

# Genetic determinants associated with response to clozapine in schizophrenia

Citation for published version (APA):

van der Horst, M. Z., Papadimitriou, G., & Luykx, J. J. (2022). Genetic determinants associated with response to clozapine in schizophrenia: an umbrella review. *Psychiatric Genetics*, 32(5), 163-170. <https://doi.org/10.1097/YPG.0000000000000320>

## Document status and date:

Published: 01/10/2022

## DOI:

[10.1097/YPG.0000000000000320](https://doi.org/10.1097/YPG.0000000000000320)

## Document Version:

Publisher's PDF, also known as Version of record

## Document license:

Taverne

## Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

## General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

[www.umlib.nl/taverne-license](http://www.umlib.nl/taverne-license)

## Take down policy

If you believe that this document breaches copyright please contact us at:

[repository@maastrichtuniversity.nl](mailto:repository@maastrichtuniversity.nl)

providing details and we will investigate your claim.

# Genetic determinants associated with response to clozapine in schizophrenia: an umbrella review

Marte Z. van der Horst<sup>a,b,c</sup>, Georgia Papadimitriou<sup>d</sup> and Jurjen J. Luykx<sup>a,b,c,e</sup>

**Objective** Clozapine response varies widely from person to person, which may be due to inter-individual genetic variability. This umbrella review aims to summarize the current evidence on associations between pharmacodynamic genes and response to clozapine treatment.

**Methods** Following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis methodology, a systematic literature search was conducted in the *PubMed* and *EMBASE* databases from inception to November 2021 to identify systematic reviews and meta-analyses of studies that examined genetic determinants of clozapine response. The quality of the reviews was assessed with the AMSTAR-2 tool.

**Results** From a total of 128 records, 10 studies representing nine systematic reviews and one meta-analysis met our inclusion criteria. The overall quality of the included studies was poor. All systematic reviews concluded that the results of primary studies were largely negative or conflicting. Most evidence was found for an association with clozapine response and rs6313 and rs6314 within *HTR2A* and rs1062613 within *HTR3A* in the serotonergic system.

## Introduction

Approximately one-third of patients diagnosed with schizophrenia do not respond to standard antipsychotic treatment and are classified as having treatment-resistant schizophrenia (TRS) (Lally and MacCabe, 2015). TRS has been associated with less functional recovery and poorer quality of life (Griffiths *et al.*, 2021), and attempts to develop therapies for TRS have been elusive.

Clozapine, the first atypical antipsychotic developed, is the only drug officially indicated for TRS. Clozapine has many advantages over other antipsychotic drugs. It acts on both positive and negative symptoms as well as on cognitive deficits associated with schizophrenia (Leucht *et al.*, 2013). Additionally, clozapine is not associated with the development of extrapyramidal symptoms or tardive dyskinesia, as is frequently observed in other (typical) antipsychotics. Furthermore, clozapine has been suggested to reduce levels of aggression and suicidality in patients diagnosed with schizophrenia (Wagner *et al.*, 2021).

**Conclusions** Conclusive evidence for associations between genetic variants and clozapine response is still lacking. Hypothesis-generating genetic studies in large, well-characterized study populations are urgently needed to obtain more consistent and clinically informative results. Future studies may also include multi-omics approaches to identify novel genetic determinants associated with clozapine response. *Psychiatr Genet* XXX: 000–000 Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

Psychiatric Genetics XXX, XXX:000–000

**Keywords:** clozapine, genetic determinants, pharmacogenetics, response, schizophrenia

<sup>a</sup>Outpatient Second Opinion Clinic, GGNet Mental Health, Warnsveld, <sup>b</sup>Department of Translational Neuroscience, <sup>c</sup>Department of Psychiatry, University Medical Center Utrecht, Utrecht University, Brain Center, Utrecht, The Netherlands, <sup>d</sup>Department of Psychology, Panteion University of Social and Political Sciences, Athens, Greece and <sup>e</sup>Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University Medical Center, Maastricht, the Netherlands

Correspondence to Marte van der Horst, MD, UMC Utrecht, Department of Psychiatry, Heidelberglaan 100, Utrecht, The Netherlands  
Tel: +088 755 5555; e-mail: m.z.vanderhorst-10@umcutrecht.nl

Received 24 February 2022 Accepted 8 June 2022

Despite these benefits, clozapine remains underutilized in up to two-thirds of patients diagnosed with TRS (Forrester *et al.*, 2015) and the mean delay in initiation reaches up to 6 years, mainly due to (the fear of) the occurrence of side effects (Howes *et al.*, 2012; Thien and O'Donoghue, 2019). In addition, treatment is complicated by substantial inter-individual differences in treatment outcome. Favorable response is associated with several clinical and demographic factors, such as younger age, more severe symptoms, previous suicide attempts, affective symptoms and use of antidepressants (Wimberley *et al.*, 2016). Genetic factors may also be associated with response, and pharmacogenetic studies are therefore investigating how genetic variation relates to variability in clozapine treatment outcome. The ultimate goal of these studies is to help develop genetic tests to predict clozapine concentration, response and risk for side effects to individualize patient treatment. These tests would help reduce concerns surrounding clozapine use and possibly enable a larger proportion of patients to receive more beneficial treatment.

Numerous pharmacogenetic studies have been conducted to understand genetic contributions to clozapine

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, [www.psychgenetics.com](http://www.psychgenetics.com).

0955-8829 Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

DOI: 10.1097/YPG.0000000000000320

response, focusing on (1) the dopaminergic system (e.g. *DRD2*, *DRD3* and *DRD4*); (2) the serotonergic system [e.g. *HTR2A*, *HTR2C*, *HTR3A* and Serotonin Transporter (*HTT*)] and (3) other neuronal systems [e.g. Tumor Necrosis Factor-alpha (*TNF $\alpha$* ) and Brain-Derived Neurotrophic Factor (*BDNF*)]. Given the lack of overview on this topic, the aim of the current umbrella review was to provide a comprehensive and up-to-date overview of the genetic determinants so far associated with clozapine response.

## Methods

### Search strategy and study selection

This umbrella review was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement guidelines and umbrella review approach (Page *et al.*, 2021). The search was performed in the *PubMed* and *EMBASE* databases on 5 November 2021, using the keywords “clozapine”, “genet\*”, “genom\*”, “genotyp\*”, “response”, and “review”. Reference lists of included studies and other relevant documents were manually searched to find additional studies.

### Eligibility and inclusion criteria

Studies were included if: (1) they were systematic reviews or meta-analyses, describing the search strategy and inclusion criteria, (2) they were focused on genetic determinants of clozapine response and (3) they were published in a peer-reviewed journal, on humans and written in English. Publications were excluded if not all study participants were definitely taking clozapine at the time of the study. Studies, where participants were on other antipsychotics, were included as long as participants were also taking clozapine.

### Data extraction

Two investigators (M.H. and G.P.) screened all titles and abstracts of the retrieved records for eligibility. Both investigators independently extracted data from the articles based on a standardized data extraction form, including author, year, study design, study aims and study outcome. Any discrepancies that arose were discussed and decisions regarding the inclusion or exclusion of the article were made on a case-by-case basis by all authors. Data were extracted from the systematic reviews and relevant supplementary materials.

### Study quality assessment

The bias and quality of the included articles were assessed using the revised Assessment of Multiple Systematic Reviews tool (AMSTAR-2) (Shea *et al.*, 2017) (Table 2). The AMSTAR-2 tool lists 16 questions to determine the methodological quality of the review. Each question is answered ‘yes’, ‘partial yes’ or ‘no’, and the overall rating criteria are as follows: zero or one noncritical weakness is defined as high quality; more than one noncritical

weakness is defined as moderate quality; one critical flaw with or without noncritical weaknesses is defined as low quality and more than one critical flaw with or without noncritical weaknesses is defined as critically low quality. M.H. and G.P. independently completed the AMSTAR-2 for each article. The inter-rater reliability between the two reviewers was 84%. Discrepancies were discussed and resolved during consensus meetings with senior author J.L.

### Data synthesis

An overview of all primary studies included in each review was made to gain insight into the overlap in primary studies included in the different reviews (Supplementary Table 1, Supplemental digital content 1, <http://links.lww.com/PG/A268>). Pooled estimates for the reviews were not calculated due to the high degree of overlap of primary studies. Instead, a narrative synthesis of the findings of each of the included reviews was conducted, categorizing the results by neurotransmitter system and gene. For each neurotransmitter system, the results of the meta-analysis were described first, followed by the results of the systematic reviews, based on decreasing publication date.

## Results

### Description of eligible articles

The database and the manual search yielded 128 articles (Fig. 1). We evaluated the full texts of 116 articles and excluded 76 articles that did not meet the inclusion criteria (Supplemental Material). Finally, 10 articles were included in this umbrella review, of which nine were systematic reviews and one study was a meta-analysis (Table 1). In total, these studies covered 109 original primary studies (Supplementary Table S1, Supplemental digital content 1, <http://links.lww.com/PG/A268>). The included reviews were published between 2005 and 2020, and the primary studies included in these reviews were published between 1993 and 2018. The number of primary studies included in each systematic review ranged from 1 to 70.

### Study quality assessment

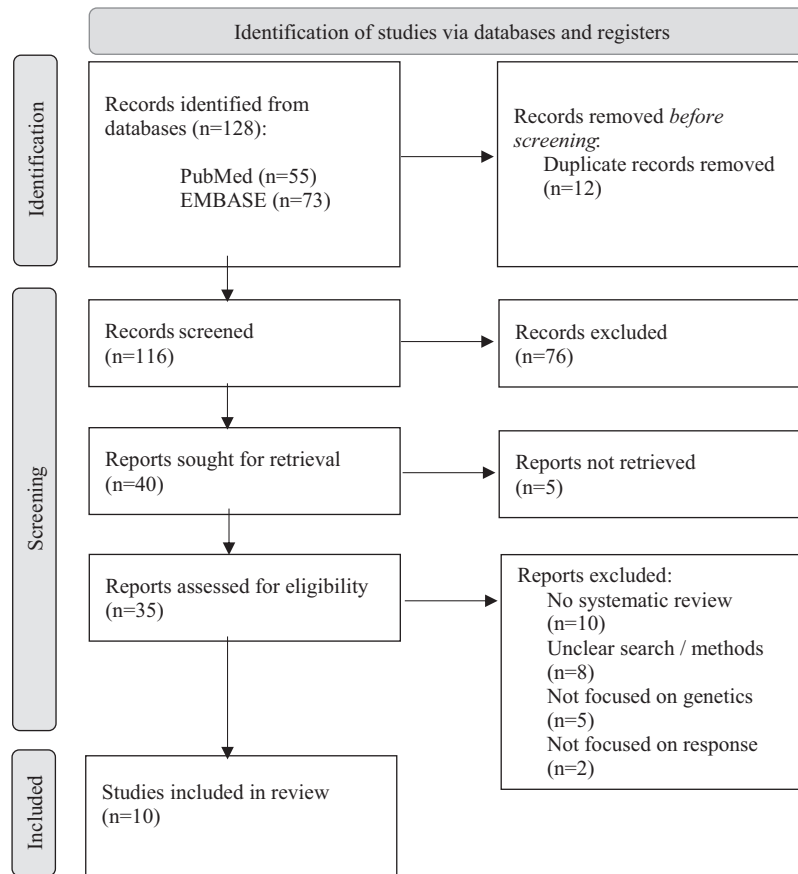
All ( $n = 10$ ) of the included reviews were rated as having critically low quality using AMSTAR-2. The main shortcomings identified by the AMSTAR-2 were that reviews did not include a list of excluded studies, did not provide sufficient details about deviations from the study protocol, and did not explore and discuss the impact of publication bias on the review. In most reviews, however, research questions, inclusion criteria and description of study characteristics were described in adequate detail.

### Genetic determinants associated with clozapine response

#### Dopaminergic system

Nine of the included reviews reported on the association between dopamine polymorphisms and clozapine

Fig. 1



Prisma flow diagram of the search.

Table 1 Study characteristics of included reviews

First author, year of publication	Study type	Searched databases	Timeframe of search	Number of primary studies included in the review
Chung and Remington (2005)	Systematic review	PubMed	1975 – June 2004	26
Dragoi <i>et al.</i> (2020)	Systematic review	PubMed, MEDLINE, Web of Science	Inception – October 2020	1
Gressier <i>et al.</i> (2016)	Systematic review and meta-analysis	PubMed, ISI Web of Knowledge, PsycINFO	Inception – May 2014	59
Kohlrausch (2013)	Systematic review	PubMed	Inception – 11 October 2012	35
Krivosy <i>et al.</i> (2015)	Systematic review	PubMed, MEDLINE	Not mentioned	7
Samanaite <i>et al.</i> (2018)	Systematic review	PubMed	Inception – 20-01-2018	70
Sriretnakumar <i>et al.</i> (2015)	Systematic review	PubMed, Ovid	January 2012 – February 2015	6
Suzuki <i>et al.</i> (2011)	Systematic review	PubMed	Inception – 31 January 2011	37
Wilffert <i>et al.</i> (2005)	Systematic review	MEDLINE, Embase	1995 – August 2002	16
Zai <i>et al.</i> (2018)	Systematic review	PubMed	2013 – 2017	10

response (Chung and Remington 2005; Wilffert *et al.*, 2005; Suzuki *et al.*, 2011; Kohlrausch, 2013; Sriretnakumar *et al.*, 2015; Gressier *et al.*, 2016; Zai *et al.*, 2018; Samanaite *et al.*, 2018; Dragoi *et al.*, 2020). In the meta-analysis by Gressier *et al.*, (2016) 22 studies focusing on the dopaminergic system were included, that were published between 1996 and 2012. They meta-analyzed four studies focusing on rs1799732 within the Dopamine Receptor

D2 (*DRD2*) gene and seven studies focusing on rs6280 within the Dopamine Receptor D3 (*DRD3*) gene and did not find an association with clozapine response for either SNP [total  $n=596$  patients, odds ratio (OR)=0.96 (95% confidence interval {CI}, 0.48–1.94), heterogeneity ( $I^2$ )=60% and total  $n=852$  patients, OR=0.79 (95% CI, 0.56–1.10),  $I^2=14$ , respectively] (Gressier *et al.*, 2016). Gressier *et al.* (2016) also included nine studies examining

the association between the Variable Number Tandem Repeat alleles of the Dopamine Receptor D4 (*DRD4*) gene and response and found that studies reported no or inconsistent associations.

Three reviews were published after the meta-analysis by Gressier *et al.* (2016) of which the most recent one was by Dragoi *et al.*, (2020). However, they only referred to the study by Gressier *et al.* (2016) and did not include any more recent studies. Zai *et al.*, (2018) focused on studies published after 2013 and concluded that there is increasing evidence for the role of dopamine systems, especially *DRD2*, in clozapine response, although this conclusion was largely based on the meta-analysis by Gressier *et al.* (2016) and on their own study, which found preliminary evidence for an association between rs2514218 and clozapine response, awaiting further replication (Zai *et al.*, 2018). Samanaite *et al.*, (2018) included 21 studies (published between 1994 and 2016) and concluded that rs6280 within *DRD3* was one of the three genetic variants (of the total 379 investigated gene variants) showing an association with clozapine response by two or more independent study groups (Samanaite *et al.*, 2018). At the same time, however, they reported that there were also studies that found no association between *DRD3* and clozapine response, including the two studies with the largest sample size (Samanaite *et al.*, 2018). The other reviews did not report any other or new findings and all reviews concluded that the findings were inconsistent (Chung and Remington 2005; Wilffert *et al.*, 2005; Suzuki *et al.*, 2011; Kohlrausch 2013; Sriretnakumar *et al.*, 2015).

#### Serotonergic system

Seven of the included reviews reported on the association between serotonin polymorphisms and clozapine response (Chung and Remington 2005; Wilffert *et al.*, 2005; Suzuki *et al.*, 2011; Kohlrausch 2013; Gressier *et al.*, 2016; Samanaite *et al.*, 2018; Zai *et al.*, 2018). In the meta-analysis by Gressier *et al.*, (2016) 22 studies on the serotonergic system were included, that were published between 1995 and 2012. They analyzed the three most investigated polymorphisms within the 5-Hydroxytryptamine Receptor 2A (*HTR2A*) gene: rs6313, rs6311 and rs6314, and observed an association between rs6313 and poor response to clozapine (total  $n=868$  patients; OR = 0.68; 95% CI, 0.49–0.93;  $I^2=7$ ) and rs6314 and better response to clozapine (total = 671; OR = 4.43; 95% CI, 1.21–16.26;  $I^2=0\%$ ) (Gressier *et al.*, 2016). However, meta-analyzing four studies on rs6311 showed no evidence of the association between this SNP and clozapine response (OR = 0.63; 95% CI, 0.35–1.15),  $I^2=56\%$ ) (Gressier *et al.*, 2016). An association with a better response to clozapine was observed within the 5-Hydroxytryptamine Receptor 3A (*HTR3A*) gene, by meta-analyzing four studies on rs1062613 (total  $n=603$ ; OR = 0.47; 95% CI, 0.24–0.93;  $I^2=50\%$ ) (Gressier *et al.*, 2016). Finally, Gressier *et al.* (2016) concluded that the 5-Hydroxytryptamine (5-*HTT*)

gene does not seem to have a significant role in the liability to clozapine response (Gressier *et al.*, 2016).

Two reviews were published after this meta-analysis. Zai *et al.*, (2018) only referred to the meta-analysis by Gressier *et al.*, (2016) and did not report any new findings. Samanaite *et al.*, (2018) included 12 studies investigating the *HTR2A* gene, but none of them was published after the meta-analysis of Gressier *et al.*, (2016) and no new findings were reported. All reviews concluded that available data are inconsistent and replication studies are missing (Chung and Remington 2005; Wilffert *et al.*, 2005; Suzuki *et al.*, 2011; Kohlrausch 2013; ). Thus far, apart from the potentially promising leads of rs6313 and rs6314 within *HTR2A* and rs1062613 within *HTR3A*, no clear evidence has been found for an association between serotonergic genes and clozapine response.

#### Glutamatergic system

Four of the included reviews reported on the potential association between glutamate polymorphisms and clozapine response (Chung and Remington 2005; Kohlrausch 2013; Gressier *et al.*, 2016; Samanaite *et al.*, 2018). The most recent review by Samanaite *et al.*, (2018) included four studies published between 2001 and 2016 and found none of the 12 investigated polymorphisms within the Glutamate Ionotropic Receptor NMDA Type Subunit 1 (*GRIN1*), Subunit 2A (*GRIN2A*) and Subunit 2B (*GRIN2B*) gene, or the Glutamate Metabotropic Receptor 3 (*GRM3*) gene to be associated with clozapine response. The other reviews did not identify any other papers on this association.

#### Histaminergic system

Three of the included reviews reported on the association between histamine polymorphisms and clozapine response (Chung and Remington 2005; Kohlrausch 2013; Gressier *et al.*, 2016). Gressier *et al.*, (2016) identified three studies on the Histamine Receptor H2 (*HRH2*) and H3 (*HRH3*) genes that were published between 2000 and 2002. Of these, one study reported an association between rs2607474 within *HRH2* and better clozapine response and the other two studies failed to replicate this finding. The other two reviews did not identify any other studies and also concluded that studies yielded negative or contradictory findings (Chung and Remington 2005; Kohlrausch 2013).

#### Adrenergic system

Five of the included reviews reported on the association between adrenergic receptor genes and response to clozapine (Chung and Remington 2005; Wilffert *et al.*, 2005; Kohlrausch 2013; Gressier *et al.*, 2016; Samanaite *et al.*, 2018). The most recent review by Samanaite *et al.*, (2018) included five studies on the Adrenoceptor Alpha 1A (*ADRA1A*), Alpha 2 (*ADRA2*) or Beta 3 (*ADRB3*) gene that were published between 2000 and 2012 and did

not find any association between adrenergic gene variants and clozapine response. The previously published reviews provided no further insight into this topic as the same primary studies were included.

#### Other gene variants

**BDNF:** Four of the included reviews reported on the association between the *BDNF* gene and clozapine response (Chung and Remington 2005; Suzuki *et al.*, 2011; Sriretnakumar *et al.*, 2015; Samanaite *et al.*, 2018). In the most recent review by Samanaite *et al.*, (2018) four studies on genetic variants in *BDNF* (mainly on the Val66Met polymorphism) were included and it was concluded that none of them showed a significant association with clozapine response. However, Sriretnakumar *et al.*, (2015) included an additional study published in 2012, which did detect a significant association between Val66Met and clozapine response (Zai *et al.*, 2012). In addition, there was a difference in interpretation of the results of a study by Hong *et al.*, (2003), about which Samanaite *et al.*, (2018) reported that an association was missing, while Sriretnakumar *et al.*, (2015) reported that the Val-allele was associated with improved response. Based on these results and the results of studies investigating the association between nonclozapine antipsychotic response and Val66Met, Sriretnakumar *et al.*, (2015) suggested that the variant's effect is quite strong. This difference may be due to the fact that Samanaite *et al.*, (2018) applied other restrictions on the percentage of clozapine users required in the primary study, as this study also included other antipsychotic users (mainly olanzapine). The reviews by Suzuki *et al.*, (2011) and Chung and Remington (2005) only mentioned the study by Hong *et al.*, (2003) and did report a positive association between Val66Met and clozapine response, but emphasized the need for replication studies.

**HLA:** Three of the included reviews reported findings on associations between the Human Leukocyte Antigen (*HLA*) gene and clozapine response (Chung and Remington 2005; Suzuki *et al.*, 2011; Samanaite *et al.*, 2018). Three primary studies reporting on this association were identified by the reviews, of which the most recent one dates back to 2001, and all reviews concluded that conflicting results were reported.

**TNF- $\alpha$ :** Four of the included reviews reported findings on the association between the *TNF- $\alpha$*  gene and clozapine response (Suzuki *et al.*, 2011; Kohlrausch 2013; Gressier *et al.*, 2016; Samanaite *et al.*, 2018). All reviews identified the same three primary studies investigating this association (the most recent of which dates from 2010), of which only one study showed a significant association between rs1800629 within *TNF- $\alpha$*  and clozapine response (Zai *et al.*, 2006). Gressier *et al.*, (2016) performed a meta-analysis on these three samples investigating rs1800629 and clozapine response and found no association [OR=0.75 (95% CI, 0.44–1.27;  $I^2=0\%$ ).

**GN $\beta$ 3:** Five of the included reviews reported findings on the association between the G Protein Subunit Beta 3 (*GN $\beta$ 3*) gene and clozapine response (Suzuki *et al.*, 2011; Kohlrausch 2013; Sriretnakumar *et al.*, 2015; Gressier *et al.*, 2016; Samanaite *et al.*, 2018). Samanaite *et al.*, (2018) included four studies published between 2005 and 2012, of which two studies reported an association between rs5443 within *GN $\beta$ 3* and higher clozapine response rates and two studies did not find any association. The four previously published reviews identified the same primary studies as Samanaite *et al.*, (2018) and reported no different or new findings.

**COMT:** Five of the included reviews referred to potential associations between the Catechol-O-Methyltransferase (*COMT*) gene and clozapine response (Suzuki *et al.*, 2011; Kohlrausch 2013; Gressier *et al.*, 2016; Samanaite *et al.*, 2018; Zai *et al.*, 2018). In total, three different primary studies were discussed in the reviews, in which 12 different SNPs were examined. A significant association with clozapine response was reported for rs4646316 and rs4680, but this could not be replicated in other studies and all reviews concluded that results were conflicting and lacked replication.

**Oxytocin:** Two of the included reviews reported on findings about the associations between genetic variants in the Oxytocin (*OXT*) gene or Oxytocine Receptor (*OXTR*) and response to clozapine (Suzuki *et al.*, 2011; Samanaite *et al.*, 2018). Both reviews identified the same primary study published in 2010, which examined several polymorphisms in *OXT* and *OXTR* and only found an association between rs2740204 within *OXT* and treatment response, which has not yet been replicated.

#### Combination of several polymorphisms

Two of the included reviews referred to association studies in multiple candidate genes to identify combinations of polymorphisms that offer the best predictability of clozapine response (Gressier *et al.*, 2016; Samanaite *et al.*, 2018). Samanaite *et al.*, (2018) described a study that investigated a logistic regression analysis with a combination of six polymorphisms (5-*HT2A* 102-T/C and His452Tyr, 5-*HT2C* -330-GT/-244-CT and Cys23Ser, 5-*HTTLPR*, H2 -1018-G/A), which found a retrospective positive predictive value of 77%, a negative predictive value of 82%, a sensitivity of 95% and a specificity of 28% in predicting clozapine response (Arranz *et al.*, 2000). Gressier *et al.*, (2016) described the same study and did not reveal any further information. In both reviews, it is noted that these results have not been replicated but still remain promising for future implications on a clinical domain.

#### Discussion

This umbrella review summarizes the current evidence on genetic determinants associated with clozapine response. Ten systematic reviews were included, one

**Table 2 Results of the AMSTAR-2 quality assessment**

	Item 1	Item 2*	Item 3	Item 4*	Item 5	Item 6	Item 7*	Item 8	Item 9*	Item 10	Item 11*	Item 12	Item 13*	Item 14	Item 15*	Item 16
Samanata <i>et al.</i> , 2018	YES	NO	NO	NO	YES	NO	YES	YES	NO	NO	NA	NA	NO	NO	NA	YES
Gressier <i>et al.</i> , 2016	YES	NO	NO	PY	YES	NO	NO	PY	PY	NO	YES	YES	YES	YES	YES	YES
Chung <i>et al.</i> , 2005	YES	NO	NO	NO	NO	NO	NO	PY	NO	NO	NA	NA	NO	NO	NA	NO
Suzuki <i>et al.</i> , 2011	YES	NO	NO	NO	NO	NO	NO	YES	NO	NO	NA	NA	NO	YES	NA	YES
Siretnakumar <i>et al.</i> , 2015	YES	NO	NO	PY	YES	NO	NO	YES	NO	NO	NA	NA	NO	YES	NA	YES
Zai <i>et al.</i> , 2018	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NA	NA	NO	NO	NA	YES
Dragoi <i>et al.</i> , 2020	YES	PY	YES	PY	YES	YES	NO	NO	NO	NO	NA	NA	NO	NO	NA	YES
Krivoy <i>et al.</i> , 2015	YES	NO	NO	PY	NO	NO	NO	PY	NO	NO	NA	NA	NO	NO	NA	YES
Willifert <i>et al.</i> , 2005	YES	NO	NO	PY	NO	NO	NO	PY	NO	NO	NA	NA	NO	NO	NA	NO
Kohlrausch, 2013	YES	NO	NO	NO	NO	NO	NO	PY	NO	NO	NA	NA	NO	NO	NA	YES

**Item 1:** Did the research questions and inclusion criteria for the review include the components of PICO?; **Item 2:** Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?; **Item 3:** Did the review authors explain their selection of the study designs for inclusion in the review?; **Item 4:** Did the review authors use a comprehensive literature search strategy?; **Item 5:** Did the review authors perform study selection in duplicate?; **Item 6:** Did the review authors perform data extraction in duplicate?; **Item 7:** Did the review authors provide a list of excluded studies and justify the exclusions?; **Item 8:** Did the review authors describe the included studies in adequate detail?; **Item 9:** Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?; **Item 10:** Did the review authors report on the sources of funding for the studies included in the review?; **Item 11:** If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?; **Item 12:** If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?; **Item 13:** Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?; **Item 14:** Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?; **Item 15:** If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?; **Item 16:** Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review? PY, partial yes. <sup>a</sup>Considered a critical item.

of which also performed meta-analyses. All systematic reviews concluded that the results of primary studies were largely negative or contradictory and no conclusive conclusions could be drawn. Reviews identified some primary studies that found significant associations of genetic determinants with clozapine response, but due to the lack of replication studies, most systematic reviews concluded that strong evidence was lacking. Most evidence for an association with clozapine response was found in the serotonergic system for rs6313 and rs6314 within *HTR2A* and rs1062613 within *HTR3A*. Three of the systematic reviews concluded that clozapine response is also associated with rs6265 within *BDNF*, although one of the other reviews rated this association as insufficiently strong and reliable.

Differences in conclusions between systematic reviews were mainly caused by the use of different inclusion criteria for primary studies, especially with regard to the characteristics of the study populations. The relatively small sample sizes and the high degree of heterogeneity in the primary studies complicate the interpretation of results. Studies that had been included were both retrospective and prospective, used different definitions of response and often did not adequately account for factors that may influence clozapine metabolism or response, such as clozapine blood levels, dose, co-medication and sociodemographic factors.

The results of this umbrella review nonetheless provide important directions for future genetic studies aimed at understanding clozapine response and optimizing clozapine treatment. A better understanding of the implication of genetic variation in clozapine response may lead to a better understanding of the molecular mechanisms involved in clozapine response. Their identification could help identify people with low or high chances of response, so that the treatment strategy can be adapted accordingly (for example, regarding the use of co-medication or the initiation of clozapine earlier or later in the disease course).

The main strength of this umbrella review is that it is the first on the subject and it provides a complete and clear overview of the evidence to date. Moreover, we adhered to stringent standards and used commonly accepted guidelines to retrieve and evaluate the reviews we included in our umbrella review. However, there were also some limitations. First, only one of the included systematic reviews performed a meta-analysis and the included reviews showed a high degree of overlap in primary studies. As a result, findings could not be pooled. Second, the results were mainly based on the results of the systematic review and meta-analysis by Gressier *et al.*, (2016) and the systematic review by Zai *et al.*, (2018) as these were the most recent and comprehensive reviews. Third, the overall quality of the included systematic reviews was considered to be low, based on the AMSTAR-2 criteria. Finally, no protocol was registered

before conducting this umbrella review, and publication bias may play a role as we searched only two databases and no gray or unpublished literature.

The advent of genome-wide association studies (GWAS) and next-generation sequencing may lead to new insights into genetic determinants associated with clozapine response. These techniques enable a systematic and unbiased analysis of genetic factors across the genome and their advent holds hope for further elucidation of common and rare genetic variations associated with clozapine response. GWAS also allow generating data for polygenic risk scores (PRS), which can be used to estimate an individual's genetic liability to a certain trait. So far, two studies used this genome-wide approach to detangle genetic factors associated with symptomatic outcomes of clozapine treatment. The first study in 123 clozapine-treated individuals used schizophrenia-PRS to detect genetic differences between responders and nonresponders but found no statistically significant results ( $P=0.06$ ) (Frank *et al.*, 2015). The other study performed PRS-analyses in a multicenter cohort of 684 clozapine-treated individuals and found that schizophrenia-PRS was most significantly and positively associated with low symptom severity during clozapine treatment ( $P=1.03 \times 10^{-3}$ , explained variance=1.85) (Okhuijsen-Pfeifer *et al.*, 2021). Compared to the lowest tertile, patients in the highest schizophrenia-PRS tertile had 1.94 times ( $P=6.84 \times 10^{-4}$ ) increased probability of low symptom severity. However, it should be noted that this study was performed cross-sectionally and a replication study has not yet been performed. In addition to genetic variants directly associated with clozapine response, genetic variants associated with clozapine metabolism are also important in the search for predictors of response. Response to clozapine treatment is partly influenced by blood levels and the large inter-individual variability in blood levels may be related to genetic factors. A GWAS in 422 clozapine-treated individuals identified a novel variant (rs28379954) within *NFIB* that was associated with reduced clozapine blood levels ( $P=5.63 \times 10^{-5}$ ,  $\beta=-0.36$ , explained variance=7.63%) (Smith *et al.*, 2020). Studies adopting standardized prospective procedures accounting for clinical, environmental and sociodemographic factors associated with clozapine response, will enable the field to achieve greater consistency in findings because the results in those domains are still conflicting. Finally, multi-omics approaches can nuance our understanding of the neurobiological mechanisms underlying clozapine response.

In conclusion, in this umbrella review we show that despite a fairly large number of studies on this topic, no single genetic factor is consistently associated with clozapine response. For a few genetic factors, weak evidence was found for associations with clozapine response, but reproducibility, sensitivity and specificity are lacking. As is the case for schizophrenia, clozapine response is unlikely to be dictated by a single gene variant, and more likely reflects additive or interacting effects at multiple genetic loci.

## Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

## References

- Arranz MJ, Munro J, Brikett J, Bolonna A, Mancama D, Sodhi M, *et al.* (2000). Pharmacogenetic prediction of clozapine response. *Lancet* **355**:1615–1616.
- Chung C, Remington G (2005). Predictors and markers of clozapine response. *Psychopharmacology (Berl)* **179**:317–335.
- Dragoi AM, Radulescu I, Năsui BA, Pop AL, Varlas VN, Trifu S (2020). Clozapine: an updated overview of pharmacogenetic biomarkers, risks, and safety-particularities in the context of COVID-19. *Brain Sci* **10**:E840.
- Forrester T, Siskind D, Winckel K, Wheeler A, Hollingworth S (2015). Increasing clozapine dispensing trends in Queensland, Australia 2004-2013. *Pharmacopsychiatry* **48**:164–169.
- Frank J, Lang M, Witt SH, Strohmaier J, Rujescu D, Cichon S, *et al.* (2015). Identification of increased genetic risk scores for schizophrenia in treatment-resistant patients. *Mol Psychiatry* **20**:913.
- Gressier F, Porcelli S, Calati R, Serretti A (2016). Pharmacogenetics of clozapine response and induced weight gain: a comprehensive review and meta-analysis. *Eur Neuropsychopharmacol* **26**:163–185.
- Griffiths K, Millgate E, Egerton A, MacCabe JH (2021). Demographic and clinical variables associated with response to clozapine in schizophrenia: a systematic review and meta-analysis. *Psychol Med* **51**:376–386.
- Hong CJ, Yu YW, Lin CH, Tsai SJ (2003). An association study of a brain-derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients. *Neurosci Lett* **349**:206–208.
- Howes OD, Vergunst F, Gee S, McGuire P, Kapur S, Taylor D (2012). Adherence to treatment guidelines in clinical practice: study of antipsychotic treatment prior to clozapine initiation. *Br J Psychiatry* **201**:481–485.
- Kohrausch FB (2013). Pharmacogenetics in schizophrenia: a review of clozapine studies. *Braz J Psychiatry* **35**:305–317.
- Krivoy A, Gaughran F, Weizman A, Breen G, MacCabe JH (2016). Gene polymorphisms potentially related to the pharmacokinetics of clozapine: a systematic review. *Int Clin Psychopharmacol* **31**:179–184.
- Lally J, MacCabe JH (2015). Antipsychotic medication in schizophrenia: a review. *Br Med Bull* **114**:169–179.
- Leucht S, Cipriani A, Spinelli L, Mavridis D, Orey D, Richter F, *et al.* (2013). Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet (London, England)* **382**:951–962.
- Okhuijsen-Pfeifer C, van der Horst MZ, Bousman CA, Lin B, van Eijk KR, Ripke S, *et al.*; GROUP (Genetic Risk and Outcome of Psychosis) investigators. (2022). Genome-wide association analyses of symptom severity among clozapine-treated patients with schizophrenia spectrum disorders. *Transl Psychiatry* **12**:145.
- Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al.* (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* **372**:n71.
- Samanaitte R, Gillespie A, Sendt KV, McQueen G, MacCabe JH, Egerton A (2018). Biological predictors of clozapine response: a systematic review. *Front Psychiatry* **9**:327.
- Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, *et al.* (2017). AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *BMJ* **358**:j4008.
- Smith RL, O'Connell K, Athanasios L, Djurovic S, Kringen MK, Andreassen OA, Molden E (2020). Correction: identification of a novel polymorphism associated with reduced clozapine concentration in schizophrenia patients—a genome-wide association study adjusting for smoking habits. *Transl Psychiatry* **10**:366.
- Sriretnakumar V, Huang E, Müller DJ (2015). Pharmacogenetics of clozapine treatment response and side-effects in schizophrenia: an update. *Expert Opin Drug Metab Toxicol* **11**:1709–1731.
- Suzuki T, Uchida H, Watanabe K, Kashima H (2011). Factors associated with response to clozapine in schizophrenia: a review. *Psychopharmacology bulletin* **44**:32–60.
- Thien K, O'Donoghue B (2019). Delays and barriers to the commencement of clozapine in eligible people with a psychotic disorder: a literature review. *Early Interv Psychiatry* **13**:18–23.
- Wagner E, Sifakis S, Fernando P, Falkai P, Honer WG, Röh A, *et al.* (2021). Efficacy and safety of clozapine in psychotic disorders—a systematic quantitative meta-review. *Transl Psychiatry* **11**:487.



- Wiffert B, Zaal R, Brouwers JR (2005). Pharmacogenetics as a tool in the therapy of schizophrenia. *Pharm World Sci* **27**:20–30.
- Wimberley T, Støvring H, Sørensen HJ, Horsdal HT, MacCabe JH, Gasse C (2016). Predictors of treatment resistance in patients with schizophrenia: a population-based cohort study. *Lancet Psychiatry* **3**:358–366.
- Zai CC, Tiwari AK, Zai GC, Maes MS, Kennedy JL (2018). New findings in pharmacogenetics of schizophrenia. *Curr Opin Psychiatry* **31**:200–212.
- Zai GCM, Zai CCH, Chowdhury NI, Tiwari AK, Souza RP, Lieberman JA, *et al.* (2012). The role of brain-derived neurotrophic factor (BDNF) gene variants in antipsychotic response and antipsychotic-induced weight gain. *Prog Neuro-Psychopharmacol Biolog Psychiatry* **39**:96–101.
- Zai G, Müller DJ, Volavka J, Czobor P, Lieberman JA, Meltzer HY, Kennedy JL (2006). Family and case-control association study of the tumor necrosis factor-alpha (TNF-alpha) gene with schizophrenia and response to antipsychotic medication. *Psychopharmacology (Berl)* **188**:171–182.