976 and *GRIN2A*						rs4532 and *DRD3* rs1394016 for BPOS change (P = 0.028). -In African Americans, positive interactions observed between *DRD2* C957T and *DRD3* Ser9Gly for BPRS change (corrected P = 0.039); *DRD1* rs686 and *DRD2 Taq*IA for BPOS % change (corrected P = 0.033); and *DRD1* rs265976 and *GRIN2A* (corrected P = 0.005) for BNEG % change
Bosia *et al.*, 2015[83]	-COMT Val158Met (rs4680) and HTR1A-1019C/G (rs6295)	107	Italian	217-248 mg/day	8 and 16 weeks	PANSS	- COMT Met allele and *5-HT1A-R* C allele predicted a better CLZ response than *COMT* Val/Val and *5-HT1A-R C allele* (P = 0.01) and *COMT* Val/Val and *5-HT1A-R* G/G (P = 0.04)
Rajagopal *et al.*, 2018[84]	-DRD4 120 bp duplication and -COMT Val158Met	93	Indian	Average dose 320 mg/day in responders and 387 in nonresponders	At least 12-weeks	BPRS	COMT Met carriers (Met/Met or Val/Met) and 120-bp allele carriers (120/120 or 120/240) showed a better CLZ response than those without these alleles (0.003)

5HT: 5 Hydroxytryptamine; ACTT: auditory consonant trigram test; BNEG: BPRS subscale for negative symptoms; bp: base pair; BPOS: BPRS subscale for positive symptoms; BPRS: brief psychiatric rating scale; CGI-I: clinical global impression improvement; CIA: clozapine-induced agranulocytosis; CIGT: category instance generation test; COMT: catechol-O-methyl transferase; COWA: controlled oral word association test; CYP: cytochrome P450; DRD1: gene for dopamine receptor-1; DRD2: gene for dopamine receptor-2; DRD3: gene for dopamine receptor-3; DRD4: gene for dopamine receptor-4; DSST: digit symbol substitution test; HLA: human leukocyte antigen; HTR1A: gene for serotonin receptor-1A; HTR2A: gene for serotonin receptor-2A; HTR2A: gene for serotonin receptor-2A; HTR3A: gene for serotonin receptor-3A; HTR6: gene for serotonin receptor-6; OR: odds ratio; PANSS: positive and negative syndrome scale; SLC6A3: gene for dopamine transporter; SLC6A4: gene for serotonin transporter.

found the T allele of the T102C polymorphism[75,86], two reported HTR2A His452Tyr (rs6314) to be associated with good clozapine response[72,74], and one found the G-1438A SNP as a significant predictor for clozapine response[73]. The HTR3A gene showed significant results in two studies[76,77]. The significant change in clozapine response with the SLC6A4 gene for the serotonin transporter was also reported in one study[78]. One study produced significant results in clozapine response with HTR6[79] and one with HTR2C[80]. In addition, four studies used more than one candidate gene to conduct a combinatorial analysis to report predictability in clozapine response[81-84]. Three GWAS studies were not included in the 32 formally reviewed studies but have been discussed in the "DISCUSSION" Section[62,87,88].

Like the genetic predictability for CIA, genetic predictors for clozapine response require significantly more research. This is despite replicated findings with some of the pharmacokinetic and pharmacodynamic genetic predictors for clozapine response, including those from the combinatorial assays. Like the genetic predictability of CIA, the genetic predictors of clozapine efficacy are also compromised by the small sample sizes, controversial findings, and lack of large genome-wide studies.

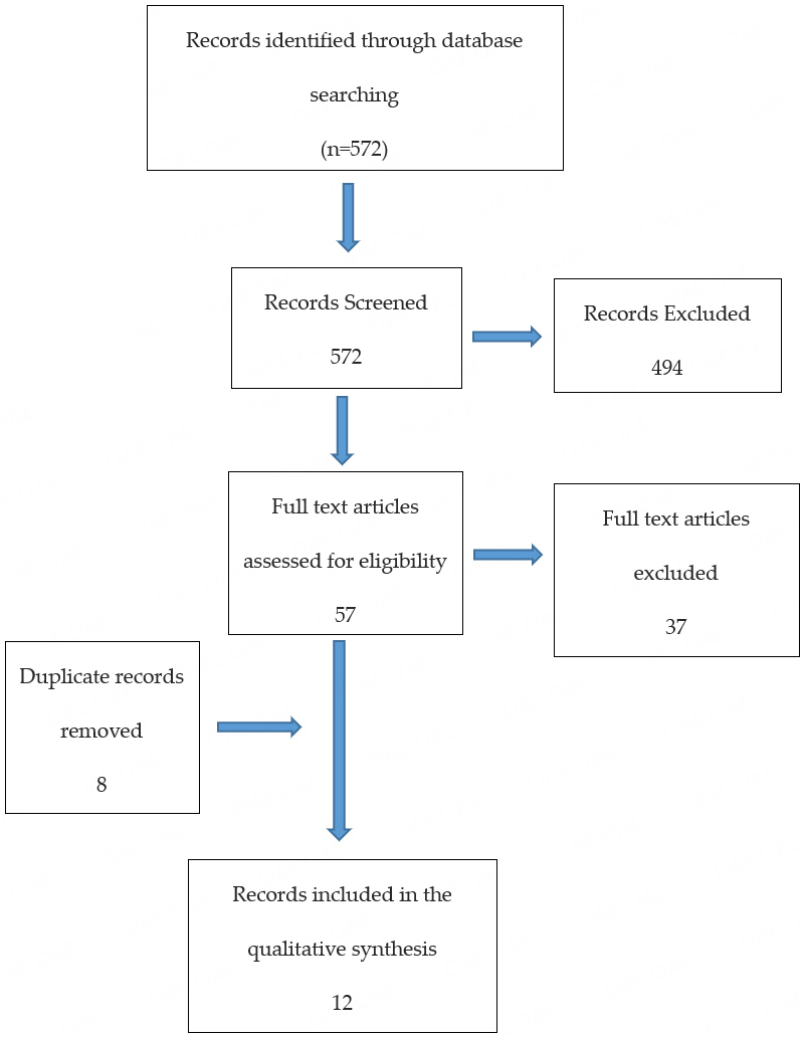

Figure 1. PRISMA flow chart for clozapine-induced agranulocytosis.

DISCUSSION

Most of the studies in this review examined the genetic biomarkers of CIA and clozapine efficacy [Table 1]. Concerning clozapine efficacy, the genetic variants involve pharmacokinetic and pharmacodynamic genetic biomarkers [Table 2]. However, most genetic studies have primarily focused on pharmacodynamic biomarkers involving immune system human leukocyte antigen (HLA) genes from the major histocompatibility system (MHC)[89] [Table 1]. Recently, a few studies have also employed a genome-wide association approach to explore which genes could potentially be associated with CIA[51-53] and[62,87,88]. Therefore, the following paragraphs will present genetic predictors of CIA and clozapine's response.

Clozapine-induced agranulocytosis

Although the bone marrow toxicity of clozapine can express as different types of hematological disorders (i.e., leukocytosis, eosinophilia, or thrombocytopenia), agranulocytosis is one of the most frequent and studied adverse effects[29]. The first case of CIA was reported in 1974 and was later found to range from benign neutropenia to fatal agranulocytosis[90,91]. Several new cases of clozapine-induced bone marrow toxicity were reported a few years later in Europe, which initiated epidemiologic and genetic research in this area[92,93]. Although the potential mechanisms underlying CIA are not entirely understood, CIA may be

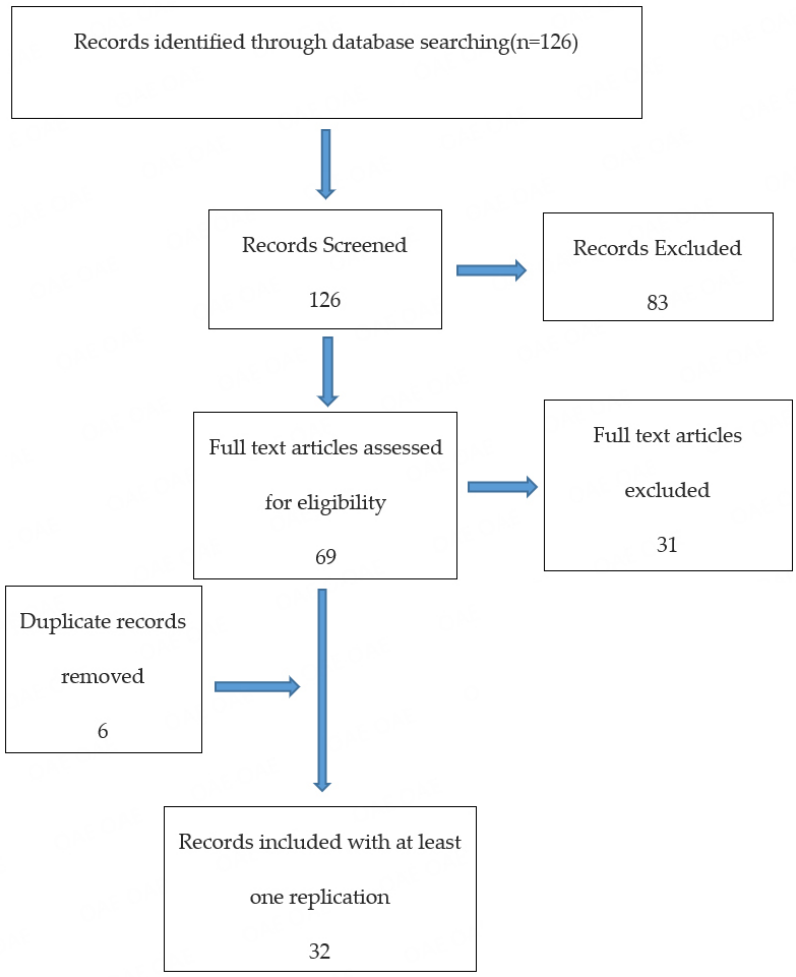

Figure 2. PRISMA flow chart for antipsychotic response with clozapine.

mediated by an autoimmune response targeting neutrophils. A direct toxic effect via nitrenium metabolites has also been suggested to play a role[90]. An indirect effect of increased clozapine levels, as may occur during acute inflammatory stress, has also been proposed[94-96]. Similar increases in clozapine levels via drug interactions or genetic deficiency in metabolism may also contribute to CIA[97-99]. However, a few studies have reported no association of cytochrome P450 enzyme variation with CIA, suggesting that CIA is not dose-dependent[100,101]. Nevertheless, elevated clozapine levels may still compromise immune defenses to combat infections, contributing to bone marrow toxicity[102]. Here it is extremely important to mention that not all cases of agranulocytosis are clozapine-related[103] and may be wrongly attributed to clozapine due to surveillance bias. For example, benign ethnic neutropenia (BEN) is associated with leukopenia that may be mislabeled as CIA in patients receiving clozapine therapy. This may be particularly true for agranulocytosis reported in a relatively large patient population without the high-risk haplotypes that develop CIA. If every case of neutropenia or agranulocytosis is misattributed to clozapine, we may not be able to develop reliable and valid genetic predictors for CIA. Therefore, future research must differentiate CIA from the other phenotypes of agranulocytosis.

Age and gender may also alter the risk for blood marrow toxicity with clozapine. The age-related effects include a 53% increase in CIA for every ten-year increase in age[104]. Thus, women may have a higher risk for

CIA than men due to an older age of onset of psychosis[105] and clozapine initiation. Although few studies have reported no gender differences in risk for CIA[20,106-108], women may have a higher risk of clozapine-induced leukopenia[109-111] than CIA[112]. Women are also prescribed fewer clozapine treatments than men[113]. Taken together, these findings suggest a complex interaction between age and gender as summarized by an earlier onset of psychosis and clozapine treatment in younger men contrasted with a later onset of psychosis and clozapine treatment in older women with a higher age-related risk for CIA.

Genetic predictors for clozapine-induced agranulocytosis

The genetic predictors for CIA are pharmacodynamic (PD) in nature, and pharmacokinetic factors have rarely been associated with CIA. The first evidence for PD genetic factors came from twin studies[114,115], followed by several studies reporting a significant association between polymorphisms in the MHC and CIA[38]. One of these early studies in a small sample reported that 83% of the patients who developed CIA carried the *HLA-B38* marker[39]. The predictability of CIA went up to 100% after 3 HLA alleles (i.e., *HLA-B38*, *HLA-DR4*, and *HLA-DQw3*) were combined compared to only 12% of controls[39]. The finding with HLA-B38 was replicated in other studies[41,44]. A couple of studies found a link between the MHC complex of HSP-70 9.0A in both Jewish[40] and non-Jewish populations[40,42]. Other genetic polymorphisms that have been reported in the Ashkenazi Jewish population, in association with CIA, include HLA-DQA1*0301, HLA-0302, HLA-0402, and HLA-DRB1*11[41]. The role of HLA-DRB1*04 in the CIA has been replicated in a small number of non-Jewish German populations[47]. However, it is worth mentioning that the genetic patterns may vary between different Jewish people due to their migration from the Middle East to many countries and intermarriages with local populations.

Although there is a relative lack of such studies in other ethnic groups, the ethnically diverse allele of HLA-DRB1*04 is reported to be less frequent in Sub-Saharan, Native and North American, and Southeast Asian subjects than the European population[116]. More importantly, a recent review and meta-analysis found a negative probability value of about 99% for CIA without the HLA-DRB1*0402 allele, which may allow relaxed blood monitoring[38]. Interestingly, the high negative predictability of the HLA-DRB1*0402 for CIA[38] is about the same as that of the HLA-B*58:01 with allopurinol[117] and of HLA-B*1511 for carbamazepine for skin reaction[118]. However, the negative predictability of CIA for HLA-DRB1*0402 is higher than that of HLA-B*5701 for abacavir reactions (i.e., 82%)[119]. Although the positive predictability of HLA-DRB1*04:02 for CIA is only about 4%, it is still higher than the positive predictability of HLA-B*15:11 for carbamazepine-induced Steven Johnson Syndrome (i.e., 1%)[120]. These findings suggest that 99% of the individuals without HLA-DRB1*0402 are unlikely to have CIA, while only about 4% of the individuals with HLA-DRB1*0402 are predicted to develop CIA. However, the low positive predictability is counterbalanced by the high negative predictability of HLA-DRB1*0402, making this allele a valuable predictive genetic test for CIA[38]. The high negative predictability of HLA-DRB1*0402 for a relatively rare occurrence of CIA suggests its clinical utility in a much larger group of patients[121].

Like the positive predictability, the ability of HLA-DRB1*04:02 to detect true positive cases of CIA (i.e., sensitivity) is also relatively low but still higher than that of the HLA-A*31:01 (23%)[122], and HLA-B*15:11 (14%)[118] for the carbamazepine-induced skin reactions. However, like the negative predictability, the ability of HLA-DRB1*04:02 to detect true negative cases (i.e., specificity) is high (i.e., 94%) but slightly lower than that of HLA-A*31:01 (i.e., 95%)[122] and HLA-B*15:02 (i.e., 99%)[118] for carbamazepine hypersensitivity. A specificity of 94% with HLA-DRB1*04:02 is interpreted as six out of 100 individuals will be at high risk for agranulocytosis, which is not that different from the actual population risk of 0.8%[123]. Therefore, specificity needs to exceed 99% to be clinically useful. Nevertheless, the predictive value and validity of an HLA-DRB1*04:02 screening test for assessing the patient risk of CIA are comparable to the existing HLA predictors currently used in clinical practice.

In another study, a link between HLA-DQB1*0201 and CIA has been documented in both Jewish and non-Jewish patient populations[43]. Association between NQ02 372 T>C and CIA was also observed but only in the Jewish population[45]. A following study in the Finnish population also found a significant correlation between HLA-A1, HLA-A28, and HLA-B16 and CIA[46]. In another study, CIA in the non-Jewish German population was associated with more MHC polymorphisms, including HLA-Cw*7, HLA-DPB*0401, HLA_DQB*0502, HLA-DRB1*0101, and HLA-DRB3*0202[47]. A later study from the same research group replicated findings with HLA-Cw*7 but no other findings from the earlier study[48]. In a recent meta-analysis, two haplotypes, HLA-DQB1*0502 and HLA_DQB1 6672 G>C, were associated with increased risk for CIA in Europeans[124]. The following study by Van der Weide *et al.* replicated the link between CIA and NQ02 polymorphisms in an earlier study[45,49]. Two prospective clozapine studies reported an odds ratio (OR) of 17 for CIA in patients with an HLA-DQB1 SNP (i.e., 2G>C) with high specificity and sensitivity rates[50]. Later studies have also reported a correlation between clozapine-induced neutropenia and two independent polymorphisms in *HLA-DQB1* (126Q), previously associated with autoimmune disease, and *HLA-B* (158T) with severe drug reactions[52].

Other genes have also been implicated in CIA in the European population, such as SNP rs149104283 on chromosome 12p12.2[51], but not in the Japanese population[125]. However, HLA-B*5901 was correlated with CIA in the Chinese population[126]. One study proposed that patients with clozapine-related neutropenia in the absence of HLA-B*59:01 may be given a clozapine retrial[53]. In addition, CIA was also associated with the tumor necrosis factor[42] but not with *CYPD26*[101] variants or cytochrome b-245 α polypeptide[127].

Although African subjects may have a better clozapine response than Europeans, a higher genetic risk for benign ethnic neutropenia (BEN), often misdiagnosed as CIA or clozapine-induced neutropenia, has resulted in frequent discontinuation of clozapine treatment[128]. Single studies have also reported ethnic differences in genes associated with CIA, such as a large British study reporting more than a two-fold higher risk of CIA in Asians than Caucasians[104]. Another study showed a protective effect with TNFb5 and an increased susceptibility with TNFd3 to CIA in schizophrenia patients with Jewish and non-Jewish backgrounds[42]. These findings suggest significant ethnic differences in genetic predictors for CIA[124,129]. In addition, some studies investigating other polymorphisms have not produced promising results, such as a weak correlation between an oxidative gene NADPH quinone 2 (*NQO2*) polymorphism and bone marrow toxicity[45] and negative findings with another oxidative gene for myeloperoxidase[101,127].

Although it took some time to initiate genome-wide research in this area, a growing number of genome-wide association studies (GWAS) have explored the genetic basis of CIA. The first GWAS supported a significant association between CIA and HLA-DQB1-126Q and HLA-B-158T[52]. Although not replicated, HLA-DQB1 (126Q) has a strong linkage disequilibrium with HLA-DQB1*05:02, one of the most replicated risk alleles for CIA[41,106]. Another study revealed a sensitivity of about 21% and specificity of about 98% for the HLA-DQB1 6672G>C polymorphism for CIA[50] [Table 1]. The second GWAS in the Japanese population reported a significant association between HLA-B*59:01 and CIA[53], with a sensitivity of about 32% and a specificity of about 95%[53]. The third GWAS in non-Jewish Europeans found SNP rs149104283 at chromosome 12p12.2 associated with CIA[51]. However, this finding was not replicated in a Japanese sample[125]. The latest GWAS conducted in the Chinese population identified a weak correlation between clozapine-related leukopenia and SNP rs11753309 near HLA-B[126]. These ethnic differences in risk alleles for CIA across GWAS further support the need for studies with multi-ethnic representation. Some of the findings from the candidate gene studies have also been endorsed by GWAS studies, such as the correlation between CIA and HLA-DBQ1 and HLA-B*5901 polymorphisms. However, most results from reviewed studies are based on small sample studies in various ethnic groups and should be interpreted cautiously

[Table 1]; some replicated polymorphisms may provide reliable genetic predictors for CIA, such as HLA-B38, HLA-DBQ1, HLA-DRB1, and HLA_DQA1.

Finally, it is worth mentioning that in patients who developed CIA or granulocytopenia, adjunctive therapy with a single dose of granulocyte stimulating factor (GSF) reduced the risk for blood toxicity during a clozapine rechallenge[130-132]. This adjunctive strategy may be investigated to see if GSF can be used prophylactically during the early weeks of clozapine treatment in vulnerable patients with high genetic risk for bone marrow toxicity. Another option is lithium, which has also been used successfully to induce leukocytosis in patients who develop neutropenia with clozapine treatment[133].

Genetic predictors of clozapine efficacy

Pharmacogenetic studies of clozapine response have focused on pharmacokinetic (PK) genetic factors primarily involving genetic variance in the CYP enzymes mediating clozapine's metabolism) and the pharmacodynamic (PD) genetic factors affecting primary neurotransmitter systems targeted by clozapine, mainly dopamine and serotonin.

Pharmacokinetic genetic factors for clozapine's efficacy

Any genetic variance in the CYP (cytochrome-P450) enzymes involved in the metabolism of antipsychotic medications may affect their efficacy and/or tolerability[134]. In this context, the most observed clinical finding is a genetically mediated increase in drug levels resulting in increased adverse effects with no apparent correlation with efficacy. Therefore, most data on PK genetic biomarkers represent adverse effects and not effectiveness. The only exception is clozapine due to an evidence-based relationship between clozapine levels and its efficacy[135]. Thus, therapeutic drug monitoring (TDM) is recommended to optimize clozapine treatment, particularly in difficult-to-treat patients with inadequate or no clozapine response or those with unusual or intolerable adverse effects on conventional clozapine doses[135,136]. Clozapine metabolism involves several CYP enzymes, particularly CYP1A2, providing the main metabolic pathway for converting clozapine to its primary biologically active metabolite, norclozapine[137]. Although inhibition of CYP1A2 in drug interactions has been associated with an increase in adverse effects, patients with highly inducible CYP1A2, as observed in smokers, may compromise clozapine efficacy by lowering its plasma levels[138]. Although one study did not[139], three other studies reported a significant correlation between the lack of clozapine response and the ultrarapid activity of CYP1A2[54-56]. This is one of the most consistent pharmacokinetic findings associated with clozapine response. However, unlike CYP1A2, the ultra-rapid activity of CYP2C19 was reported to be associated with an improvement in clozapine response[57]. In addition, genetic variance in the p-glycoprotein transporter gene (ABCB1) has been correlated with the effectiveness of clozapine in a couple of studies[58,140] and another antipsychotic medication, risperidone, in one study[141].

Pharmacodynamic genetic factors for clozapine efficacy

The findings from genetic studies investigating genetic variance in pharmacodynamic (PD) genetic factors, such as transporters, neuropeptides, and receptors, have been inconsistent, perhaps due to inadequate study samples failing to capture relatively rare polymorphisms. Genome-wide association Studies (GWAS) provide an effective alternative to investigate relatively rare genetic polymorphisms affecting clozapine response, but these studies require large sample sizes and can be enormously expensive, explaining their scarcity in psychiatry. Therefore, the following paragraphs review significant findings from studies using the candidate gene approach investigating the relationship between major neurotransmitter systems and clozapine response [Table 2].

Dopamine-associated genes

Dopamine is the primary neurotransmitter system mediating the antipsychotic efficacy of all antipsychotic medications. Therefore, several studies have replicated findings from studies that examined the relationship between clozapine efficacy and genetic variance in various dopamine receptors genes, such as DRD1[68,69], DRD2[62-65], and DRD3[59-61], and DRD4[66,142].

The first study investigating DRD1 found a positive clozapine response in individuals with rs4532 2/2 and a diminished response in those with rs4532 1/2 after five weeks of clozapine treatment[69]. The second study reported novel findings showing clozapine nonresponse with SNP, rs265976 A/C and haplotype T-G-A (rs265981, rs4532, rs686) in African Americans but a better response with haplotype T-G-G haplotype in Caucasians after at least six months of clozapine treatment[68]. However, the findings from the later study[68] may carry more weight due to the larger sample size of 232 and more extended clozapine treatment than the first study [Table 2].

The first study investigating the DRD2 genes reported a significant correlation between 141C Ins/Del and a 10-week clozapine efficacy with a fivefold improvement in psychosis in Del- carriers as compared to Del+ carriers[65]. The second study reported 3 DRD2 SNPs (Taq1A, Taq1B, and rs1125394) as predictors of clozapine response after at least six months of clozapine treatment in African Americans[64]. The same group replicated findings with 2 SNPs (Taq1B and rs1125394) in a smaller sample of African American patients[63]. A comprehensive metanalysis has supported the relationship between the allelic variance in *D2* -141C Del and TaqIA2 and response from antipsychotics, including clozapine[143]. But a recent meta-analysis reported a lack of relationship between 141C Ins/Del and clozapine response[144]. Allelic variants, *D2* -141C Del and TaqIA2, have also been correlated with inadequate clozapine response with other antipsychotic medications across various ethnic groups[145-148]. Thus, various genetic polymorphisms in DRD2 have been the most replicated findings with the clozapine response in the dopamine system. At the same time, some studies have also found genetic variance in the promotor areas of DRD2, DRD3, and DRD4 associated with antipsychotic efficacy[149,150].

Regarding the DRD3 gene, the first study found a higher frequency of the genotype Ser-9/Ser-9 (rs6280) in patients who responded to at least three months of clozapine treatment versus those who did not[59]. The second study in the Pakistani sample replicated these findings in a smaller sample[60]. However, a meta-analysis failed to find any relationship between clozapine efficacy and Ser-9-Gly[144]. In the second study[61], at least six months of clozapine treatment was significantly associated with clozapine response to the positive symptoms with the DRD3 polymorphism in A allele (i.e., rs2134655) in Caucasians (N = 183) and the clozapine response to the negative symptoms with the DRD3 polymorphism in T allele (i.e., rs1394016) in African Americans (N = 49)[61].

Although multiple studies have examined the DRD4 gene[149,150], only two reported variances in DRD4 associated with clozapine efficacy[66,142]. The first study reported that the frequencies of 5 alleles and 5/5 genotype in the variant number tandem repeat of the DRD4 48 bp were higher among the clozapine nonresponders than the responders after two weeks of clozapine treatment[142]. The second study in African Americans documented a significant correlation between 120-bp and intron I 142/140 bp genotype and lack of clozapine efficacy after six months of clozapine treatment and a significant positive relationship between 48 bp repeat and clozapine efficacy in a Caucasian sample (N = 183)[66]. However, genetic variance in DRD5 was not correlated with clozapine efficacy[66].

Genetic polymorphisms in other dopamine-related genes were also investigated, such as genetic variance in the enzyme, Catechol-O-methyltransferase (COMT), which metabolizes dopamine[151], and the dopamine transporter/reuptake pump gene (i.e., SLC6A3). For example, the first study with COMT Val108/158Met polymorphism (rs4680) found a more significant improvement in attention and verbal fluency domains in patients with hetero- or homozygosity for the Met allele than the Val/Val group after clozapine treatment[70]. These findings were also supported by a meta-analysis[152], which showed that patients with met homozygosity were more likely to respond to antipsychotic treatment. A couple of other studies have also correlated genetic variance in COMT with antipsychotic efficacy[153,154].

The study investigating the dopamine transporter gene (SLC6A3) reported that the 71T allele of rs2975226 (T-71A) occurred more frequently in the clozapine responders than the nonresponders after 8-weeks of clozapine treatment[71]. However, another study failed to replicate this finding with SLC6A3[155].

Serotonin-associated genes

Several studies have examined clozapine response to genetic variance in serotonin receptors, such as the serotonin receptor 2A (HTR2A), the serotonin receptor 2C (HTR2C), and the serotonin receptor 6 (HTR6[156]. However, the HTR2A gene is one of the most investigated genes concerning clozapine response, and several studies have produced positive findings[149,150]. Two of these studies replicated better clozapine response with the HT2RA T102C variant[75,86]. The HTR2A 102-T/C variance was confirmed by a meta-analysis[157]. In addition, a review reported correlations between HTR2A variants 102-C, -1438-G, and Tyr452 and clozapine response[134]. Another study reported reduced antipsychotic response with the HRT2A 102-C/C genotype in Caucasian patients[158]. This finding is consistent with a more frequent occurrence of homozygosity for the C102 allele in clozapine nonresponders compared to the responders[75]. At the same time, another meta-analysis reported a better clozapine response in patients with the C allele hetero- or homozygosity than the T allele or TT genotype[144]. This finding is consistent with a higher N100 amplitude in Chinese patients with 102C/C genotypes that reduced significantly after clozapine treatment suggesting improved sensory processing[86]. One reviewed study was primarily designed for a combinatorial analysis to examine the interaction between HRT2A and other serotonin-associated genes, HRT2C and SCL6A4[81]. The next study reported a more frequent occurrence of the Tyr452 (rs6314) allele of the HTR2A gene in clozapine nonresponders than in responders[72]. This result with Tyr452 (rs6314) allele was reproduced in subjects who received at least 6-month treatment with clozapine[74]. The following study reported a positive association between CLZ response and the G-1438/A-1438 genotype and G-1438/A allele of the HTR2A gene and a negative association with homozygosity for the G-1438A (rs6311) allele[159]. However, a meta-analysis did not find an association between the G-1438A polymorphism and clozapine efficacy[144]. While other studies associated the HTR2A 1438-A/A genotype with treatment resistance for negative symptoms in a French study[160] and *5-HT2A* -1438-G allele with psychotic relapse in an Algerian study[161]. The combination of potent 5H2A and D4 effects is one of the mechanisms for unique clozapine's efficacy[156,162].

Variance in HTR2C receptors has been documented in multiple studies[149,150]. However, the first two observed a positive association between the 5HTR2C Cys23Ser and clozapine efficacy after > three months of clozapine treatment[80]. But a meta-analysis did not support this finding in a Caucasian sample[144]. The second study replicating earlier results was designed to primarily investigate gene-gene interactions between three polymorphisms, Ser allele of Cys23Ser (rs6318) in the HTR2C, HRT2A, and SLC6A4 gene to predict clozapine response[81].

Genetic polymorphisms in the HTR3A gene have also been researched in multiple studies[149,150], but only two studies produced significant findings[76,77]. The first study demonstrated that the T allele of HTR3A

rs1062613 was associated with clozapine response after > 6 months of treatment[76]. The second study reported an association between the T allele of HTR3A rs106263 and the G allele of HTR3A rs2276302 with the clozapine response after patients were stable on clozapine dose for at least three months[77]. A meta-analysis has also reported a correlation between the T allele of rs1062613 and an improved clozapine efficacy[144]. However, not all studies have reported a significant relationship between clozapine response and genetic variance in HTR3B and 5HTR5[76,81,163,164].

With regards to 5HTR1A, one study, primarily designed to examine the interaction between COMT and HTR1A genes, reported a significant association between 5HTR1A 1019C/G (rs6295) polymorphism and clozapine response for 8 and 16 weeks of treatment with a more significant improvement in subjects with the G homozygosity as compared to the C homozygosity[83]. However, HTR1A polymorphism, *5-HT1A*-1019 G, was associated with lower efficacy with other second-generation antipsychotic medications in various ethnic groups[165-167].

Some studies have also examined the correlation between clozapine response and the HTR6 gene[58,79,168]; however, only one of these studies found any significant correlation between a genetic variance in HRT6 and clozapine efficacy[79]. This study demonstrated that the C267T polymorphism in the HTR6 gene was more frequent in clozapine responders than in nonresponders after at least two months of clozapine treatment. One of the negative studies with the effects of the HRT6 variant also reported no effects of HTR7 pro279leu polymorphism on the clozapine response[168].

Another pharmacodynamic genetic factor studied in the serotonin system is the SLC6A4, which is the gene for the serotonin transporter protein[78,81,169,170]. One of these studies reported a significant relationship between the short allele of HTTLPR/rs25531) in the promoter area of the SLC6A4 and the clozapine response in a Caucasian sample[78]. However, while examining gene-gene interactions, another study reported only a trend between a genetic variance in SLC6A4 and clozapine efficacy in a larger sample than the earlier study[171]. In addition, a few studies have also produced negative findings[172,173].

Although multiple other reports have also documented a relationship between specific PD genetic markers and antipsychotic efficacy, most findings are generally without replication raising questions about the clinical utility. In addition, a few studies have also produced negative results for various serotonin targets[149,150]. Nevertheless, the overall data support the critical role of the serotonin system in clozapine's efficacy, which requires replication in future large-sample genetic studies.

Combinatorial genetic assays examining the interplay between multiple candidate genes have also assessed clozapine efficacy. Four studies with significant findings were reviewed in the paper[81-84]. The first study reported a combinatorial analysis of different polymorphisms, offering one of the most robust predictors of clozapine response[81]. In this study, six different polymorphisms showing the strongest association with clozapine efficacy included 5-HT2A 102-T/C, 5HT2A His452Tyr, 5-HT2C 330-GT/244-CT, 5HT2C Cys23Ser, 5-HTTLPR H2-1018-G/A. This combinatorial analysis provided a positive predictability of 76% and a negative predictability of 82%, along with a sensitivity of about 96% and a specificity of 38%. However, another study did not replicate these findings[169]. The second study reported the most substantial effect of gene-gene drug interactions between DRD1 rs686 and DRD3 Ser9Gly on clozapine efficacy for a minimum of six months in Caucasian subjects[82]. The third combinatorial study demonstrated an additive effect of COMT Val158Met and HTR1A-1019 C/G on the clozapine treatment response when taken for 8 and 16 weeks[83]. Similarly, the final study[84] documented a significant effect of a gene interaction between DRD4 120-bp duplication and COMT Val158Met on clozapine efficacy when taken for not less than three

months[84]. Combinatorial analysis of two candidate genes, COMT rs6269 and HTR2C rs3813929, in a large sample of 995 subjects also predicted antipsychotic response, including clozapine[174].

Finally, few GWAS have found a relationship between specific polymorphisms and antipsychotic efficacy, such as sharing genetic variance linked to antipsychotic response and risk for schizophrenia in two GWAS[88,175]. One GWAS consortium study in Caucasians observed an association between homozygosity for the minor A-allele of rs2535629 in the inter-alpha-trypsin inhibitor heavy chain H3 gene and improvement in negative symptoms of schizophrenia but not in the total BPRS scores after 6 month treatment with clozapine[87]. Another genome-wide consortium study found a significant correlation between DRD2 SNP rs2514218 and clinical response to clozapine after six months of clozapine therapy[62].

Overall, significantly more research is warranted before pharmacogenetics can be cost-effective in psychiatric patients. Nevertheless, available commercial genetic assays may still help improve patient outcomes in treatment-refractory populations to decrease healthcare costs and enhance medication adherence[176]. In addition, pharmacogenetic testing with psychotropic medications is becoming less expensive and increasingly covered by major insurance companies. This could explain a steady increase in genetic testing, particularly in the treatment-refractory population[177,178].

In conclusion, Adding genetic screening for CIA to clozapine response has a greater potential than either of the two to identify the most appropriate patients for clozapine treatment without exposing potential clozapine nonresponders to bone marrow toxicity. Despite growing evidence for the genetic basis of CIA and clozapine response, genetic testing is not ready for prime time. More research is warranted on large samples of diverse populations to develop more sensitive and precise genetic predictors of CIA and clozapine response. However, most research in this area has focused on a candidate gene approach in small ethnic groups not powered to detect relatively rare genetic predictors across various ethnic groups. Although a few studies have attempted to employ combinatorial analyses to add more significance to the findings from candidate gene studies, there is a need to continue the paradigm shift from the candidate genes to the genome-wide approach. Genome-wide studies provide the first logical step to generate novel hypotheses that can be later confirmed in a more targeted candidate gene approach to improving the predictability of genetic biomarkers for CIA and clozapine response.

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Not applicable.

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