DECREASING THE IMPACT OF TREATMENT RESISTANCE IN SCHIZOPHRENIA: IDENTIFYING NOVEL MOLECULAR TARGETS/PATHWAYS TO INCREASE TREATMENT EFFICACY

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DECREASING THE IMPACT OF TREATMENT RESISTANCE IN SCHIZOPHRENIA: IDENTIFYING NOVEL MOLECULAR TARGETS/ PATHWAYS TO INCREASE TREATMENT EFFICACY

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Editorial: Decreasing the Impact of Treatment Resistance in Schizophrenia: Identifying Novel Molecular Targets/Pathways to Increase Treatment Efficacy

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Editorial on the Research Topic

Decreasing the Impact of Treatment Resistance in Schizophrenia: Identifying Novel Molecular Targets/Pathways to Increase Treatment Efficacy

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Schizophrenia (SCZ) is a severe mental disorder with a prevalence of about 1% in the general population (Owen et al., 2016). Antipsychotics remain the mainstay for the treatment of core symptoms of SCZ. However, a substantial proportion of patients (20–30%) present little or no response to these treatments, with naturalistic data pointing to even higher rates of treatment resistant schizophrenia (TRS) (>50%) in community mental health settings (Beck et al., 2019). In addition, even an higher rate of patients shows suboptimal response to antipsychotics (Samara et al., 2019). This population of patients is heavily burdened by higher rates of persistent symptoms, longer duration of hospital admissions and higher treatment costs compared to patients responsive to antipsychotics (Carbon and Correll, 2014). TRS patients show more severe psychopathology, more impaired cognitive functioning, and poorer psychosocial adjustment, which result in worst community functioning, compared to non-TRS patients (Iasevoli et al., 2016). This makes the clinical and socioeconomic impact of TRS enormous. In this context, the early identification of individuals who might present treatment resistance in any phase of the illness course is vital. However, predictive models, either based on clinical or biological data, have not reached yet the accuracy threshold needed to be tested (and eventually validated) in clinical settings. It is conceivable that only models informed by multiple sources of data (phenotypic, genomic, transcriptomic, epigenomic, microbiome) will reach clinically relevance. For instance, a recent study showed that predictive methods, machine learning and polygenic risk scores (PRS), applied to genomic data were unable to discriminate with sufficient accuracy TRS patients form healthy controls (Vivian-Griffiths et al., 2019).

In this scenario, we believed to be timely a Research Topic summarizing the state of the art of "omics", neurobiological and clinical research in the area of TRS. A series of studies focused on the role of genetic determinants in modulating TRS (Sagud et al.; Pisanu and Squassina; Vita et al.), response to antipsychotics, including to the mainstay treatment for TRS, clozapine (Bosia et al.; Koopmans et al.; Numata et al.), as well as the onset of side effects, such as clozapine-induced agranulocytosis and tardive

dyskinesia (Numata et al.; Zai et al.). Specifically, Sagud et al. investigated whether haplotypic and genotypic association of COMT rs4680 and rs4818 polymorphisms modulated the risk of TRS in 931 Caucasian patients diagnosed with SCZ, of whom 270 met the criteria for TRS. They found a gender specific effect of COMT rs4680 and rs4818 polymorphisms with women carrying the G-allele having a lower risk of TRS (Sagud et al.). Pisanu and Squassina performed a critical appraisal of the literature on genetics of TRS, focusing on the ability of genomic data to inform predictive models of TRS. These authors found a lack of genetic markers showing adequate prediction accuracy in TRS. Furthermore, they highlighted the presence of contrasting findings on the power of PRS and machine-learning approaches in discriminating between TRS and non-TRS individuals (Pisanu and Squassina). Vita et al. performed a systematic review with the aim of summarizing the genetic and neuroimaging correlates of TRS and uncovering the underlying neurobiological mechanisms of such resistance. The authors showed that, although interesting data have come from (pharmaco)genetics and neuroimaging investigations as well as from the analysis of their interacting effects, few converging findings are available that describe the antipsychotic treatment response and resistance mechanisms in SCZ (Vita et al.).

Another set of studies focused on the role of genetic variation in predicting response to antipsychotics (Bosia et al.; Koopmans et al.; Numata et al.). Bosia et al. investigated the relationship between metabolic syndrome and cognition, testing for the moderating effect of the Sterol Regulatory Element Binding Transcription Factor 1 (SREBF-1) rs11868035 genetic polymorphism. To this end, they studied 172 outpatients with SCZ assessed for metabolic parameters and neurocognitive measures, with 138 patients, who completed cognitive remediation therapy (CRT), re-evaluated for cognition. They showed the negative impact of metabolic syndrome on executive functions and global cognitive outcome after CRT and revealed the significant effect of SREBF-1 polymorphism, with a higher prevalence of metabolic syndrome and worse processing speed performance among G/G homozygous subjects, compared to the A allele carriers (Bosia et al.). Koopmans et al. evaluated the effect of dose adjustment of antipsychotics to the CYP2D6 genotype and phenotype, in patients diagnosed with SCZ already receiving psychopharmacological treatment. These authors did not identify benefit for the patients from dose adjustment based on the CYP2D6 genotype or phenotype in terms both of efficacy and of safety (Koopmans et al.). Finally, Numata et al. examined the existing literature on genetic predictors of clozapine response. They highlighted that meta-analytical evidence indicate that only three SNPs (rs6313 and rs6314 in the HTR2A gene and rs1062613 in the HT3A gene) are significantly associated with clozapine response (Numata et al.). Concerning genetic predictors of side effects, Numata et al. also summarized genomic findings pointing to the association of genetic variants within the MHC region as well as to a series of other chromosomal regions, with the onset of clozapine induced agranulocytosis. Furthermore, Zai et al. showed a strong significant association of the G allele of the rs2445142 polymorphism within the Perlecan (HSPG2) gene with the risk of tardive dyskinesia.

The area of "omics" research was nicely complemented by the systematic review of Cuomo et al. investigating the possible relationships between microbiome, schizophrenia and treatment resistance. They also summarized the findings exploring the hypothesis that microbiome modulation with probiotics and prebiotics could influence illness severity and clinical response to antipsychotics (Cuomo et al.). This study confirmed that the microbiome is substantially altered in SCZ patients, with pathological reduced variability of the amount and distribution of its components (Cuomo et al.). Furthermore, it highlighted the lack of data on microbiome alteration in TRS as well as the presence of encouraging findings on the use of prebiotics/probiotics in SCZ (Cuomo et al.).

Neurobiological underpinnings of TRS were examined in three studies (Crocker and Tibbo; Rampino et al.; de Bartolomeis et al.). Crocker and Tibbo reviewed the evidence on the role of white matter abnormalities in the brain in the symptomology of psychotic disorders. They confirmed that white matter deficits correlate with treatment resistance in SCZ and proposed that putative myelin-enhancing therapies, including n-3 PUFA, minocycline, clemastine, and sulfasalazine among the others, would be potential candidates for large-scale clinical trials in SCZ as well as for targeting TRS (Crocker and Tibbo). Furthermore, Rampino et al. analyzed the analogies between the established dopaminergic pathophysiological hypothesis of SCZ and the available findings coming from candidate gene and genome-wide association studies. These authors observed that the prevalence of genes involved in dopamine transmission among those associated with treatment responsiveness in SCZ is flagrant and suggested that the further examination of genes that are more distal to dopamine signaling, and are nonetheless associated with SCZ risk and drug responsiveness, may provide a new roster of targets for drug development (Rampino et al.). Finally, de Bartolomeis et al. explored the role of glycine, a non-essential amino acid that plays a critical role in both inhibitory and excitatory neurotransmission, and its related pathways in TRS. These authors highlighted that converging findings point to the pharmacological augmentation of glutamatergic transmission through glycine signaling enhancement as a valuable option to restore the function of prefrontal cortex to control dopamine release, offering a potentially useful strategy in TRS (de Bartolomeis et al.).

Finally, a set of studies focused on the role of clinical determinants of TRS (Thomas-Brown et al.; Barlati et al.; Bozzatello et al.). Thomas-Brown et al. reported on the loss of efficacy of antipsychotic drugs on sleep when concomitant use of cannabis was present, showing that in these cases risperidone was more beneficial than haloperidol in improving sleep efficiency. Barlati et al. systematically reviewed the evidence on factors that can modulate the response to cognitive remediation (CR) interventions in SCZ. The authors identified a series of predictors, such as age, duration of illness, premorbid adjustment, baseline cognitive performance, intrinsic motivation, hostility among the others, that can inform personalized approaches to cognitive

remediation in SCZ and can increase the rates of treatment responsiveness (Barlati et al.). Finally, Bozzatello et al. systematically reviewed the evidence on clinical predictors of TRS. They found that a series of factors such as lower premorbid functioning, lower level of education, negative symptoms from first psychotic episode, comorbid substance use, younger age at onset, lack of early response, non-adherence to treatment, longer duration of untreated psychosis, can inform treatment decision making and lead to a personalized approach in TRS (Bozzatello et al.).

In conclusion, this Research Topic has offered a perspective of the most promising advancements in the area of TRS. These data might guide the development of predictive tools of TRS, and help identifying new molecular targets and pathways to expand the pharmacological armamentarium. This is crucial in psychiatry, where the pharmacological pipeline has lagged behind in the past decade, and it is even more important in the presence of treatment resistance, particularly in a severe and chronic mental illness such as SCZ.

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MM and BC took part in drafting the article, revising it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Haplotypic and Genotypic Association of Catechol-*O*-Methyltransferase rs4680 and rs4818 Polymorphisms and Treatment Resistance in Schizophrenia

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Treatment-resistant schizophrenia (TRS) continues to be a challenge. It was related to different factors, including alterations in the activity of brain dopaminergic system, which could be influenced by the dopamine-degrading enzyme, catechol-*O*-methyltransferase (COMT). Variants of the *COMT* gene have been extensively studied as risk factors for schizophrenia; however, their association with TRS has been poorly investigated. The aim of the present study was to determine the haplotypic and genotypic association of *COMT* rs4680 and rs4818 polymorphisms with the presence of TRS. Overall, 931 Caucasian patients diagnosed with schizophrenia (386 females and 545 males) were included, while 270 participants met the criteria for TRS. In males, no significant haplotypic and genotypic associations between *COMT* rs4680 and rs4818 polymorphisms and TRS were detected. However, genotypic analyses demonstrated higher frequency of *COMT* rs4680 AA genotype carriers compared to G-allele carriers ($p = 0.033$) and higher frequency of *COMT* rs4818 CC genotype carriers than G-allele carriers ($p = 0.014$) in females with TRS. Haplotype analyses confirmed that the presence of the G allele in females was associated with lower risk of TRS. In women with TRS, the high activity G-G/G-G haplotype was rare, while carriers of other haplotypes were overrepresented ($p = 0.009$). Such associations of *COMT* rs4680 and rs4818 high-activity (G variants), as well as G-G/G-G haplotype, with the lower risk of TRS in females, but not in males, suggest significant, but sex-specific influence of *COMT* variants on the development of treatment-resistance in patients with schizophrenia. However, due to relatively low number of females, those findings require replication in a larger sample.

Keywords: treatment-resistant schizophrenia, *COMT* rs4680 polymorphism, *COMT* rs4818 polymorphism, *COMT* rs4680 and rs4818 haplotype, sex-specific association

INTRODUCTION

Schizophrenia is a severe psychiatric disorder. Antipsychotics are the first-line agents in the treatment of schizophrenia, but the clinical response is highly variable. Between 23% (Demjaha et al., 2017) and 47% (Vlatkovic et al., 2018) of patients met the criteria for treatment-resistant schizophrenia (TRS), although the definition has varied across different studies (Howes et al., 2017). In spite of more than 60 years of the widespread use of antipsychotics, TRS continues to present an enormous challenge (Šagud, 2015; Lally et al., 2016). While altered dopaminergic function is the main feature of schizophrenia (Lally et al., 2016; Nedic Erjavec et al., 2017; Nikolac Perkovic et al., 2017; Pruessner et al., 2017), patients with TRS might have distinct dopamine changes, such as lower dopamine synthesis capacity in the striatum (Kim et al., 2017), lower density of dopaminergic synapses in the caudate nucleus (Roberts et al., 2009), and a decrease in the dopamine transporter protein expression (Purves-Tyson et al., 2017), compared to patients who responded to antipsychotics. There is an urgent need to distinguish TRS from non-TRS using genetic or other markers (Šagud, 2015; Lally et al., 2016; Gillespie et al., 2017) as early as possible, in order to provide the best possible treatment for an individual patient.

Catechol-*O*-methyltransferase (COMT) is an important enzyme that degrades catecholamines including dopamine. COMT regulates dopamine availability primarily in the prefrontal cortex (PFC), where the presence of dopamine transporters is scarce (Bilder et al., 2004). Variants of the *COMT* gene have been extensively studied as risk factors for schizophrenia (Egan et al., 2001). Among various polymorphisms of the *COMT* gene, rs4680 and rs4818 significantly affect COMT activity and therefore prefrontal dopamine levels and function. The rs4680 (A > G substitution) or Val108/158Met is the most common functional *COMT* polymorphism in which a G/A substitution results in valine (Val) to methionine (Met) replacement at codon 158 for membrane-bound (MB) COMT, and at codon 108 for the soluble (S) COMT. The Met (A) variant has been associated with a lower thermostability, fourfold lower functional enzyme activity (Lachman et al., 1996), lower protein expression (Chen et al., 2004), and higher dopamine activity compared to Val (G) variant. Previously observed association between *COMT* rs4680 and schizophrenia (Egan et al., 2001; González-Castro et al., 2016) was not confirmed in a meta-analysis (Munafo et al., 2005), or in a cohort with large number of ethnically homogeneous Caucasians in our previous study (Nikolac et al., 2013). Another frequently studied polymorphism of the *COMT* gene is a synonymous polymorphism rs4818, with a C/G substitution (Leu/Leu) at codon 86 of S-COMT or at codon 136 of MB-COMT (Roussos et al., 2008). The G variant of the *COMT* rs4818 polymorphism is associated with greater COMT activity and therefore lower prefrontal dopamine activity (Roussos et al., 2008). It has been reported that *COMT* rs4818 is responsible for the larger variation in the COMT activity than the *COMT* rs4680 polymorphism (Nackley et al., 2006). Some studies demonstrated that *COMT* rs4818 polymorphism was not associated with schizophrenia (Chen C.Y. et al., 2011; Li et al., 2012); however, it is transmitted together with *COMT* rs4680 polymorphism in a haploblock (Hirasawa-Fujita et al., 2018). Contradictory findings exist for the association of *COMT* haplotypes and schizophrenia or its symptoms (Chen C.Y. et al., 2011; Li et al., 2012). Haplotype including *COMT* rs4818 G allele (with rs740603/G allele) was linked to negative symptoms of schizophrenia (Li et al., 2012). However, no significant association of several functional *COMT* polymorphisms and haplotypes with schizophrenia or with psychopathological symptoms was found (Chen C.Y. et al., 2011). Moreover, there were no differences between patients with and without TRS in the whole-blood gene expression of 13 genes, including *COMT* gene (Moretti et al., 2018).

Despite a considerable amount of research on the association of *COMT* rs4680 variants with the response to antipsychotics (Huang et al., 2016), only four studies addressed this *COMT* polymorphism in relation to TRS (Inada et al., 2003; Bosia et al., 2015; Terzić et al., 2016; Rajagopal et al., 2018). These studies yielded inconsistent results, had relatively small sample sizes and the data were not separately analyzed for males and females, although sex-specific associations with *COMT* rs4680 have been reported. For example, the overexpression of the rs4680 GG genotype was found in Spanish males with schizophrenia compared to general population, with no such differences in females (Hoenicka et al., 2010). The presence of one or two A allele in the *COMT* rs4680 elevated the risk of violence in male, but not female patients with schizophrenia (Singh et al., 2012). On the other hand, when *COMT* rs4680 was investigated in Slovenian suicide victims, the AA genotype was more common in the group of control males than in males who committed suicide, and in the control males versus males who committed suicide with violent methods, while again no differences were observed among females (Pivac et al., 2011). The interpretation of the influence of this *COMT* functional polymorphism in psychiatric disorders is therefore complicated by sex-, but also by ethnic-related differences in allele distributions. Whereas Caucasians had similar frequencies of the A and G alleles, the G allele was more common in Asian and other populations (Palmatier et al., 1999). In contrast to these findings, the A allele was associated with bipolar disorder in Asian but not in Caucasian subjects (Taylor, 2018). Moreover, in the meta-analysis of case-control studies, the presence of the G allele of the *COMT* rs4680 was associated with schizophrenia in Caucasian, but not in Asian population, although the data were not analyzed by gender (González-Castro et al., 2016). In addition, only a few studies investigated *COMT* rs4818 polymorphism and the response to antipsychotics (Gupta et al., 2009; Xu et al., 2015; Shi et al., 2017).

Since there is inconclusive or insufficient evidence on the association of *COMT* rs4680 and rs4818 polymorphisms with TRS, especially regarding gender and ethnic differences, the aim of this study was to evaluate genotypic and haplotypic association of the *COMT* rs4680 and rs4818 and TRS in ethnically homogeneous Caucasian subjects of both sexes. Our hypothesis was that patients with TRS have the overrepresentation of rs4680 A allele, as well as rs4818 C allele, compared to non-TRS patients,

and that the observed associations with *COMT* are gender-specific.

MATERIALS AND METHODS

Subjects

This cross-sectional study included 931 biologically unrelated Caucasian patients with schizophrenia, 585 males and 386 females, who gave their consent to participate and met the inclusion criteria, and were considered eligible. Subjects were recruited from the University Hospital Centre Zagreb, University Psychiatric Hospital Vrapce, Zagreb, and Neuropsychiatric Hospital Dr. Ivan Barbot, Popovaca, Croatia. Inclusion criteria were in- and out-patients aged 18–65 years, diagnosed with schizophrenia for at least 5 years. Diagnosis was confirmed using the Structured Clinical Interview (SCID; First et al., 1995) based on the DSM-IV criteria [American Psychiatric Association [APA], 1994]. Exclusion criteria were intellectual disabilities, patients with first-episode psychosis and/or no previous treatment with antipsychotics, substance abuse and dependence in the previous three months, any comorbid severe somatic or neurological disorder and patients who had no available detailed medical records with complete psychiatric medication history. After inclusion, all patients underwent complete diagnostic evaluation. Clinical Global Impression-Severity (CGI-S) scale was used to assess the severity of patients' clinical condition (Guy, 1976). Patients were evaluated using structured interview for the Positive and Negative Syndrome Scale (PANSS) including the PANSS positive, PANSS negative and PANSS general psychopathology subscale (Kay et al., 1987). Schizophrenic patients were subdivided into 574 smokers (i.e., current smokers) and 354 non-smokers (i.e., never smokers and former smokers), whereas for three patients smoking status was not defined. Besides nicotine dependence, no other co-morbid substance abuse or dependence was present. All patients were Caucasians of Croatian origin. The patients were treated with different antipsychotic medication: olanzapine, either as monotherapy or antipsychotic combination (5–20 mg/day), clozapine (300–800 mg/day), risperidone (2–6 mg/day), fluphenazine (5–15 mg/day), haloperidol (4–15 mg/day), promazine (400–500 mg/day), alone or combined with benzodiazepines, i.e., diazepam (2–10 mg/day). Mean dose of antipsychotic medication, calculated into chlorpromazine equivalent doses, was 309.5 ± 263.5 mg/day (range 50–1600 mg/day). Although the concept of TRS is widely used, there is a lack of consensus how to define it. In our study, patients were classified in TRS or non-TRS group according to criteria proposed by Suzuki et al. (2012), which refer to the failure of at least two antipsychotics, given at ≥600 mg chlorpromazine equivalents (Inada and Inagaki, 2015) for more than consecutive 6 weeks, assessed retrospectively. Out of 931 patients, 270 of them met the criteria for TRS and 661 were non-TRS patients. At the time of assessment, psychiatrists were not aware of the genetic test results. The study was approved by the Ethics Committees of the University Hospital Centre Zagreb, University Psychiatric Hospital Vrapce, and Neuropsychiatric Hospital Dr. Ivan Barbot, Popovaca, Croatia, and was carried out in accordance with the Helsinki declaration (1975), as revised in 1983. All patients have signed informed consent prior to study procedures.

Genotyping

The *COMT* rs4680 (assay ID: C_25746809_50) and rs4818 (assay ID: C_2538750_10) genotypes were determined using DNA isolated from the blood samples with a salting out method (Miller et al., 1988). Genotyping was performed using the primers and probes from the TaqMan® Drug Metabolism Genotyping Assays (Applied Biosystems, Foster City, CA, United States) on ABI Prism 7300 Real time PCR System apparatus (Applied Biosystems, Foster City, CA, United States), according to the procedures described by Applied Biosystems. The 10 μL reaction volume contained 30–100 ng of DNA. Around 10% of randomly selected samples were genotyped again as a quality control for genotyping assays.

Statistical Analyses

Data were analyzed using Graph Prism version 7.00 (GraphPad Software, Inc.). Data distribution normality was determined with the Kolmogorov–Smirnov normality test. Due to the lack of a normal distribution, Kruskal–Wallis analysis of variance (ANOVA) and Dunn *post hoc* were used to assess differences in age, chlorpromazine equivalent doses, PANSS total, positive, negative, and general psychopathology scores between different groups of patients. The Hardy–Weinberg equilibrium (HWE) was determined using χ^2-test (Rodriguez et al., 2009). Smoking status, as well as genotype and haplotype distributions, between male and female patients with TRS and non-TRS, were also compared using χ^2-test (Rodriguez et al., 2009). To assess which category was a major contributor to rejecting the null hypothesis, standardized residuals (*R*; Field et al., 2012) were calculated. Haploview software v. 4.2 (Barrett et al., 2005) was used to determine LD pairwise values for *COMT* rs4818 and rs4680. Loci are considered to be in linkage disequilibrium if D' coefficient is >0.80. Best-estimate haplotype pair for every patient was assigned by PLINK v. 1.07 software using the expectation–maximization algorithm (Purcell et al., 2007). Besides genotypic and haplotypic analyses, additional genetic models (González-Castro et al., 2016) were evaluated: dominant model (G carriers, i.e., GG + GA vs. AA) and recessive model (A carriers, i.e., GA + AA vs. GG) for the *COMT* rs4680, as well as dominant model (C carriers, i.e., CC + CG vs. GG) and recessive model (G carriers, i.e., GG + GC vs. CC) for the *COMT* rs4818. For individual SNP analysis the *p*-value (0.05/2 = 0.025) was corrected because two SNPs were compared and the results were considered significant if $p < 0.025$. G*Power 3 Software (Faul et al., 2009) was used to determine a priori sample size. For a χ^2-test [with $\alpha = 0.025$; with expected small effect size = 0.2; power $(1 - \beta) = 0.800$], the required sample size was $N = 288$ with $df = 2$; or $N = 238$ for $df = 1$. For Kruskal–Wallis ANOVA, the *p*-value (0.05/4 = 0.0375) was corrected because of four groups into

$\alpha = 0.0375$; with expected small effect size = 0.15; and power $(1 - \beta) = 0.800$]; the required sample size was 528. As the study included 931 participants, it had adequate sample size and statistical power to detect significant differences among the groups.

RESULTS

Clinical and Demographic Data

Treatment-resistance (TRS) differed significantly ($\chi^2 = 69.694$; $df = 1$; $p < 0.001$) between male and female patients, since males were more frequently treatment resistant (79.6%) than females (20.4%), and the lowest number of female TRS patients ($R = 5.4$) significantly contributed to this significance. Therefore, in further analyses all TRS and non-TRS patients were subdivided according to gender (**Table 1**).

Dunn *post hoc* analysis performed following Kruskal–Wallis ANOVA confirmed significant difference ($p < 0.001$) in age, chlorpromazine equivalent doses, PANSS total, positive, negative, and general psychopathology scores between male and female patients in TRS, as well as in non-TRS group. Moreover, there were also significant differences ($p < 0.001$) between male patients with TRS and non-TRS, as well as between female patients with TRS and non-TRS, in chlorpromazine equivalent doses, PANSS total, positive, negative, and general psychopathology scores, but not in the age.

As shown in **Table 1**, the distribution of smokers and non-smokers was also significantly different ($p < 0.0001$) between male and female patients with TRS and non-TRS. Significantly ($R = 2.86$) lower frequency of female non-smokers in non-TRS group contributed to this difference. Males smoked more often than females in both TRS ($\chi^2 = 10.55$; $df = 1$; $p = 0.0012$) and no-TRS group ($\chi^2 = 15.27$; $df = 1$; $p < 0.0001$); however, there were no significant differences between male patients with TRS and non-TRS, as well as between female patients with TRS and non-TRS.

Genotype Analysis

In male patients with schizophrenia, in the TRS group, *COMT* rs4818 ($\chi^2 = 1.333$; $df = 1$; $p = 0.248$) and *COMT* rs4680 ($\chi^2 = 0.929$; $df = 1$; $p = 0.335$) genotypes distributions did not deviate from HWE. In male patients who were not treatment resistant (i.e., in non-TRS group), no departure from HWE was found for *COMT* rs4818 ($\chi^2 = 0.447$; $df = 1$; $p = 0.503$) and *COMT* rs4680 ($\chi^2 = 1.492$; $df = 1$; $p = 0.222$) genotypes. In female TRS patients, no significant deviation from HWE in *COMT* rs4818 ($\chi^2 = 1.603$; $df = 1$; $p = 0.206$) and *COMT* rs4680 ($\chi^2 = 0.197$; $df = 1$; $p = 0.657$) genotypes distributions was detected. Among female patients in non-TRS group, frequencies of *COMT* rs4818 ($\chi^2 = 0.981$; $df = 1$; $p = 0.322$) and *COMT* rs4680 ($\chi^2 = 0.114$; $df = 1$; $p = 0.736$) genotypes did not deviate from HWE.

There were no significant differences in the frequency of the genotypes or in the dominant or recessive model for the *COMT* rs4818 and COMT rs4680 between male patients with or without TRS (**Table 2**).

In female patients (**Table 3**) subdivided into TRS and non-TRS groups, significant differences were found in the frequency of the *COMT* rs4818 genotypes (CC, CG, and GG; $p = 0.014$) and in the dominant model (C carriers, i.e., CC + CG vs. GG; $p = 0.008$). The distribution of the genotypes in the recessive model (G carriers, i.e., GG + GC vs. CC; $p = 0.043$) for the *COMT* rs4818 did not differ significantly between female TRS and non-TRS groups (**Table 3**). Further analysis revealed that the C carriers were five times more likely to be in the TRS group than GG homozygotes [odds ratio (OR) (C carriers/GG) = 5.748; 95% confidence interval (CI) (1.362–24.251), $z = 2.381$; $p = 0.017$] in female patients with schizophrenia. Similar frequency of the *COMT* rs4680 genotypes (GG, GA and AA), and the genotypes in the recessive (A carriers, i.e., GA + AA vs. GG) or dominant (G carriers, i.e., GG + GA vs. AA) model was found in female TRS and non-TRS patients (**Table 3**). Although a difference in the distribution of the G carriers vs. AA homozygotes was not significant ($p = 0.033$) due to correction ($p = 0.025$), female carriers of AA genotype of the *COMT* rs4680 were slightly more frequent in TRS group than G carriers ($R = 1.7$; OR (AA homozygotes/ G carriers) = 1.917, 95% CI (1.046–3.515); $z = 2.105$; $p = 0.035$). These results showed that female AA homozygotes had almost double chance to develop TRS when compared to G carriers in female patients with schizophrenia (**Table 3**).

Haplotype Analysis

To further examine the association of *COMT* rs4818 and rs4680 polymorphisms with TRS in male and female patients, a haplotype analysis was performed. As shown in **Figure 1**, a high degree of linkage disequilibrium ($D' = 0.88$) was revealed for *COMT* rs4818 and rs4680 polymorphisms.

Table 4 shows the frequencies of the *COMT* (rs4818-rs4680) haplotypes in all patients subdivided in TRS and non-TRS group. The most common haplotype was C-A, followed by G-G haplotype. There was a significant difference ($p = 0.037$) in the distribution of G-G haplotype among TRS and non-TRS patients, demonstrating that G-G haplotype was less frequently represented in TRS than in non-TRS group (**Table 4**).

In the analysis of the *COMT* rs4818-rs4680 haplotypes, all patients were subdivided into carriers and non-carriers of the particular haplotype. For two of the most common haplotype (C-A and G-G) groups, subjects were additionally subdivided into "homozygotes" for the particular haplotype (carriers of the two same haplotype groups), individuals that carry only one of the examined haplotype, and non-carriers of the tested haplotype. Because of the low frequency of the "homozygotes", for another two haplotype groups (C-G and G-A), patients were divided only into carriers and non-carriers.

Haplotype analysis showed a lack of significant difference in the frequency of the particular haplotypes between male TRS and non-TRS patients (**Table 5**).

In female (**Table 6**) patients with schizophrenia, haplotype distribution differed significantly between TRS and non-TRS

TABLE 1 | The demographic and clinical data of male and female patients with schizophrenia subdivided in TRS and non-TRS groups.

	TRS (*N* = 270)		Non-TRS (*N* = 661)		
	Males (*N* = 215)	**Females (*N* = 55)**	**Males (*N* = 330)**	**Females (*N* = 331)**	
Age (years) (median, 25 and 75 percentile)	39 (30,45)	48 (38,58)	40 (31,49)	50 (39,57)	$H = 106.086$; $df = 3$; $p < 0.001$*; Kruskal–Wallis ANOVA
Smokers, *N* (%)	155 (72.09%)	27 (49.09%)	220 (66.67%)	172 (51.96%)	$\chi^2 = 198.8$; $df = 3$; $p < 0.0001$*; χ^2-test
PANSS total scores (median, 25 and 75 percentile)	130 (115,143)	106 (98,124)	105 (92,123)	90 (75,102)	$H = 324.222$; $df = 3$; $p < 0.001$*; Kruskal–Wallis ANOVA
PANSS positive scores (median, 25 and 75 percentile)	34 (30,39)	28 (26,32)	26 (22,33)	22 (17,26)	$H = 300.511$; $df = 3$; $p < 0.001$*; Kruskal–Wallis ANOVA
PANSS negative scores (median, 25 and 75 percentile)	34 (28,37)	27 (23,30)	26 (23,31)	23 (19,26)	$H = 248.191$; $df = 3$; $p < 0.001$*; Kruskal–Wallis ANOVA
PANSS general psychopathology scores (median, 25 and 75 percentile)	62 (56,69)	54 (48,63)	52 (45,59)	45 (38,51)	$H = 279.877$; $df = 3$; $p < 0.001$*; Kruskal–Wallis ANOVA
Chlorpromazine equivalent doses, mg/per day (median, 25 and 75 percentile)	700 (500,1000)	500 (400,750)	500 (300,750)	400 (200,500)	$H = 136.508$; $df = 3$; $p < 0.001$*; Kruskal–Wallis ANOVA

*Results are presented as medians with 25 and 75 percentile. N is the number of patients. *Significant differences between male and female TRS and non-TRS patients. Non-TRS, non-treatment-resistant schizophrenia; PANSS, Positive and Negative Syndrome Scale; TRS, treatment-resistant schizophrenia.*

TABLE 2 | The distribution of the *COMT* rs4818 and rs4680 genotypes in male patients with schizophrenia subdivided into TRS and non-TRS groups.

Male patients (*N* = 545)		TRS (*N* = 215)		Non-TRS (*N* = 330)		
		N	**Frequency (%)**	***N***	**Frequency (%)**	
COMT rs4818 genotypes	CC	60	27.9	83	25.2	$\chi^2 = 2.315$; $df = 2$;
	CG	99	46.0	171	51.8	$p = 0.314$; χ^2-test
	GG	56	26.0	76	23.0	
C carriers	C carriers	159	74.0	254	77.0	$\chi^2 = 0.911$; $df = 1$;
	GG	56	26.0	76	23.0	$p = 0.340$; χ^2-test
G carriers	G carriers	155	72.1	247	74.8	$\chi^2 = 0.671$; $df = 1$;
	CC	60	27.9	83	25.2	$p = 0.413$; χ^2-test
COMT rs4680 genotypes	AA	85	39.5	119	36.1	$\chi^2 = 1.741$; $df = 2$;
	AG	95	44.2	167	50.6	$p = 0.419$; χ^2-test
	GG	35	16.3	44	13.3	
A carriers	A carriers	180	83.7	286	86.7	$\chi^2 = 0.645$; $df = 1$;
	GG	35	16.3	44	13.3	$p = 0.422$; χ^2-test
G carriers	G carriers	130	60.5	211	63.9	$\chi^2 = 0.511$; $df = 1$;
	AA	85	39.5	119	36.1	$p = 0.475$; χ^2-test

Results are presented as numbers and %. N is the number of patients. COMT, catechol-O-methyl-transferase; C carriers of the COMT rs4818, the combined CC and CG genotype; G carriers of the COMT rs4818, the combined GG and GC genotype; G carriers of the COMT rs4680, the combined GG and AG genotype; A carriers of the COMT rs4680, the combined AA and AG genotype; non-TRS, non-treatment-resistant schizophrenia; TRS, treatment-resistant schizophrenia.

patients. Among female schizophrenia patients, haplotype G-G was detected less frequently in TRS group ($R = 1.5$; $p = 0.011$) compared to non-TRS group. Detailed analysis showed that female non-carriers of the haplotype G-G were 2 times more likely to be treatment resistant than female carriers of the haplotype G-G (OR (non-carriers/GG carriers) = 2.1015; 95% CI (1.179–3.743); $z = 2.522$; $p = 0.012$). Haplotype analysis confirmed genotyping results since haplotype frequency of G-G/G-G, G-G/* and */* haplotypes between female TRS and non-TRS patients differed significantly ($p = 0.009$). Namely, presence of the G allele was "protective" against TRS. The high activity G-G/G-G haplotype was the rarest haplotype (3.6%) in female patients with TRS, followed by G-G/** haplotype, while carriers of any other haplotype than G-G were overrepresented (56.4%) in female patients with TRS in comparison to non-TRS female patients (**Table 6**).

TABLE 3 | The distribution of the *COMT* rs4818 and rs4680 genotypes in female patients with schizophrenia subdivided into TRS and non-TRS group.

Female patients (*N* = 386)		**TRS (*N* = 55)**		**Non-TRS (*N* = 331)**		
		N	**Frequency (%)**	***N***	**Frequency (%)**	
COMT rs4818 genotypes	CC	28	50.9	121	36.6	$\chi^2 = 8.525$; *df* = 2;
	CG	25	45.5	151	45.6	$p = 0.014$*; χ^2- test
	GG	2	3.6	59	17.8	
C carriers	C carriers	53	96.4	272	82.2	$\chi^2 = 7.136$; *df* = 1;
	GG	2	3.6	59	17.8	$p = 0.008$*; χ^2-test
G carriers	G carriers	27	49.1	210	63.4	$\chi^2 = 4.100$; *df* = 1;
	CC	28	50.9	121	36.6	$p = 0.043$; χ^2-test
COMT rs4680 genotypes	AA	20	36.4	76	23.0	$\chi^2 = 5.263$; *df* = 2;
	AG	25	45.5	162	48.9	$p = 0.072$; χ^2-test
	GG	10	18.2	93	28.1	
A carriers	A carriers	45	81.8	238	71.9	$\chi^2 = 2.370$; *df* = 1;
	GG	10	18.2	93	28.1	$p = 0.124$; χ^2-test
G carriers	G carriers	35	63.6	255	77.0	$\chi^2 = 4.534$; *df* = 1;
	AA	20	36.4	76	23.0	$p = 0.033$; χ^2-test

*Results are presented as numbers and %. N is the number of patients. *Significant difference in the distribution of the CC, CG, and GG genotypes; or of the C carriers and GG homozygotes, between TRS and non-TRS patients. COMT, catechol-O-methyl-transferase; C carriers of the COMT rs4818, the combined CC and CG genotype; G carriers of the COMT rs4818, the combined GG and GC genotype; G carriers of the COMT rs4680, the combined GG and AG genotype; A carriers of the COMT rs4680, the combined AA and AG genotype; non-TRS, non-treatment-resistant schizophrenia; TRS, treatment-resistant schizophrenia.*

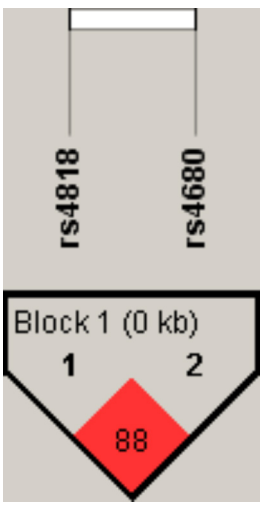

FIGURE 1 | Linkage disequilibrium (LD) plot with D' values characterizing haplotype blocks in COMT gene. The plot was generated by Haploview 4.2 software. Block definition followed the rules of solid spine of LD. D' values multiplied by 100 are shown as a number in the diamonds.

DISCUSSION

Clinical Differences Between Patients With TRS and Non-TRS

Our findings of four times higher prevalence of TRS in male compared to female patients are in contrast to results from Danish patients (Wimberley et al., 2017) who had higher rates of TRS in female patients. However, this study used different methodology, such as the determination of TRS by a treatment-based proxy (Wimberley et al., 2017). In our study, both male and female patients with TRS had significantly higher PANSS total, positive, negative, and general psychopathology scores, as well as higher chlorpromazine equivalent doses in comparison to male and female patients in non-TRS group. This is in agreement with previous studies reporting that patients with TRS had more severe symptomatology as measured by PANSS (Moretti et al., 2018) and received higher total antipsychotic dose presented as chlorpromazine equivalents, compared to patients with non-TRS (Hotta et al., 2011; de Bartolomeis et al., 2018; Moretti et al., 2018). In the present study, the concentration of antipsychotics in plasma was not measured. While about one-third of patients with TRS had sub-therapeutic or non-detectable antipsychotic plasma levels (McCutcheon et al., 2018), non-adherence, or only partial adherence in some of our patients with TRS cannot be ruled out. However, unlike the latter article which excluded patients on long-acting antipsychotics (McCutcheon et al., 2018), in the present study about a quarter of patients received different depot antipsychotics (data not shown), which provided continuous drug delivery. Furthermore, while the majority of patients with TRS were hospitalized at the time of the assessment, antipsychotic intake was monitored by hospital stuff. Given the pronounced difference (an average of 100–200 mg/per day) in chlorpromazine equivalents between individuals with and without TRS, some of those patients might developed the antipsychotic-induced dopamine suprasensitivity psychosis (DSST). Although the presence of neurological disorders was exclusion criteria, rating scales for the assessment of movement disorders were not performed. Therefore, while subjects with pronounced extrapyramidal symptoms or tardive dyskinesia were not included, subtle movement disorders, indicative of DSST (Chouinard et al., 2017), might have gone undetected in some individuals. In the present study, the prevalence of

TABLE 4 | The distribution of *COMT* rs4680-rs4818 haplotypes in all patients with schizophrenia subdivided into TRS and non-TRS group.

Haplotype	All patients	TRS, Non-TRS	
	Frequency (%)	Frequency (%)	
C-A	48.0	49.4, 47.4	$\chi^2 = 0.585$; $p = 0.444$; χ^2-test
G-G	36.3	32.7, 37.8	$\chi^2 = 4.436$; $p = 0.037$*; χ^2-test
C-G	13.5	14.7, 13.0	$\chi^2 = 1.019$: $p = 0.313$; χ^2-test
G-A	2.2	3.2, 1.8	$\chi^2 = 3.422$; $p = 0.064$; χ^2-test

*Results are presented as %. COMT, catechol-O-methyl-transferase; non-TRS, non-treatment-resistant schizophrenia; TRS, treatment-resistant schizophrenia. *Significant differences in the frequency of G-G haplotype between TRS and non-TRS patients.*

TABLE 5 | Haplotype frequencies of *COMT* rs4680 and rs4818 polymorphisms in male patients with schizophrenia subdivided according to the TRS.

Male patients (*N* = 565)		TRS (*N* = 215)		Non-TRS (*N* = 330)		
		N	Frequency (%)	*N*	Frequency (%)	
Haplotype C- A	C-A/C-A	52	24.2	75	22.7	$\chi^2 = 2.494$; *df* = 2;
	C-A/*	102	47.4	178	53.9	$p = 0.287$; χ^2-test
	/	61	28.40	77	23.3	
C-A carriers	C-A carriers	154	71.6	253	76.7	$\chi^2 = 1.748$; *df* = 1;
	Non-carriers	61	28.4	77	23.3	$p = 0.186$; χ^2-test
Haplotype G-G	G-G/ G-G	30	14.0	43	13.0	$\chi^2 = 1.730$; *df* = 2;
	G-G/*	92	42.8	160	48.5	$p = 0.421$; χ^2-test
	/	93	43.3	127	38.5	
G-G carriers	G-G carriers	122	56.7	203	61.5	$\chi^2 = 1.231$; *df* = 1;
	Non-carriers	93	43.3	127	38.5	$p = 0.267$; χ^2-test
C-G carriers	C-G carriers	49	22.8	77	23.3	$\chi^2 = 0.022$; *df* = 1;
	Non-carriers	166	77.2	253	76.7	$p = 0.883$; χ^2-test
G-A carriers	G-A carriers	12	5.6	9	2.7	$\chi^2 = 2.862$; *df* = 1;
	Non-carriers	203	94.4	321	97.3	$p = 0.091$; χ^2-test

**Other haplotypes. Results are presented as numbers and %. N is the number of patients. COMT, catechol-O-methyl-transferase; non-TRS, non-treatment-resistant schizophrenia; TRS, treatment-resistant schizophrenia.*

TABLE 6 | Haplotype frequencies of *COMT* rs4680 and rs4818 polymorphisms in female patients with schizophrenia subdivided according to the TRS.

Female patients (*N* = 386)		TRS (*N* = 55)		Non-TRS (*N* = 331)		
		N	Frequency (%)	*N*	Frequency (%)	
Haplotype C-A	C-A/C-A	17	30.9	71	21.5	$\chi^2 = 4.198$; *df* = 2;
	C-A/*	28	50.9	161	48.6	$p = 0.123$; χ^2-test
	/	10	18.2	99	29.9	
C-A carriers	C-A carriers	45	81.8	232	70.1	$\chi^2 = 3.201$; *df* = 1;
	Non-carriers	10	18.2	99	29.9	$p = 0.074$; χ^2-test
Haplotype G-G	G-G/G-G	2	3.6	53	16.0	$\chi^2 = 9.318$; *df* = 2;
	G-G/*	22	40.0	152	45.9	$p = 0.009$#; χ^2-test
	/	31	56.4	126	38.1	
G-G carriers	G-G carriers	24	43.6	205	61.9	$\chi^2 = 6.544$; *df* = 1;
	Non-carriers	31	56.4	126	38.1	$p = 0.011$#; χ^2-test
C-G carriers	C-G carriers	15	27.3	81	24.5	$\chi^2 = 0.198$; *df* = 1;
	Non-carriers	40	72.7	250	75.5	$p = 0.656$; χ^2-test
G-A carriers	G-A carriers	3	5.5	8	2.4	$\chi^2 = 1.572$; *df* = 1;
	Non-carriers	52	94.5	323	97.6	$p = 0.210$; χ^2-test

**Other haplotypes. Results are presented as numbers and %. N is the number of patients. COMT, catechol-O-methyl-transferase; non-TRS, non-treatment-resistant schizophrenia; TRS, treatment-resistant schizophrenia. #Significant differences in the frequency of G-G/G-G, G-G/* and */* haplotypes between TRS and non-TRS patients.*

smoking was higher in males than females, in both patients with TRS and non-TRS. This is in line with our previous data showing higher prevalence of smoking in male (64%) than in female (46%) patients with schizophrenia (Nikolac et al., 2013), and agrees with the data from general population, given that the men had higher smoking prevalence than woman in European countries, including Croatia (Gallus et al., 2014). Men with schizophrenia also had higher rates of nicotine dependence and different smoking habits compared to healthy men (Nikolac et al., 2013; Šagud et al., 2018). However, the rate of smoking in our study was similar in patients with TRS and non-TRS, which is consistent with the finding from the smaller sample of 21 patients with TRS and 20 patients with non-TRS (Mouchlianitis et al., 2016). Although patients with schizophrenia have the highest known rates of smoking (de Leon and Diaz, 2005), confirmed by the 62.8% of smokers found in schizophrenia patients in our previous study (Nedic Erjavec et al., 2017), our results suggest that treatment-resistance does not further increase this rate. However, smoking might be related to more severe clinical presentation in patients with TRS, since smokers with TRS had higher PANSS total scores and negative subscale scores, and performed significantly worse on the problem solving cognitive task, compared to TRS patients who did not smoke (Iasevoli et al., 2013). The relationship between schizophrenia and smoking is complex (Šagud et al., 2009) given that smoking status was also related to lower total PANSS scores and the PANSS general psychopathology scores in patients with schizophrenia (Nedic Erjavec et al., 2017). Smoking might contribute to treatment resistance by decreasing serum levels of clozapine and olanzapine, but the dose increase might overcome those effects (Tsuda et al., 2014).

Gender-Related Differences in the COMT rs4680 and rs4818 Genotypic and Haplotypic Association With Treatment Resistance

Our results confirmed gender-related differences in the genotypic and haplotypic association of the *COMT* rs4680 and rs4818 and treatment resistance in patients with schizophrenia. Namely, our study revealed that: (1) in male patients with schizophrenia, there were no significant haplotypic and genotypic associations between *COMT* rs4680 and rs4818 and treatment-resistance; (2) in female patients with TRS, AA genotype carriers of the *COMT* rs4680 were nominally more frequently present compared to G carriers, whereas CC carriers of the *COMT* rs4818 were significantly more frequent than G carriers; (3) in female patients with TRS, the high activity G-G/G-G *COMT* haplotype was rare, followed by G-G/** haplotype, while carriers of any other than G-G haplotype were overrepresented, in comparison to female patients with non-TRS. To the best of our knowledge, this is the first report to document that the presence of high-activity (G variants) of the *COMT* rs4680 and rs4818, and *COMT* G-G/G-G haplotype, appears to be associated with lower risk of TRS in female patients with schizophrenia, while no such associations were observed in men. These findings suggest the significant, but gender-specific associations of *COMT* variants with the development of treatment-resistance in schizophrenia.

Gender-Related Association Between the COMT rs4680 Genotype and Treatment Resistance

Gender differences were previously noticed in COMT activity (Chen et al., 2004) as well as in distribution of the *COMT* rs4680 genotypes in healthy individuals (Gurvich and Rossell, 2015; El-Hage et al., 2017) and patients with schizophrenia (Bollettini et al., 2017). There is also evidence of distinct, sex-dependent brain changes related to *COMT* rs4680 polymorphism (Bollettini et al., 2017; El-Hage et al., 2017). Female patients with schizophrenia, carriers of the *COMT* rs4680 AA genotype, had smaller volumes of caudate, putamen, and pallidum, while male patients, homozygous for the Met allele showed higher or similar subcortical volumes compared to other groups (Bollettini et al., 2017). In healthy volunteers, male GG homozygotes had higher white matter integrity compared to A carriers, whereas no differences were observed in females (El-Hage et al., 2017). In healthy women, carriers of the *COMT* rs4680 AA genotype had reduced and GG genotype carriers had superior cognitive flexibility, whereas in men no association with cognition was found (Gurvich and Rossell, 2015). Those studies suggested that AA homozygosity, specifically in women, might be adversely associated with cognitive functioning (Gurvich and Rossell, 2015) and subcortical brain volumes (Bollettini et al., 2017), which could be also related to the association of *COMT* rs4680 polymorphism with treatment-resistance observed only in female patients with schizophrenia in the present study. On the other hand, some studies have not observed associations of *COMT* rs4680 polymorphism with age or gender (Walder et al., 2010; Collip et al., 2011; Armbruster et al., 2012).

Four studies have addressed the association between *COMT* rs4680 and TRS so far. Two of them have included only patients with TRS (Bosia et al., 2015; Rajagopal et al., 2018) whereas two other trials included patients with both TRS and non-TRS (Inada et al., 2003; Terzić et al., 2016). The discrepancies across the studies might arise from sex-differences, ethnic differences, diversities in the populations studied, limited power and small sample sizes, as well as different methodology such as the various definitions of treatment-resistance and measurements of psychopathology. In contrast to our results, TRS patients, carriers of the AA or GA genotypes of the *COMT* rs4680, but who were also carriers of one or two DRD4 120-bp alleles (120/240 and 120/120), experienced better response to clozapine than TRS patients, carriers of the GG genotype (Rajagopal et al., 2018). This study included 93 TRS patients of South Indian ethnicity, but did not divide patients according to gender. Corresponding to our results in male patients with schizophrenia, *COMT* rs4680 genotypes were not associated with better response to clozapine in TRS (Rajagopal et al., 2018). Since in our study the presence of the A allele was associated with TRS in female but not in male patients, either different number of patients, the fact that we did not evaluate gene-gene interaction with the DRD4 120-bp alleles, or ethnic differences (Rajagopal et al., 2018), might explain

these different results. In line with the results from the study evaluating 107 treatment-resistant Italian patients (Bosia et al., 2015), carriers of the *COMT* rs4680 GG genotype have shown better response to clozapine (in reducing negative symptoms), compared to patients with the GA and AA genotypes (Bosia et al., 2015). In our study, this slight association was observed only in female but not in male patients. Since the cited study (Bosia et al., 2015) did not evaluate gender specific association with TRS, and included much smaller sample, we might speculate that this non-significant association (due to correction) of the AA genotype with TRS might be presumably related to female gender. Our results on the link between the AA genotype and TRS in female patients with schizophrenia also agree with the data from the 100 Japanese patients with schizophrenia (Inada et al., 2003). In this study, patients with TRS had marginally higher frequency of the A variant of *COMT* rs4680 polymorphism, and the odds ratio for the AA genotype in TRS was 4.392 (Inada et al., 2003). Patients with the *COMT* rs4680 AA genotype also received higher chlorpromazine equivalent doses compared to carriers of the GA and GG genotypes (Inada et al., 2003). Although the sample size was small (only eight patients had *COMT* rs4680 AA genotype), and patients were not evaluated according to gender (Inada et al., 2003), these results are in line with our female data. In agreement with our data in males, but in contrast to data obtained in female patients with or without TRS, no difference in the *COMT* rs4680 genotype frequency was detected in 138 patients: 44 treatment-resistant and 94 treatment-responsive patients from Slovenia, who were not divided by gender (Terzić et al., 2016).

Gender-Related Association Between the COMT rs4818 Genotype and Treatment Resistance

While *COMT* rs4680 is among the most frequently investigated polymorphisms in treatment response to psychotropic drugs, only a few studies investigated *COMT* rs4818 polymorphism and the response to antipsychotics (Gupta et al., 2009; Xu et al., 2015; Shi et al., 2017). In our study the presence of the CC genotype of the *COMT* rs4818 in female, but not in male group of patients with TRS, was found significantly more frequently than the presence of the GC and GG genotypes. In line with our findings, in the large Shanghai cohort of 995 Chinese patients with schizophrenia, C carriers of the *COMT* rs4818 had more frequently poor response to quetiapine (Xu et al., 2015). The association between *COMT* rs4818 and treatment response to risperidone was also reported in 288 Shanghai patients with schizophrenia (Shi et al., 2017), but opposed to our and their previous (Xu et al., 2015) results, the G/C allele frequency was similar between good and poor responders (Shi et al., 2017).

Gender-Related Association Between the COMT rs4680 and rs4818 Haplotype and TRS

The findings of the present study that carriers of the high activity G-G/G-G haplotype were more frequently observed in non-TRS female group, whereas female carriers of any other haplotype than G-G were overrepresented in the group of patients with TRS, suggest that presence of the G allele might be associated with decreased risk of TRS. This association was also confirmed in Shanghai cohort (Xu et al., 2015). In contrast to our data, among 117 patients with schizophrenia of the southern Indian origin, the *COMT* haplotype C-A (rs4818-r4680) was observed more often in responders to risperidone, compared to non-responders (Gupta et al., 2009). However, these data were not analyzed by gender, and the treatment-response was defined only by the clinical global impression scale (Gupta et al., 2009).

Our results showed that presence of the high activity (i.e., G variants) of the *COMT* was associated with lower risk of TRS in female patients. Likewise, negative symptoms of schizophrenia were less severe in female patients carrying the high activity *COMT* variants (rs740603 (G)-rs4818 (G) haplotype), but this effect was not observed in male patients with schizophrenia (Li et al., 2012). If this finding will be confirmed in larger studies and meta-analyses, women with schizophrenia who are carriers of the *COMT* rs4680 and rs4818 low-activity haplotypes, might require different treatment approach, such as early clozapine initiation (Siskind et al., 2017), clozapine augmentation with different antiepileptic drugs (Zheng et al., 2017), or electroconvulsive therapy (ECT) (Vuksan et al., 2018).

The Association Between the High-Activity COMT Haplotype and Treatment Response in Female Patients With Schizophrenia: Possible Explanations

COMT activity plays a key role in the regulation of dopamine activity in PFC, while its role in the regulation of striatal dopamine turnover is less important due to higher abundance of dopamine transporter in this region (Bilder et al., 2004). In line with this hypothesis, in healthy subjects, *COMT* rs4680 GG carriers had lower dopamine tone in cortical and limbic regions, compared to carriers of the GA or AA genotypes, while no changes of dopamine tone were detected in the striatal regions (Slifstein et al., 2008). However, striatal dopamine turnover seems to be altered in patients with TRS (Kim et al., 2017). While patients with schizophrenia generally exhibited elevated striatal dopamine synthesis capacity compared to healthy controls (Fusar-Poli and Meyer-Lindenberg, 2013), individuals suffering from TRS had lower capacity of dopamine synthesis in striatum (Kim et al., 2017). This finding was observed in TRS patients treated with clozapine (Kim et al., 2017) and in patients treated with other antipsychotics (Demjaha et al., 2012), in comparison to patients who responded to antipsychotic treatment. Although COMT has a minor role in metabolizing striatal dopamine, modifications of COMT activity may affect dopamine signaling also in the striatum, as shown by the data from animal models (Simpson et al., 2014; Tammimaki et al., 2016), and from some (Boot et al., 2011) but not all (Slifstein et al., 2008) human reports. Considering that compensatory mechanisms might influence dopamine function in striatum (Simpson et al., 2014), the relationship between prefrontal dopamine availability, modulated by *COMT* rs4680, and striatal dopamine tone (Bilder et al., 2004; Ceaser et al., 2013) was proposed. In post-mortem brain samples of individuals without psychiatric disorders,

carriers of the *COMT* rs4680 GG genotype had greater expression of tyrosine hydroxylase mRNA in mesencephalic dopamine neurons than GA genotype carriers, particularly in neuronal populations that project to the striatum, suggesting higher dopamine synthesis in striatal regions of GG homozygotes (Akil et al., 2003).

Although these studies did not account the possible gender differences (Akil et al., 2003; Slifstein et al., 2008), we might speculate that females with AA genotype of the *COMT* rs4680 might have had higher prefrontal and compensatory lower striatal dopamine levels, which were reported in patients with TRS (Kim et al., 2017). The dopamine synthesis capacity in striatum was proposed as a biomarker for TRS (Kim et al., 2017). In the presence of higher dopamine stimulation from PFC, such as in the *COMT* rs4680 AA homozygous subjects, dopamine release might decrease in striatum in an attempt to protect the brain from excessive dopaminergic stimulation, and this mechanism might be related to treatment-resistance. According to our findings, this hypothesis might be only relevant for female patients with schizophrenia. Given the COMT-inhibiting properties of estradiol (McDermott et al., 2015), decreased COMT activity in women (Chen et al., 2004), and large sex-differences in dopaminergic cortical pathways in preclinical model (Kritzer and Creutz, 2008), it could be hypothesized that women have higher PFC dopamine levels than men, carrying the same *COMT* genotype. Such greater dopaminergic stimulation in females could lead to a gender-specific hyperdopaminergic overdrive in PFC, and consequently to decreased dopamine levels in striatum, which might eventually predispose women to TRS. This proposal fits the presumption that some patients do not respond to treatment because they do not exhibit elevated dopamine input in striatum (Demjaha et al., 2012; Kim et al., 2017), while increased dopamine stimulation in striatum is the target for antipsychotic drugs (Fusar-Poli and Meyer-Lindenberg, 2013). However, the current study did not measure dopamine levels.

The evidence of sexual dimorphism of *COMT* gene is still inconclusive, but continues to accumulate (Harrison and Tunbridge, 2008). The findings regarding sex-dependent associations of COMT with various clinical and biological features have been reported in diverse populations (Rybakowski et al., 2006; Lang et al., 2007; Chen C. et al., 2011; Jacobs and D'Esposito, 2011; Klebe et al., 2013; Koike et al., 2018), as well as in preclinical trials (Gogos et al., 1998; Laatikainen et al., 2013; Sannino et al., 2015). In general, sexually dimorphic effects of *COMT* gene variations are complex, and range from robust to subtle, depending on the parameters, which were measured.

Great amount of the data demonstrating gender differences in association of *COMT* gene with various personality traits, phenotypes, cognitive domains and behaviors, came from studies that enrolled healthy individuals (Eley et al., 2003; Enoch et al., 2003; Olsson et al., 2005; Stein et al., 2005; Kim et al., 2006; Barnett et al., 2007; Lang et al., 2007; de Castro-Catala et al., 2015; Costa et al., 2016). However, sexual dimorphism of *COMT* gene has been also reported in various neuropsychiatric disorders such as anxiety disorders, depression, attention deficit hyperactivity disorder, and obsessive-compulsive disorder (Karayiorgou et al., 1997, 1999; Domschke et al., 2004, 2007; Poyurovsky et al., 2005; Denys et al., 2006; Rothe et al., 2006; Pooley et al., 2007; Cao et al., 2014; Akutagava-Martins et al., 2016). In addition, our results are in line with other studies demonstrating sex-specific associations of *COMT* variants in patients with schizophrenia (Shifman et al., 2002; Dempster et al., 2006; Rybakowski et al., 2006; Lee and Kim, 2011). All these data suggest that *COMT* gene variations might contribute to the sex differences in brain function and structure (Domschke et al., 2012; White et al., 2014; Sannino et al., 2015, 2017; Elton et al., 2017), and consequently result in sexual dimorphism in the predisposition to various neuropsychiatric disorders. Sex-specific effects of COMT gene are usually attributed to transcriptional regulation by estrogens, and it has been suggested that reciprocal and partly genotype-influenced interactions between COMT and estrogens may be relevant to sexual dimorphism (Harrison and Tunbridge, 2008). Although the biological reason why COMT association is sex-specific is still not clear, it has been hypothesized that COMT genotype may modulate the role of estrogens in brain function and dysfunction (Seeman, 1997), while estrogens affect COMT activity and its pathophysiological consequences by influencing the COMT gene expression (Harrison and Tunbridge, 2008). However, additional mechanisms are also possible. Nevertheless, it is known from various studies that sex differences in the genetic architecture of many human traits and psychiatric disorders are common. Namely, in addition to COMT, sexually dimorphic genetic associations with psychiatric phenotypes have been reported also for other autosomal genes such as HTR2A (Enoch et al., 2001), MTHFR (Sazci et al., 2005; Kempisty et al., 2006), and AC7 (Hines et al., 2006). Therefore, our findings reporting sex-specific associations of COMT variants with the development of treatment-resistance in schizophrenia also contribute to the knowledge in this field.

Limitations of the Study

Limitation of the study is the fact that treatment-resistance in schizophrenia patients was determined retrospectively. Another limitation of this investigation is the lower number of female then male patients, which resulted in a limited representation of some of the genotypes after the gender-stratified analysis was conducted. Therefore, the obtained results require replication in a larger sample.

CONCLUSION

Our findings reveal complex and gender-dependent genotypic and haplotypic associations between *COMT* rs4680 and rs4818 and TRS. In males with schizophrenia, treatment-resistance was not associated with the *COMT* rs4818 and rs4680 genotypes or haplotypes. In contrast, as far as we are aware, this is the first study to show that in female patients with schizophrenia, the presence of high-activity (G variants) of the *COMT* rs4680 and rs4818 polymorphisms, and the presence of the G-G/G-G haplotype, is associated with the lower risk of TRS. These findings extend previously reported gender-related association of *COMT*

rs4680 variants and TRS, and for the first time detect gender-dependent association of *COMT* rs4818 polymorphism with treatment-resistance in patients with schizophrenia. Accordingly, we might speculate that determination of the *COMT* rs4680 and rs4818 genotypes and haplotypes early in the course of treatment might help in the prediction of the treatment-resistance in female patients with schizophrenia. However, those findings must be interpreted with caution, given that the number of included females was substantially lower than the number of males. Therefore, investigation of the genotypic and haplotypic association between *COMT* SNPs rs4680 and rs4818 and TRS in the larger sample of females with schizophrenia is warranted.

AUTHOR CONTRIBUTIONS

MS did the research idea, patient recruitment, data collection, assessment by rating scales, organization of blood sampling, data interpretation, article preparation, and final draft approval. LT did experimental work, processing of blood samples, DNA isolation, genotyping, statistical analysis, and final draft approval. SU did patient recruitment, data collection, assessment by rating scales, organization of blood sampling, and final draft approval. MNP, MK, and GNE did experimental work, processing of blood samples, DNA isolation, genotyping, and final draft approval. MZ did patients recruitment, data collection, assessment by rating scales, organization of blood sampling, and final draft approval. OK, BVC, AMP, and NM did patient recruitment, data collection, assessment by rating scales, and final draft approval. DSS did experimental work, collection of blood samples, DNA isolation, genotyping, proof reading, and final draft approval. IR did data collection, and final draft approval. NP did design of the study, data analysis and interpretation, article preparation, and final draft approval.

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Risperidone Provides Better Improvement of Sleep Disturbances Than Haloperidol Therapy in Schizophrenia Patients With Cannabis-Positive Urinalysis

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A high percentage of persons with Schizophrenia also uses *Cannabis* and this may potentially alter the therapeutic benefits of the antipsychotic medications prescribed. The aim of this study was to assess the impact of *Cannabis* usage on antipsychotic therapy of sleep disturbances in schizophrenia subjects. Male subjects, ≥18 years, admitted to the University Hospital of the West Indies psychiatric ward between October 2015 and October 2016, and diagnosed with schizophrenia were recruited for the study. Following written informed consent to the study, subjects were prescribed either risperidone monotherapy or haloperidol monotherapy orally for 14 days and classified as *Cannabis* users (CU) or non-users (non-CU), with presence/absence of *Cannabis* metabolite in urine samples. After 1 week of admission, subjects wore the Actiwatch-2 device, to record sleep data for 7 consecutive nights. Inferential statistical analysis involved non-parametric tests, expressed as median and inter-quartile ranges (IQR), with $p<0.05$ considered statistically significant. Fifty subjects were assessed, with a median (IQR) age of 28 (14) years. Majority (30; 60%) were CU, displaying longer sleep latency (SL) than non-CU when receiving haloperidol; but equivalent SL when receiving risperidone. In comparison to non-CU, the CU also displayed longer time in bed, but shorter durations asleep, awoke more frequently during the nights and for longer durations, whether receiving haloperidol or risperidone. This resulted in lower sleep efficiency for the CU (<85%) compared to the non-CU (≥85%). Over the study period, sleep efficiency was significantly improved for non-CU receiving either risperidone ($p = 0.032$) or haloperidol ($p = 0.010$); but was only significantly improved with risperidone for the CU ($p = 0.045$). It is apparent that the presence of *Cannabis* may be impacting the therapeutic benefits of antipsychotic drugs on sleep. In schizophrenia subjects with concomitant *Cannabis* use, risperidone is more beneficial than haloperidol in improving sleep efficiency.

Keywords: *Cannabis*, risperidone, haloperidol, schizophrenia, actigraphy, sleep

INTRODUCTION

The prognosis of schizophrenia is often worsened with *Cannabis* use (Foti et al., 2010; Manrique-Garcia et al., 2014), mainly through potentiation of the well-established dopamine dysregulations in schizophrenia (Bossong et al., 2009, 2015). High percentages of *Cannabis* users (CU) report that it promotes sleep (Cousens and DiMascio, 1973; Schofield et al., 2006; Schaub et al., 2008; Goonawardena et al., 2012; Walsh et al., 2013); however, polysomnographic sleep assessment show relatively inconsistent sleep-promoting properties of *Cannabis* (Gates et al., 2014, 2016). Pharmacological intervention in schizophrenia requires long-term administration of antipsychotic drugs (Mercolini et al., 2007; Fumagalli et al., 2009; Uchida et al., 2011), which block the dopaminergic system. In early polysomnographic studies, typical antipsychotic drugs, such as haloperidol (Clarenbach et al., 1978; Taylor et al., 1991; Maixner et al., 1998; Dursun et al., 1999), demonstrate sleep-promoting effects and improve sleep maintenance/continuity, mainly through the reduction of sleep latency (SL) and frequency of awakening, prolongation of sleep time and an increase in sleep efficiency in healthy subjects and schizophrenia patients (Taylor et al., 1991; Benson, 2006; Cohrs, 2008; Anderson and Bradley, 2013). In contrast to typical antipsychotics, the atypical agents, including risperidone, have demonstrated greater improvement of sleep efficiency, due to the higher affinity for serotonin 5-$HT_{2A/2C}$ receptors, which are involved in controlling sleep quality (Dursun et al., 1999; Ichikawa et al., 2001; Miller, 2004; Cohrs, 2008; Anderson and Bradley, 2013). Furthermore, schizophrenia patients treated with risperidone display better sleep quantity, sleep quality, and general functioning compared to patients treated with typical antipsychotic drugs (Dursun et al., 1999; Yamashita et al., 2002; Giménez et al., 2007; Apiquian et al., 2008; Wichniak et al., 2011).

It is possible for *Cannabis* use to alter the clinical benefits of antipsychotic drugs (Thomas et al., 2015; Patel et al., 2016; Foglia et al., 2017); however, there is a paucity of information evaluating the impact of *Cannabis* use on the sleep outcomes of schizophrenic patients being treated with antipsychotics. Through actigraphy, this study examined the differences in sleep parameters in schizophrenia patients treated with risperidone vs. haloperidol and the impact of *Cannabis* use. Sleep quality can be estimated through sleep efficiency percentage, which incorporates the ratio of total sleep time and total time in bed; both of which can be altered by the SL, wake after sleep onset (WASO) duration and number of awakening (Shrivastava et al., 2014; Reed and Sacco, 2016; Sathyanarayana et al., 2016).

METHODS AND SUBJECTS

Ethical approval was obtained from the University of the West Indies Ethics Committee. Males of at least 18 years of age were recruited from the University Hospital of the West Indies' psychiatric ward between October 2015 and October 2016, if they met the *Diagnostic and Statistical Manual of Mental Disorders, 5th edition* (DSM-V) criteria for schizophrenia, schizophreniform disorder or brief psychotic disorder, as assessed by trained psychiatrists managing each patient. Psychiatric assessments were conducted by three psychiatrists. Written informed consent was obtained from each subject's relative/guardian on day 1. After consent, urine samples (5 mL) were collected in sterile containers and screened for the possible use of *Cannabis*, using the SD BIOLINE drug of abuse kit, based on the analysis for 11-nor-Δ^9-tetrahydrocannabinol-9-Carboxylic acid; the main metabolite of Δ^9-tetrahydrocannabinol, with a detection limit of 50 ng/mL. Subjects were classified as *Cannabis* users (CU) or non-users (non-CU), with a positive or negative kit result, respectively. Psychiatrists managing subjects were blinded from the *Cannabis* result.

Subjects were hospitalized for the 2-weeks study period, and recruited if administered either risperidone or haloperidol, orally at a dose prescribed by the assigned psychiatrists. Therapy could be flexibly adjusted within the therapeutic range as clinically warranted (risperidone, 6–8 mg/day; haloperidol, 10–20 mg/day). In addition, all enrolled subject received daily administrations of benztropine, 2 mg/day. Subjects were excluded from the study if they were receiving any other concomitant medications. Subjects who presented with diagnoses of other central nervous system disorders, mental retardation, somatic diseases, trauma or brain injury and primary sleep disorders were also excluded. Female subjects were excluded to control for the hormone-dependent confounding differences with *Cannabis* use (Craft et al., 2013; Castelli et al., 2014) and sleep disturbances (Sharkey et al., 2014). Demographic information collected from each subject's docket included age, ethnicity, marital status, occupational status and educational level. History of psychosis (schizophrenia, schizophreniform disorder, or brief psychotic disorder) and previous substances abused (*Cannabis*, tobacco, alcohol, other), if any, were also recorded upon admission to the psychiatric ward.

After 7 days of antipsychotic therapy, only subjects remaining on monotherapy and confirmed by psychiatrist as displaying mild to moderate symptoms (scores < 53), using the Brief Psychiatric Rating Scale (BPRS) were then given the Actiwatch-2 device (Respironics, Inc., Murrysville, PA, United States) to wear on the non-dominant wrist. This device recorded sleep data in each subject for seven consecutive nights, 8 h each night, from 10:00 P.M. to 6:00 A.M. Computer scoring of actigraphy-recorded sleep parameters were performed using Respironics Actiware software, version 5.70.0. The software default setting of immobile minutes was used as the sleep interval detection algorithm. Immobility was determined if the activity counts (AC) was <4 in a 1 min epoch. The data were analyzed using medium wake thresholds (40 AC), with 10 min of immobility set for sleep onset and sleep end. Sleep parameters were calculated during the sleep/rest period (when AC was <40 AC threshold), which was set three different ways: (1) automatic scoring of major rest intervals by the software; (2) pre-determined bedtimes and wake-up times for each subject; (3) manual setting based on surrounding activity level. With the manual setting, the start and end of the sleep period were set close to the pre-determined times on the ward, providing that the AC were not >500 for 2 min, or $>1{,}000$ for

5 min, at the start. End of sleep was set when the AC increased to >0 for 5 min, without an epoch scored as sleep. A subject was likely scored as sleep when immobile for 3 h or more. While each of these parameters may have been manipulated by the user, sleep/wake analysis was performed automatically by the software. The scorer was blinded to *Cannabis* status when manually setting the rest intervals. Sleep parameters measured include SL, frequency of awakening, duration of WASO and total sleep time (TST).

DATA AND STATISTICAL ANALYSIS

Sleep efficiency percentage (SE%) is a good overall descriptor of a night's sleep, as it reflects the percentage of time in bed actually spent sleeping (Shrivastava et al., 2014; Reed and Sacco, 2016; Sathyanarayana et al., 2016). SE% was calculated as the percentage of the ratio TST/Time spent in bed (TIB); with TIB calculated as the sum of SL, TST, and WASO. Additionally, sleep efficiency scores were classified as good-quality sleep (SE% $\geq$ 85%) or poor-quality sleep (SE% < 85%) (Reed and Sacco, 2016; Sathyanarayana et al., 2016). The power of the study was calculated using SE% for CU and non-users, prescribed either haloperidol or risperidone. The power for SE% was >80%, which was adequate. Daily drug dosages were converted to chlorpromazine equivalent (CPZE) doses (Danivas and Venkatasubramanian, 2013) to facilitate drug dosage comparisons.

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS v.20) for Windows (SPSS, Inc., Chicago, IL, United States), with the level of significance set at $p < 0.05$. Demographic data and continuous variables were evaluated using descriptive statistics and expressed as medians and IQR, frequencies (n) and percentages, as appropriate. Subjects were divided into four groups: CU vs. non-CU receiving haloperidol therapy and CU vs. non-CU receiving risperidone therapy. Inferential statistics involved use of Spearman Rho to determine correlations between *Cannabis* grouping and the actigraphy-measured sleep parameters, Kruskal-Wallis test for comparisons across all four groups, with *post hoc* analysis between groups using Bonferroni corrections, and Friedman test for the change in SE% over the seven nights.

RESULTS

Table 1 gives demographic information of the 50 male subjects of Afro-Caribbean descent who completed the study. The sample population consisted of 30 subjects (60%) presenting with first-time psychotic episodes (acute psychosis/drug-naive). The remaining 20 subjects (40%) had undergone outpatient therapy or had previously been treated in an inpatient setting. Duration of illness was the only variable significantly different between the acute vs. relapse subjects [0 (2) vs. 11 (1); $p = 0.024$]. Based on self-declaration, initiation of *Cannabis* use was from a median (IQR) age of 14 (2) years. Majority of subjects ($n = 30$, 60%) used *Cannabis* within one week of admission to the psychiatric ward and were classified as CU, with urinalysis. Eighteen CU subjects received risperidone and 12 received haloperidol. The remaining 20 subjects were grouped as non-CU, with 14 receiving risperidone and 6 receiving haloperidol. CU subjects received significantly higher doses of haloperidol compared to non-CU subjects ($p = 0.001$) and CU subjects receiving risperidone ($p = 0.001$). There was no significant difference in dose between CU and non-CU receiving risperidone. By day seven of therapy, there was no significant difference in the BPRS score of schizophrenia symptoms between CU and non-CU receiving haloperidol [30 (6) vs. 28 (9); $p = 0.335$] or risperidone [30 (9) vs. 30 (10); $p = 0.722$].

According to the rank order by Cohen and Holliday (1996), Spearman Rho analysis showed that for subjects receiving haloperidol, *Cannabis* use was moderately correlated to SL ($r = 0.432$, $p = 0.017$), but showed low correlations with TST ($r = -0.335$, $p = 0.020$), WASO duration ($r = 0.308$, $p = 0.031$) and frequency of awakening ($r = 0.300$, $p = 0.038$). This trend was similar for subjects receiving risperidone, with moderate and negative correlation of *Cannabis* use to TST ($r = -0.433$, $p = 0.017$), moderate and positive correlation to WASO duration ($r = 0.412$, $p = 0.024$) and low and positive correlation to frequency of awakening ($r = 0.381$, $p = 0.035$); but no correlation with SL ($r = 0.336$, $p = 0.064$).

Sleep parameters for CU and non-CU, receiving either haloperidol or risperidone are presented in **Figure 1**. Analysis across the groups showed significant differences for SL ($p = 0.048$), TST ($p = 0.038$), frequency of awakening ($p = 0.022$) and WASO ($p = 0.030$). For the subjects receiving haloperidol,

TABLE 1 | Demographic characteristics of study participants.

Demographic data		Total ($N = 50$)
Diagnosis (*n*)		Schizophrenia (37), Schizophreniform disorder (7), brief psychotic disorder (6)
Age, years, Median (IQR)		24 (8)
Marital status (*n*)		Single (47), married (1), common-law (2)
Highest education level (*n*)		Primary (6), secondary (41), tertiary (3)
Occupational status (*n*)		Unemployed (48), employed (2)
History of substance use (self-declaration)		*Cannabis* (35), alcohol (27), tobacco/Cigarettes (42), cocaine (2)
Day 7 CPZE dose (mg/day), Median (IQR)		
Haloperidol	CU ($n = 12$)	1,000 (375)[a]
	Non-CU ($n = 6$)	750 (500)
Risperidone	CU ($n = 18$)	300 (25)[b]
	Non-CU ($n = 14$)	300 (0)[b]

[a]$p < 0.05$ *vs. non-CU receiving haloperidol.* [b]$p < 0.05$ *vs. subjects receiving haloperidol. CPZE, Chlorpromazine Equivalent; CU, Cannabis users; non-CU, non-users of Cannabis.*

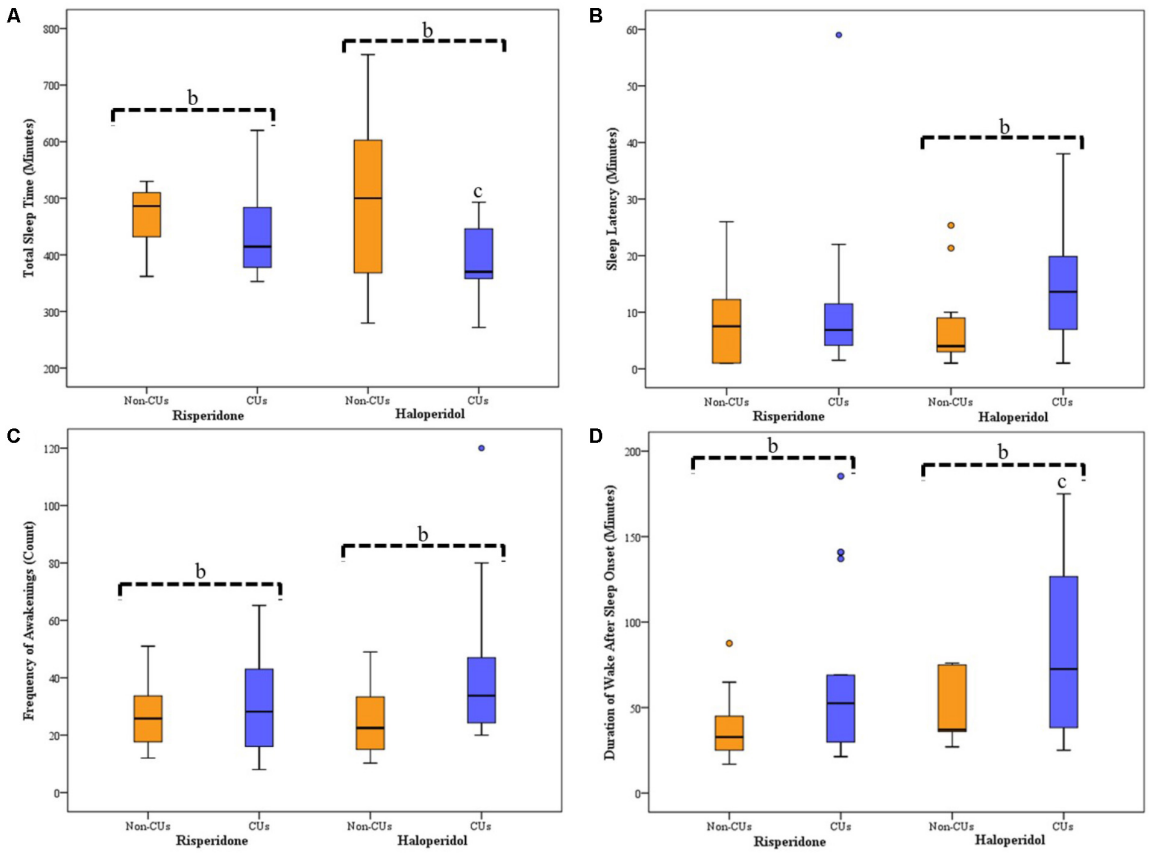

FIGURE 1 | Average Sleep for Seven Consecutive Nights of Actigraphy Recording for CU and Non-CU Receiving either Haloperidol or Risperidone. Box-plots represent median (thick, dark horizontal lines), IQR (boxes), minimum and maximum (whiskers) of each sleep parameter recorded using actigraphy. Circles represents outliers in sleep data. [b]Significant differences between CU and non-CU groups. [c]Significant difference between CU treatment groups. **(A)** TST was significantly less for CU than non-CU when receiving either haloperidol ($p = 0.015$) or risperidone ($p = 0.035$); there was significantly less TST between CU receiving haloperidol and CU receiving risperidone ($p = 0.045$). **(B)** SL was significantly longer for CU than non-CU when receiving haloperidol ($p = 0.009$), but not when receiving risperidone. **(C)** Frequency of awakening after sleep onset was significantly more for CU than non-CU when receiving either haloperidol ($p = 0.017$) or risperidone ($p = 0.004$). **(D)** WASO duration was significantly longer for CU than non-CU when receiving either haloperidol ($p = 0.005$) or risperidone ($p = 0.020$); there was significantly longer WASO between CU receiving haloperidol and CU receiving risperidone ($p = 0.022$). CU, *Cannabis* user; non-CU, non-user of *Cannabis*; SL, sleep latency; TST, total sleep time; WASO, wake after sleep onset.

CU subjects displayed significantly longer SL than non-CU subjects [14 (15) vs. 4 (7) minutes; $p = 0.009$] and had significantly less TST [314 (115) vs. 487 (101) minutes; $p = 0.015$]. During sleep, CU subjects had more frequency of awakening [34 (27) vs. 23 (24); $p = 0.004$] and longer WASO durations [73 (94) vs. 37 (42) minutes; $p = 0.020$]. For subjects receiving risperidone, there was no difference in the SL between groups [8 (15) vs. 6 (8) minutes; $p = 0.088$], but CU subjects had significantly less TST [370 (100) vs. 500 (259) minutes; $p = 0.035$], more frequency of awakening [28 (28) vs. 22 (18); $p = 0.017$] and longer WASO durations [53 (75) vs. 34 (21) minutes; $p = 0.005$] than non-CU subjects.

Sleep parameters were not significantly different between the antipsychotic groups for non-CU subjects. Comparisons between CU groups showed no difference in SL and frequency of awakening; however, CU subjects receiving risperidone had longer TST ($p = 0.045$) and shorter WASO duration ($p = 0.022$) than CU subjects receiving haloperidol.

SE% for the seven consecutive nights of actigraphy recording for the CU and non-CU receiving either haloperidol or risperidone are presented in **Figure 2**. For non-CU subjects, sleep quality was consistently good (≥85%) over the seven nights and Friedman's analysis showed significant improvements in SE% when receiving haloperidol ($n = 6$; $\chi^2 = 16.82$, $p = 0.010$, $df = 6$) or risperidone ($n = 14$; $\chi^2 = 13.84$, $p = 0.032$, $df = 6$). Good sleep quality and significant improvement in SE% was also recorded for CU subjects receiving risperidone ($n = 18$; $\chi^2 = 12.90$, $p = 0.045$, $df = 6$). Sleep quality was consistently poor for CU subjects receiving haloperidol and there was no improvement recorded over the 7 days ($n = 12$; $\chi^2 = 2.78$, $p = 0.180$, $df = 6$).

DISCUSSIONS

Risperidone and haloperidol can improve sleep outcomes in schizophrenia patients (Taylor et al., 1991; Wetter et al., 1996;

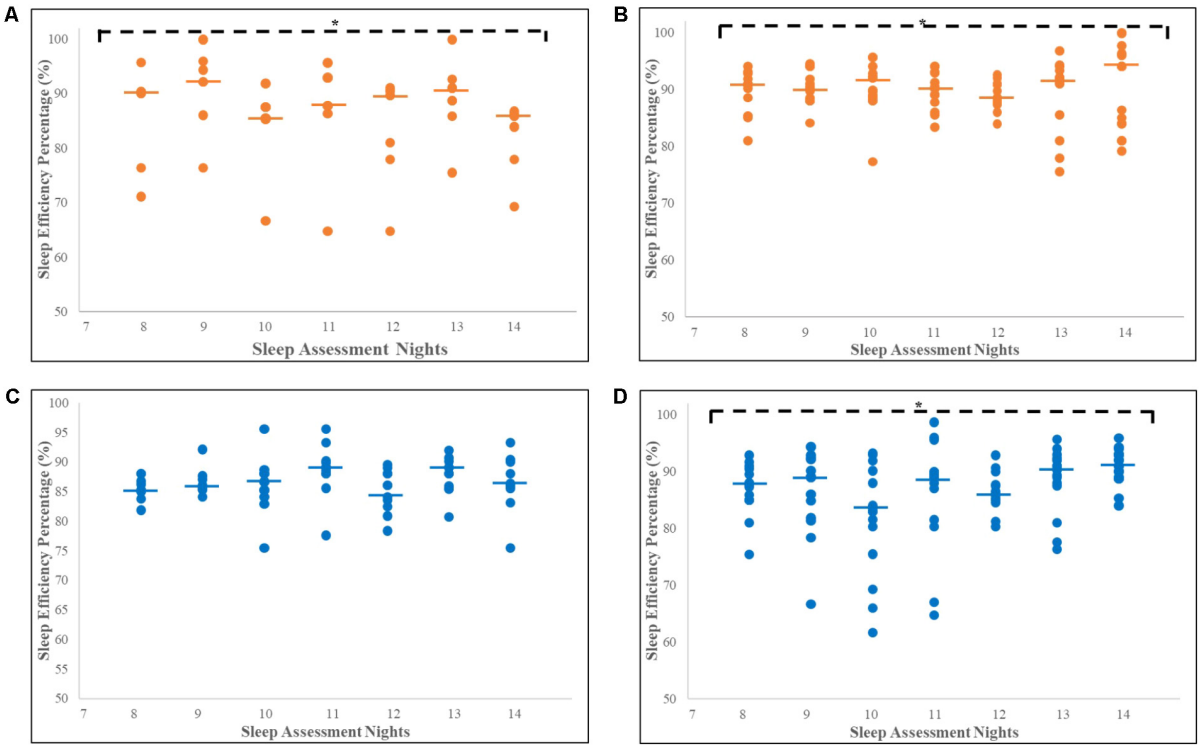

FIGURE 2 | Increase in sleep efficiency percentage for seven consecutive nights for CU and non-CU receiving either haloperidol or risperidone. Each dot represents individual SE% for each subject and the thick horizontal lines indicate the median, on each assessment night. *Significant increase in SE% over 7 days of recording. **(A)** Significant increase in median SE% for non-CU receiving haloperidol ($n = 6$; $\chi^2 = 16.822$, $p = 0.010$, $df = 6$). **(B)** Significant increase in median SE% for non-CU receiving risperidone ($n = 14$; $\chi^2 = 13.84$, $p = 0.032$, $df = 6$). **(C)** No significant increase in median SE% for CU receiving haloperidol ($n = 12$; $\chi^2 = 2.78$, $p = 0.180$, $df = 6$). **(D)** Significant increase in median SE% for CU receiving risperidone ($n = 18$; $\chi^2 = 12.90$, $p = 0.045$, $df = 6$). Trend analyses were conducted using Friedman's tests, with $p < 0.05$ representing significance. CU, *Cannabis* User; non-CU, non-user of *Cannabis*; SE%, Sleep Efficiency Percentage.

Maixner et al., 1998; Dursun et al., 1999; Haffmans et al., 2001, 2004; Yamashita et al., 2002; Miller, 2004; Monti and Monti, 2004). Similarly, in this study, both risperidone and haloperidol showed improvement in sleep outcomes over the 7 days among schizophrenic subjects not exposed to *Cannabis*. Contrastingly, subjects exposed to *Cannabis* use within 1 week of admission, displayed less total sleep time, with more frequent awakening and longer WASO duration, whether being treated with haloperidol or risperidone; thus, suggesting that the presence of *Cannabis* may be impacting the therapeutic benefits of both drugs on sleep. Converting the antipsychotic doses to CPZEs, our study found haloperidol therapy involved larger doses than risperidone therapy. For subjects exposed to *Cannabis*, the haloperidol dose requirements were much larger when compared with the dose given to non-users. This was not so for risperidone, as the dose was similar between non-users and subjects exposed to *Cannabis*.

Since last use of *Cannabis* by subjects in this study was more than 7 days before actigraphy measurements, the findings may be consistent with signs of *Cannabis* withdrawal on sleep (Bolla et al., 2008, 2010; Babson and Bonn-Miller, 2014; Gates et al., 2014, 2016; Babson et al., 2017). Sleep disturbance is a prominent *Cannabis* withdrawal symptom, usually noted after two nights (Bolla et al., 2008), and progresses over the first 2 weeks (Bolla et al., 2010) of abstinence. Budney et al. (2003) examined the impact of *Cannabis* withdrawal in 18 current users with 12 previous users of *Cannabis* as controls. Users abstained from smoking (confirmed through urine assay for *Cannabis* metabolites) and were assessed using Sleep Inventory questionnaires, administered by telephone daily for 50 days. The study reported sleep difficulty with *Cannabis* use, which peaked 2–6 days after abstinence and remained elevated over a course of 45 days.

Our findings are the first to use actigraphy readings to show that the benefits of haloperidol and risperidone can be significantly impacted by the use of *Cannabis*. Interestingly, risperidone therapy showed better sleep outcomes with recent use of *Cannabis*, as SL was equivalent to non-users. Also, CU awoke for longer durations after sleep onset and thus, slept less, with poor sleep quality when treated with haloperidol. Furthermore, only risperidone was successful at increasing the sleep efficiency among these subjects for the 7 days of observation.

Our findings among the subjects involved in this study may have significant implications, as they suggest risperidone may provide better clinical outcomes on sleep in schizophrenic patients who use *Cannabis;* more so in the Jamaican setting where *Cannabis* use among schizophrenic patients has been established to be high (Knight, 1976; Thomas et al., 2015). However, the positive confirmatory test for *Cannabis* is only able to detect

acute *Cannabis* exposure; providing no measure of the history of *Cannabis* use, as documented by self-report of most subjects in this study. Subjects also provided self-report of using alcohol, tobacco and cocaine, which are possible confounders. Despite the presence of these substances and the time of admission not being assessed, the inpatient setting for the study period prevented access. Additionally, some of the subjects in this study previously received therapy with antipsychotics, which could also be a confounder. An antipsychotic-free control group would provide greater comparisons. However, in this setting, this would be unethical since all subjects were experiencing psychosis when admitted to the ward.

CONCLUSION

Cannabis use can attenuate the benefits of haloperidol and risperidone therapy on sleep in patients being treated for schizophrenia. The better sleep outcomes of risperidone support further examination of its possible superiority over haloperidol. However, the sample size was small, and a larger sample is recommended to confirm findings.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the University of the West Indies/University Hospital of the West Indies/Faculty of Medical Sciences Ethics committee guidelines for conducting research. The protocol was approved by the University of the West Indies Ethics Committee. Relatives/Guardians of all subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

The study was designed by MG-W, JM, and WA. P-GT-B reviewed the literature and wrote the protocol for the study. JM, CS, and WA aided in the recruitment of the study participants and performed the psychiatric assessments. Patient recruitment and assessments, sample preparations and assays and the data analyses were conducted by P-GT-B, who also made initial interpretations and wrote the first draft of the manuscript. All authors further interpreted and discussed the findings and contributed to the final version of the manuscript.

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No Effect of Dose Adjustment to the *CYP2D6* Genotype in Patients With Severe Mental Illness

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Background: The CYP2D6 enzyme is involved in the metabolism of numerous psychopharmacological drugs. Guidelines recommend how to adjust the dose of medication based on the *CYP2D6* genotype.

Aims: To evaluate the effect of dose adjustment to the *CYP2D6* genotype and phenotype, in patients with severe mental illness (SMI) already receiving psychopharmacological treatment.

Methods: A total of 269 psychiatric patients (on the island Curaçao) receiving antipsychotic treatment were genotyped for *CYP2D6*. Of these, 45 patients were included for dose adjustment according to the clinical guideline of the Royal Dutch Association for the Advancement of Pharmacy, i.e., 17 CYP2D6 poor metabolizers, 26 intermediate metabolizers, and 2 ultrarapid metabolizers. These 45 patients were matched for age, gender, and type of medication with a control group of 41 patients who were CYP2D6 extensive metabolizers (i.e., with a normal CYP2D6 function). At baseline and at 4 months after dose adjustment, subjective experience, psychopathology, extrapyramidal side-effects, quality of life, and global functioning were assessed in these two groups.

Results: At baseline, there were no differences between the groups regarding the prescribed dosage of antipsychotics, the number of side-effects, psychiatric symptoms, global functioning, or quality of life. After dose adjustment, no significant improvement in these parameters was reported.

Conclusion: In psychiatric patients with SMI already receiving antipsychotic treatment, dose adjustment to the *CYP2D6* genotype or phenotype according to the guidelines showed no beneficial effect. This suggests that dose adjustment guidelines are currently not applicable for patients already using antipsychotics.

ClinicalTrials.gov: Cost-effectiveness of CYP2D6 and CYP2C19 Genotyping in Psychiatric Patients in Curacao; Identifier: NCT02713672; https://clinicaltrials.gov/ct2/show/NCT02713672?term=CYP2D6&rank=5

Keywords: CYP2D6, severe mental illness (SMI), guidelines, dose adjustment, genotyping, psychopharmacology, personalized medicine

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INTRODUCTION

The cytochrome P450 isozymes, in particular CYP2D6, is responsible for the biotransformation of many psychopharmacological drugs (1, 2). Substrates of CYP2D6 include first generation antipsychotics, selective serotonin receptor inhibitors and tricyclic antidepressants[1]. Based on genetic variation, patients can be divided into poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultrarapid metabolizers (UM). The recommended dosages of psychopharmacological medication that are metabolized by this enzyme are based on the metabolism of the most common genotype, i.e., the EM type (i.e., a normal CYP2D6 function). However, because the plasma level of a drug is related to the genotype, the same dosage will probably lead to a higher plasma level in PMs and IMs, as compared to EMs, and to a lower plasma level in UMs as compared to EMs. The plasma level is often related to the effectiveness of the drug and the risk of dose-related side-effects (3–7). Also, when physicians prescribe a drug metabolized by CYP2D6 without taking into account the genotype, the hospital stay is longer (and the costs higher) in patients with a PM and UM profile (8).

Clinical guidelines recommend dose adjustment according to the *CYP2D6* genotype (9–11). However, the current guidelines do not differentiate between patients that start vs. those that are already receiving psychopharmacological treatment. Patients with severe mental illness (SMI) are especially known to suffer from problems with adverse drug reactions, lack of medication effect, and new models of care are warranted (12–16). In a study in patients with SMI, more adverse drug events and higher costs were found in the extreme metabolizer groups (17). In a cost analysis study it was found that genotyping in patients with schizophrenia could lead to lower treatment costs (18).

Genotyping in patients with SMI could potentially individualize treatment, reduce side-effects in slower metabolizers and increase treatment effects in rapid metabolizers. Until now, it remains unclear whether routine *CYP2D6* genotyping is efficacious in patients with SMI already undergoing psychopharmacological treatment and evidence of clinical utility of *CYP2D6* genotyping in patients being prescribed antipsychotics is lacking (19–21). We hypothesized that dose adjustment of antipsychotics to the *CYP2D6* genotype and phenotype would be beneficial regarding side-effects, psychiatric symptoms, quality of life, and/or global functioning. The aim of the present study was to evaluate the effect of dose adjustment to the *CYP2D6* genotype and phenotype, in patients with SMI already receiving psychopharmacological treatment. The dose adjustment group consisted of patients with a PM, IM, or UM profile using antipsychotics metabolized by CYP2D6, whereas the control group consisted of patients with an EM geno-/phenotype. The effect of dose adjustment of the antipsychotics on psychopathological symptoms, side-effects, and well-being was evaluated.

METHODS

Patients

This study was performed on the Caribbean island, Curaçao: this is one of the western Leeward Antilles in the Caribbean with about 160,000 inhabitants[2]. Patients were recruited via the *Klinika Capriles* (the psychiatric hospital on the island), the psychiatric ward of the local prison (FOBA), and the psychiatric outpatient clinic (*Psychiaters Maatschap Antillen*).

After being informed about the study procedures, all patients signed written informed consent. Inclusion criteria were: Antillean ethnicity (defined in line with the concepts used by the Central Office of Statistics in the Netherlands, as birth on the former Netherlands Antilles and birth of at least one parent on the former Netherlands Antilles); age $\geq$18 years; use of an antipsychotic or antidepressant drug; able and written informed consent. All participants in both groups received a token for 25 Netherlands Antillean Guilder (about US $13) if they completed the study.

All DNA samples were genotyped July 2012 for *CYP2D6* *1, *2, *3, *4, *5, *6, *7, *8, *9, *10, *17, *29, *41 and gene duplication in the Erasmus Medical Center (Rotterdam, the Netherlands) and grouped according to the predicted phenotype for *CYP2D6* as described earlier (22).

Diagnoses, demographic information and information on psychiatric and somatic medication was derived from the electronic patient file. The following psychiatric drugs were considered to have a major dependence on the CYP2D6 enzyme for their elimination: amitriptyline, aripiprazole, atomoxetine, clomipramine, imipramine, haloperidol, nortriptyline, paroxetine, pimozide, risperidone, venlafaxine, and zuclopenthixol[1] (9, 10).

Based on the *CYP2D6* genotype or phenotype, patients were selected who were recommended a dose adjustment of their psychopharmacological medication according to the guideline of the Royal Dutch Association for the Advancement of Pharmacy (updated until July 2013). In this guideline, recommendations were developed for 53 drugs based on a systematic review of the literature. In CYP2D6 PM, IM, or UM patients, using medication metabolized by CYP2D6, it is advised to switch to a drug that is not metabolized by CYP2D6. An alternative is to adjust the dosage with dose reductions of respectively 25–50% of the original dose in IMs and PMs (9, 10).

To increase the power of the present study, patients who were PM or IM based on inhibiting medication were also included in the dose adjustment group (23).

Patients using strong CYP2D6 inhibitors (bupropion, cinacalcet, fluoxetine, paroxetine, quinidine) according to Flockhart's interaction table, were classified as being PM[1] (23, 24).

The selected patients were matched for age, gender and type of medication with a control group of patients who were CYP2D6 extensive metabolizers.

[1]Flockhart DA. P450 Drug Interaction Table (Division of Clinical Pharmacology, Indiana University). July 12, 2013. Available at: http://medicine.iupui.edu/clinpharm/ddis/main-table/ (Accessed 2013).

[2]Central Bureau of Statistics Curaçao. Available at: http://www.cbs.cw. Accessed 2017.

All prescribed antipsychotics were calculated to a "defined daily dose" (DDD) as reported by the World Health Organization (WHO) (25). This is a unit of measurement and defined as the assumed average maintenance dose per day for a drug used for its main indication in adults. The total equivalent of the DDD was calculated for every patient. The study protocol was approved by the Institutional Review Board of Maastricht University (the Netherlands) and ethical approval to collect DNA samples was received according to local policies by the Institutional Review Board of the Klinika Capriles (Curaçao).

The study was registered in an international trial registry at http://www.clinicaltrials.gov (NCT02713672). All procedures were in accordance with the ethical standards of the Declaration of Helsinki 1975 (as revised in 1983).

Assessments

Each patient underwent a thorough assessment of psychopathology, subjective experience, extrapyramidal symptoms, quality of life, global functioning, and metabolic parameters at baseline (T0) (November–December 2014) and at 4 months after dose adjustment (T1) (April–June 2015). Information about drug and alcohol use was registered.

The severity of the patient's psychopathology was assessed with the Brief Psychiatric Rating Scale (BPRS) (26). Extrapyramidal symptoms were assessed with the St. Hans Rating Scale (SHRS) (27). Akathisia was measured with the Barnes Rating Scale for drug-induced akathisia (BARS) (28, 29). Quality of life was assessed with the EQol 5-D (EQ 5-D) (30). Global functioning was assessed with the WHO Disability Assessment Schedule 2.0 36-item proxy-administered version (WHODAS 2.0) and was administered with a personal caregiver (31, 32); scores were recalculated to a standardized score. In all above-mentioned scales, higher scores indicate more severe symptoms. Subjective experience was measured with the Subjective Well-Being Under Neuroleptics Scale (SWN-20) (33). Scores were recalculated, with higher scores indicating a higher level of well-being. For patients who were unable to read Dutch, the questions were translated into Papiamento (a local language). The investigator, a resident in psychiatry, was trained by professionals in scoring the SHRS, BARS and the WHODAS 2.0. Patients receiving depot medication were measured the same number of days after administration of the depot at T0 and at T1.

Secondary outcome measurements were metabolic parameters (blood pressure, body mass index (BMI), waist size, cholesterol, HDL, LDL, triglycerides, glucose, HbA1Cl, and prolactin). Also in 31 of the 60 patients receiving antipsychotics metabolized by CYP2D6 (dose adjustment group $n = 22$, control group $n = 9$), the plasma levels of antipsychotics were measured.

Procedures

After baseline measurements, another psychiatrist in training prescribed the dose adjustments. A standard procedure for dose adjustments was followed. Lowering the dose was done in steps according to a local protocol[3] (34). Tranquilizing medication with inhibitory activity was replaced by benzodiazepines. By removing the inhibiting medication, no further dose reduction was necessary in these patients. Complex cases were discussed with the research team during a regular meeting and individual dose adjustment plans were made.

[3]Switching antipsychotics available at: http://wiki.psychiatrienet.nl/index.php/SwitchAntipsychotics. (Accessed 2014).

Statistical Analysis

Analyses were performed with IBM SPSS statistics (version 22). Differences between groups were tested with a Chi-square test for dichotomous variables and an independent *t*-test for continuous variables. ANOVA was used to compare means between the geno-/phenotypes. Non-parametric tests were used for variables not normally distributed (i.e., SHRS, BARS, EQ 5-D). Differences at T1 that were present at T0 were corrected for in an ANCOVA model. The relation between geno-/pheno and DDD was investigated with Kendall's tau. All tests were two-tailed. The significance level was set at $p < 0.05$. After Bonferroni correction, the significance level used was $p < 0.005$ (0.05/11).

To analyse all aspects of deterioration, psychiatric well-being was evaluated on (one of) three scored items: i.e., deterioration was defined as a specific report by caregivers or a physician, or a >5-point increase on the BPRS, or on the WHODAS 2.0.

A *post-hoc* power analysis showed that a 25% reduction of the psychiatric symptoms, or a 30% reduction of the symptoms measured by WHODAS 2.0, or a 75% reduction of the Parkinson symptoms, results in a power of 80% at a significance level of 0.05.

RESULTS

Baseline Characteristics

The study population consisted of 269 long-term psychiatric patients, of which 94% was diagnosed with a psychotic disorder. Other diagnoses were major depressive disorder, bipolar disorder, substance abuse and intellectual disability. No regular drug or alcohol use was reported in any of the questionnaires. A quarter of the patients was outpatient. The majority (92%) was admitted in a long-term treatment facility or institutional housing at the start of genotyping and had a long treatment history. Every patient was genotyped 2.5 years before the start of the intervention, and for at least this period in treatment. Patients were stable on antipsychotic treatment for at least 2.5 years, three patients switched once from antipsychotic during this treatment period.

Genotyping succeeded in 231 participants. Failure of genotyping was due to the low quality DNA obtained from the buccal swabs. Frequencies of PMs, IMs, and UMs were similar to those found in an Antillean population without psychiatric disease (22); Caucasian populations (22); and in patients using antipsychotic medication not metabolized by CYP2D6.

In total 111 out of 269 patients were using medication metabolized by CYP2D6. In total, 153 patients were prescribed two types of antipsychotics and 24 patients used antidepressant medication. Because the admitted patients received their medication from the nurses and two third of the patients were using depot medication we could account for medication adherence in 90% of the included patients. **Figure 1** presents an overview of the patient selection and study procedure.

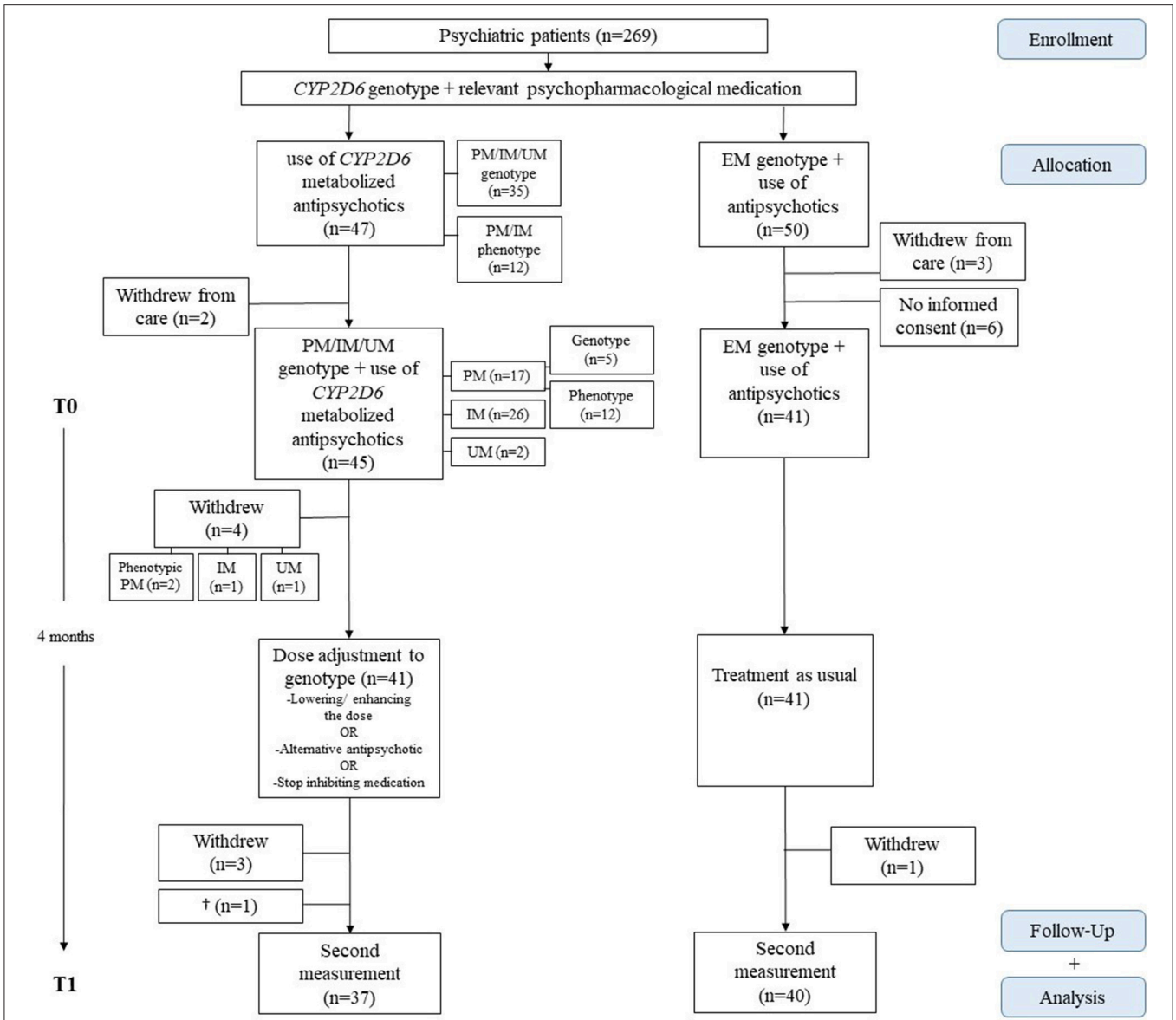

FIGURE 1 | Study procedure and inclusion of participants. PM, poor metabolizer; IM, intermediate metabolizer; UM, ultrarapid metabolizer; EM, extensive metabolizer.

The medication in the dose adjustment group was haloperidol ($n = 15$), risperidone ($n = 21$), and zuclopenthixol ($n = 9$).

Out of 41 patients, 16 patients received a dose reduction. The mean dose reduction was 0.60 DDD, (1.4 DDD in PMs, 0.54 DDD in IMs) which equals a reduction from 5 to 2 mg risperidone. Other patients stopped with their inhibiting medication or received alternative antipsychotic medication. **Table 1** presents the patient characteristics and the outcome of measurements at T0 and T1.

Four months after dose adjustment (T1), 81 (94%) patients were assessed for the follow-up measurements; one patient had died of cancer, two patients had withdrawn from psychiatric care, and two patients did not want to participate a second time.

Baseline (T0)

At baseline, no differences were found between the geno-/phenotype and the mean prescribed dose of antipsychotics as shown in **Figure 2**. In the dose adjustment group the mean DDD was 1.65 (SD 0.83) and in the control group it was 1.92 (SD 0.97) (Ns). PMs, IMs and UMs were prescribed the same amounts of psychopharmacological medication as the EMs.

Second, we found no difference in dose-dependent adverse drug reactions between the normal and extreme metabolizers. Movement disorders were equally distributed in both groups. There were no differences in metabolic parameters.

Third, no differences were found between the normal and extreme metabolizers for psychiatric symptoms, subjective

TABLE 1 | Clinical characteristics, baseline, and delta scores of the dose adjustment and control group.

	T0 dose adjustment group $n = 45$ Mean (*SD*)	T0 control group $n = 41$ Mean (*SD*)	*p*-value*	T1-T0 dose adjustment group $n = 37$ Mean (*SD*)	T1-T0 control group $n = 40$ Mean (*SD*)	*p*-value*
Male (*n*)	30	25	0.58			
Female (*n*)	15	16	0.58			
Age (years)	52.4 (12.0)	50.3 (10.8)	0.15			
Depot medication	30	27	0.59			
Outpatient	14	9	0.34			
Defined daily dose	1.65	1.92	0.17			
BPRS: 24 items (1–7)	1.79 (0.51)	1.66 (0.43)	0.26	−0.26 (0.26)	−0.17 (0.27)	0.15
SWN-20: 20 items (1–6)	4.59 (0.95)	4.45 (1.02)	0.59	0.29 (0.66)	−0.06 (0.57)	0.04
WHODAS 2.0: 32 items (1–5) (standardized total score)	32.06 (16.28)	30.40 (16.89)	0.69	5.47 (17.50)	2.93 (10.63)	0.52
EQ 5-D: 5 items (1–3)	1.30 (0.31)	1.31 (0.38)	0.78	−0.07 (0.37)	−0.05 (0.24)	0.74
Dyskinesia SHRS: 18 items: (0–6)	0.61 (0.70)	0.78 (0.87)	0.58	0.19 (0.55)	0.045 (0.57)	0.33
Parkinsonism SHRS: 10 items (0–6)	0.97 (1.12)	1.00 (1.33)	0.70	0.47 (0.76)	0.04 (0.72)	0.05
Dystonia SHRS: 2 items (0–6)	0.06 (0.34)	0.27 (1.05)	0.36	0 (0.51)	−0.20 (1.00)	0.70
BARS: 3 items (0–3)	0.10 (0.40)	0.37 (0.73)	0.01	0.13 (0.54)	−0.26 (0.70)	0.25**
Blood pressure (mmHg)	124/81 (14/10)	125/79 (17/11)	0.60	1.50 (15.48) /−0.76 (9.72)	−1.89 (15.29) /−0.76 (9.72)	0.37
BMI	26.6 (6.4)	27.3 (6.7)	0.66	−0.05 (1.51)	−0.64 (1.45)	0.12
Cholesterol (mg/dl)	159.8 (37.7)	160.0 (34.4)	0.99	8.8 (22.6)	6.4 (13.6)	0.63
HDL (mg/dl)	45.4 (15.1)	42.4 (10.7)	0.34	3.5 (6.5)	1.2 (6.3)	0.19
LDL (mg/dl)	92.7 (33.8)	92.1 (31.3)	0.94	6.4 (19.8)	8.1 (20.6)	0.76
Triglyceride (mg/dl)	104.5 (41.4)	111.6 (56.7)	0.57	−5.7 (28.4)	−6.6 (38.6)	0.93
Prolactin (ng/ml)	37.3 (62.1)	20.2 (14.4)	0.22	−13.0 (37.5)	2.6 (10.0)	0.11
Fasting glucose (mg/dl)	112.0 (25.1)	103.2 (32.0)	0.24	−4.1 (14.8)	−4.2 (17.4)	0.98
HbA1c %	4.5 (1.5)	5.4 (1.5)	0.33	−0.1 (0.0)	−0.08 (0.43)	0.95

**Significance level after Bonferroni correction $p < 0.005$. **ANCOVA test corrected for differences at T0 that were present at T1; BPRS, Brief Psychiatric Rating Scale; SWN-20, Subjective Well-being Under Neuroleptics; WHODAS, World Health Organization Disability Assessment Schedule; SHRS, St. Hans Rating Scale; BARS, Barnes Rating Scale for drug-induced akathisia.*

well-being and quality of life. There were no differences in psychiatric symptoms as measured by subscales of the BPRS.

Because only 13 of the patients worked, the four items of the WHODAS 2.0 concerning work were omitted. Patients with a PM genotype or phenotype scored higher on the WHODAS 2.0 than the IMs (PM 41.5, IM 26.6, EM 29.1, UM 34.0) ($p = 0.007$); however, this difference was not significant after Bonferroni correction ($p = 0.005$).

Fourth, no significant differences were found between the dose adjustment and the control group in mean therapeutic drug plasma levels of antipsychotics metabolized by CYP2D6 (analyzed with the Mann-Whitney *U*-test). The mean therapeutic plasma levels at T0 of respectively the dose adjustment and the control group were for haloperidol 0.0015 mg/l (*SD* 0.0013) ($n = 4$) and 0.0023 mg/l (*SD* 0.0006) ($n = 3$) ($p = 0.35$); risperidone 0.0173 mg/l (*SD* 0.0164) ($n = 9$) and 0.0073 mg/l (*SD* 0.0081) ($n = 3$) ($p = 0.34$); zuclopenthixol 0.0143 mg/l (SD 0.0167) ($n = 9$) and 0.0160 mg/l (*SD* 0.0227) ($n = 2$) ($p = 0.91$). In the dose adjustment and control group there was a linear incremental relationship between dose and plasma level (data not shown).

Separate analyses were performed with the exclusion of the 12 patients with a PM profile who were selected due to inhibiting medication (data not shown); however, this exclusion had no effect on the results. There were no differences in outcomes between males and females (data not shown).

Effect of Dose Adjustment

No significant effect of dose adjustment was found on psychiatric symptoms, quality of life, or global functioning. Of the 41 patients

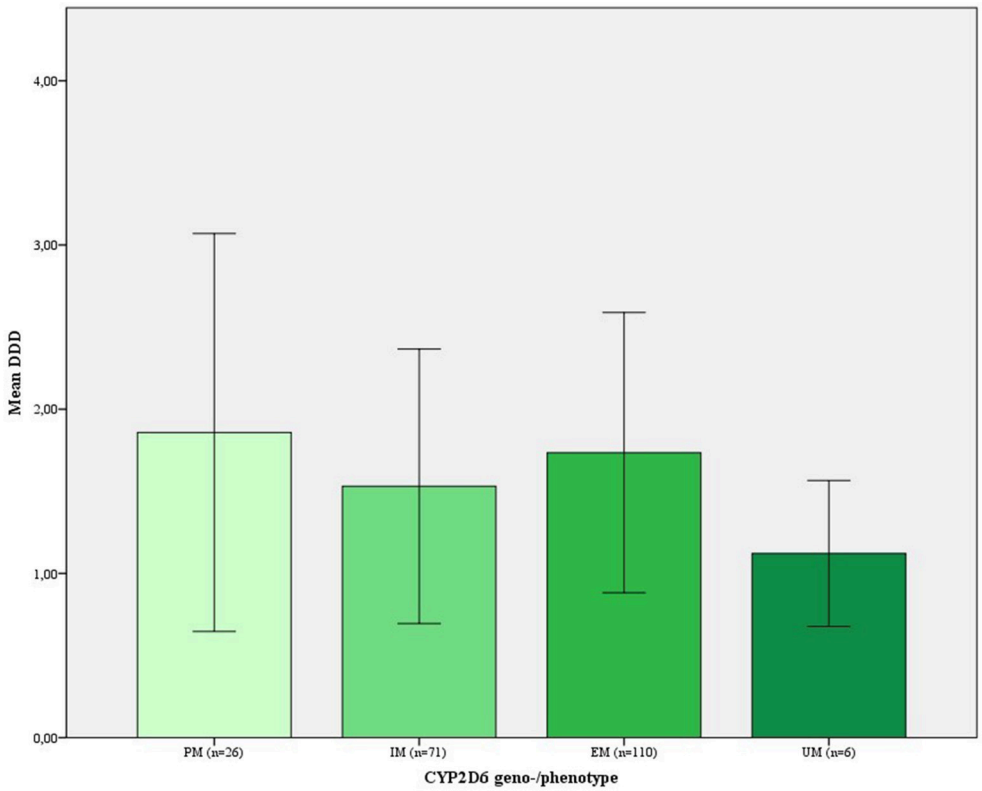

FIGURE 2 | Prescribed dose of antipsychotics in DDD per CYP2D6 geno-/phenotype group at baseline. DDD, defined daily dose; PM, poor metabolizer; IM, intermediate metabolizer; UM, ultrarapid metabolizer; Error Bars, ±1 SD.

receiving dose adjustment, six returned to the original dose of the antipsychotics because of deterioration after dose adjustment. Deterioration of psychiatric symptoms resulted in two clinical admissions of outpatients of the dose adjustment group, whereas no admissions were reported in the control group; this difference was not significant.

There was no significant difference in deterioration in psychiatric symptoms between the two groups. In the dose adjustment group, 16 patients (39%) showed a decline in one of three aspects (defining deterioration) compared with 14 patients (34%) in the control group. In patients who deteriorated, the mean prescribed dose of antipsychotics (in DDD) after dose adjustment was equivalent to the DDD in patients who remained stable (data not shown). **Table 1** shows the mean changes in scores after dose adjustment (T1-T0). There were no differences in outcomes between males and females (data not shown). **Table 2** shows the individual therapeutic plasma levels of antipsychotics (mg/l) metabolized by CYP2D6 of the nine patients in the dose adjustment group who participated in the measurements at T0 and T1.

Effect of Dose Adjustment on Side-Effects and Well-being

Dose adjustment did not result in a significant improvement of parkinsonism, dyskinesia, dystonia, or akathisia. There was a slight improvement (6%) in well-being as measured by the SWN-20. However, this is not considered a clinical relevant finding and is not significant after Bonferroni correction.

Effect of Dose Adjustment on Metabolic Parameters

Table 1 shows changes in metabolic parameters from baseline until after dose adjustment; no significant differences were found between the two groups.

DISCUSSION

This study rejected the hypothesis that patients with SMI on antipsychotic treatment in a clinical setting benefit from dose adjustment based on the *CYP2D6* genotype or phenotype. Importantly, at baseline, no differences were found in the severity of side-effects or global/psychiatric functioning between the dose adjustment group (with PM, IM, and UM) and control group (with EM). There was no effect of dose adjustment on these parameters.

We expected before we started the study that during years of treatment, clinicians would have optimized dosages to the geno-/phenotype based on side-effects or effectiveness of the drugs used. However at baseline, the CYP2D6 PMs, IMs, and UMs used the same amount of antipsychotics as the EMs.

TABLE 2 | Therapeutic plasma levels of antipsychotics (mg/l) metabolized by CYP2D6 of the nine patients in the dose adjustment group measured at T0 and T1.

	Dose adjustment (%)	Plasma level change (%)	T0			T1			T1–T0		
			H	R	Z	H	R	Z	H	R	Z
Participant 1	−25				0.0120			Undetectable*			
Participant 2	−25	−54		0.0130			0.0060			−0.0070	
Participant 3	−25	−44			0.0250			0.0140			−0.0110
Participant 4	−25				Undetectable*			0.0050			
Participant 5	STOP			0.0350			Undetectable*				
Participant 6	STOP	−65		0.0260			0.0090			−0.0170	
Participant 7	STOP	0	0.0010			0.0010			0.0000		
Participant 8	STOP				Undetectable*			Undetectable*			
Participant 9	STOP				Undetectable*			0.0110			

**All patients were using depot medication and medication adherence was guaranteed, therefore a lab result of 0 was interpreted as undetectable. H, Haloperidol; R, Risperidone; Z, Zuclopenthixol; STOP, stopped with inhibiting medication.*

This finding motivated us to find out if dose adjustment could improve the clinical picture. A possible explanation for the absence of an effect, which could also explain the deterioration of some patients, is that long-term use of antipsychotics induces structural brain changes and the brain adapts to the changed dopamine levels (35). It is suggested that antipsychotics play a role in the progressive reduction of brain size and enlargement of ventricular spaces in patients with schizophrenia, which is associated with involuntary movement disorders (36, 37). Studies show that patients with long-term antipsychotic treatment have a threefold increase in loss of dopamine terminals in the substantia nigra (15% per decade vs. 5% in healthy controls) which is suggested to play a role in persistent parkinsonism and tardive dyskinesia (38). Additionally, it is reported that only 3% of patients discontinuing movement disorder-causing agents, resolved spontaneously from tardive syndromes and a reduction of the dosage of antipsychotics did not decrease the severity of parkinsonism (39, 40). It could be that in this clinical population, a dose adjustment to CYP2D6 might have had an effect in an earlier disease stadium but after years of treatment has come too late.

Another possible explanation for these findings is that in both our study groups, the baseline dosage of antipsychotics may have been so high (average DDD 1.65) that D2 receptor occupancy exceeded the optimal window for subjective well-being and to forestall extrapyramidal side-effects (41, 42). This could explain why we found no differences in the prevalence of movement disorders and subjective well-being between the dose adjustment group and control group. However, no improvements in extrapyramidal and psychiatric symptoms were found in our patients using lower dosages of antipsychotics (DDD 1.0, after reduction DDD 0.5).

Lastly, the role of the *CYP2D6* genotype as a major factor in the metabolization of antipsychotics might be overestimated. The present study supports this hypothesis by showing no differences in plasma levels of drugs in the different phenotypes. Another clinical study showed, that a proportion of healthy individuals with a PM genotype are phenotypically EMs as measured by CEIBA metabolization (43).

In clinical practice, in patients with SMI, common factors as co-morbidities, inflammation, age, smoking, and drug/alcohol use, could cause conversion of genotypic PMs into phenotypic EMs and the other way around (44–49). This undetected phenomenon, named phenoconversion, might explain the negative outcome effects in the present study.

Although a small group of patients ($n = 14$) remained stable with lower dosages of antipsychotics, no patients improved in clinical symptoms. This relatively minor saving in direct costs, did not weigh up to the costs of genotyping a large group of patients ($n = 269$).

Strengths and Limitations

This is the first study which prospectively investigated the clinical utility of dose adjustment to the *CYP2D6* genotype or phenotype in patients on antipsychotic medication (20). The representativity was high as we were able to approach all psychiatric patients, with a homogenous African-Caribbean background in a restricted area, i.e., we included all three psychiatric institutes on the island of Curaçao. When including patients, no selection was made regarding the type of psychiatric care, medication, presence of side-effects or treatment response. This has resulted in a heterogeneous group of patients, representative for a general clinical population. It allowed us to analyse the effects of genotyping and dose adjustment in a clinical setting and has led to results with practical clinical value. We have no treatment history of the patients more than 2.5 years before the start of the dose adjustment but we know from clinical practice that patients with SMI make several switches in antipsychotics during the course of their illness and treatment. Earlier studies, in a larger population from this same clinic (40, 50) show this is also true for this population.

Although a large cohort of 269 psychiatric patients was genotyped, only a small number of patients (45) had an extreme geno-/phenotype and used medication metabolized by CYP2D6;

therefore, a randomized controlled trial design was not possible. A control group was used to investigate differences between normal and extreme metabolizers before dose adjustment and to monitor possible effects from time. Furthermore, the design of the study, with a rater who was blinded to whether a patient was in the dose adjustment group or the control group, reduced possible expectation bias.

Ideally we would have analyzed the effect of dose adjustment in patients using only one type of antipsychotic medication, due to the small numbers of extreme metabolizers this was not possible. Because all the investigated antipsychotics are metabolized by CYP2D6 as reported in the Flockhart table, it is very unlikely that this accounted for the absence of an effect.

At last, the Food and Drugs Administration provides a list of strong and weak inhibitors and, by inclusion of patients with a PM/IM phenotype based on interacting medication, greatly improved the prediction of the correct phenotype and has increased the power of the study (23, 24). *Post-hoc* power analysis showed that the number of included patients was high enough to demonstrate clinical relevant results. Moreover, not one out of 45 patients showed an evident improvement in side-effects, psychiatric symptoms or functioning after dose adjustment and six patients returned to their original doses, due to deterioration of psychiatric symptoms. This supports the conclusion that adjustment of the dose based on the *CYP2D6* geno-/phenotype had no effect.

DATA AVAILABILITY STATEMENT

Datasets are available on request: The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

AK, DV, IP, PG, RvS, PvH, and HH study conception and design. AK, DV, and PG acquisition of data. AK and DV analysis and interpretation of data. AK drafting of manuscript. AK, DV, IP, PG, RvS, PvH, and HH critical revision.

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Investigation of the *HSPG2* Gene in Tardive Dyskinesia – New Data and Meta-Analysis

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Tardive dyskinesia (TD) is a movement disorder that may occur after extended use of antipsychotic medications. The etiopathophysiology is unclear; however, genetic factors play an important role. The Perlecan (*HSPG2*) gene was found to be significantly associated with TD in Japanese schizophrenia patients, and this association was subsequently replicated by an independent research group. To add to the evidence for this gene in TD, we conducted a meta-analysis specific to the relationship of *HSPG2* rs2445142 with TD occurrence, while also adding our unpublished genotype data. Overall, we found a significant association of the G allele with TD occurrence (p = 0.0001); however, much of the effect appeared to originate from the discovery dataset. Nonetheless, most study samples exhibit the same trend of association with TD for the G allele. Our findings encourage further genetic and molecular studies of HSPG2 in TD.

Keywords: pharmacogenetics, tardive dyskinesia, schizophrenia, perlecan/heparan sulfate proteoglycan 2 (HSPG2), meta-analysis

INTRODUCTION

Schizophrenia is a serious long-term psychiatric disorder treated primarily with antipsychotic drugs. However, each intervention has side effects and in the case of typical antipsychotics, such as haloperidol and perphenazine, tardive dyskinesia (TD) is one of them. TD is a severe, potentially irreversible movement disorder that is characterized by athetoid movements that affect mainly

the mouth, tongue, jaw, and muscles for facial expressions; it may also affect the upper limbs, lower limbs, neck and trunk. These movements may persist even after medication has been withdrawn and can worsen with age (Solmi et al., 2018). Between 20 and 25% of chronic schizophrenia patients treated with first-generation antipsychotics develop TD (Margolese et al., 2005; Tarsy and Baldessarini, 2006). The risk of TD has been associated with older age, female sex, longer duration of antipsychotic treatment, and use of older, typical, first-generation antipsychotics such as haloperidol, perphenazine, and chlorpromazine (Martino et al., 2018). Atypical antipsychotics, such as clozapine, olanzapine, and quetiapine, are associated with a lower risk of developing TD (Solmi et al., 2018). While TD rates have declined with increased use of atypical antipsychotics, TD risk has not been completely eliminated (Correll and Schenk, 2008).

The cause of TD remains unclear. The mechanism of TD development has been hypothesized to involve supersensitivity of the nigrostriatal dopaminergic pathway, damage to neurons by free radical overproduction, and dysregulation of the GABAergic system (Lee and Kang, 2011). Familial occurrence of TD also supports a genetic component in TD (Weinhold et al., 1981; Yassa and Ananth, 1981; Müller et al., 2001). As such, a number of findings have emerged from candidate gene studies (Zai et al., 2018a). For example, the dopamine D2 receptor (*DRD2)* gene has been a primary candidate (Zai et al., 2007a; Bakker et al., 2008). More recently, there is growing evidence that the vesicular monoamine transporter 2 (VMAT2/SLC18A2) gene may be associated with TD, including association findings from genetic studies (Tsai et al., 2010; Zai et al., 2013) and promising findings from clinical trials on the VMAT2 inhibitors deutetrabenazine and valbenazine as treatment for TD (Anderson et al., 2017; Factor et al., 2017; Fernandez et al., 2017; Hauser et al., 2017). However, the associated genetic markers had only moderate effect sizes, suggesting additional genetic factors likely contribute to TD susceptibility. A number of genome-wide association studies (GWASs) of TD have been conducted (Aberg et al., 2010; Greenbaum et al., 2010; Syu et al., 2010), leading to a number of novel candidate genes, including the Perlecan-coding gene *HSPG2* (also known as Heparan Sulfate Proteoglycan 2; HGNC ID: 5273; at 1p36.12).

The *HSPG2* rs2445142 G allele was found to be the risk allele for TD in a GWAS on 86 Japanese schizophrenia patients with treatment-resistant TD and 186 without. This allele was also associated with an increase in gene expression in human prefrontal cortical tissues (Syu et al., 2010). Administrations of the typical antipsychotic haloperidol in mice increased HSPG2 expression after 4 weeks, but decreased expression after 50 weeks (Syu et al., 2010). More importantly, *Hspg2* deficiency led to fewer vacuous chewing movements in a mouse model of TD (Syu et al., 2010). The *HSPG2* association was replicated in a refined sample from the CATIE trial, consisting of 179 schizophrenia patients of European ethnicity (Greenbaum et al., 2012), as well as a sample of Jewish Israeli schizophrenia patients (Greenbaum et al., 2012). Thus, to further validate these findings, we conducted an association study of *HSPG2* rs2445142 with TD and followed up with a meta-analysis.

MATERIALS AND METHODS

Subjects

For this meta-analysis, we added two datasets to the currently available data from the literature. The first dataset included 217 participants from two samples (Canada, United States) for which the sample characteristics have been described previously (Basile et al., 1999; Zai et al., 2007b, 2017). Briefly, participants were enrolled from one site in Canada and three sites in the United States: Center for Addiction and Mental Health in Toronto, Toronto, ON, Canada (Dr. G. Remington, N = 94); Case Western Reserve University in Cleveland, Cleveland, OH, United States (Dr. H. Y. Meltzer, N = 63); Hillside Hospital in Glen Oaks, Glen Oaks, NY, United States (Dr. J. A. Lieberman, N = 48); and University of California at Irvine, Irvine, CA, United States (Dr. S. G. Potkin, N = 12). Participants had DSM-III-R or DSM-IV diagnoses of schizophrenia or schizoaffective disorder (American Psychiatric Association, 2000); individuals with type II diabetes, head injury with loss of consciousness, or seizure disorder were excluded from the study. Patients recruited in the United States (HYM, JAL, SGP) had no prior exposure to atypical antipsychotics, while the chronic patients from Canada (GR) may have been on either typical or atypical antipsychotics. Overall, all patients had been exposed to typical antipsychotic medication for at least 1 year before TD assessment. The rate of TD was not significantly different between the United States (38%) and Canadian (43%) samples (p = 0.58), and was lower, albeit not significantly, in males (36%) versus females (49%) in the collective sample (p = 0.11).

Our second dataset consisted of schizophrenia or schizoaffective disorder patients from a naturalistic pharmacogenetic study [The Individualized Medicine: Pharmacogenetics Assessment and Clinical Treatment (IMPACT, N = 20 TD cases and 41 TD-negative controls at baseline)] (Herbert et al., 2017).

The classification of TD was based on the Schooler and Kane criteria, using the Abnormal Involuntary Movement Scale (AIMS) or the modified Hillside Simpson Dyskinesia Scale (HSDS) for the 48 patients recruited from the Hillside Hospital (Schooler and Kane, 1982; Basile et al., 1999). Thus, presence of TD included at least one moderate rating or at least two mild ratings on the first seven items of the AIMS (Schooler and Kane, 1982). Because of previous findings of a higher rate of TD in patients of African ancestry compared to those of European ancestry, we analyzed our self-reported African (N = 30, 11 of which were classified as having TD) and European (N = 187, of which 76 were positive for TD) subjects separately (Jeste and Caligiuri, 1993; Solmi et al., 2018). AIMS scores were available for 155 European patients and 26 African patients. Our European sample has over 80% power to detect an odds ratio of 2.07 [α = 0.05, allele frequency = 0.2, additive model; Quanto v1.2.3; (Gauderman and Morrison, 2006)]. This study was carried out in accordance with the recommendations of Tri-Council

Policy Statement 2 and Good Clinical Practice. The protocol was approved by the individual institutional research ethics boards. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Genotyping and Analysis

We genotyped the *HSPG2* rs2445142 single-nucleotide polymorphism by polymerase chain reaction amplification with the TaqMan genotyping assay C__16231131_10 (Thermo Fisher Scientific) following the manufacturer's protocol, followed by genotype determination in the ViiA 7 Real-Time PCR System. Ten percent of the genotypes were repeated for quality assurance, and no mismatches were observed.

Tests for deviation from Hardy–Weinberg Equilibrium were conducted using Haploview (Barrett et al., 2005). We conducted logistic regression analysis of TD occurrence and linear regression analysis of log-transformed AIMS scores using the entire sample, including age and sex as covariates (SPSS).

We carried out the meta-analysis using the R package 'meta' with the Mantel-Haenszel fixed-effect method. Using search terms "tardive" and "HSPG2" in PubMed, Scopus, and Web of Science, we found 11 studies, of which six were excluded because they represented review articles (Lee and Kang, 2011; MacNeil and Muller, 2016; Lanning et al., 2017; Zai et al., 2018a,b), one was excluded because it was not a gene association study (Seeman and Tinazzi, 2013), one was excluded because the article was in Japanese (Arinami and Inada, 2011), and one was excluded because the underlying diagnosis was not specified (Bakker et al., 2012). We were able to obtain allele counts for TD cases and controls for the meta-analysis from two studies (Syu et al., 2010; Greenbaum et al., 2012). For the Greenbaum et al. (2012) study, there was also genotype data on rs878949, which is a proxy marker for rs2445142 in a sub-group of the CATIE sample. We included our TD samples (Canada-European, United States-European, Canada/United States-African American, and PGX-European) in the meta-analysis (**Table 1**).

RESULTS

The genotypes for *HSPG2* rs2445142 did not deviate significantly from Hardy–Weinberg Equilibrium ($p > 0.05$). Our analysis of *HSPG2* rs2445142 in our samples did not yield significant findings with TD occurrence or severity as measured by AIMS ($p > 0.05$). In the CAMH European sample, the marker was not associated with log-transformed total AIMS scores ($t = 0.162$, $p = 0.872$) or TD occurrence [Odds Ratio (adjusted for sex and age) = 1.08, 95% confidence interval: 0.61–1.89; Wald = 0.028; $p = 0.868$]. Similarly, in the CAMH IMPACT sample, the marker was not associated with log-transformed total AIMS scores ($t = 1.122$, $p = 0.267$) or TD occurrence [Odds Ratio (adjusted for sex and age) = 1.22, 95% confidence interval: 0.47–3.17; Wald = 0.168; $p = 0.682$].

We performed a meta-analysis of *HSPG2* rs2445142 in TD occurrence including the Syu et al. (2010) discovery sample, Greenbaum et al. (2012) Israeli and selected CATIE samples, and our Canada (European), United States (European), Canada/United States (African American), and IMPACT (European) samples, totaling 324 TD cases and 515 TD-negative controls. The G-allele was significantly associated with TD [fixed-effects model: OR(G) = 1.51, 95% confidence interval: 1.22–1.86; $p = 0.0001$; Heterogeneity $p = 0.23$], and no significant heterogeneity was observed among the studies included in the meta-analysis (**Table 2**). In addition, the test for Funnel plot asymmetry indicated that the meta-analysis did not suffer from significant publication bias ($p = 0.116$). Meta-regression analyses found age to have a significant effect on our findings [$Q(df = 1) = 7.62$; $p = 0.006$], while sex appeared to have a trend effect [$Q(df = 1) = 3.18$; $p = 0.075$]. Results from the sensitivity analysis showed that the Syu et al. (2010) dataset contributed to most of the association signal as well as heterogeneity (**Table 3**). Nonetheless, the direction of effect remained the same throughout the sensitivity analysis.

DISCUSSION

We have conducted an association study and meta-analysis of the *HSPG2* rs2445142 marker in TD occurrence. The finding of the G allele being associated with risk for TD supports a role of this marker in TD, though similar to the meta-analysis of *DRD2* rs1800497 in TD, the effect size of 1.507 was not substantial, thus supporting the notion that TD risk reflects multiple genetic factors.

TABLE 1 | Demographic information on the samples included in the meta-analysis.

Study/sample	N (TD cases/TD -negative controls)	Ethnicity	Mean age (years)	Males/females
Syu et al. (2010)	86/186	Japanese	56.57	164/110
Greenbaum et al. (2012) Israeli	73/91	Jewish	48.7	89/75
Greenbaum et al. (2012) CATIE*	75/101	European	41.5	145/31
Zai et al. (2013) Canada**	40/52	European	42.48	59/33
Zai et al. (2013) United States**	24/31	European	34.75	39/16
Zai et al. (2013) Canada/United States**	6/13	African American	29.74	15/4
Herbert et al. (2017) IMPACT**	20/41	European	41.2	46/15

**For the CATIE sample, rs878949 genotypes were used as proxies for rs2445142 genotypes.*
***Previously unpublished data on HSPG2 rs2445142.*

TABLE 2 | Results from meta-analysis of HSPG2 rs2445142 with TD occurrence.

Study	Odds ratio	95%-Confidence interval	%Weight (fixed)	%Weight (random)
Syu et al. (2010)	2.249	1.545–3.272	25.5	26.1
Greenbaum et al. (2012) Israeli	1.599	0.981–2.607	18.2	19
Greenbaum et al. (2012) CATIE	1.232	0.791–1.918	25.1	21.5
Zai et al. (2013) Canada*	1.035	0.535–2.003	12.2	12.3
Zai et al. (2013) United States*	1.073	0.471–2.446	7.7	8.5
Zai et al. (2013) Canada/United States*	0.857	0.218–3.371	3.1	3.4
Herbert et al. (2017) IMPACT*	1.189	0.541–2.611	8.1	9.2
Fixed-effects model[a]	1.507	1.220–1.861	100	
Random-effects model[b]	1.442	1.111–1.873		100

[a]*Fixed-effects model p = 0.0001;* [b]*random-effects model p = 0.006.*
**Previously unpublished data on HSPG2 rs2445142.*

TABLE 3 | Results from sensitivity analysis under fixed-effects model.

Sample omitted	Odds ratio	95%-Confidence interval	*p*-Value	Tau^2	I^2 (%)
Syu et al. (2010)	1.2525	0.970–1.618	0.0847	0	0.00
Greenbaum et al. (2012) Israeli	1.4863	1.176–1.878	0.0009	0.0596	37.90
Greenbaum et al. (2012) CATIE	1.5987	1.257–2.033	0.0001	0.0428	29.40
Zai et al. (2013) Canada	1.5726	1.259–1.965	<0.0001	0.0299	25.70
Zai et al. (2013) United States	1.5431	1.241–1.920	<0.0001	0.0396	32.60
Zai et al. (2013) Canada/United States	1.5278	1.234–1.892	0.0001	0.0376	32.90
Herbert et al. (2017) IMPACT	1.5349	1.233–1.911	0.0001	0.0453	35.40

There are a number of points to consider for the present study. First, the persistence of TD case and TD control status was only assessed in two of the seven included samples (Syu et al., 2010; Greenbaum et al., 2012). Longer term longitudinal observations that include examinations of the fluctuation patterns of TD may help strengthen the genetic findings. In addition, results from the sensitivity analysis indicated that most of the signal may be coming from the discovery sample (Syu et al., 2010), and the observed effect may diminish with subsequent studies. Thus, international efforts are need to provide additional independent replications in large samples, especially for genetic associations with small effect sizes. Moreover, the minor allele frequencies differed across ethnicities, and findings may also be more relevant for East Asian samples in which the original findings were found. Replication studies on patients of various ethnicities may provide insight into whether the genetic association is stronger in East Asians than other populations.

Mutations in the *HSPG2* gene have been observed in patients with Schwartz-Jampel syndrome (chondrodystrophic myotonia), which is an autosomal recessive disorder characterized by bone dysplasia and myotonia (Nicole et al., 2000; Arikawa-Hirasawa et al., 2002a; Stum et al., 2006). This association was supported in mice with reduced expression of perlecan (Arikawa-Hirasawa et al., 1999; Rodgers et al., 2007; Stum et al., 2008). In addition, somatic mutations in *HSPG2* have also been associated with aging of skeletal muscles (Franco et al., 2018), which are coated by a perlecan-containing basement membrane. Perlecan has been found at the neuromuscular junction and is required for acetylcholinesterase clustering at the synapse (Arikawa-Hirasawa et al., 2002b; Guerra et al., 2005; Singhal and Martin, 2011). Because acetylcholinesterase terminates synaptic transmission through the breakdown of acetylcholine, mutations in *HSPG2* may prevent the degradation of acetylcholine, leading to muscle over-excitation (Bordia et al., 2016). Perlecan is also a part of the basement membrane extracellular matrix that makes up part of the blood–brain barrier (Roberts et al., 2012; Marcelo and Bix, 2014). Its C-terminal domain V fragment may play a neuroprotective role following ischemic stroke (Lee et al., 2011). Further work investigating the role of perlecan in neuromuscular junctions and neuroprotection as well as exploring the perlecan-related biological pathways through GWAS approaches will improve our understanding of the potential role this protein plays in TD.

AUTHOR CONTRIBUTIONS

CZ, AT, NK, NF, and JK contributed to the design of the project. CZ, FL, JYL, DH, AS, SC, AA, MS, SS, MT, LG, BL, AV, SP, JAL, HM, and GR contributed to data collection. CZ, AT, VdL, GZ, MM, and DM contributed to data analysis. CZ wrote the first draft of the manuscript. All authors reviewed the manuscript.

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REVIEW
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Clozapine Pharmacogenetic Studies in Schizophrenia: Efficacy and Agranulocytosis

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Clozapine is an efficacious atypical antipsychotic for treatment-refractory schizophrenia. Clinical response and appearance of adverse events vary among individual patients receiving clozapine, with genetic and non-genetic factors potentially contributing to individual variabilities. Pharmacogenetic studies investigate associations between genetic variants and drug efficacy and toxicity. To date, most pharmacogenetic studies of clozapine have been conducted through candidate gene approaches. A recent advance in technology made it possible to perform comprehensive genetic mapping underlying clinical phenotypes and outcomes, which allow novel findings beyond biological hypotheses based on current knowledge. In this paper, we will summarize the studies on clozapine pharmacogenetics that have extensively examined clinical response and agranulocytosis. While there is still limited evidence on clozapine efficacy, recent genome-wide studies provide further evidence of the involvement of the human leukocyte antigen (HLA) region in clozapine-induced agranulocytosis.

Keywords: clozapine, schizophrenia, clinical response, agranulocytosis, pharmacogenetics, SNP, GWAS, review

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INTRODUCTION

Approximately 30% of patients with schizophrenia are treatment-resistant (Meltzer, 1997). Clinical practice guidelines recommend clozapine in treatment-refractory schizophrenia (Warnez and Alessi-Severini, 2014), given that it has been shown to be superior for those resistant to treatment (Siskind et al., 2016). Clozapine may cause serious adverse events, such as agranulocytosis, cardiomyopathy, and myocarditis (Alvir et al., 1993; De Berardis et al., 2012; Alawami et al., 2014), so careful monitoring is needed during clozapine treatment. Clinical response and the presence of adverse events vary among individuals taking clozapine. Although the molecular mechanisms of clozapine action are still unclear (Yamamori et al., 2013, 2014; Hung et al., 2017; Kinoshita et al., 2017; Lee et al., 2017; Nakazawa et al., 2017), twin studies suggest that genetic factors may contribute to the variance in therapeutic and adverse effects of clozapine (Vojvoda et al., 1996; Horácek et al., 2001; Theisen et al., 2001; Wehmeier et al., 2005; Anil Yagcioglu et al., 2011).

Pharmacogenetic studies investigate the associations between genetic variants and drug efficacy and toxicity (Jorgensen and Williamson, 2008). To date, a number of pharmacogenetic studies of clozapine have been reported. In this paper, we summarize the studies on clozapine pharmacogenetics that have extensively examined clinical response and agranulocytosis.

Clinical Response to Clozapine

Response rate for clozapine ranges from 32% in the short term to 39% in the long term among those with treatment-resistant schizophrenia (Siskind et al., 2017). Pharmacogenetic studies of clinical response to clozapine have focused on variations in the genes involved in the metabolism of clozapine [pharmacokinetics, e.g., the cytochrome P 450 (CYP) enzyme family] and the affinities of clozapine (pharmacodynamics, e.g., dopaminergic and serotonergic receptors). Positive candidate gene studies of the clinical response to clozapine are shown in **Table 1**.

Candidate Gene Approach

CYP enzyme related genes

Clozapine is metabolized primarily by *CYP1A2*, with additional contributions from *CYP2C19*, *CYP2D6,* and *CYP3A4* (Urichuk et al., 2008). To date, genetic variants in the *CYP1A2*, *CYP2C19*, *CYP2D6*, and *CYP3A4* genes as well as in the *CYP3A5*, *CYP3A7*, *CYP3A43*, and ATP binding cassette subfamily B member 1 (*ABCB1*) genes have been investigated in clozapine response studies (Arranz et al., 1995b; Eap et al., 2004; Balibey et al., 2011; Lee et al., 2012; Rajkumar et al., 2013b; de Brito et al., 2015; Piatkov et al., 2017). Five studies found significant associations between the clozapine treatment response and genetic variants in the *CYP1A2*, *CYP2C19*, and *ABCB1* genes (Eap et al., 2004; Balibey et al., 2011; Lee et al., 2012; de Brito et al., 2015; Piatkov et al., 2017). Eap and colleagues suggested that the *CYP1A2*1F/*1F* genotype may be associated with low plasma levels of clozapine and a lack of response to clozapine ($N = 4$) (Eap et al., 2004). Balibey and colleagues demonstrated that a positive response rate of clozapine was significantly lower in patients carrying the *CYP1A2*1F/*1F* genotype compared to those with at least one wild type allele for *CYP1A2* ($N = 97$) (Balibey et al., 2011). De Brite and colleagues replicated these findings ($N = 54$) (de Brito et al., 2015). Piatkov and colleagues demonstrated that homozygote *CYP2C19*17* carriers were five times more likely to exhibit improvement on clozapine treatment ($N = 45$) (Piatkov et al., 2017). Furthermore, Lee and colleagues demonstrated that rs7787082 G and rs10248420 A alleles in the *ABCB1* gene were more common in non-responders ($N = 96$) (Lee et al., 2012).

Dopamine related genes

Clozapine exhibits a relatively high affinity for the dopamine D4 receptor (*DRD4*) and relatively lower affinities for the dopamine D1 receptor (*DRD1*), the dopamine D2 receptor (*DRD2*), and the dopamine D3 receptor (*DRD3*) (Meltzer, 1994). The combination of relatively high D1, low D2, and very high 5-HT2 receptor occupancy rates is unique to clozapine (Mauri et al., 2014). To date, genetic variants in the *DRD1*, *DRD2*, *DRD3*, *DRD4, and* dopamine D5 receptor (*DRD5)* genes as well as in dopamine - related genes, Catechol-O-methyltransferase (*COMT*) and solute carrier family 6 member 3 (*SLC6A3*) have been investigated in clozapine response studies, with positive associations observed in the *DRD1, DRD2, DRD3, DRD4*, *COMT*, and *SLC6A3* genes.

With respect to the *DRD1* gene (Potkin et al., 2003; Hwang et al., 2007; Lee et al., 2012), two studies found significant associations between genetic variants and the clozapine response (Potkin et al., 2003; Hwang et al., 2007). Potkin and colleagues demonstrated that rs4532 2/2 carriers were more likely to have a positive clozapine response following 5 weeks of treatment, while rs4532 1/2 carriers demonstrated a diminished clozapine response ($N = 15$) (Potkin et al., 2003). Hwang and colleagues demonstrated that rs265976 AC carriers were more likely to be non-responders to clozapine with a minimum of 6 months treatment in an African American sample ($N = 49$). They also investigated an association between three-marker haplotype (rs265981, rs4532, and rs686) and clozapine response, demonstrating that the T-G-A haplotype was associated with poor response in a Caucasian sample ($N = 183$), while the T-G-G haplotype was associated with better response in an African American sample ($N = 49$) (Hwang et al., 2007). With respect to the *DRD2* gene (Arranz et al., 1998a; Malhotra et al., 1999; Hwang et al., 2005, 2006; Lee et al., 2012), three studies found significant associations between genetic variants in this gene and the clozapine response (Malhotra et al., 1999; Hwang et al., 2005, 2006). Malhotra and colleagues investigated an association between 141C Ins/Del (rs1799732) and clozapine response in a 10 week treatment, demonstrating that Del- subjects had a five-fold greater reduction in psychotic symptoms as compared to Del+ subjects ($N = 72$) (Malhotra et al., 1999). Additionally, Hwang and colleagues investigated an association between 12 single nucleotide polymorphisms (SNPs) in the *DRD2* gene and clozapine response for a minimum of 6 months treatment, identifying 3 SNPs (Taq1A, Taq1B, and rs1125394) only in an African-American sample ($N = 49$) and several haplotypes in both Caucasian ($N = 183$) and African-American samples ($N = 49$) as being predictive of a clozapine response (Hwang et al., 2005). This same group later investigated the effect of the same 12 SNPs in the *DRD2* gene on clozapine response evaluated by overall, positive, and negative symptoms in smaller sample set ($N = 35$) and demonstrated that 2 SNPs (Taq1B and rs1125394) were associated with overall and positive symptom response to clozapine in an African-American sample (Hwang et al., 2006). However, a recent meta-analysis (total $N = 596$) suggests no association between 141C Ins/Del and a clozapine treatment response (Gressier et al., 2016). With respect to the *DRD3* gene (Gaitonde et al., 1996; Shaikh et al., 1996; Malhotra et al., 1998; Scharfetter et al., 1999; Arranz et al., 2000b; Barlas et al., 2009; Hwang et al., 2010; Lee et al., 2012), three studies found significant associations between genetic variants and the clozapine response (Shaikh et al., 1996; Scharfetter et al., 1999; Hwang et al., 2010). Shaikh and colleagues investigated an association between Ser-9-Gly polymorphism (rs6280) in the *DRD3* gene and clozapine response for at least 3 months of treatment ($N = 79$), demonstrating that the genotype Ser-9/Ser-9 was more frequent in the non-responders than in responders (Shaikh et al., 1996). Scharfetter and colleagues replicated this finding in patients treated with clozapine for 6 months ($N = 32$) (Scharfetter et al., 1999). However, a recent meta-analysis (total $N = 852$) suggests no association between Ser-9-Gly and a clozapine treatment response (Gressier et al., 2016). Hwang and colleagues demonstrated an association of better clozapine response for a minimum of 6 months with the A allele of

TABLE 1 | Positive findings of pharmacogenetic studies of clinical response to clozapine.

Authors	Gene	Positive candidate genetic variants	Sample size	Ethnicity	CLZ dose	Treatment Duration	Clinical outcome
CYTOCHROME P450 (CYP) ENZYME RELATED GENES							
Eap et al., 2004	*CYP1A2*	*1F	33 (4/29)	Four patients who were non-responder to clozapine in Lausanne, Konigsfelden, and Essen	Four non-responder patients: dose range 450–800 mg/day before augmentation treatment		CGI
Balibey et al., 2011	*CYP1A2*	*1F	97	Turkish	Mean dose: 308 ± 92 mg/day	18 weeks	BPRS
de Brito et al., 2015	*CYP1A2*	*1F	54	Brazilian	Mean dose of the non-responders: 593 ± 114 mg/day, mean dose of the responders: 535 ± 116 mg/da	About 2 years	BPRS
Piatkov et al., 2017	*CYP2C19*	*17	45	Australian (Caucasian, Asian, Pacific Islander, others)	No information	3 months and 12 months	No information
Lee et al., 2012	*ABCB1*	rs7787082, rs10248420	96	Korean	Mean dose: 319.0 ± 133.1 mg/day	More than 6 months	CGI
DOPAMINE RELATED GENES							
Potkin et al., 2003	*DRD1*	A-48G	15	Caucasian and African American	Mean dose: 460 ± 11 mg/day	5 weeks	BPRS
Hwang et al., 2007	*DRD1*	rs265976 in African-American samples	232	Caucasian and African American	No information	A minimum of 6 months	BPOS
Malhotra et al., 1999	*DRD2*	−141C Ins/Del	72	No information	No information	10 weeks	BPRS
Hwang et al., 2005	*DRD2*	Taq1A, Taq1B, rs1125394 in African-American samples	232	Caucasian and African American	No information	A minimum of 6 months	BPRS
Hwang et al., 2006	*DRD2*	Taq1B, rs1125394 in African-American samples	132	Caucasian and African American	No information	A minimum of 6 months	BPRS, BPOS
Huang et al., 2016	*DRD2*	rs2514218 in Caucasian subsamples	208	Caucasian and African American	No information	6 months	BPRS
Shaikh et al., 1996	*DRD3*	Ser9Gly (rs6280)	133	Caucasian	Dose range: 150–900 mg/day	At least 3 months on a stable regime of clozapine	GAS
Scharfetter et al., 1999	*DRD3*	Ser9Gly (rs6280)	32	Pakistani	Maximum dose: 600 mg/day	6 months	BPRS
Hwang et al., 2010	*DRD3*	rs2134655 in Caucasian samples, rs1394016 in African-American samples	232	Caucasian and African American	No information	A minimum of 6 months	BPOS, BNEG
Zhao et al., 2005	*DRD4*	48 bp variant number tandem repeat	81	Chinese	Dose range: 200–450 mg/day	At least 2 months treatment after clinical stabilization	PANSS
Hwang et al., 2012	*DRD4*	120-bp tandem repeat and 142bp/140bp in African American samples, 48 bp repeat in Caucasian samples	232	Caucasian and African American	No information	6 months	BPRS, BPOS

(Continued)

TABLE 1 | Continued

Authors	Gene	Positive candidate genetic variants	Sample size	Ethnicity	CLZ dose	Treatment Duration	Clinical outcome
Woodward et al., 2007	*COMT*	Val108/158Met	86	Caucasian and African American	No information	6 weeks and 6 months	CIGT, COWAT, DSST
Xu et al., 2010	*SLC6A3*	rs2975226	160	Chinese	Mean dose: 415 ± 97 mg/day	At least 8 weeks	BPRS
SEROTONIN RELATED GENES							
Arranz et al., 1995a	*HTR2A*	T102C (rs6313)	149	Caucasian	Dose range: 125–600 mg/day	Stable for at least 3 months after clinical stabilization	GAS
Arranz et al., 1996	*HTR2A*	His452Tyr (rs6314)	153	Caucasian	Dose range: 125–600 mg/day	No information	GAS
Masellis et al., 1998	*HTR2A*	His452Tyr (rs6314)	185	Caucasian, African American, and Asian	No information	A minimum of 6 months	BPRS, CGI
Arranz et al., 1998b	*HTR2A*	G-1438A (rs6311)	274	Caucasian	Dose range: 125–600 mg/day	At least 3 months	GAS
Yu et al., 2001	*HTR2A*	T102C (rs6313)	99	Chinese	No information	No information	ERPs to auditory stimuli
Sodhi et al., 1995	*HTR2C*	Cys23Ser (rs6318)	162	Caucasian	Dose range: 125–600 mg/day	Stable for at least 3 months following clinical optimization	GAS
Yu et al., 1999	*HTR6*	C267T	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
Souza et al., 2010	*HTR3A*	rs1062613	140	Caucasian and African American	No information	A minimum of 6 months	BPRS
Rajkumar et al., 2012	*HTR3A*	rs1062613, rs2276302	101	Indian	Mean dose: 304.84 ± 119.04 mg/day	Stable dose regiments of clozapine for at least 12 weeks	BPRS
Kohlrausch et al., 2010	*SLC6A4*	HTTLPR/rs25531	116	Brazilian (European ancestry)	Mean dose: 540.91 mg/day	The same dose of clozapine at least 3 months	BPRS
GENE-GENE INTERACTIONS							
Arranz et al., 2000b	*HTR2A, HTR2C, SLC6A4*	His452Tyr (HTR2A), G-1438A (HTR2A), T102C (HTR2A),−330-GT/-244-CT (HTR2C), 5-HTTLPR (SLC6A4)	200	Caucasian	No information	No information	GAS
Hwang et al., 2011	*DRD1, DRD3*	rs686 (DRD1)-Ser9Gly (DRD3) in a Caucasian sample	232	Caucasian and African American	No information	A minimum of 6 months	BPRS
Bosia et al., 2015	*COMT, HTR1A*	Val158Met (rs4680) (COMT),−1019C/G (rs6295) (HTR1A)	107	Italian	The dosage was titrated up to 250mg/day and further augmentations or reductions were made on the basis of clinical response and plasma levels.	8 weeks and 16 weeks	PANSS
Rajagopal et al., 2018	*DRD4, COMT*	120-bp deplication (DRD4)-Val158Met (COMT)	93	Indian	No information	At least 12 weeks on a stable doses	BPRS

CGI, Clinical Global Impression; BPRS, Brief Psychiatric Rating Scale; BPOS, positive symptom subscale (4 items of BPRS); BNEG, negative symptom subscale (3 items of BPRS); GAS, Global Assessment Scale; PANSS, Positive and Negative Symptom Scale; CIGT, Category Instance Generation Test; COWAT, Controlled Oral Word Association Test; DSST, Digit Symbol Subtest; ERP, event-related potential.

rs2134655 in a Caucasian sample ($N = 183$) and the T allele of rs1394016 in an African-American sample ($N = 49$) (Hwang et al., 2010). With respect to the *DRD4* gene (Shaikh et al., 1993, 1995; Kerwin et al., 1994; Rao et al., 1994; Rietschel et al., 1996; Kohn et al., 1997; Kaiser et al., 2000; Zhao et al., 2005; Hwang et al., 2012; Lee et al., 2012; Rajagopal et al., 2018), two studies found significant associations between genetic variants and the clozapine response (Zhao et al., 2005; Hwang et al., 2012). Zhao and colleagues investigated an association between the 48 bp variant number tandem repeat (VNTR) polymorphism in the *DRD4* gene and clozapine response for at least 2 months of treatment after clinical stabilization ($N = 81$), demonstrating that the frequencies of 5 allele and 5/5 genotype were higher among the non-responders than in responders ($N = 81$) (Zhao et al., 2005). Hwang and colleagues demonstrated an association between 120-bp 1-copy allele and intron I (G)n 142/140 bp genotype and poor clozapine responders for 6 months of treatment in an African American sample ($N = 49$), and an association between 48 bp repeat polymorphism and better clozapine response in a Caucasian sample ($N = 183$) (Hwang et al., 2012). With respect to the *DRD5* gene, there were no significant associations between genetic variants and the clozapine response (Hwang et al., 2012). With respect to the *COMT* gene (Woodward et al., 2007; Bosia et al., 2015; Rajagopal et al., 2018), two studies found a significant association between the *COMT* Val108/158Met polymorphism (rs4680) and the clozapine response (Woodward et al., 2007; Bosia et al., 2015). Woodward and colleagues investigated an association between this polymorphism and clozapine response for 6 weeks and 6 months of treatment ($N = 86$), demonstrating that both the Met/Met and Val/Met groups showed greater improvement in attention and verbal fluency domains compared to the Val/Val group (Woodward et al., 2007). Bosia and colleagues demonstrated a greater improvement in the Val/Val group compared to both the Val/Met and Met/Met groups in the negative symptom response for 8 and 16 weeks of treatment with clozapine ($N = 107$) (Bosia et al., 2015). With respect to the *SLC6A3* gene, Xu and colleagues demonstrated that the 71T allele of rs2975226 (T-71A) in the *SLC6A3* gene occurred more frequently in the responders than in non-responders following at least 8 weeks of treatment with clozapine (Xu et al., 2010).

Serotonin related genes

Clozapine has a high affinity for the 5-hydroxytryptamine receptor 2A (*HTR2A*), the 5-hydroxytryptamine receptor 2C (*HTR2C*), the 5-hydroxytryptamine receptor 6 (*HTR6*), and the 5-hydroxytryptamine receptor 7 (*HTR7*) (Meltzer, 1994). To date, genetic variants in the *HTR2A, HTR2C, HTR6,* and *HTR7* genes as well as in 5-hydroxytryptamine receptor 1A (*HTR1A*), 5-hydroxytryptamine receptor 3A (*HTR3A*), 5-hydroxytryptamine receptor 3B (*HTR3B*), 5-hydroxytryptamine receptor 5A (*HTR5A*), and solute carrier family 6 member 4 (*SLC6A4*) genes have been investigated in clozapine response studies, with positive associations observed in the *HTR2A, HTR2C, HTR6, HTR1A, HTR3A*, and *SLC6A4* genes.

With respect to the *HTR2A* gene (Arranz et al., 1995a, 1996, 1998b, 2000b; Masellis et al., 1995, 1998; Nöthen et al., 1995; Malhotra et al., 1996a; Lin et al., 1999; Schumacher et al., 2000; Yu et al., 2001; Lee et al., 2012), six studies found significant associations between genetic variants and the clozapine response (Arranz et al., 1995a, 1996, 1998b, 2000b; Masellis et al., 1998; Yu et al., 2001). Arransz and colleagues investigated an association between the T102C polymorphism (rs6313) in the *HTR2A* gene and clozapine response for at least 3 months of treatment following clinical stabilization ($N = 149$), demonstrating that homozygosity for the C102 allele was more frequent in the non-responders than in the responders (Arranz et al., 1995a). Yu and colleagues demonstrated an association between the 102C/C genotypes and higher N100 amplitude following clozapine treatment using event-related potentials to auditory stimuli ($N = 98$) (Yu et al., 2001). Consistent with these findings, a recent meta-analysis (total $N = 868$) suggests a significant association between the CC genotype and poor clozapine treatment response (Gressier et al., 2016). Arransz and colleagues also investigated an association between the his452tyr polymorphism (rs6314) in the *HTR2A* gene and clozapine response ($N = 153$), demonstrating that the Tyr452 allele occurred more frequently in the non-responders than in the responders (Arranz et al., 1996). Masellis and colleagues replicated this finding in subjects who received clozapine for a minimum of 6 month ($N = 185$) (Masellis et al., 1998). Consistent with these findings, a recent meta-analysis (total $N = 671$) suggests a significant association between C allele or C carriers or CC genotype and better clozapine treatment response compared to T allele or T carriers or TT genotype (Gressier et al., 2016). Arranz and colleagues also examined an association between the G-1438A polymorphism (rs6311) in the *HTR2A* gene and clozapine response ($N = 274$), demonstrating that homozygosity for the G-1438 allele was more frequent in the non-responders than the responders (Arranz et al., 1998b). However, a recent meta-analysis (total $N = 547$) suggests no association between the G-1438A polymorphism and clozapine treatment response (Gressier et al., 2016). With respect to the *HTR2C* gene (Sodhi et al., 1995; Malhotra et al., 1996b; Rietschel et al., 1997; Masellis et al., 1998; Arranz et al., 2000b; Schumacher et al., 2000), two studies found a significant association between genetic variants and the clozapine response (Sodhi et al., 1995; Arranz et al., 2000b). Sodhi and colleagues investigated an association between the Cys23Ser polymorphism (rs6318) in the *HTR2C* gene and clozapine response for at least 3 months treatment after clinical stabilization ($N = 162$), demonstrating that Ser allele carriers were more likely to show a response to clozapine (Sodhi et al., 1995). However, a recent meta-analysis suggests no association between the Cys23Ser polymorphism and clozapine treatment response in a Caucasian sample (Gressier et al., 2016). With respect to the *HTR6* gene (Yu et al., 1999; Masellis et al., 2001; Lee et al., 2012), one study found a significant association between a genetic variant and the clozapine response (Yu et al., 1999). Yu and colleagues examined the association between the C267T polymorphism in the *HTR6* gene and clozapine response for at least 8 weeks of treatment ($N = 99$), demonstrating that the 267T/T genotype was more frequent in responders than in non-responders (Yu et al., 1999). With respect to the *HTR7* gene, there was not a

significant association between the pro279leu polymorphism and the clozapine response (Masellis et al., 2001). With respect to the *HTR1A* gene (Masellis et al., 2001; Bosia et al., 2015), one study found a significant association between a genetic variant and the clozapine response (Bosia et al., 2015). Bosia and colleagues examined the association between the −1019C/G (rs6295) polymorphism in the *HTR1A* gene and clozapine response for 8 and 16 weeks of treatment ($N = 107$), demonstrating a greater improvement in the G/G group compared to the C/C group (Bosia et al., 2015). With respect to the *HTR3A* gene (Arranz et al., 2000b; Gutiérrez et al., 2002; Souza et al., 2010; Lee et al., 2012; Rajkumar et al., 2012), *two* studies found significant associations between genetic variants and the clozapine response (Souza et al., 2010; Rajkumar et al., 2012). Souza and colleagues demonstrated the T allele of rs1062613 in the *HTR3A* gene was associated with a good clozapine treatment response when used for at least 6 months ($N = 140$) (Souza et al., 2010). Similarly, Rajkumar and colleagues investigated an association of rs1062613 and rs2276302 in the *HTR3A* gene with the clozapine response, demonstrating that the T allele of rs106263 and the G allele of rs2276302 were associated with a good clozapine treatment response when presented with a stable dose for at least 12 weeks ($N = 101$) (Rajkumar et al., 2012). Consistent with these findings, a recent meta-analysis suggested that there may be an association between the T allele of rs1062613 and an improved response to clozapine (Gressier et al., 2016). With respect to the *HTR3B and HTR5A* genes (Arranz et al., 2000b; Birkett et al., 2000; Gutiérrez et al., 2002; Souza et al., 2010), there were no significant associations between genetic variants and the clozapine response. With respect to the *SLC6A4* gene (Arranz et al., 2000a,b; Schumacher et al., 2000; Tsai et al., 2000; Kohlrausch et al., 2010), two studies found significant associations between genetic variants and the clozapine treatment response (Arranz et al., 2000b; Kohlrausch et al., 2010). Arranz and colleagues demonstrated a significant association between the biallelic polymorphism in the promoter region of the *SLC6A4* gene, 5-HTTLPR, and the clozapine response in a Caucasian sample ($N = 200$) (Arranz et al., 2000b), and they found a similar trend in a larger sample ($N = 268$) ($p = 0.08$) (Arranz et al., 2000a). Kohlrausch and colleagues demonstrated that the short allele of HTTLPR/rs25531 occurred more frequently in the non-responders than in responders (Kohlrausch et al., 2010) ($N = 116$).

Glutamatergic receptors

Accumulating evidence suggests the potential for clozapine to act on glutamatergic neurotransmission (Heresco-Levy, 2003). To date, genetic variants in the solute carrier family 1 member 2 (*SLC1A2*), solute carrier family 6 member 9 (*SLC6A9*), glutamate ionotropic receptor AMPA type subunit 1 (*GRIA1*), glutamate metabotropic receptor 2 (*GRM2*), and glutamate decarboxylase 1 (*GAD1*) genes, glutamate ionotropic receptor NMDA type subunit 1 (*GRIN1*), glutamate ionotropic receptor NMDA type subunit 2A (*GRIN2A*), glutamate ionotropic receptor NMDA type subunit 2B (*GRIN2B*), have all been examined in clozapine treatment response studies, with no significant associations observed following correction for multiple testing (Hong et al., 2001; Hwang et al., 2011; Taylor et al., 2016, 2017).

Gene-Gene Interactions

Several trials have been performed to investigate a gene-gene interaction between multiple candidate genes and the clinical response to clozapine. Arranz and colleagues examined an association between 19 genetic polymorphisms in nine clozapine-targeted receptor subtypes and a neurotransmitter transporter and the response to clozapine, demonstrating that the strongest association was with a combination of six polymorphisms (*HTR2A* T102C, *HTR2A* his452tyr, *HTR2C*−330-GT/-244-CT, *HTR2C* Cys23Ser, *SLC6A4* 5-HTTLPR, and *H2*−1018-G/A) ($N = 200$) (Arranz et al., 2000b). However, Schumacher et al. could not replicate this finding (Schumacher et al., 2000). Hwang and colleagues demonstrated the most straightforward gene-gene- interaction effect of *DRD1* rs686 and *DRD3* Ser9Gly on the clozapine treatment response when taken for a minimum of 6 months in a Caucasian sample ($N = 183$) (Hwang et al., 2011). Bosia and colleagues demonstrated an additive effect of *COMT* Val158Met and *HTR1A*−1019 C/G on the clozapine treatment response when taken for 8 and 16 weeks ($N = 107$) (Bosia et al., 2015). Similarly, Rajagopal and colleagues demonstrated a significant gene-gene- interaction effect of *DRD4* 120-bp duplication and *COMT* Val158Met on the clozapine response when taken for at least 12 weeks ($N = 93$) (Rajagopal et al., 2018). Furthermore, Xu and colleagues conducted multivariate interaction analysis using 77 SNPs of 25 genes and demonstrated that the combination of rs6269 in the *COMT* gene and rs3813929 in the *HTR2C* gene may work as predictor to improve the clinical antipsychotic response (risperidone, clozapine, quetiapine, and chlorpromazine) in a large sample set ($N = 995$) (Xu et al., 2016).

Non-candidate Gene Approach

To date, the authors are unaware of any genome-wide association studies (GWAS) of clozapine efficacy that have been published. Recent pharmacogenetics GWAS suggest genetic overlap between antipsychotic response and susceptibility to schizophrenia (Ikeda et al., 2015; Ruderfer et al., 2016), and two genetic variants, identified by GWAS as schizophrenia risk loci (Cross-Disorder Group of the Psychiatric Genomics Consortium., 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium., 2014), have shown to be associated with a significant clinical response to clozapine (Brandl et al., 2016; Huang et al., 2016). Randl and colleagues demonstrated a significant association between rs2535629 in the inter-alpha-trypsin inhibitor heavy chain H3 (*ITIH3*) gene and an improvement of negative symptoms after 6 months of clozapine treatment ($N = 105$) (Brandl et al., 2016). Additionally, Huang and colleagues demonstrated a significant association between rs2514218, located upstream of the *DRD2* gene, and clozapine response for 6 months of treatment ($N = 208$) (Huang et al., 2016).

Clozapine-Induced Agranulocytosis

The occurrence of clozapine-induced agranulocytosis (CIA) is 0.8% at 1 year of administration of clozapine with reduced

incidence after the first 6 months of clozapine treatment (Alvir et al., 1993). A recent meta-analysis suggests that 3.8% of patients exposed to clozapine will develop mild neutropenia (with an absolute neutrophil count of <1,500/μl; Myles et al., 2018). A number of genetic studies of CIA and clozapine-induced agranulocytosis/granulocytopenia (CIAG) using samples from patients with schizophrenia have been conducted (Lieberman et al., 1990; Claas et al., 1992; Corzo et al., 1995; Yunis et al., 1995; Theodoropoulou et al., 1997; Turbay et al., 1997; Amar et al., 1998; Valevski et al., 1998; Meged et al., 1999; Dettling et al., 2000, 2001a,b, 2007; Lahdelma et al., 2001; Ostrousky et al., 2003; Mosyagin et al., 2004, 2005; Athanasiou et al., 2011; Anil Yagcioglu et al., 2016; van der Weide et al., 2017), many focusing on genetic variants in the genes within the major histocompatibility complex (MHC) region. Positive candidate gene studies of CIA and CIAG are shown in **Table 2**.

Candidate Gene Approach

Human leukocyte antigen (HLA) genes

In patients developing CIA ($N = 6$) compared with controls ($N = 25$), Lieberman and colleagues demonstrated that the occurrence of *HLA-B38* as well as the haplotype of *HLA-B38*, *DR4*, and *DQw3*, was more frequent (Lieberman et al., 1990). The same laboratory subsequently analyzed them separately by Ashkenazi Jewish patients and non-Jewish patients, conforming their previous finding in the Ashkenazi Jewish samples and demonstrating a significant association of *HLA-B7* and *DR2* with CIA in non-Jewish patients (Yunis et al., 1995). They also demonstrated that the variants in the heat-shock protein 70 (*HSP-70*) and tumor necrosis factor (*TNF*) genes were associated with CIA (Corzo et al., 1995; Turbay et al., 1997). Additionally, Amar and colleagues demonstrated that *HLA-DQB1*020* significantly occurred more frequently in subjects who developed CIAG compared to those who did not develop those complications (5 CIAG cases vs. 13 controls) (Amar et al., 1998). Valevski and colleagues replicated the involvement of *HLA-B38* in CIA in Jewish Israeli samples (11 CIA cases vs. 50 controls) (Valevski et al., 1998). Dettling and colleagues demonstrated a significant association of *HLA-Cw*7*, *DQB*0502*, *DRB1*0101*, and *DRB3*0202* with CIA in non-Jewish Caucasian samples (31 CIA cases vs. 77 controls) (Dettling et al., 2001b). The same laboratory also demonstrated a significantly higher frequency of *HLA-DRB5*02* and *HLA-DQB*0502* in patients who developed CIA (Dettling et al., 2001a). Later, the same laboratory performed an association study of CIA by combining the HLA class I and class II genetic variants, demonstrating that *HLA-DRDB5*0201* and several haplotypes of the HLA genetic variants were associated with CIA (Dettling et al., 2007). Furthermore, Lahdelma and colleagues demonstrated that the *HLA-B16* allele occurred more frequently in patients who developed CIAG (22 CIAG cases vs. 120 healthy controls) (Lahdelma et al., 2001), while Athanasiou and colleagues demonstrated that the *HLA-DQB1* "REC 21G" occurred more frequently in patients who developed CIA compared with controls in two independent cohorts (Athanasiou et al., 2011).

Non-human leukocyte antigen (HLA) genes

Ostrousky and colleagues demonstrated that four polymorphisms in the dihydronicotinamide riboside quinone oxidoreductase 2 (*NQO2*) gene were associated with CIA (18 CIA cases vs. 80 controls) (Ostrousky et al., 2003). Additionally, Mosyagin and colleagues demonstrated that myeloperoxidase (*MPO*)−463AA carriers occurred more frequently in patients who developed CIA compared with AG and GG-carriers combined (49 CIA cases vs. 78 controls) (Mosyagin et al., 2004). Furthermore, Athanasiou and colleagues showed that *DRD1*, neurotensin receptor 1 (*NTSR1*), and β chain of colony-stimulating factor 2 receptor (*CSF2RB*) as well as *HLA-DQB1* and *HLA-C* were associated with CIA (33 CIA cases vs. 54 controls) (Athanasiou et al., 2011). Van der Weide and colleagues demonstrated that *NQO2* 154AA and *ABC1* 3435TT occurred more frequently in patients who developed CIA compared with controls (31 CIA cases vs. 241 controls) (van der Weide et al., 2017). They also showed that for patients who developed neutropenia ($N = 38$), compared to controls ($N = 241$), *ABCB1* 3435TT and homozygosity for glutathione S-transferase theta 1 (*GSTT1*)null occurred more frequently, but glutathione S-transferase mu 1 (*GSTM1*)null occurred less frequently (van der Weide et al., 2017).

Non-candidate Gene Approach

Tiwari and colleagues conducted exome sequence analysis and did not find any genetic variants associated with CIA in Finnish patients after Bonferroni correction (13 CIA cases and 11 cases with severe neutropenia vs. 26 controls) (Tiwari et al., 2014). Meanwhile, Goldstein and colleagues conducted GWAS, whole-exome sequencing, and HLA allele imputation and were able to show that *HLA-B* 158T and *HLA-DQB1* 126Q were associated with CIAG (Goldstein et al., 2014). Legge and colleagues utilized GWAS, imputed HLA alleles, exome array, and copy-number variation analyses in a European population and subsequently combined the data of Goldstein et al. (2014) and Legge et al. (2017). In their meta-analysis of GWAS, they demonstrated that rs149104283, an intronic transcript of *SLCO1B3* and *SLCO1B7*, was associated with clozapine-induced neutropenia. The authors of this paper conducted GWAS of CIAG in Japanese samples (50 CIAG cases vs. 2,905 controls) and identified rs1800625 in the HLA region as the CIAG candidate locus (Saito et al., 2016). A classical HLA analysis was subsequently conducted, demonstrating a significant association of *HLA-B*59:01* with CIAG. However, we failed to replicate the risk SNPs on clozapine-associated neutropenia previously identified by Legge et al. (2017) in a Japanese population (Saito et al., 2017).

DISCUSSION

In the present article, we summarize clozapine pharmacogenetic studies, separated by clinical response and CIA. To date, pharmacogenetic studies of clozapine in schizophrenia have been mostly conducted through candidate gene approaches. Genetic variants in the CYP enzyme family, dopamine, and serotonin receptor genes have been extensively examined as pharmacokinetic and pharmacodynamics candidates related to

TABLE 2 | Positive findings of pharmacogenetic studies of clozapine-induced agranulocytosis.

Authors	Candidate genes/genetic variants	Sample size	Ethnicity	Clinical outcome	Definition of Agranulocytosis/Granulocytopenia
HUMAN LEUKOCYTE ANTIGEN (HLA) GENES					
Lieberman et al., 1990	*HLA-B38, HLA-B38-DR4-DQw3*	6 cases and 25 controls	Mainly Jewish ancestry	CIA	A total white blood cell count of $<3 \times 10^9$/L and an absolute polymorphonuclear leukocyte count of $<0.5 \times 10^9$/L
Yunis et al., 1995	*HLA-B38, HLA-DR4, HLA-B38-DR4-DQw3, HLA-DRB1*0402, HLA-DQB1*0302, HLA-DQA1*0301, HLA-DQB1*0301, HLA-DRB1*11, DRB1*0402-DRB4*0101-DQB1*0302, DQA1*0301* in Jewish patients, *HLA-B7, HLA-DR2, HLA-DR2-DQw1, HLA-B7-DR2-DQw1, HLA-DQB1*0502, HLA-DQA1*0102, DRB1*1601-DRB5*02-DQB1*0502-OQA1*0102* in non-Jewish patients	31 cases and 52 controls	White European Jerish and non-Jerish	CIA	Absolute neutrophil count $<500/mm^3$
Corzo et al., 1995	*HSP-70*	32 cases and 43 controls	Jerish and non-Jerish	CIA	No information
Turbay et al., 1997	*TNF*	33 cases and 33 controls	White European Jerish and non-Jerish	CIA	Absolute neutrophil count $<500/\mu L$
Amar et al., 1998	*HLA-DQB1*0201*	5 cases and 13 controls	Jerish and non-Jerish	CIAG	Agranulocytosis: total white blood cell count $<3{,}000\ mm^3$ and absolute polymorphonuclear count $<500\ mm^3$ Granulocytopenia: total white blood cell count $<3{,}500\ mm^3$ and absolute polymorphonuclear count $<1{,}000\ mm^3$
Valevski et al., 1998	*HLA-B38*	11 cases and 50 controls	Israeli Jerish	CIA	A granulocyte count of $<500\ mm^{-3}$
Dettling et al., 2001b	*HLA-Cw*7, HLA-DQB*0502, HLA-DRB1*0101, HLA-DRB3*0202*	31 cases and 77 controls	Non-Jewish German Caucasian	CIA	Absolute neutrophil count of <500 per mm^3
Dettling et al., 2001a	*HLA-DQB*0502, HLA-DRB5*02*	30 cases and 77 controls	Non-Jewish German Caucasian	CIA	Absolute neutrophil count of $<500 \times 10^9$/L
Dettling et al., 2007	*HLA-DRB5*0201, HLA-Cw7-B18, HLA-Cw7-B39, HLA-DRB5*0201-DRB4*000, HLA-Cw7-B18-DRB5*000, HLA-Cw7-B39-DRB5*000, HLA-Cw7-B44-DRB5*000*	42 cases and 75 controls	Non-Jewish German Caucasian	CIA	Absolute neutrophil count of <500 per mm^3
Lahdelma et al., 2001	*HLA-A1, HLA-A28, HLA-B16*	22 cases and 19 controls or 120 controls	Finnish	CIAG	Agranulocytosis: neutrophil granulocytes $<0.5 \times 10^9$/L Granulocytopenia: neutrophil granulocytes $<1.5 \times 10^9$/L

(Continued)

TABLE 2 | Continued

Authors	Candidate genes/genetic variants	Sample size	Ethnicity	Clinical outcome	Definition of Agranulocytosis/Granulocytopenia
NON-HUMAN LEUKOCYTE ANTIGEN (HLA) GENES					
Athanasiou et al., 2011	*HLA-DQB1*	Cohort I:33 cases and 54 controls Cohort II:49 cases and 78 controls	Cohort I:United States, Russia, and South Africa Cohort II:non-Jewish German Caucasian	CIA	absolute neutrophil count ≤500 cells/mm^3
Ostrousky et al., 2003	*NQO2*	18 cases and 80 controls	Jerish	CIA	A neutrophil count <500 cells/ul
Mosyagin et al., 2004	*MPO*	49 cases and 78 controls	German White	CIA	Absolute neutrophil count of <500 per mm^3
Athanasiou et al., 2011	*DRD1*, *CSF2RB*, *NTSR1*	33 cases and 54 controls	United States, Russia, and South Africa	CIA	Absolute neutrophil count ≤500 cells/mm^3
van der Weide et al., 2017	*ABCB* C3435T (rs1045642) and *NQO2* G1541A for CIA, *ABCB1* C3435T, *GSTT1*, and *GSTM1* for neutropenia	69 cases and 241 controls	Dutch	CIA, neutropenia	CIA: at least one neutrophil count ≤500 ul^{-1} Neutropenia: at least one neutrophil count between 500 ul^{-1} and 1500 ul^{-1} or two neutrophil counts <2,000 ul^{-1}

CIA, Clozapine-induced Agranulocytosis; CIAG, Clozapine-induced agranulocytosis/Clozapine-induced granulocytopenia.

clozapine efficacy. Although there is limited evidence, the most recent meta-analyses indicate that only three SNPs (rs6313 and rs6314 in the *HTR2A* gene and rs1062613 in the *HT3A* gene) are associated with a significant clozapine response (Gressier et al., 2016). Genetic variants in the genes within the MHC region have been extensively examined with respect to CIA. The results of these candidate studies indicate the HLA variants are implicated in developing CIA. A comprehensive screening of genetic variants linked to a significant clinical response to clozapine and CIA is critical because the selection of candidate genes is restricted to current knowledge about underlying biological mechanisms (Adkins et al., 2011). Recently, the field of clozapine pharmacogenetics has shifted from a candidate gene approach to a genome-wide approach. A genome-wide approach has successfully identified novel genes that are associated with CIA and provided further evidence of the involvement of the HLA variants in CIA (Goldstein et al., 2014; Saito et al., 2016). On the other hand, there are no known GWAS looking at the clinical response to clozapine, although there are several GWAS looking at the antipsychotic treatment response in patients with schizophrenia (McClay et al., 2011; Drögemöller et al., 2016; Ruderfer et al., 2016; Li et al., 2017; Yu et al., 2018). Yu and colleagues conducted GWAS in a large cohort (n = 3,792) and detected five loci (*CNTNAP5*, *MEGF10*, *PCDH7*, *SLC1A1*, and *TNIK*) associated with general antipsychotic treatment response (Yu et al., 2018). Interestingly, these loci did not include *DRD2,* although D2 receptor blockade in the brain is a general pharmacodynamic property of antipsychotics (Mauri et al., 2014).

Pharmacogenetic testing has the potential to help improve patient outcome, lower healthcare costs, and increase patient medication adherence (Gardner et al., 2014). Pharmacogenetic testing in psychiatry is not yet a standard of practice, however, its utilization is steadily increasing (Eum et al., 2016; Fabbri et al., 2018). Several studies have, indeed, assessed the clinical utility for risk genetic variants of CIA. The sensitivity and specificity of the *HLA-DQB1* 6672G.>C polymorphism for CIA in patients treated with clozapine, identified though candidate approach, was 21.5 and 98.4%, respectively (Athanasiou et al., 2011). The sensitivity and specificity of the *HLA-B*59:01* for CIA, identified by GWAS, was 31.8 and 95.3%, respectively (Saito et al., 2016) and the sensitivity and specificity of *HLA-B* 158T and *HLA-DQB1* 126Q polymorphisms for CIA, identified by GWAS and whole-exome sequencing (Goldstein et al., 2014), was 41 and 85%, respectively (Girardin et al., 2018). Clinical application guidelines require HLA allele testing for CIA to have a sensitivity of ∼50% (Girardin et al., 2018), therefore none of these have reached an acceptable threshold for clinical application. Conversely, we examined the diagnostic performance of non-risk allele (alleles except for *HLA-B*59:01*) on non-CIA among CIG patients and demonstrated a moderate, positive predictive value for detecting non-CIA subjects in the CIG group without the risk allele, suggesting a potential candidate for re-challenging with clozapine treatment in a Japanese population (Saito et al., 2016). Based on this finding, a re-challenging with clozapine following neutropenia in a patient with a low risk of CIAG (*HLA-B*52:01/52:01*) was successfully conducted (Yamaki et al., 2017).

The decision regarding clozapine re-challenge or withdrawal in case of CIAG should be based on careful consideration of risk factors, which can be facilitated by genetic testing in the future (Wicinski and Weclewicz, 2018). Further efforts to identify strong and reproducible genetic variants related to the clinical response to clozapine and CIA are needed to develop appropriate pharmacogenetic testing of clozapine.

The major issue of pharmacogenetics studies is inconsistent findings among studies. The discrepancies between these studies might be caused by statistical issues (i.e., sample size, multiple testing) and methodological issues (i.e., study design, phenotype definition, genetic polymorphism, population stratification) (Ross et al., 2012). Indeed, each sample size of the clozapine pharmacogenetic studies was relatively small. Large samples are needed to have enough statistical power to detect the effects of genetic variants on clinical outcomes by creating a clozapine consortium (Saito et al., 2016, 2017) or performing meta-analyses (Gressier et al., 2016; Legge et al., 2017). A sample size of more than 900 participants will be needed in a pharmacogenetic study if a common variant is anticipated with a large effect (Ross et al., 2012). Additionally, most of the clozapine pharmacogenetic studies did not adjust for the multiple statistical comparisons, resulting in type I error. To adjust for multiple testing, a false discovery rate (FDR) correction will be useful. Furthermore, selection bias and information bias are confounding factors in both prospective cohort and case-control studies (Ross et al., 2012). Clinical responses to clozapine have been determined by several different evaluation scales, including the Clinical Global Impressions Improvement (CGI-I) score, the global assessment scale (GAS), the Brief Psychiatric Rating Scale (BPRS), and Positive and Negative Symptom Scale (PANSS). Linkage disequilibrium (LD) varies among ethnic population, which may affect cross-subpopulation comparisons when causal SNPs are not directly genotyped but rather captures by proxy SNPs (Ross et al., 2012). Population stratification occurs when ethnic subpopulations within the entire study population differ in terms of genotype frequency and risk of disease (Thomas and Witte, 2002). In addition, clozapine doses and treatment length as well as types of antipsychotics preceding clozapine administration differ among studies. Although the clozapine treatment length in clinical response studies ranges from 5 weeks to more than 6 months, Lieberman and colleagues reported that 76% of patients responded to clozapine administered up to 52 weeks and slow clozapine responders experienced 70% of their total improvement after 12 weeks of treatment with clozapine (Lieberman et al., 1994). Standardized treatment protocols and evaluations of clinical outcomes will be needed. Furthermore, both non-genetic and genetic factors play an important role in the clinical outcome. For example, age at onset, gender, severity of the illness, negative symptoms, extrapyramidal side effects, clozapine concentration, history of catatonia, smoking, hypersomnolence, and cognitive deficits have all been associated with the clozapine treatment response (Perry et al., 1991; Lieberman et al., 1994; Miller et al., 1994; Umbricht et al., 2002; Semiz et al., 2007; Rajkumar et al., 2013a). To take these factors in analyses will be needed.

In conclusion, a number of clozapine pharmacogenetic studies have been performed based on candidate gene approaches. However, there is heterogeneity across studies and their results have been inconsistent. Reproducible genetic variants with large effect related to the clinical response to clozapine and CIA have not been detected so far. This field is beginning to shift from candidate gene approaches to more a comprehensive strategy, such as GWAS and whole genome sequencing, which will make it possible not only to identify novel genetic variants related to clinical outcomes, but also to analyze the effects of multiple genes on clinical outcomes. Extensive effort is required to apply pharmacogenetic information in clinical practice for a personalized medicine strategy of clozapine treatment.

AUTHOR CONTRIBUTIONS

SN and HU selected the articles and wrote the first draft of the manuscript. RH and TO supervised and contributed to the editing the manuscript. All authors have approved the final manuscript.

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SYSTEMATIC REVIEW
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The Microbiome: A New Target for Research and Treatment of Schizophrenia and its Resistant Presentations? A Systematic Literature Search and Review

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Background: The gastrointestinal system hosts roughly 1,800 distinct phyla and about 40,000 bacterial classes, which are known as microbiota, and which are able to influence the brain. For instance, microbiota can also influence the immune response through the activation of the immune system or through the release of mediators that are able to cross the brain blood barrier or that can interact with other substances that have free access to the brain, such as tryptophan and kynurenic acid, which is a metabolite of tryptophan and which has been involved in the pathogenesis of schizophrenia.

Objectives: This paper reviews the possible relationships between microbiome, schizophrenia and treatment resistance. Given the possibility of a role of immune activation and alterations, we also describe the relationship between schizophrenia and immune inflammatory response. Finally, we report on the studies about the use of probiotic and prebiotics in schizophrenia.

Methods: Cochrane library and PubMed were searched from the year 2000 to 2018 for publications about microbiome, immune-mediated pathology, schizophrenia and neurodevelopmental disorders. The following search string was used: (microbiome or immune mediated) AND (schizophrenia OR neurodevelopmental disorder). Associated publications were hand-searched from the list of references of the identified papers. A narrative review was also conducted about the use of probiotics and prebiotics in schizophrenia.

Results: There exists a close relationship between the central nervous system and the gastrointestinal tract, which makes it likely that there is a relationship between schizophrenia, including its resistant forms, and microbiota. This paper provides a summary of the most important studies that we identified on the topic.

Conclusions: Schizophrenia in particular, remain a challenge for researchers and practitioners and the possibility of a role of the microbiome and of immune-mediated

pathology should be better explored, not only in animal models but also in clinical trials of agents that are able to alter gut microbiota and possibly influence the mechanisms of gastrointestinal inflammation. Microbiome targeted treatments have not been well-studied yet in patients with mental illness in general, and with schizophrenia in particular. Nonetheless, the field is well worth of being appropriately investigated.

Keywords: microbiome, schizophrenia, resistant, inflammation, probiotics, gut, immune, interleukin

INTRODUCTION

Human body is colonized by various bacteria and the majority of them are within intestines, ranging from <105 bacteria per gram of digesta in the upper parts of the small intestine, to >1,012 bacteria per gram of digesta in the large intestine (15). Indeed, the gastrointestinal system hosts roughly 1,800 distinct phyla and about 40,000 bacterial classes, which are known as microbiota (Sherwin et al., 2016).

Microbiota main component include the following phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actino-bacteria, Fusobacteria, and Verrucomicrobia. Those microorganisms play important role in maintaining homeostasis and their imbalance may lead to various diseases (Eckburg et al., 2005). Newer research has made it clear that the maintenance of gut homeostasis is important for the prevention and treatment of various neurological diseases, possibly including schizophrenia (Galland, 2014; Severance et al., 2015a). The link between the gastrointestinal system and the brain is bidirectional and it is performed through several pathways. As the brain might affect functioning of the gut, modify microbial habitat and hence influence the microbiota composition (Bruce-Keller et al., 2018), at the same time, any disturbance of the microbial flora on the surface of intestinal mucosa might lead to a number of neuropsychiatric conditions, including schizophrenia (Petra et al., 2015; Zhu et al., 2017). Based on the above observations, the relationship between the brain and the gut has become a target for the research on the pathogenesis and treatment of several illnesses, including schizophrenia. Although collecting and storing stool samples from individuals with schizophrenia is challenging, interesting results have been found though the study of the oropharyngeal microbiome (Castro-Nallar et al., 2015; Bruce-Keller et al., 2018).

Alterations in the microbiota composition might contribute to symptoms of schizophrenia through an immune response or through the release of mediators, such as, amino acids, that are able to cross the brain blood barrier or that are able to interact with other substances that have free access to the brain. For instance, it is well-known that the microbiota can influence plasma level and metabolism of tryptophan and kynurenic acid, which is a metabolite of tryptophan (Bruce-Keller et al., 2018; Gao et al., 2018). This influence has been suggested as a possible contributor to the pathogenesis of schizophrenia. Of interest, kynurenic acid is a NMDA receptor antagonist which has been involved in the pathogenesis of schizophrenia, thus confirming the possibility of the gut to influence the brain (Erhardt et al., 2003; Balu, 2016). Also, microbiota can change host physiology through the production of metabolites such as, 5-hydroxytryptophan and γ-aminobutyric acid (GABA). For instance, Bifidobacteria and Lactobacilli can generate of GABA, the Bacillus family can generate dopamine and noradrenaline, and Escherichia can generate noradrenalin and 5-HT. In addition, germs like Clostridium sporogenes decarboxylate tryptophan to tryptamine, preventing the absorption of this essential amino acid (Wall et al., 2014; O'Mahony et al., 2015).

Converging lines of evidence indicate a link between schizophrenia and immune activation (Dickerson et al., 2017; van Kesteren et al., 2017), a hypothesis that dates back to the 1950s (Severance et al., 2012). Alterations of the immune system have been described in individuals with schizophrenia, including T-cell activation, increased plasma cytokines, chemokines, and increased acute phase reactants (Kronfol and Remick, 2000; Bruce-Keller et al., 2018). Of interest, antipsychotic medications may exert anti-inflammatory effects (Maier et al., 2018) and non-steroid anti-inflammatory agents can reduce symptom severity in patients with an altered immune response (Zheng et al., 2017).

This paper reviews the possible roles of the microbiome in the development and evolution of schizophrenia, with the ultimate goal to explore the possibility of developing new treatments, especially for those patients with treatment resistant presentations. Although studies on the fecal microbiome in schizophrenia and large multi-sites placebo-controlled trials of medications able to modify gut microbiota or inflammation in patients with schizophrenia are still lacking, this line of research is exciting and may hold promise.

METHODS

Searches

Cochrane library and PubMed were searched from the year 2000 to 2018 for publications about microbiome, immune-mediated pathology, schizophrenia and neurodevelopmental disorders. The following search string was used: (microbiome or immune mediated) AND (schizophrenia OR neurodevelopmental disorder). Associated publications were hand-searched from the list of references of the identified papers. We included "neurodevelopmental disorder" in our preliminary search string despite the recent challenges to the neurodevelopmental hypothesis of schizophrenia, given that neurodevelopmental abnormalities may nonetheless contribute to- or share pathophysiological mechanism with- schizophrenia (Owen et al., 2011). Registries of clinical trials in controlled trials.com and clinical trial.gov were also scrutinized.

Study Selection

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) course of action (**Figure 1**). We deliberately chose a broad search string, given that the research on the relationship between schizophrenia and microbiome is still in its infancy and that we wanted to search as many papers as possible. However, the main criteria to select the articles for this review were the following: the study pertained to microbiome; a schizophrenia subgroup (or sample) was part of the study; the study outcomes for the schizophrenia subgroup (or sample) were presented. Hence, the majority of selected papers resulted from studies pertaining to the relationship between microbiome, schizophrenia and the inflammatory-immunity response system. Although the papers pertaining to review studies, animal research, non-original studies such as, letters to the editor, book reviews and editorials, were excluded from the in-depth analysis, they were still considered for our preliminary studies and discussion, and quoted as necessary. As a second step, we conducted a narrative review about the use of probiotics and prebiotics in schizophrenia.

Quality Assessment

First-grade studies included research on schizophrenic's microbiota. The second grade included studies of gastrointestinal inflammation, infection and antimicrobial drugs in schizophrenia that could be related to microbiome.

RESULTS

This section reports the most relevant studies pertaining to: (1) the relationship between microbiome and schizophrenia; (2) the relationship between schizophrenia and immune inflammatory response; and (3) studies about probiotic and prebiotics, with special reference to schizophrenia.

Relationship Between Microbiome and Schizophrenia

Castro-Nallar and colleagues compared the oropharyngeal microbiome of schizophrenic patients and healthy subjects and found that certain types of bacteria were significantly predominant in patients suffering of this mental condition. In addition, they observed differences in the amount and distribution of species, as well as different metabolic pathways. In schizophrenic patients lactic acid bacteria and metabolite transport system, respectively, were predominant (Castro-Nallar et al., 2015). Yuan et al. (2018) evaluated the alterations in microbiota in 41 drug naïve, first episode patients with schizophrenia, after 24-weeks of treatment with risperidone. Compared to healthy controls, schizophrenic patients had significantly lower number of fecal Bifidobacterium, Escherichia coli and Lactobacillus. Conversely, they had significantly higher number of fecal Clostridium coccoides. After 24-weeks of treatment with risperidone, a significant increase in the numbers of fecal Bifidobacterium and E. coli was observed. Also, the patients showed a significant decrease in the number of fecal Clostridium coccoides and Lactobacillus. The authors concluded that drug naïve, first episode patients with schizophrenia show abnormalities in microbiota composition and observed that risperidone treatment caused significant changes in fecal bacteria. Also, they hypothesized that those changes were associated with the metabolic changes induced by risperidone.

Schwarz et al. (2018) evaluated the differences in fecal microbiota between 28 individuals diagnosed with first-episode psychosis (half of whom received a diagnosis of schizophrenia by the study 1-year follow up assessment) and 16 matched health controls. They found that psychotic patients had higher number of Lactobacillus bacteria. Of interest, the subgroup of patients that showed the strongest differences in microbiota coincided with the subjects who had a poorer response after up to 12 months of treatment.

Shen et al. (2018) evaluated the difference in gut microbiota between 64 patients with schizophrenia and 53 healthy controls and found a higher number of Proteobacteria, Succinivibrio, Megasphaera, Collinsella, Clostridium, Klebsiella and Methanbrevibacter and a lower number of Blautia, Coprococcus, Roseburia in patients with schizophrenia compared to healthy controls. Interestingly, the authors observed that 12 microbiota could be used as diagnostic factors to distinguish the patients with schizophrenia from the control cohort. Yolken et al. (2015) investigated the relationship between schizophrenia and bacteriophage genomes. They enrolled 41 persons with schizophrenia and 33 healthy controls. They came to conclusion that Lactobacillus phage phiadh was significantly more prevalent in the oropharynx of patients with schizophrenia. The presence of this microorganism also correlated with immunological disorders and valproate administration in the study group.

Severance et al. (2013) measured serological surrogate markers of bacterial translocation [soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP)] in two cohorts. The first one included 141 patients suffering from schizophrenia, 75 with bipolar disorder and 78 controls. In the other cohort there were 78 with antipsychotic naïve first-episode schizophrenia and 38 with medicated first-episode schizophrenia. They found that soluble CD14 was more prevalent in patients with schizophrenia and both sCD14 and LBP correlated with CRP in that group. Critchley and Harrison (2013) evaluated the impact of visceral homeostasis on both physiological and mental capacities of the brain and added to the studies showing that microbiota might affect gut-brain axis at any age, leading to neurodevelopmental or neurodegenerative conditions (Dinan and Cryan, 2017).

Relationship Between Schizophrenia and Immune Inflammatory Response

Several studies have pointed to an association between schizophrenia the immune-inflammatory response system (IRS), For instance, it has been observed that people living with schizophrenia had more prevalent inflammatory cytokine L1 receptor antagonists (Severance et al., 2012). Also, Chengappa et al. (1995) showed that Caucasian patients suffering from schizophrenia with HLA B8/DR3 had impaired proliferative lymphocyte response. Ganguli et al. (1994) discovered elevated IL-6 levels in patients with schizophrenia. Maes et al. (1997a,b) wrote about immunological response in schizophrenia. Arolt

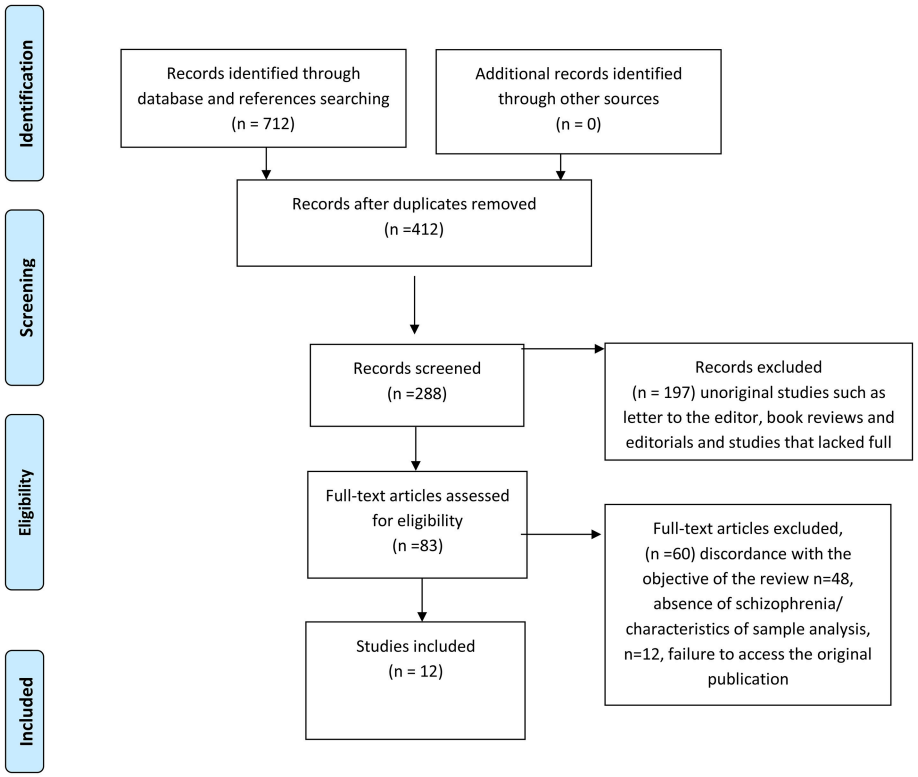

FIGURE 1 | Prisma Flow Diagram.

et al. (1997) and Rothermundt et al. (1998) published similar findings about the correlation of schizophrenia with abnormal immunological response.

In a study involving 17 individuals with treatment-resistant schizophrenia (TRS) 14 patients with schizophrenia showing response to antipsychotic treatment and seven normal controls, IL-6 was significantly higher in individuals with schizophrenia than in healthy volunteers (HV), IL-1RA was significantly higher in the TRS individuals than in HV, whereas schizophrenic patients who were not treatment resistant showed intermediate values. The authors concluded that schizophrenia in general, and TRS in particular, are characterized by cell-mediated immunity activation, primarily in the monocytic arm (Maes et al., 2000).

Lin et al. (1998) examined serum Clara Cell Protein (CC16), an endogenous protein with anti-inflammatory and immunosuppressive properties, interleukin-6 (IL-6), IL-6 receptor (IL-6R), and IL-1R antagonist (IL-1RA) in subjects with schizophrenia and normal controls. IL-6 and IL-6R were significantly higher in patients with schizophrenia than in normal controls and IL-6 was significantly higher in TRS than in normal controls, whereas patients with non-resistant schizophrenia TRS had intermediate values. Serum CC16 was significantly higher in normal volunteers and schizophrenic patients without a positive family history than in schizophrenic patients with a family history for psychoses. A significant inverse relationship was found between CC16 and IL-6 and IL-6R in patients with schizophrenia, but not in normal volunteers, once again pointing to the role of inflammation in schizophrenia, as indicated by higher IL-6 and IL-6R serum level, which may be linked to lower serum CC16. Müller et al. (1993) studied 55 patients with schizophrenia and observed a relationship between cellular immune parameters and the course of the psychopathological symptoms. It remains to be established what the cause is for IRS activation. IRS activation could be due to autoimmune responses (DeLisi et al., 1985; Margutti et al., 2006) or a microbial (i.e., viral) infection or reactivation (DeLisi and Crow, 1986). Kelly et al. (2017) suggested a strong role of the immune system in the development of schizophrenia; at the gene level, there is evidence about B-lymphocyte lineages that are included in the acquired immunity and major histocompatibility complex being related to this condition. Findings of schizophrenic patients with elevated levels of peripheral cytokines further confirm the possibility of a primary role of inflammatory processes. Benros et al. (2011) investigated if autoimmune diseases combined with exposures to severe infections may increase the risk of schizophrenia. They analyzed data from nationwide population-based registers in Denmark for the period from 1977 to 2006 and linked persons with autoimmune diseases and infections with individuals with diagnosis of a schizophrenia spectrum disorder in the Danish Psychiatric Central. They concluded that autoimmune disease might increase the risk of schizophrenia by 29%, and 60% in case of infection.

Dickerson et al. (2014) recently reviewed the literature concerning the immunity system, schizophrenia and bipolar disease and focused on two studies reporting differences in the oro-pharyngeal microbiota between schizophrenia cases and controls. The authors also pointed to studies showing higher rates of GI inflammation in patients with schizophrenia or bipolar disorder and on investigations focused on the relationship between psychiatric disorders and increased use of antibiotics, possibly mediated by antibiotic induced changes in microbiome.

Borovčanin et al. (2012) analyzed levels of serum type-1 cytokines, type-2 cytokines, type-17 cytokines and regulatory cytokines in 88 drug-naïve patients with first episode psychosis, 45 patients with schizophrenia in relapse and in the control group of 36 healthy persons. Patients with schizophrenia had elevated levels of IL-4 and increased production of TGF-β, which suggested the link between this psychiatric condition and chronic inflammatory processes. It is noteworthy that autoimmune conditions such as, celiac disease, i.e., a condition resulting from the interaction between certain dietary components and altered structure of the gastrointestinal tract, may be linked to schizophrenia. This link was first observed in epidemiological studies, and followed by research about common HLA predisposition (Severance et al., 2016). Efforts have been made to identify the role of the certain foods and inflammatory processes in bowels, and thus severity of symptoms of schizophrenia (Kraft and Westman, 2009).

Studies About Probiotic and Prebiotics, With Special Reference to Schizophrenia

In light of recent findings about the link between gut microbiota and schizophrenia, and the role of environmental factors in the development of this illness, there might be a possibility of using probiotics (containing species just as Lactobacillus and Bifidobacteria) in treatment of inflammatory processes within the gastrointestinal system, with positive effects of the symptoms of schizophrenia. Such treatment with "psychobiotics" could become a breakthrough in the management of mental illnesses (Saulnier et al., 2013; Sarkar et al., 2016; Deans, 2017). Severance and associates explored the possible relationship between food antigen-associated immune activation in patients with schizophrenia and gastrointestinal inflammation. They enrolled 193 subjects with non-recent and 67 with recent onset of schizophrenia, while there were 207 persons in the control group. They revealed food antigen antibodies and gastrointestinal inflammation in both schizophrenia groups (Severance et al., 2012).

In a study published in 2015, the same authors explored the link between dietary agents (wheat gluten and bovine milk casein) and immune response in blood and CSF samples in 105 patients with first episode of schizophrenia and 61 persons in the control group. In the experimental group IgG as a response to dietary proteins were significantly higher in both serum and CSF (Severance et al., 2015b).

Preliminary yet interesting information is emerging from clinical trials with probiotics in the treatment of schizophrenia (Bruce-Keller et al., 2018). Microbiome transplants from donor mice fed with high-fat diet showed that high fat-shaped microbiota disrupted cognitive, exploratory, and stereotypical/impulsive behaviors (Bruce-Keller et al., 2015). Other studies involving animal models demonstrated that probiotics may improve cognition, mood, anxiety, while improving neural activity and signaling (Sudo et al., 2004; Desbonnet et al., 2010; Bravo et al., 2011; Smith et al., 2014; Bruce-Keller et al., 2018). Also, mice studies have shown the ability of probiotics to promote hypothalamic synaptic plasticity and prevent decreases in hippocampal neurogenesis induced by stress (Ait-Belgnaoui et al., 2014). Dietary trans and saturated fats, may increase intestinal inflammation (Deopurkar et al., 2010; Okada et al., 2013), which results in a decrease of commensal Bacteroidetes and increase of pathogenic Enterobacteriaceae and Proteobacteria (Lupp et al., 2007; Stecher et al., 2007; Pédron and Sansonetti, 2008).

Karakuła-Juchnowicz et al. (2016) reviewed the role of the food antigens in schizophrenia, the use of diet modification, as well as antibiotics and probiotics as the possible treatment solutions. Probiotics are microorganisms, usually Lactobacilli and/or Bifidobacteria (Messaoudi, 2011; Tillisch et al., 2013; Steenbergen, 2015; Sarkar et al., 2016). Prebiotics are non-digestible carbohydrates that increase beneficial microbiota. Prebiotics may improve emotional affect and modulate stress responses (Schmidt et al., 2015). Randomized trials have shown efficacy of probiotics on mood (Messaoudi, 2011; Steenbergen, 2015) as well as the ability to reduce responses to stress (Kato-Kataoka, 2016). However, other studies have produced controversial results and therefore more trials are needed to completely demonstrate efficacy, to identify the specific strains that are most beneficial, as well as the correct dose and treatment duration (Doron and Snydman, 2015; Bruce-Keller et al., 2018). Similarly, large, controlled and well-powered studies about the efficacy of prebiotics are warranted (Bruce-Keller et al., 2018).

Tomasik et al. studied probiotic in schizophrenia and found their significant impact in reducing von Willebrand factor and increasing brain-derived neurotrophic factor (BDNF), monocyte chemotactic protein-1 (MCP-1), T-cell-specific protein RANTES, and macrophage inflammatory protein-1 beta (MIP-1) beta. Also, they found that that probiotic were related to regulation of intestinal immune and epithelial cells and suggested that supplementation of probiotics may improve control of gastrointestinal leakage in patients with schizophrenia (Tomasik et al., 2015).

Dickerson et al. (2014) performed a randomized, double-blind, placebo-controlled study and enrolled 65 patients with schizophrenia who were first treated with double blind probiotic or placebo for 14 weeks. Although no significant differences between probiotics and placebo groups were found in terms of changing schizophrenia symptoms severity, probiotics reduced the likelihood to develop severe bowel difficulty over the course of the trial.

Severance et al. (2017) conducted a randomized, placebo-controlled, longitudinal pilot study and explored the use of probiotics in treatment of both yeast gut infection and psychiatric symptoms 56 patients with schizophrenia. Probiotics

were associated with a decrease of Candida albicans antibody levels as well as a decrease in gastrointestinal symptoms in male subjects and a trends for improvement in positive schizophrenia symptoms in males who received probiotics and were seronegative for C. albicans.

DISCUSSION

We reviewed the relationship between microbiome and schizophrenia as a means to speculate on the possibility that microbiome alterations play a role in schizophrenia in general, and in treatment resistant schizophrenia in particular. This field of investigation is in its initial phase but the potential role of the trillions of viruses, bacteria, and fungi that inhabit most of our body, from the gastrointestinal tract to the upper and lower airways, oral cavity, skin, urogenital tract, and even tissues once thought to be sterile, such as, blood or the eyes, is likely and worth being further explored. For instance, Castro-Nallar, Shen and Yolken's studies showed clear differences in the amount and/or distribution of germs species, as well as different metabolic pathways. However, only few studies have explored the relationship between microbiota and schizophrenia and—to our knowledge—no study has evaluated the relationship between microbiota and treatment resistance in schizophrenia. However, a hint came from Schwarz and colleagues' study. In fact, these authors correlated the numbers of Lactobacilli with severity along different symptom domains and observed that patients with the strongest microbiota differences were also those who showed poorer response to treatment.

We believe that the scarce data that is already available may be helpful to formulate new hypotheses and to stimulate further research toward a better understanding of the contribution of microbiome and immune-mediated abnormalities to schizophrenia and treatment resistant schizophrenia.

Although the study of microbiomes is still in its infancy in psychiatry, other medical disciplines have already evolved to a better understanding of its role and potential therapeutic implications. For instance, the role of microbiome alterations in a potentially lethal intestinal infection caused by Clostridium difficilis has been clearly established. In fact, it is now clear that Clostridium difficilis spreads and becomes dangerous only when antibiotics destroy the gut's ordinary bacterial residents that otherwise prevent it from overgrowing. Interestingly, when specific antibiotics against Clostridium difficilis fail, a fecal transplant from a healthy gut, able to provide bacteria able to suppress Clostridium difficilis may be highly effective (Brandt, 2012; Burke and Lamont, 2013; Gens et al., 2013). However, the efficacy of fecal transplantation for psychiatric disorders in general, and schizophrenia in particular, is yet to be appropriately tested, despite encouraging preliminary data. For instance, an open-label trial (Kang et al., 2017) demonstrated that children with autism treated with fecal transplantation showed improved behavior.

Clearly, more studies on the role of the microbiome, probiotics, prebiotics and fecal transplantation in schizophrenia in general, and its resistant forms in particular, are in order.

CONCLUSIONS

We are still far from considering the possibility of microbiome targeted treatments in patients with mental illness in general, and with schizophrenia in particular. Nonetheless, TRS remains a challenge for researchers and practitioners and the possibility of a role of the microbiome and of immune-mediated pathology should be better explored, not only in animal models but also in clinical trials of agents that are able to alter gut microbiota and possibly influence the mechanisms of gastrointestinal inflammation. In fact, given that alterations in the immune system (e.g., interleukins mediated inflammatory processes and in T-cell functions), have been considered among the neurobiological correlates of treatment resistant schizophrenia (Altamura et al., 2005), the possibility of agents that are able to modulate such processes should be better explored. More studies on the relationship between schizophrenia and immune system alterations, as well as on the gut microbiome and on the possibility that agents such as, probiotics may contribute to the treatment of inflammatory processes within the gastrointestinal system, and exert positive effects on the symptoms of schizophrenia that are otherwise resistant to the classic medications, are warranted.

AUTHOR CONTRIBUTIONS

All authors contributed to conceptualization, interpretation of reviewed papers, drafting and review of the present paper. AC, AG, and AF also selected the papers that were the object of this review.

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Confused Connections? Targeting White Matter to Address Treatment Resistant Schizophrenia

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Despite development of comprehensive approaches to treat schizophrenia and other psychotic disorders and improve outcomes, there remains a proportion (approximately one-third) of patients who are treatment resistant and will not have remission of psychotic symptoms despite adequate trials of pharmacotherapy. This level of treatment response is stable across all stages of the spectrum of psychotic disorders, including early phase psychosis and chronic schizophrenia. Our current pharmacotherapies are beneficial in decreasing positive symptomology in most cases, however, with little to no impact on negative or cognitive symptoms. Not all individuals with treatment resistant psychosis unfortunately, even benefit from the potential pharmacological reductions in positive symptoms. The existing pharmacotherapy for psychosis is targeted at neurotransmitter receptors. The current first and second generation antipsychotic medications all act on dopamine type 2 receptors with the second generation drugs also interacting significantly with serotonin type 1 and 2 receptors, and with varying pharmacodynamic profiles overall. This focus on developing dopaminergic/serotonergic antipsychotics, while beneficial, has not reduced the proportion of patients experiencing treatment resistance to date. Another pharmacological approach is imperative to address treatment resistance both for response overall and for negative symptoms in particular. There is research suggesting that changes in white matter integrity occur in schizophrenia and these may be more associated with cognition and even negative symptomology. Here we review the evidence that white matter abnormalities in the brain may be contributing to the symptomology of psychotic disorders. Additionally, we propose that white matter may be a viable pharmacological target for pharmacoresistant schizophrenia and discuss current treatments in development for schizophrenia that target white matter.

Keywords: psychosis, white matter, treatment resistance, treatment refractory, schizophrenia, neuropharmacology, neuroimaging

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The current evidence based approach to treating psychotic disorders is to identify patients early in the disease process and apply a comprehensive pharmacological, psychological and supportive program to change the disease trajectory and improve outcomes (Addington et al., 2013). Despite the clear improvements in patient prognosis with this approach, there is still a cohort within the early phase psychosis population that will not respond to treatment, reflected also in a similar rate of treatment resistance in more established psychosis. Treatment resistance can cause significant personal, family, and societal burden, requiring increased rates of hospitalizations, longer lengths

of stay while in hospital, and significant use of other resources (Revicki, 1999; Kennedy et al., 2014). To improve outcomes in this group it is important to have an agreed definition of treatment resistance as this will allow confidence in the research to further the understanding of the biological underpinnings of treatment resistance, informing future treatment strategies.

Treatment resistance criteria as outlined by Kane et al. (1988), with subsequent modifications following, has been well accepted in both clinical and research spheres. It tends to be the definition most used in clinical and drug trial research and includes a minimum requirement of exposure to two different antipsychotics (from different classes) at adequate dose, each for at least 4–6 weeks, without at least a 20% reduction in either positive or negative symptoms as measured by standardized rating scales, and no period of good functioning within the past 5 years (Kane et al., 1988). This definition was initially tested and applied to patients with established and more lengthy illnesses, and modifications over the years has allowed its use in all phases of illness. A recent suggestion, to encompass early phase of illness and to move away from the focus on chronicity, is to define "clozapine eligibility" rather than treatment resistance (see, Williams et al., 2017). The Kane criteria however encompasses the clinical picture of persistent illness and continuous symptoms despite adequate treatment.

Other definitions of lack of treatment responsiveness used in research tend to be variants of the Kane criteria and can include failure to respond to two consecutive rounds of pharmacotherapy of adequate duration (6–8 weeks) and dosage (between 400 and 600 mg per day chlorpromazine equivalents) (Suzuki et al., 2012; Howes et al., 2017). Another commonly used criterion is from the American Psychiatric Association which defines treatment resistance simply as a lack of significant symptom improvement following at least two different trials of antipsychotic medications at therapeutic doses with each treatment round lasting at least 6 weeks (Lehman et al., 2004). A third paradigm that is often used for defining treatment resistance uses the criteria for remission from the Schizophrenia Working Group (Andreasen et al., 2005). In this approach, those individuals who have not met criteria for sustained remission are defined as treatment resistant. Interestingly, a new consensus guideline on terminology has been released from the APA but it has not yet been used in any published research studies (Howes et al., 2017).

While similar, the variations in the criteria used have led to differences in estimations of the prevalence of treatment resistance. Treatment resistant patients may constitute approximately 23% of patients in first episode or early phase populations with the additional observation that 84% of these individuals are potentially treatment resistant from onset of treatment (Demjaha et al., 2017). The percentage of individuals with treatment resistance in later phase or chronic schizophrenia ranges from 5 to 50% but generally a value of roughly 30% is accepted (Juarez-Reyes et al., 1995; Essock et al., 1996; Meltzer, 1997; Lehman et al., 2004; Howes et al., 2017). Within the treatment resistant population, an additional 10–20% may be considered ultra resistant and this is usually defined by a resistance to clozapine treatment (Kane et al., 1988; Juarez-Reyes et al., 1995; Essock et al., 1996).

The current gold standard for treatment of pharmacoresistance is clozapine (Van Sant and Buckley, 2011; Elkis and Buckley, 2016). Despite some uncertainty around the best timing for clozapine initiation, clozapine use in treatment resistant schizophrenia is evidence based and thus reflected in standards of care around identification and treatment of treatment resistance (e.g., National Institute for Health and Care Excellence, 2014; Abidi et al., 2017). Response rates of 60–77% are seen in patients though there are questions regarding its superiority and low rates of use in early phase patients (Williams et al., 2017; Thien et al., 2018). While response rates to clozapine are significant, they are not 100%, indicating that there exists another population of patients with schizophrenia that are resistant even to clozapine. Individuals with schizophrenia may come to a treatment resistant state by being inherently resistant to treatment, while others may lose the effectiveness of antipsychotics after multiple relapses. Importantly, while there are potential gains with clozapine in individuals with treatment resistance, these gains may not be actualized for some individuals who are not be able to tolerate clozapine due to its side effects. The potential for agranulocytosis requires regular blood monitoring and side effects such as sedation, weight gain, and hypersalivation acts as barriers to its use (Mortimer et al., 2010). Clearly more options are needed to address pharmacotherapy in schizophrenia, including more options in treatment resistant cases.

RATIONALE FOR A FOCUS ON BRAIN WHITE MATTER (WM) IN TREATMENT RESISTANCE

There is not a large body of research examining possible mechanisms behind the development of pharmacoresistance (reviewed in Gillespie et al., 2017). The existing studies have identified several possible mechanisms with a focus on the examination of neurotransmitter systems, which have informed the pharmacology of current therapeutic strategies. The dopaminergic, glutamatergic and serotonergic systems have all been studied in this regard with the dominant dopamine hypothesis underlying the development for many of the current antipsychotics. However, while the dopamine hypothesis, and for that matter other neurotransmitter systems may play a role in treatment resistance (Lau et al., 2013), these systems do not fully explain treatment-resistant schizophrenia (Demjaha et al., 2014; Mouchlianitis et al., 2016a).

Despite uncertainty regarding the underlying causes of schizophrenia and certainly treatment resistant schizophrenia; there are features of the pathophysiology of psychotic disorders that may inform new targets for pharmacological intervention. Schizophrenia has been proposed to be a dysconnectivity syndrome based on cognitive and functional fMRI research (Stephan et al., 2009; Friston et al., 2016). The major connections within the brain are seen structurally as white matter tracts (WM), myelinated axons that move signals between the hemispheres, lobes and gyri of the brain. There is a growing body of evidence, including our own work and the work

of others (Palaniyappan et al., 2013; Iwabuchi et al., 2015; Kumar et al., 2015; Crocker et al., 2017), suggesting that a disturbance in neuronal connectivity between different brain regions, rather than abnormalities restricted to individual brain regions, may be responsible for the clinical symptoms and cognitive dysfunctions observed in psychosis (Zhang et al., 2013). This raises the important question of not only the role of WM in the pathophysiology of psychosis, but its role in the more difficult clinical setting of treatment resistance. Inherent in this discussion is the subsequent pharmacological focus on WM as a potential treatment target.

There is not an extensive body of WM neuroimaging studies completed to date in treatment resistant psychotic patients. In this review, we examine the existing literature with respect to WM changes in the context of psychosis and treatment resistance. While this is not a large body of literature and more research clearly needs to be done, existing studies can be used to inform a discussion of how we may be able to use these findings to re-focus our efforts for effective pharmacological treatments to ultimately improve treatment response. Both animal and human studies that have investigated pharmacological WM targets will be discussed.

METHOD

This is a narrative review examining the possible role of WM in treatment resistant schizophrenia and its putative utility as a therapeutic target. However, elements of systematic review structure were used to ensure that the literature was comprehensively searched for research around this topic.

Search Strategy

The databases searched were Pubmed, PsycINFO, and Web of Science. For the location of papers examining white matter in treatment resistant patients; search terms included schizophrenia or psychosis and treatment resistance or pharmacoresistant or refractory. Results were then further refined by searching for white matter, connectivity or myelination or diffusion tensor imaging or MRI or magnetic resonance imaging or voxel based morphometry. For articles related to pharmacological treatments and genetics involved in white matter; search terms included white matter and treatment resistant or treatment response or refractory. Articles in both English and French were included in the searches. Ninety-Five Relevant articles and conference proceedings published between 1995 and 2018 were identified. References and abstract listings were screened for eligibility. No abstract proceedings were included in the final literature set. Then all identified studies underwent title and abstract screening followed by full text review. Further articles were identified by scrutinizing the reference lists of included articles. Inclusion criteria were English or French Language, a defined published/peer reviewed criteria for characterization of treatment resistance and specific inclusion of white matter measures.

AN OVERVIEW OF THE EVIDENCE FOR WM CHANGES BEING RELATED TO PSYCHOSIS SYMPTOMOLOGY

WM abnormalities have been shown to be affected in schizophrenia, including connectivity changes. However, what is the evidence that WM changes correlate with psychosis in individuals responding to treatment and its symptomology? This topic alone could constitute a review article, but to give context for the work in treatment resistant patients, we touch on the key points here. Comprehensive coverage of this topic can be found in recent reviews by others (Dietsche et al., 2017; Parnanzone et al., 2017).

It may first be instructive to consider the mechanisms by which WM can be altered in adult individuals. Myelination begins after 30 weeks gestation but occurs mainly in the post-natal period and is largely complete by young adulthood. Over the past couple of decades extensive research has been done to show that myelination is a dynamic process in the adult brain (Wang and Young, 2014; Almeida and Lyons, 2017), a process that can be affected by various mechanisms. There are thus changes in white matter myelination and oligodendrocytes that occur in adults and have been reported to malfunction in various disease states. Neuroinflammatory processes may lead to WM damage, most often associated with multiple sclerosis resulting in T2-hyperintense lesions that tend to be reduced in number and volume in interferon-beta treated patients (Kaunzner and Gauthier, 2017). The degree of myelin ensheathing has also been associated with abnormal processing speed in studies examining cognitive function in individuals undergoing chemotherapy (Matsos et al., 2017). In examining some of the neurotransmitter systems thought to be involved in schizophrenia, both glutamate and dopamine signaling have been found to have effects on WM. Excitotoxic damage to white matter by glutamate excitotoxicity is another phenomenon that can damage WM and has recently been reported to be associated with vesicular glutamate release (Doyle et al., 2018). Dopaminergic signaling itself is also associated with activity dependent myelination (Roy et al., 2007). Not only does the thickness of the myelin coating on axons affect conductance speed but synaptic activity influences the activity and replacement of oligodendrocytes in the brain throughout life (Almeida and Lyons, 2017). While the overall plan of WM tracts may not be altered after adolescent brain development ceases, there is evidence that fine tuning of the pathways may continue as myelin internodes continue to be created into adulthood (Wang and Young, 2014; Saifetiarova et al., 2017; Snaidero and Simons, 2017).

White matter may play a role in schizophrenia in several ways. First there is the neurodevelopmental hypothesis for schizophrenia which posits that mis-wiring the cortex including the WM connections is the underlying pathology of schizophrenia, to be clear, wholesale rewiring of WM is not likely to be affected by the treatments that are being proposed here (Fatemi and Folsom, 2009). Processes such as those outlined in the previous paragraph may be dysfunctional in schizophrenia though. There is evidence from genetic and post-mortem studies of the interaction with white matter and schizophrenia.

DISC1 (disrupted-in-schizophrenia-1) which was identified from genetic studies of a large cohort in Scotland (Zhang et al., 2006), is now known to negatively affect differentiation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes (Hattori et al., 2014). Post-mortem tissue from the dorsolateral prefrontal cortex has been shown in several studies to have downregulated expression of genes that relate to the function of myelin and oligodendrocytes (Hakak et al., 2001; Aston et al., 2004). Neuregulin 1 (NRG-1), a gene involved in regulating oligodendrocyte development and function is also implicated in schizophrenia (Papaleo et al., 2016; Mostaid et al., 2017) There is some evidence for neuroinflammation playing a role in schizophrenia as inflammatory mediators such as IL-10 as a promoter polymorphism in this gene has been shown to be a risk factor for schizophrenia development (He et al., 2006).

Changes in gray matter and brain volume have been well studied in schizophrenia. Less well studied is the trajectory of potential WM changes in the disorder over time (Dietsche et al., 2017). Early studies focused on changes in WM by examining the amount of WM in the brains of affected individuals using structural magnetic resonance imaging (MRI) scans. This early research approach suffered from two main issues. One, most of the studies were done at 1.5T where WM to gray matter junctions can be blurred leading to inaccurate quantitation (Chu et al., 2016, 2017). Second, our current understanding about connections within the brain suggests that dramatic changes in WM are not needed to affect integrity of neural pathways and network nodes within the brain (Rutgers et al., 2008; Englander et al., 2013; De Marco et al., 2017). For these reasons, not observing changes in WM volume on structural MRI does not assure that WM is not affected.

Current research is focused on using specialized magnetic resonance imaging techniques including diffusion tensor imaging and WM mapping techniques such as T1 mapping and R1 mapping. Diffusion Tensor Imaging (DTI) is often used to examine human *in vivo* WM non-invasively as it can provide an index of the cellular level integrity of WM tissue (Beaulieu, 2002). This neuroimaging method is based on water diffusion, with the variable of fractional anisotropy (FA) indicating a preferred direction of water diffusion in the region of interest (Mori and Zhang, 2006; Nucifora et al., 2007). When FA is found to be reduced in a disease condition, it broadly suggests reduced WM integrity (Ruest et al., 2011). Another technique that addresses the above is T1-weighted/T2-weighted imaging (T1-w/T2-w) using specialized sequences such as MPRAGE and MC-DESPOT (Glasser and Van Essen, 2011); in addition to fast scanning times (important in clinical studies) there are correlations with myelin content with low inter-subject variability (Glasser and Van Essen, 2011; Ganzetti et al., 2014). More recently when examined with a network based analysis, diffusion tensor imaging has shown overall decreases in FA that were widespread; greater than 50% of the cortico-cortical and cortico-subcortical white matter tracts were damaged in patients with schizophrenia and schizoaffective disorder (Klauser et al., 2017). Additionally, there are studies demonstrating an association of deficits in white matter integrity with core cognitive deficits including processing speed in treatment responding patients (Karbasforoushan et al., 2015; Kochunov et al., 2017) Additionally, work in non-treatment resistant patients shows WM alterations in the fornix connections suggesting a mechanism by which WM changes could affect memory (Fitzsimmons et al., 2009; Abdul-Rahman et al., 2011) So, while not clear if white matter changes are a cause or an effect of some underlying pathology, it is clear that white matter integrity is affected in schizophrenia. An overview of these processes that may play a role, or importantly be a subsequent treatment target in schizophrenia, are shown in **Figure 1**.

We have some evidence that WM changes may be related to changes over time with disease progression in non-resistant patients. The focus on the role of white matter in psychotic disorders is a recent development based on improvements in imaging techniques. There are studies that have examined correlations between WM integrity and the presence of various symptoms present in psychotic disorders (Dietsche et al., 2017; Parnanzone et al., 2017). In patients without treatment resistance, there is evidence that the degree of WM abnormalities is correlated with severity of positive symptoms, primarily reported in DTI and combined structural MRI and DTI studies (Chan et al., 2010; Bracht et al., 2014; Whitford et al., 2014, 2015). Thus, suggesting a direct relationship between white matter integrity and disease course. Examples that are relevant to treatment resistance but did not study it directly, include a recent DTI study that has shown cingulum bundle WM changes in chronic schizophrenia that may be associated specifically with persistent delusions (Oestreich et al., 2016). Another recent study showed severity of psychotic symptoms in hospitalized patients was related to reductions in WM volume measured by T1 structural MRI imaging in the medial portion of the left superior frontal area (Banaj et al., 2018). WM changes were also reported to be present at the onset of illness and potentially able to differentiate the FEP trajectory between what will be schizophrenia or not schizophrenia (Keymer-Gausset et al., 2018). Though treatment resistance was not specifically examined in this study, this could be evidence of how integral WM is to the disease process in schizophrenia. T1 mapping has recently been applied successfully in chronic schizophrenic with results correlating to clinical measures (Iwatani et al., 2015). A recent DTI study in patients at different points in their illness discovered evidence for progressive deterioration of connections over disease course (Di Biase et al., 2017). Additionally, work done with fMRI suggests that the functional brain networks that support higher order cognitive ability in individuals with schizophrenia undergo accelerated aging (Sheffield et al., 2016). As we believe functional networks are underpinned by structural networks, there is a basis for considering that the more damaged the WM, the worse the clinical course. In the next section, we consider the direct evidence for this assumption.

WHAT EVIDENCE DO WE HAVE FOR WM CHANGES IN SCHIZOPHRENIA BEING RELATED TO TREATMENT RESISTANCE?

Neuroimaging research has predominantly examined gray matter in relation to treatment resistance in schizophrenia (reviewed in Mouchlianitis et al., 2016b). Our literature search for papers

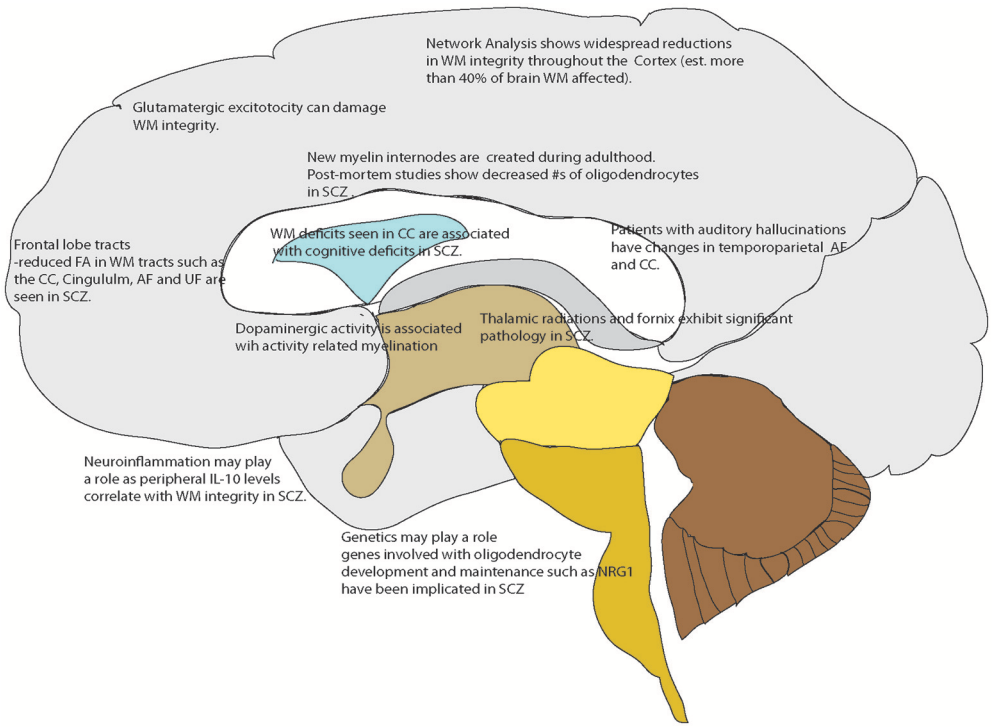

FIGURE 1 | Overview of White matter changes reported in schizophrenia or related to mechanisms known to be affected in schizophrenia that could serve as drug targets. Points referenced in the text.

that examined WM in treatment resistant patients resulted in finding studies that generally measured white matter changes as an adjunct to examination of gray matter content. Overall our search resulted in 15 papers relevant to the topic (**Table 1**). These papers are neuroimaging studies utilizing the methodologies of structural MRI, diffusion imaging, and T1 mapping. The variety of approaches taken and the small sample sizes in many of these studies highlight the need for further research on this potential new target for treatment of pharmacoresistance in schizophrenia. However, this body of literature overall shows potential for targeting WM in treatment.

Molina et al. (2008) examined WM structure and volume using MRI in treatment resistant, responsive and health controls (Molina et al., 2008). Thirty patients (21 were male) were treatment resistant using the Kane criteria (Kane et al., 1988). Increased WM content in treatment resistant was seen at baseline relative to responders and healthy controls. Longitudinal imaging done in a subset of their subjects (25–28 month interval between two scans) saw a significant decrease in WM in treatment resistant patients relative to healthy controls, controlling for sex as there was a significant sex difference between groups. However, at follow-up the healthy control group consisted of only 11 subjects (5 women/6 men) and the treatment resistant group 13 subjects (4 females/9 males) which limits their longitudinal findings (Molina et al., 2008). Anderson et al. (2015) examined WM by voxel based morphometry in treatment resistant subjects using the APA 2004 criteria (Lehman et al., 2004) and with an imaging sequence using a higher field strength (3T) magnet that more clearly demarcates the WM to gray matter margins (Anderson et al., 2015). The study reported significant differences compared to healthy controls with responders having significantly less WM than controls ($p < 0.019$) and ultra-treatment resistant having significantly less than HC ($p < 0.007$). While treatment resistant patients trended toward less WM overall, this group had the largest standard deviation and the result did not reach significance (Anderson et al., 2015). Whole brain WM quantitation by structural MRI was also completed comparing 52 treatment resistant schizophrenia patients to 182 treatment resistant major depressive disorder patients and 76 healthy controls. The treatment resistant schizophrenia group had significantly less whole brain WM as compared to HC and MDD patients ($p < 0.000$). This large effect size may have been related to the larger sample size of this study (Maller et al., 2012). The same group published another study examining the structure of the corpus callosum between a subset of the treatment resistant schizophrenia patients, treatment resistant major depressive disorder patients, and healthy controls (Sun et al., 2009). Their analysis divided the corpus callosum into 5 equidistant segments with differences in segments being observed between groups. This was interpreted as being suggestive of aberrant intrahemispheric connections in the treatment resistant group (Sun et al., 2009).

Hoptman et al. (2005) examined WM in the context of overt aggression in a treatment resistant cohort ($n = 49$; 43 M:6 F); controlled for substance use). They reported that larger orbital frontocortex WM volumes bilaterally were associated with

TABLE 1 | List of White matter and Pharmacoresistance Clinical Studies identified.

Authors (publication year)	Method	Comparison done	Summary of relevant findings
Hoptman et al., 2005	Structural MRI (1.5T T1-weighted IR prepared SPGR sequence or T1-weighted MPRAGE). Volumetric analysis	49 resistant to symptoms	Larger orbital frontocortex WM volumes bilaterally were associated with higher aggression scores on the overt aggression scale.
Mitelman et al., 2006	Diffusion Tensor Imaging (1.5T 7 directions, 7.5 mm non isotropic). Fractional Anisotropy (FA). TBSS.	53 resistant 51 responding 41 healthy controls	Right hemisphere showed FA reductions in resistant compared to responders.
Mitelman et al., 2007	Diffusion Tensor Imaging (1.5T 7 directions, 7.5 mm non isotropic). Fractional Anisotropy (FA). Tract analysis by ROI coordinates.	53 resistant 51 responding 41 healthy controls	Differences were seen between responders and resistant in corpus callosum and bilaterally in the fronto-occipital fasciculus. Other changes in left hemisphere only in optic radiation, and rostral segment of anterior limb of internal capsule. Right hemisphere were associated tracts were associated with more PANSS positive scores and negative symptoms inversely associated decreased FA both hemispheres.
Molina et al., 2008	Structural MRI (1.5T T1-weighted 3D gradient echo sequence). ROI volumetrics.	30 resistant 19 responding 44 healthy controls	Increased WM in TR at baseline relative to R and HC in frontal, parietal and occipital lobe. Longitudinal imaging done in a subset (25-28 mo interval between two scans) saw significant decrease in WM in TR relative to R in same lobes as above.
Mitelman et al., 2009	Structural MRI (1.5T T1-weighted 3D SPGR) and diffusion imaging (7 directions)	For structural 65 schizophrenia 16 healthy controls For DTI 17 resistant 17 responder 15 healthy controls	TR patients at baseline had a smaller, and more elongated corpus callosum and lower average FA. During 4 year follow-up, CC in TR patients declined in size but a smaller decline in FA than responders.
Sun et al., 2009	Structural MRI (1.5T T1-weighted IR prepared SPGR sequence). ROI volumetrics.	42 resistant 45 resistant major depressive disorder (MDD) 30 healthy controls	Corpus callosum divided into 5 equidistant segments. Differences in segments were observed between groups and this was interpreted as suggestive of aberrant intrahemispheric connections.
Luck et al., 2011	Diffusion Tensor Imaging (1.5T, 60 directions, 2.2 mm isotropic). FA.	24 resistant 20 responder 30 healthy controls	Resistant had greater decrements in FA in the uncinate fasciculus (UF) and superior longitudinal fasciculus (SLF) as compared to responders and healthy controls. FA values in SLF inversely correlated to several negative symptoms in PANSS. FA correlated to blunted affect only in UF.
Maller et al., 2012	Structural MRI (1.5T T1-weighted IR prepared SPGR sequence). ROI volumetrics.	52 resistant schizophrenia (TR) 182 resistant major depressive disorder (MDD) 76 healthy controls	TR had significantly less whole brain WM as compared to HC and MDD patients ($p < 0.000$).
Holleran et al., 2014	Diffusion Tensor Imaging (1.5T 64 directions, 2.5 mm isotropic). TBSS. Fractional Anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD) measures.	19 resistant (clozapine naïve) 19 healthy controls	Significantly reduced FA (and increased RD) in the genu, body, and splenium of the corpus callosum, the right posterior limb of the internal capsule, right external capsule, and the right temporal inferior longitudinal fasciculus. Decrease in splenium correlated to illness duration.
Reis Marques et al., 2014	Diffusion tensor imaging (3T 32 directions, 2.4 mm isotropic). Baseline and 12 week followup scans. FA and TBSS.	33 resistant 30 responders 52 healthy controls	Resistant lower FA than both responders and healthy controls. Most pronounced in uncinate, cingulum, and corpus callosum. FA increased in both patient groups with antipsychotic treatment. FA values correlated with PANSS total.
Anderson et al., 2015	Structural MRI (3T T1-weighted MPRAGE). Voxel based morphometry.	19 resistant 15 ultra-resistant 18 responders 20 healthy controls	Whole brain voxel based morphometry showed significant differences in 2 comparisons with responders having significantly less WM than controls ($p < 0.019$) and ultra-treatment resistant having significantly less than HC ($p < 0.007$).
Psomiades et al., 2016	Diffusion tensor imaging (1.5T, 24 directions). FA and tractography examined.	26 resistant with auditory verbal hallucinations 12 resistant with persistent negative symptoms but no hallucinations	FA values were significantly higher in the left arcuate fasciculus (LAF) in resistant patients with hallucinations than in no AVH but negative symptoms resistant patients. Correlation of FA value in the LAF and the severity of auditory verbal hallucinations ($p < 0.05$).

(Continued)

TABLE 1 | Continued

Authors (publication year)	Method	Comparison done	Summary of relevant findings
Chen et al., 2018	Diffusion tensor imaging (3T 25 directions). FA and MD	20 resistant 20 responders	Pilot study in First episode patients who were responsive or resistant after 1 year. White matter "impairment" found in right temporal lobe and right occipital lobe. No correlation of decreased FA to symptoms
Huang et al., 2018	3T T1 weighted MPRAGE and diffusion spectrum imaging. Analysis of 76 white matter tracts.	41 resistant 50 responders 50 healthy controls	Differences were found between patient groups and healthy controls for several tracts. Comparison of resistant to responder showed 4 tracts that were significantly different (right fornix, bilateral uncinated fasciculi, temporal pole callosal fibers) further these tracts correlated with negative PANSS scores.
Vanes et al., 2018	T1 mapping (3T mcDespot) Myelin water fraction and cognitive testing	22 Resistant 21 Responsive 24 healthy controls	Resistant and responsive patients showed reduced myelin water fraction compared to HC in bilateral fronto-occipital fasciculi but no difference between patient groups. Callosal Myelin water fraction was associated with cognitive control in patients.

higher aggression scores on the overt aggression scale (Hoptman et al., 2005). For this study, treatment resistance was defined as persistence of positive symptoms with typical antipsychotic treatment (600 mg chlorpromazine equivalents or higher) and a functional criteria of a "poor level of functioning" for the previous 2 years was included. This criteria was based on the work of others (Volavka et al., 2002).

T1 WM mapping using a MC-DESPOT sequence has also been reported. Treatment resistant and responsive patients showed reduced myelin water fraction compared to HC in bilateral fronto-occipital fasciculi but there was no difference between the patient groups. Callosal myelin water fraction was also associated with degree of cognitive control during the Stroop task in patients, however there were no difference in the Stroop scores between the two patient groups (Vanes et al., 2018). This study was limited due to small group sizes. There were also no patient demographics given in this paper as the referenced table is missing at time of writing, so it was difficult to judge how ill each patient group was. The groups were divided by a score of at least 4 on at least two of the PANSS (Kay et al., 1987) positive scale for the treatment resistant group and a score of 3 or less on all items of the PANSS for the responder group.

Diffusion tensor imaging studies have reported on treatment resistant patients both in first episode patient populations and more established schizophrenia. Two papers that compared treatment resistant and responder groups with health controls were completed by Mitelman et al. (2006, 2007). One study focused on FA values in 40 Brodmann's areas and the other compared fiber integrity in the same group of patients. Treatment resistance was defined by the criteria of Keefe (Keefe et al., 1987) and tract based spatial statistics (TBSS) were used. TBSS is an automated method of analyzing FA values from different scans by aligning FA values to allow group-wise comparison with a reduction in bias. The right hemisphere of treatment resistant patients showed FA reductions in comparison to patients who responded to treatment, both patient groups had long standing disease and substance abuse (current or historic) was an exclusion criteria (Mitelman et al., 2006). The data from this study was then extended into another paper examining WM tracts. When tracts were compared between treatment resistant and responsive individuals, there were differences in regions of the corpus callosum and bilaterally in the fronto-occipital fasciculus [thought to be involved in semantic processing (Martino et al., 2010)]. The findings in the fronto-occipital fasciculus should be interpreted with caution as the extent and connectivity of this tract is under debate (Bao et al., 2017). Other WM changes were observed in the left hemisphere only and located in the optic radiation, and the rostral segment of anterior limb of internal capsule. Interestingly this group compared changes in WM globally to symptoms as well in their analysis. WM values in the right hemisphere tracts were associated with more PANSS positive scores and negative symptoms were inversely associated with decreased FA in both hemispheres (Mitelman et al., 2007). The same group later performed a longitudinal study again using the criteria of Keefe to define treatment resistance (Keefe et al., 1987) and examined patients with structural and DTI measures (Keefe et al., 1987; Mitelman et al., 2009). In retrospect, we would now be more concerned with the small sample size of this longitudinal DTI study (Melicher et al., 2015) as well as the unbalanced gender ratios; however, the results are quite interesting as they span a 4 year time period comparing treatment responding, treatment resistant and healthy control subjects. The corpus callosum of the treatment resistant patients at baseline was smaller, more elongated and possibly more caudally positioned with lower FA observed in comparison to treatment responsive subjects. Four years later, these non-responders had significant decreases in corpus callosum dimensions but with less decline in FA as compared to responders. This could suggest that dorsoventral thinning was driving the changes in corpus callosum size in treatment resistant subjects and the position changes could be secondary to ventricular enlargement and gray matter loss, as opposed to representing a different arrangement of tracts (Mitelman et al., 2009). Another DTI TBSS study found significantly reduced FA (and increased RD) in the genu, body, and splenium of the corpus callosum, the right posterior limb of the internal capsule, right

external capsule, and the right temporal inferior longitudinal fasciculus. Decreased FA in the splenium correlated to illness duration (Holleran et al., 2014) and treatment resistance in this study was defined as failure to respond to two antipsychotic medications (one of which was atypical) and prolonged period of moderate to severe symptoms as defined by the PANSS (Kay et al., 1987). Another short DTI communication found significant differences suggesting WM impairment in the right temporal and occipital lobes in treatment resistant patients as compared to those in remission (Chen et al., 2018). This study defined treatment resistance using China's schizophrenia treatment guidelines and was a pilot study in first episode patients who were responsive or resistant after 1 year of treatment (Chinese Medical Association, 2003). While FA, RD and MD were measured, impairment was not defined other than to say $p < 0.05$ between groups and there was no correlation of decreased FA to symptoms. However, it was not clear how the symptoms were analyzed, for example if the total PANSS score was considered or if subscores were also compared (Chen et al., 2018).

In Psomiades et al. (2016) investigation, two groups of treatment resistant patients were compared, one with auditory verbal hallucinations and one without but with enduring negative symptoms (Psomiades et al., 2016). Treatment resistance was defined in a manner similar to the APA guidelines as the presence of symptoms after two well-conducted antipsychotic drug treatment trials with sufficient doses and duration. FA values were significantly higher in the left arcuate fasciculus (the pathway connecting the frontal lobe with the temporal lobe) in resistant patients with hallucinations as compared to the treatment resistant patients who had enduring negative symptoms (Psomiades et al., 2016). This study brings forward the possibility that WM deficiencies may be specific to the symptomology that is resisting treatment.

There are two other studies that examined WM integrity as it related to treatment outcome in first episode patients. The first study determined resistant status 6 months after scanning and was defined as by the criteria of the remission in Schizophrenia Working group (Andreasen et al., 2005). The treatment refractory group had greater decrements in FA in the uncinate fasciculus (UF) and superior longitudinal fasciculus (SLF) as compared to treatment responders and healthy controls. An exploratory analysis was done to compare diffusion values to FA, and in the SLF an inverse correlation was seen to several negative symptoms from the PANSS (blunted affect, social withdrawal and lack of spontaneity). By contrast, FA correlated to blunted affect only in the UF (Luck et al., 2011). The second study used DTI to try to predict clinical course in first episode psychosis patients. This study used the criteria of remission from the Schizophrenia Working group (Andreasen et al., 2005). The treatment refractory group had lower FA values than both responders and healthy controls at baseline scanning. The decreased values were most significant in the uncinate, cingulum, and corpus callosum. FA values increased in both patient groups with antipsychotic treatment at the follow-up scan. Also FA values negatively correlated with PANSS total scores (Reis Marques et al., 2014). A potential weakness of this study is that the 12 week follow-up for determination of treatment resistance would not reach the threshold for the definition of treatment resistance by other guidelines' criteria (Lehman et al., 2004; Abidi et al., 2017).

A very recent intriguing work was reported by Huang et al. (2018). They used a particular MRI protocol for structural imaging that shows WM very clearly at 3T, which was a T1 weighted MPRAGE in conjunction with diffusion spectrum imaging. Responding and resistant patients were compared to healthy controls as well as each other. Treatment resistance was defined as by the criteria of the remission in Schizophrenia Working group (Andreasen et al., 2005). Analysis of 76 WM tracts was conducted and differences were found between patient groups and healthy controls for several tracts. Comparison of resistant to responder showed 4 tracts that were significantly different (right fornix, bilateral uncinated fasciculi, temporal pole callosal fibers). Further, these tracts correlated with negative PANSS scores.

Complementing the *in vivo* neuroimaging studies already discussed, there is also one paper examining myelination in the substantia nigra in 14 post-mortem samples. Six samples were from treatment resistant subjects and 6 samples were from treatment responsive individuals (a further 2 samples were unknown for treatment response) and these were compared to 9 normal controls. Though this is a small sample size study, tissue from the substantia nigra of treatment resistant patients showed aberrant myelination characterized by increased G ratio (associated with decreased myelin thickness), axons without cytoplasm, and protrusions into the myelin sheath (Walker et al., 2018). The patients in both groups had an average duration of disease of 24 years so while treatment exposure is likely to have been different between groups, these results suggest cellular level changes that may be integral to treatment resistance.

These 15 studies reviewed here are small in number and have methodological differences (including small sample sizes and differences in definition of treatment resistance). However, the signals coming out of this body of work offer the important possibility of WM as another potential target for pharmacological research for schizophrenia as well as treatment resistant schizophrenia.

PHARMACOLOGICAL WM TARGETS IN TREATMENT RESISTANT SCHIZOPHRENIA: ANIMAL (PRECLINICAL) STUDIES

A challenge of conducting studies in treatment resistant schizophrenia patients is the potential for confounding of results through medication exposure over the disease course. This is an important consideration in neuroimaging of treatment resistant patients who conceivably have had extensive medication exposure and this has not always accounted for in neuroimaging studies to date. An alternative approach to this problem is to examine medication effects directly on white matter in preclinical models. It is worth noting that no animal model fully recapitulates all the symptom domains of schizophrenia

but these models allow examination of particular mechanisms of the disease process and potential treatments under controlled conditions.

Exposure of C57BL/6 mice to cuprizone results in demyelination and behaviors that resemble some of those seen in schizophrenia. Cuprizone is a copper chelating agent that when included in the rodent diet for weeks will result in mice developing widespread demyelination, oligodendrocyte loss and myelin breakdown which is similar to changes seen in post-mortem brains of individuals who had schizophrenia (Gudi et al., 2014; Walker et al., 2018). This model was used to test the effects of haloperidol, clozapine, quetiapine on WM recovery and remyelination (Xu et al., 2011). This study examined WM recovery by histological examination using myelin basic protein and anti-glutathione-transferase-pi immunostaining. They found that recovery of WM was still impaired after all three antipsychotic drug treatments with none of the three treatments promoting WM recovery, suggesting that typical and atypical antipsychotics do not act on WM (Xu et al., 2011). This is not surprising given what we know about the mechanism of action of these three drugs and further affirms that an alternative approach to promoting WM recovery is needed. A similar lack of recovery was seen in behavioral testing related to negative symptomology. Potentially this could also help explain why current AP reduce positive symptoms preferentially to negative symptoms. Examining antipsychotic effects directly on WM in preclinical models is important as there is no clear way to balance patients with varying pharmaceutical exposure over the course of their illness in neuroimaging studies, especially in treatment resistant patients who conceivably have had extensive medication exposure.

Reduced numbers of mature oligodendrocytes and increased numbers of microglia are also seen in the cuprizone model of demyelination in C57BL/6 mice (Zhang et al., 2018). This is similar to what is observed post-mortem in people who suffered from schizophrenia (Vikhreva et al., 2016). However, when mice were given N-acetylcysteine, an antioxidant that is a nutrition supplement, at doses of 100 mg/kg/day or greater these reactive immune changes were not seen (Zhang et al., 2018). Changes in interleukin-1 beta and tumor necrosis factor alpha were also significantly decreased with N-acetylcysteine treatment (Zhang et al., 2018). This work is particularly intriguing as a food supplement N-acetylcysteine is readily available and appears to have a wide safety margin. Acetylcysteine is a simple modified amino acid that is used in treating acetaminophen overdose, it has not been associated with serum enzyme elevations during therapy or with episodes of clinically apparent liver injury (National Institutes of Health, 2018). There are some concerns regarding shelf life for the stability of the active ingredients that should be kept in mind, however, as reports show N-acetylcysteine begins to breakdown within 96 h of exposure to air (McEvoy, 2012).

Another preclinical model of schizophrenia is the *St8sia2-/-* mouse (Angata et al., 2004). This mouse model has both behavioral evidence (Krocher et al., 2015) as well evidence from human studies that the gene is part of a susceptibility region with studies that link polymorphisms in the *St8sia2* gene to schizophrenia (Mcauley et al., 2012; Yang et al., 2015; Mandelli et al., 2016). ST8SIA2 is a polysialyltransferase that has as its targets neural cell adhesion molecule 1 (NCAM1) and cell adhesion molecule 1 (CADM1). Polysialylation is a process known to be involved in brain development and ST8SIA2 may be involved in myelin formation as its paralog ST8SIA4 has been shown to do (Koutsoudaki et al., 2010). Examination of myelination and oligodendrocytes in the *St8sia2-/-* mouse model showed lower myelin content, smaller malformed axons and a higher percentage of undifferentiated oligodendroglia (Szewczyk et al., 2017). There may be a role for *St8sia2* in oligodendrocyte differentiation and this could lead to deficits in myelination which in turn can affect axon structure and degeneration. Valproic acid has been shown to downregulate *St8sia2*. This property of valproic acid has been used in experimental autoimmune encephalitis to drive creation of larger numbers of oligodendrocyte precursors (OPCs) and then follow this treatment with Oct4 expressing lentiviral particles which induce differentiation of OPCs, thus resulting in increased numbers of myelinating oligodendrocytes (Dehghan et al., 2016). This is potentially a new approach for promoting myelination in adults and while the focus has been on multiple sclerosis for these types of treatments, the case could be made for treating treatment refractory schizophrenia in the same way. Multiple sclerosis has been shown to be associated with some overlap with schizophrenia symptomology and genetics (Arneth, 2017). Another approach shown in experimental models to improve WM integrity is the injection of extracellular vesicles after experimental stroke in rats (Otero-Ortega et al., 2017). Extracellular vesicles are complexes that are secreted by mesenchymal stem cells after brain insults such as stroke and may hold regenerative properties that are associated with stem cell treatment (Marote et al., 2016). Administration of extracellular vesicles in the rat subcortical infarct stroke model promoted axonal sprouting, oligodendrocyte formation, remyelination, but more importantly tract connectivity was seen (Otero-Ortega et al., 2017). This is potentially a very exciting approach as all of these processes could be useful in repairing the WM changes in treatment resistant schizophrenia.

Another potential avenue for treating WM deficits is suggested by recent work that examined catatonia in both schizophrenia and mice. Catatonia is a psychomotor syndrome that has not been well understood despite being seen across several neuropsychiatric disorders. WM involvement in catatonia is suggested by the association of catatonic signs with reduced expression of 2′-3′-cyclic nucleotide 3′-phosphodiesterase (CNP) which is a myelin associated protein (Hagemeyer et al., 2012). Janova et al. examined the percentage of a loss of function CNP single nucleotide polymorphisms in individuals with catatonia signs within the Gottingen Research Association for Schizophrenia database and found that there was an association between the two (Janova et al., 2018). Extending this work in a preclinical model, catatonia signs could be blocked in *Cnp-/-* mice treated with PLX5622 which pharmacologically blocks colony-stimulating factor 1 receptor and results in glial cell depletion. This group went on to examine WM inflammation in *Cnp-/-* mice by magnetic resonance spectroscopy of a region of

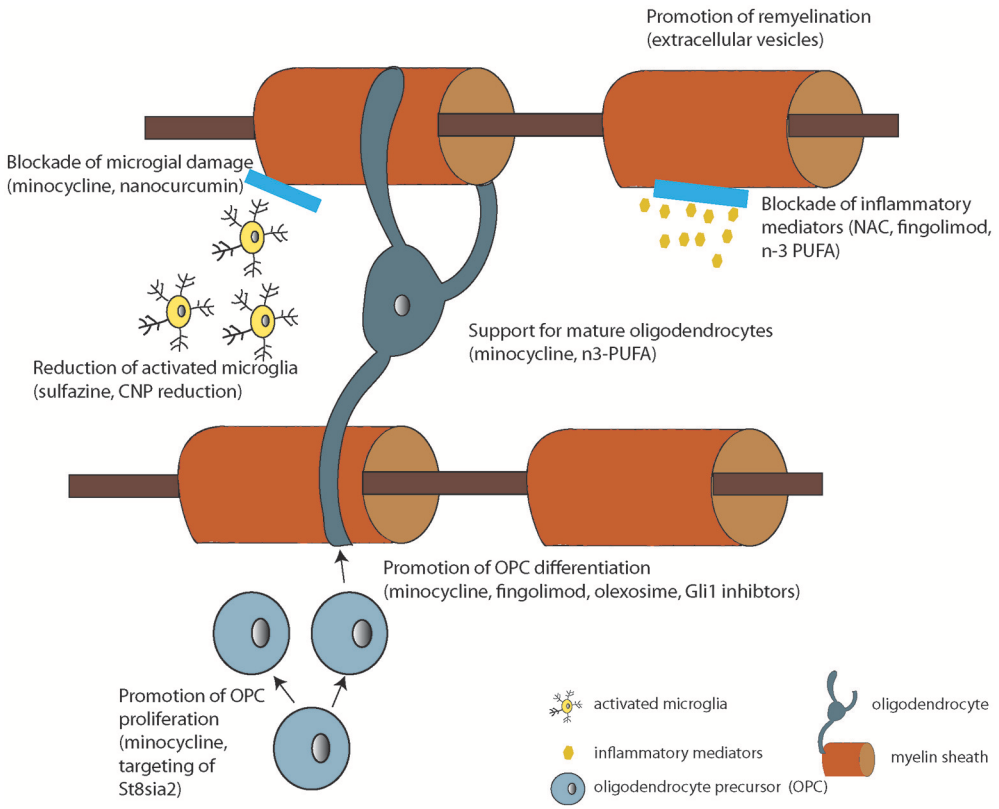

FIGURE 2 | Diagram summarizing the cellular or molecular mode of action of the compounds under development listed in **Table 2** (where known).

the corpus callosum. Myoinositol levels, a marker for glial cell activation, were reduced with PLX5622 treatment in the *Cnp-/-* mice suggesting that neuroinflammation plays a role in the WM processes associated with catatonia (Janova et al., 2018; Pease-Raissi and Chan, 2018). Overall these preclinical studies provide some insight into the possible mechanisms by which WM may play a mechanistic role in schizophrenia and in particular facets of the disorder that are more frequently treatment refractory.

PHARMACOLOGICAL WM TARGETS IN TREATMENT RESISTANT SCHIZOPHRENIA: HUMAN STUDIES

Based on the literature reviewed here, there are WM deficits that correlate with treatment resistance in schizophrenia. While other mechanisms of pharmacoresistance are still possible for any particular patient, if we consider WM as a target for therapy, there are options that are in development for human use. In fact, myelin enhancing strategies have been under investigation in human subjects for many years as effective treatments for multiple sclerosis are sought. Thus, repurposing and investigating these approved therapeutics currently in use for other medical conditions for treatment resistant patients is a reasonable approach. More specifically, putative myelinenhancing therapies would be potential candidates for large-scale clinical trials in schizophrenia. These include myelin-enhancing agents such as n-3 PUFA (Chen et al., 2014), minocycline (Rodgers et al., 2013), clemastine (Liu et al., 2016), polyphenols (Ghaiad et al., 2017), and potential neuro/myeloreparative agents such as sulfasalazine (Kim et al., 2015), nano-curcumin (Mohajeri et al., 2015), stem cell enhancing therapies such as Gli-1 inhibitors (Samanta et al., 2015), immunodmodulators such as fingolimod [FTY720, approved for use in MS (Kipp and Amor, 2012)], olexosime (Magalon et al., 2016) and retinoid receptor activators such as pioglitazone (Natrajan et al., 2015; Palaniyappan, personal comm.) (Summarized in **Figure 2** and **Table 2**).

(n-3) polyunsaturated fatty acids (n-3 PUFA) are dietary components and are a family of fatty acids mainly found in oily fish and fish oil supplements. Myelin sheaths are formed from the cell membranes of oligodendrocytes which contain polyunsaturated fatty acids. A sufficient quantity of n-3 PUFA in the diet was found to increase WM integrity and executive function in a study of healthy elderly (Virtanen et al., 2008). There is reason to believe that these fatty acids may also play a role in psychotic disorders. Lower total PUFA concentration in the membranes of erythrocytes was associated with lower fractional anisotropy (FA) measured by DTI in the corpus callosum and bilateral parietal, occipital, temporal and frontal WM in a study in early psychosis patients (Peters et al., 2013). An additional mechanism for how these compounds could help in treatment

TABLE 2 | List of potential therapeutics targeting white matter for treatment resistant schizophrenia.

Compound/drug (IUPAC name)	Proposed mode of action	Current stage of development relative to psychosis treatment
Minocycline (2*E*,4*S*,4a*R*,5aS,12a*R*)-2-(Amino-hydroxy-methylidene)-4,7-bis(dimethylamino)-10,11,12a-trihydroxy-4a,5,5a,6-tetrahydro-4*H*-tetracene-1,3,12-trione)	Protection of white matter by blocking microglial damage to white matter, promoting proliferation and maturation of oligdendroglial precursor cells and preservation of mature oligodendrocytes.	FDA approved and available by prescription. Multiple trials with some focus on treatment resistance: "Adjunctive Minocycline in Clozapine Treated Schizophrenia Patients" (clinicaltrials.gov id NCT01433055), "Minocycline Augmentation of Clozapine for Treatment Resistant Schizophrenia" (clinicaltrials.gov id NCT02533232), "The Benefit of Minocycline on Negative Symptoms in Schizophrenia: Extent and Mechanisms" (clinicaltrials.gov id NCT02928965), "Minocycline add-on to Antipsychotics for the Treatment of Negative and Cognitive Symptoms in Schizophrenia" (clinicaltrials.gov id NCT02907437)
Fingolimod (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol)	Mimic of sphingosine 1-phosphate (S1P)	FDA approved as a treatment for multiple sclerosis and available by prescription. Phase 2 clinical trial: "Fingolimod in schizophrenia patients (STEP)" (clinicaltrials.gov id NCT0177970)
Pioglitazone (5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione)	peroxisome proliferator activated receptor gamma (PPARgamma) agonist	FDA-approved as diabetes treatment and available by prescription. Phase 4 trial: "Pioglitazone as a Treatment for Lipid and Glucose Abnormalities in Patients With Schizophrenia" (clinicaltrials.gov id NCT00231894)
N-acetylcysteine (also called NACS; (2R)-2-acetamido-3-sulfanylpropanoic acid)	Antioxidant, shown to reduce interleukin-1 beta and tumor necrosis factor alpha in preclinical demyelination models.	Dietary supplement with multiple trials for psychosis underway: Phase 4 trial for "Treatment of Cognitive and Negative Symptoms in Schizophrenia With N-acetylcysteine" (clinicaltrials.gov id NCT02505477) and Phase 2 "N-Acetyl-Cysteine (NAC) in Early Phase Schizophrenia Spectrum Psychosis" (clinicaltrials.gov id NCT01354132)
Nano-curcumin [(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione]	Reduction in inflammation by several mechanisms including inhibiting NF-kappaB and induction of iNOS, reduces myelin and blood brain barrier damage, may improve microRNA profile	Dietary supplement with clinical trials relevant to treatment resistant schizophrenia: Phase 2 trial "Curcumin as a Novel Treatment to Improve Cognitive Dysfunction in Schizophrenia" (clinicaltrials.gov id NCT02104752), and "Curcumin Addition to Antipsychotic Treatment in Chronic Schizophrenia Patients" (clinicaltrials.gov id NCT02298985).
(n-3) polyunsaturated fatty acids (n-3 PUFA)	Increasing oligodendrocyte membrane integrity and blocking inflammatory damage to myelin	Dietary supplement. One trial found to examine cardiovascular disease which is also relevant to individuals with psychosis "Fish Oil-derived N-3 Polyunsaturated Fatty Acids and Extracellular Vesicles (HI-FIVE)" (clinicaltrials.gov id NCT03203512).
Clemastine fumarate ((2*R*)-2-{2-[(1*R*)-1-(4-chlorophenyl)-1-phenylethoxy]ethyl}-1-methylpyrrolidine)	Enhanced oligodendrocyte progenitor differentiation, may reverse some negative epigenetic changes	FDA approved as an allergy treatment. Available over the counter in most jurisdictions. No clinical trials for psychosis registered currently.
Olexosime {(NZ)-N-[(8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-ylidene]hydroxylamine}	Targeting proteins of the outer mitochondrial membrane and prevention of permeability transition pore opening mediated by oxidative stress. Promotion of oligodendrocyte maturation	Was in development for Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA). Roche announced it was discontinuing development in June 2018. No clinical trials for psychosis registered currently.
Sulfasalazine ((3Z)-6-oxo-3-[[4-(pyridin-2-ylsulfamoyl)phenyl]hydrazinylidene]cyclohexa-1,4-diene-1-carboxylic acid)	Inhibition of CD44v-xCT (cystine transporter, reduction of the number of macrophages and microglia	FDA approved as Antirheumatic and gastrointestinal treatment. Available by prescription. No clinical trials for psychosis registered currently.
Gli1 inhibitors (such as 5-fluorouracil, methotrexate, cisplatin, vismodegib)	Promotes differentiation of stem cells into mature oligodendrocytes	Preclinical development
Extracellular Vesicles derived from stem cells	Promotion of axonal sprouting, oligodendrocyte formation, remyelination with tract connectivity	Preclinical development
Reduced expression of 2′-3′-cyclic nucleotide 3′-phosphodiesterase (CNP)	Blockade of microglial activation	Preclinical development
Targeting of *St8sia2*	Promotion of stem cell production of oligodendrocyte precursors.	Preclinical development

resistant patients is suggested by work in an Experimental autoimmune encephalomyelitis model of demyelination, n-3 PUFA was able to block the release of inflammatory mediators by microglia and keep the M2 phenotype thus blocking microglial damage to myelin (Chen et al., 2014). Thus n-3 PUFA could help by increasing oligodendrocyte membrane integrity and blocking inflammatory damage to myelin.

Minocycline is in the family of tetracycline antibiotics and is used as an anti-acne treatment. It crosses the blood-brain-barrier and is immunomodulatory which led to it being studied for use in relapsing remitting MS (Rodgers et al., 2013). This immunomodulatory role and the research linking the possibility of negative symptoms with neuroinflammation has led to a number of clinical trials examining minocycline as an add-on to second-generation antipsychotic treatment. A recent meta-analysis of eight RCTs with minocycline used as an adjunctive medication concluded that minocycline appeared to be superior to placebo for positive, negative and general symptom scores, exhibiting a good safety profile (Xiang et al., 2017). Interestingly, minocycline also appears to protect WM by both blocking microglial damage to WM and promoting proliferation and maturation of oligdendroglial precursor cells (Schmitz et al., 2014). It is thus also positioned to be investigated for use in treatment resistant schizophrenia.

Clemastine fumarate is an ethanolamine-derivative, and a first generation histamine H1 antagonist used for allergic rhinitis. Based on its ability to stimulate oligodendrocyte differentiation, clemastine is being considered as a treatment for MS and is in phase 2 testing (Green et al., 2017). One small note is often made in reference to use of clemastine in that similar to other antihistamines, it is known to cause drowsiness. Clemastine is particularly interesting as a possible treatment for treatment refractory schizophrenia as not only does it enhance oligodendrocyte progenitor differentiation but it also causes epigenetic changes which may be important in the overall disease course (Liu et al., 2016).

Polyphenols such as green tea polyphenol mixture (GTPP) and its active ingredient, epigallocatechin-3-gallate (EGCG), prevent both the neurite outgrowth-inhibiting activity and growth cone-collapsing activity of the C-terminal domain of Nogo-A, which is derived from myelin (Gundimeda et al., 2015). Another polyphenol, resveratrol, which is a stilbenoid polyphenol, and known to pass the blood brain barrier, has been shown to reverse cuprizone-induced demyelination, and improved mitochondrial function in preclinical work (Ghaiad et al., 2017). However, results in humans with a 6 month trial of resveratrol examining inflammatory mediators and brain structure have not been impressive to date (Huhn et al., 2018).

Sulfasalazine is a pro-drug that is converted to 5-Aminosalicylic Acid (5-ASA) and is used to treat ulcerative colitis and rheumatoid arthritis. Sulfasalazine treatment promoted remyelination in the CNS of a transgenic zebrafish model of NTR/MTZ-induced demyelination by reducing the number of macrophages/microglia, suggesting an immunomodulatory function of sulfasalazine in remyelination (Kim et al., 2015). Sulfasalazine inhibits the CD44v-xCT (cystine transporter) which is crucial for growth and viability and required for synthesis of intracellular glutathione. CD44+ microglia are involved in neuroinflammatory processes (Matsumoto et al., 2012). However, caution may be in order as trials with this drug in MS patients have shown limited effects and a significant side effect profile (Shirani et al., 2016).

Nano-curcumin is a more bioavailable form of curcumin. Curcumin is a polyphenolic phytochemical that has antioxidant properties. Many claims have been made to its effectiveness for a variety of conditions. However, it has been shown to reduce inflammation by several mechanisms including inhibiting NF-kappaB and induction of iNOS (Xie et al., 2011). In a rat experimental autoimmune encephalomyelitis (EAE) model, polymerized nano-curcumin was shown to reduce myelin damage and blood brain barrier breakdown (Mohajeri et al., 2015). Another particularly intriguing therapeutic target is the ability of nano-curcumin to restore microRNA expression in a small cohort of relapsing-remitting MS patients (Dolati et al., 2018). MicroRNAs have been shown to be involved in both the pathogenesis of schizophrenia, affect drug metabolizing enzymes and are altered by antipsychotic treatment (Swathy and Banerjee, 2017). Curcumin has been tested as an adjunct to fluoxetine in major depressive disorder with no significant improvement in depression scores but also no safety concerns (Sanmukhani et al., 2014). Curcumin may act on several systems to improve treatment response and nano-curcumin makes use of this phytochemical therapeutically possible.

Neural stem cell based approaches are another possible avenue to repair WM. Gli-1 inhibitors act upon a pool of neural stem cells that express Gli1 and when Gli1 expression is repressed, these cells differentiate into oligodendrocytes (Samanta et al., 2015).

Fingolimod [FTY720, an immunodmodulator approved for use in MS (Kipp and Amor, 2012)], is believed to mimic sphingosine 1-phosphate (S1P) *in vivo* and a lipid mediator that acts through G protein coupled receptors and can cross the blood-brain-barrier (Chun and Hartung, 2010). Receptors for S1P are also present in the CNS. There is a listing for phase two clinical trial examining fingolimod in schizophrenia patients (STEP) active in the clinical trial registry that was due to finish recruiting patients in Dec 2017 (See **Table 2**). A recent study from a phase 3 trial in MS patients showed significantly less ventricular volume enlargement and less WM loss with fingolimod as compared to placebo (Gaetano et al., 2018). This raises a possibility for fingolimod to modify not only WM loss but the pathological ventricular enlargement seen in schizophrenia which would be an exciting possibility.

Olexosime (TRO19622) is a novel cholesterol-oxime mitochondrial-targeted neuroprotective compound that acts by targeting proteins of the outer mitochondrial membrane and prevents permeability transition pore opening mediated by, among other things, oxidative stress. It was originally designed for movement disorders such as Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) where it was thought to have potential to prevent mitochondrial rupture (Lenglet et al., 2014). Olesoxime has been shown to promote myelination through action on oligodendrocytes to accelerate maturation and remyelination in preclinical models of demyelination (Magalon et al., 2016).

Pioglitazone, an FDA-approved peroxisome proliferator activated receptor gamma (PPARgamma) agonist, was originally designed as a diabetes drug but has found further targets in MS. As a diabetes drug, it works by increasing the body's sensitivity to insulin, a potential bonus for patients with metabolic issues in addition to psychosis symptomology. Pioglitazone has been shown through diffusion tensor imaging to reduce lesion formation in normal appearing WM in relapsing remitting MS patients (Shukla et al., 2010). It has also been shown to decrease WM lesions in the corpus callosum in a stroke model in hypertensive rats and reduced microglia proliferation in the same model (Lan et al., 2015). Both of which would be beneficial in treatment of WM deficits in treatment resistant patients.

A number of these agents are suitable for drug repurposing and repositioning applications, which greatly enhances the lab-to-clinic transition (Ashburn and Thor, 2004). Repurposing RCTs are already underway for some of these agents [e.g., fingolimod (fingolimod in Schizophrenia clinicaltrials.gov)] and pioglitazone (Iranpour et al., 2016). Of these minocycline, which predominantly limits neuronal damage by promoting oligodendrocyte progenitor proliferation and preserving mature oligodendrocytes (Guimaraes et al., 2010; Schmitz et al., 2012; Ma et al., 2015; Scheuer et al., 2015), and pioglitazone which promotes antioxidant defense of oligodendrocytes (Bernardo et al., 2009) have already shown promise in treating psychosis (Chaudhry et al., 2012; Iranpour et al., 2016). Further work is needed to see if an association exists between extensive WM changes and pharmacoresistance, but if it does then these individuals can be specifically targeted for clinical trials of myeloprotection (Palaniyappan, personal comm.).

CONCLUSION

Schizophrenia is highly heterogeneous in presentation leading some to propose, based on symptoms and even genome wide association studies, that we might be better served to re-consider "The Schizophrenias" (Peralta and Cuesta, 2011; Schizophrenia Working Group Of The Psychiatric Genomics Consortium, 2014). The rich tapestry of variation in schizophrenia is a challenge for researchers but is also an opportunity for developing personalized medicine strategies. Differential treatment response is one of these areas of disease variation that may point the way to designing a personalized plan and shed light on the etiology behind the disorder.

Ultimately, a combination of clinical profile, imaging and possibly as we move forward pharmacogenomics will likely be required to identify what therapeutic approach best suits each individual pharmacoresistant patient. The observation that greater than 80% of the individuals who are treatment resistant are already resistant at the first episode psychosis stage of schizophrenia, gives further credence to the idea that there is more than one type of schizophrenia and clearly targeting the dopaminergic system is not sufficient for 30% or greater of the patient population. WM is a viable therapeutic target for this portion of the patient population. The studies cited here are observational so it is unclear if the WM damage is related to a cause of schizophrenia or the result of damage from some underlying metabolic process; however, in either case improvement of WM integrity in pharmacoresistant patients was overall associated with an improvement in symptomology in the studies that examined this outcome (**Table 1**). Further examination of either repurposing WM modulating drugs from other demyelinating diseases or moving those in preclinical work forward to examine in treatment resistant patients should be a goal moving forward.

AUTHOR CONTRIBUTIONS

CC and PT wrote the abstract. CC performed the literature search, wrote the first draft and drew the figures. PT edited the manuscript and tables for submission and accuracy. Both authors read and approved the final version.

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CORRECTION
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Corrigendum: Confused Connections? Targeting White Matter to Address Treatment Resistant Schizophrenia

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A Corrigendum on

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In the original article Palaniyappan, personal comm. was not cited in the article. The citation has now been inserted in *Pharmacological WM Targets in Treatment Resistant Schizophrenia: Human Studies*, paragraphs 1 and 11 and should read:

Paragraph one:

Based on the literature reviewed here, there are WM deficits that correlate with treatment resistance in schizophrenia. While other mechanisms of pharmacoresistance are still possible for any particular patient, if we consider WM as a target for therapy, there are options that are in development for human use. In fact, myelin enhancing strategies have been under investigation in human subjects for many years as effective treatments for multiple sclerosis are sought. Thus, repurposing and investigating these approved therapeutics currently in use for other medical conditions for treatment resistant patients is a reasonable approach. More specifically, putative myelinenhancing therapies would be potential candidates for large-scale clinical trials in schizophrenia. These include myelin-enhancing agents such as n-3 PUFA (Chen et al., 2014), minocycline (Rodgers et al., 2013), clemastine (Liu et al., 2016), polyphenols (Ghaiad et al., 2017), and potential neuro/myeloreparative agents such as sulfasalazine (Kim et al., 2015), nano-curcumin (Mohajeri et al., 2015), stem cell enhancing therapies such as Gli-1 inhibitors (Samanta et al., 2015), immunodmodulators such as fingolimod [FTY720, approved for use in MS (Kipp and Amor, 2012)], olexosime (Magalon et al., 2016) and retinoid receptor activators such as pioglitazone (Natrajan et al., 2015; Palaniyappan, personal comm.) (Summarized in Figure 2 and Table 2).

Paragraph 11:

A number of these agents are suitable for drug repurposing and repositioning applications, which greatly enhances the lab-to-clinic transition (Ashburn and Thor, 2004). Repurposing RCTs are already underway for some of these agents [e.g., fingolimod (fingolimod in Schizophrenia clinicaltrials.gov)] and pioglitazone (Iranpour et al., 2016). Of these minocycline, which

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predominantly limits neuronal damage by promoting oligodendrocyte progenitor proliferation and preserving mature oligodendrocytes (Guimaraes et al., 2010; Schmitz et al., 2012; Ma et al., 2015; Scheuer et al., 2015), and pioglitazone which promotes antioxidant defense of oligodendrocytes (Bernardo et al., 2009) have already shown promise in treating psychosis (Chaudhry et al., 2012; Iranpour et al., 2016). Further work is needed to see if an association exists between extensive WM changes and pharmacoresistance, but if it does then these individuals can be specifically targeted for clinical trials of myeloprotection (Palaniyappan, personal comm.).

The authors apologize for these errors and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Improving Cognition to Increase Treatment Efficacy in Schizophrenia: Effects of Metabolic Syndrome on Cognitive Remediation's Outcome

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Cognitive impairment, typically more severe in treatment resistant patients, is considered a hallmark of schizophrenia and the prime driver of functional disability. Recent evidence suggests that metabolic syndrome may contribute to cognitive deficits in schizophrenia, possibly through shared underlying mechanisms. However, results are still contradictory and no study has so far examined the influence of metabolic syndrome on cognitive outcome after cognitive remediation therapy (CRT). Based on these premises, this study aims to investigate the relationship between metabolic syndrome and cognition, specifically considering cognitive outcome after treatment. Secondary objectives include the analysis of the association between cognitive impairment and psychopathological status and, in a subgroup of patients, the evaluation of the effect of Sterol Regulatory Element Binding Transcription Factor 1 (SREBF-1) rs11868035 genetic polymorphism, previously associated with metabolic alterations, on both cognition and metabolic syndrome. One-hundred seventy-two outpatients with schizophrenia were assessed for metabolic parameters and neurocognitive measures and 138 patients, who completed CRT, were re-evaluated for cognition. A subsample of 51 patients was also genotyped for rs11868035 from peripheral blood sample. Results show a negative impact of metabolic syndrome on executive functions and global cognitive outcome after CRT. Data also revealed a significant effect of SREBF-1 polymorphism, with a higher prevalence of metabolic syndrome and worse processing speed performance among G/G homozygous subjects, compared the A allele carriers. Overall these findings support the hypothesis that metabolic alterations may hamper the capacity to restore cognitive deficits, as well as they highlight the need to further explore possible converging mechanisms underlying both cognitive and metabolic dysfunction. At the clinical level, results point to the importance of a comprehensive assessment including the metabolic status of patients and of individualized strategies addressing metabolic dysfunction in order to potentiate treatment outcome in schizophrenia.

Keywords: psychosis, neuroremediation, neuropsychology, rehabilitation, metabolic alterations

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INTRODUCTION

Cognitive deficits are core features of schizophrenia, detectable in at least 75% of patients (1). The cognitive impairment is the prime driver of significant disabilities in occupational, social, and economic functioning in patients with schizophrenia (2) and still represents one of the most critical dimension in schizophrenia treatment. Standard antipsychotic drugs have only minimal effects on neuropsychological performance, even in the case of good antipsychotic response (3). Although the relationship between the degree of cognitive impairment and the severity of psychotic symptoms is not so straight-forward, treatment-resistant patients exhibit significantly poorer neurocognitive performance on a broad range of cognitive domains, such as selective attention, verbal memory, processing speed, and executive functions, compared to treatment responders (4–6). On the other hand, interventions aimed at improving cognition also showed some positive effects on different symptoms domains (7, 8). So far non-pharmacological interventions, more specifically cognitive remediation therapy (CRT), represent the gold-standard treatment for cognitive impairment. Several studies proved CRT efficacy and recently it has been demonstrated that, in certain patients, it may lead to a proper functional recovery, through the achievement of cognitive performance falling into "normal" range (9). However, it's important to stress the concept that results vary across subjects and are influenced by several individual variables, including genetic underpinning (10–15). Identifying factors affecting cognitive performance and thus putative predictors of cognitive remediation is of major importance in order to personalize interventions and improve outcome. In this view, recent literature suggested a role of metabolic syndrome (MetS). MetS, consisting of a set of cardio-metabolic alterations, has been shown to increase the risk of cognitive decline in the general population, probably because of its effect on cerebral circulation (15–18). This issue of MetS is particularly relevant in patients with schizophrenia, also because of its increased prevalence in this population (19, 20). Although recent studies explored the contribution of MetS to cognitive deficits in patients with schizophrenia (21), results are still conflicting. On the one hand, Lindenmayer et al. reported how schizophrenia patients with MetS showed significant cognitive impairments in several cognitive domains (22). On the other hand, results of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study suggested an unexpected relationship between elevated cholesterol and triglycerides levels and better cognitive functioning (23). Moreover, it is important to note that the relationship between cognitive deficits and metabolic alterations might be bidirectional, since impaired neurocognitive abilities (which lead to poor decision making and unhealthy lifestyle) may contribute to the higher prevalence of MetS among patients with schizophrenia. Remarkably, despite this evidence, the effect of MetS on the outcome of CRT in patients affected by schizophrenia, has never been investigated.

The relationship between MetS and cognition might be mediated also by genetic factors. Among these, the Sterol Regulatory Element Binding Transcription Factor 1 (SREBF-1) gene, regulating the expression of Sterol Regulatory Element-Binding Protein 1 (SREBP1) is of particular interest. On the one hand, SREBP1 is fundamental for the biosynthesis of fatty acids, cholesterol, triglycerides and phospholipids and abnormal SREBP-1 activity has been linked with obesity, insulin resistance and type 2 diabetes (24). On the other hand, lipid homeostasis is also essential in the central nervous system to guarantee the integrity of myelin membranes, as they are highly enriched for cholesterol, phospholipids and glycosphingolipids, thus suggesting a link between SREB1 regulation and brain function (25). More in details, Chen et al. found that genes involved in the lipogenic pathway were suppressed in dysbindin knockout mice (a genetic model of schizophrenia) and that maturation of SREBP1 was necessary for induction of an immediate early gene,known to influence cognitive functioning and especially critical for learning and memory (26). This evidence thus points out to a role of SREBP1 modulation in brain development and cognitive function, with possible implications for schizophrenia.

In sum, current literature highlights that MetS may aggravate the cognitive deficits in patients with schizophrenia and that this effect may be mediated by shared genetic factors. Moreover, these data provide a rationale for the hypothesis that MetS may hamper the capacity to restore cognitive deficits through cognitive remediation interventions.

Based on these premises, this study aims at investigating the effect of MetS on cognitive abilities and, innovatively with respect to previous literature, on cognitive outcome after CRT. Secondary objectives include the analysis of the relationship between the cognitive deficit and psychopathological status and, in a subgroup of patients, the evaluation of the effect of SREBF1 rs11868035 genetic polymorphism on both cognitive functioning and MetS.

MATERIALS AND METHODS

Sample

This is a monocentric retrospective study, enrolling 172 patients diagnosed with schizophrenia according to DSM-IV-TR (27) criteria, followed at the Disease Unit for Psychotic Disorders of IRCCS San Raffaele Hospital, Milan, Italy.

After a complete description of the study, informed consent to participation was obtained. The protocol followed the principles of the Declaration of Helsinki and was approved by the local Ethical Committee.

To be included, patients had to satisfy DSM-IV-TR diagnostic criteria for schizophrenia and the following conditions:

- No significant changes in psychopathologic status (requiring hospitalization or major change in pharmacologic treatment) in the 3 months prior to evaluations.
- No evidence of substance dependence or abuse, comorbid diagnoses on Axis I or II, epilepsy, or any other major neurological illness or perinatal trauma, or mental retardation.

One-hundred and thirty-eight subjects underwent 3 months of three 1-h sessions a week of Computer-assisted Cognitive Remediation Therapy performed with the Cogpack Software® (28). The protocol is detailed in previous studies (29, 30). In brief,

sessions consisted of domain-specific neurocognitive exercises, aimed at training the cognitive functions impaired in the patient. CRT was administered by trained psychologists, whose role was to motivate patients and assist them in completing exercises and trying different strategies, without giving them the solutions to the exercises.

Assessment

All patients were assessed for psychopathology, neurocognitive performance and metabolic parameters. In the subsample of patients treated with CRT, neurocognition was evaluated both at baseline and within 2 weeks from the end of the intervention, while metabolic parameters were collected within a 3 months range before starting CRT.

Psychopathology was assessed by means of the Positive and Negative Syndrome Scale (PANSS), administered by trained psychiatrists.

Neuropsychological functioning was assessed with the Brief Assessment of Cognition in Schizophrenia (BACS). The BACS is a neuropsychological battery, designed in two versions (A and B) to evaluate patients before and after rehabilitation programs, without the results being influenced by recall. The entire battery, lasting ∼30 min, consists of the following tests: verbal memory (words recall); working memory (digit sequencing); psychomotor speed and coordination (token motor task); processing speed (symbol coding); verbal fluency (production of words after semantic and literal cue); executive functions (Tower of London). From raw scores of each BACS subtest, both adjusted scores (corrected for age, education, and sex) and equivalent scores were derived, based on normative values for the Italian population (31). Equivalent scores are ranked into a 5-point interval scale, in which 0 sets the limit for pathological performance, 1 is considered as a borderline value, 2 and 3 indicate intermediate "normal" performance and 4 is equal or better than the median value. A global measure of cognitive efficiency (Cognitive Index) is obtained from the equivalent scores mean with a cut-off of 1, in which a score lower than 1 is indicative of global cognitive deficit, while a score of 1 or higher is considered within the normal range.

Data on metabolic parameters (waist circumference measured in centimeters, triglycerides, HDL-cholesterol measured from blood tests, systolic, and diastolic blood pressure measured in sitting position and fasting glycaemia) were collected from clinical records of patients. The presence of metabolic syndrome has been identified based on measurements according to the ATPIIIA criteria (32).

Genotyping

Peripheral venous blood samples were collected and DNA was extracted from whole blood by a manual extraction, using the "Illustra blood genomic Prep Midi Flow kit" (GE Healthcare, Milan, Italy). To identify the single nucleotide polymorphism G/A rs11868035, a standard Polymerase Chain Reaction (PCR) was performed with the following primers: 5′-GAGGAGGCT TCTTTGCTGTG-3′ and 5′-GGGTCAGTTGTCCCTTCTCA-3′. The PCR was carried out in a 10 μl volume containing 150 ng genomic DNA, 1 μl of 1 × Hot Master Taq Buffer with Mg++ (Eppendorf), 0.1 μl of each primer [50 uM], 1 μl of dNTPs [200 μM], 0.1 μl of Hot Master Taq [5 U/μl] (Eppendorf) and 0.5 μl of Dimethyl sulfoxide (DMSO). After an initial step of 3 min at 94°C, 35 cycles of amplification (30 s at 94°C, 30 s at 57°C, and 30 s at 70°C) and a final extension step of 6 min at 70°C were performed. The amplified fragment was then purified by Multi-Screen Colum Loader (MILLIPORE), filled up and packaged with Sephadex G-50 (Sigma-Aldrich's) to remove residual PCR reagents. An aliquot of purified PCR product was then used to perform sequencing reaction, using DYEnamic ET Dye Terminator Cycle Sequencing Kit (GE Healthcare, Milan, Italy). In its turn, sequencing reaction product was purified following the abovementioned protocol, to remove the excess of fluorescent dyes not incorporated in the DNA fragment and then loaded onto a 48 capillaries genetic analyzer (MegaBace 500, GE Healthcare, Milan, Italy).

For the analysis, patients were grouped in G/G homozygous vs. carriers of the A allele, as in previous literature (33).

Data Analysis

First, Analysis of Variance (ANOVA) and Chi Squared Test for dichotomous variables, were performed to evaluate significant differences in socio-demographic, clinical and cognitive measures at baseline between patients grouped according to the presence or absence of MetS.

Second, ANOVA was performed to evaluate significant differences in PANSS scores between patients grouped according to the presence or absence of global cognitive impairment based on the BACS Cognitive Index cut-off.

Third, a Repeated Measures ANCOVA was conducted to evaluate the effect of MetS on changes in cognitive measures after CRT, with adjusted scores of each BACS subtest and the Cognitive Index pre and post-CRT as dependent variables, the diagnosis of MetS (present vs. absent) as independent variable, duration of illness as covariate and time as fixed factor. Tukey Unequal Number HSD *post-hoc* test followed.

Last, the possible influence of SREBF-1 rs11868035 on both metabolic parameters and cognitive performance at baseline was investigated in a subsample of 51 patients. In details, Chi Squared test was used to assess differences in the prevalence of Metabolic Syndrome between genotype groups (G/G homozygous vs. A carriers), while ANOVA was performed to evaluated differences in metabolic indices and BACS subtests' adjusted scores as well as the Cognitive Index.

RESULTS

Effect of Metabolic Syndrome on Clinical and Cognitive Measures at Baseline

Clinical and Socio-Demographic Variables

In the whole sample analyzed, composed by 172 subjects, of which 107 males and 65 females, we observed a prevalence of Metabolic Syndrome of 22%.

Clinical and socio-demographical variables among MetS and non-MetS patients are detailed in **Table 1**. As noted in the table, there are no significant differences between the two groups, except for therapy distribution, showing that patients undergoing

TABLE 1 | Clinical and socio-demographical variables of patients, stratified by Metabolic Syndrome's diagnosis.

	No MetS	MetS	ANOVA/Chi squared test
SEX			
Males	75.70%	24.30%	$\chi^2 = 0.80$ $p = 0.37$
Females	81.50%	18.46%	–
Age	33.23 ± 10.48	35.10 ± 8.70	$F = 1.03$ $p = 0.31$
Education (years)	11.87 ± 2.78	11.21 ± 2.39	$F = 1.77$ $p = 0.18$
Onset	23.65 ± 6.83	23.54 ± 5.47	$F = 0.01$ $p = 0.92$
Duration of Illness	9.48 ± 8.45	11.48 ± 8.43	$F = 1.60$ $p = 0.2$
PANSS			
Positive scale	17.14 ± 5.41	15.64 ± 4.88	$F = 1.66$ $p = 0.2$
Negative scale	21.31 ± 6.10	22.61 ± 7.12	$F = 0.85$ $p = 0.35$
General scale	37.06 ± 8.31	35.21 ± 7.01	$F = 1.11$ $p = 0.29$
Total score	75.52 ± 14.99	73.46 ± 14.33	$F = 0.40$ $p = 0.52$
TREATMENT			
Clozapine	67.31%	32.69%	$\chi^2 = 4{,}86$ $p = 0.02$*
Others	82.50%	17.50%	–

**Significant p-value.*

TABLE 2 | Cognitive measures (Brief Assessment of Cognition in Schizophrenia-BACS adjusted scores) in patients stratified by metabolic syndrome's diagnosis.

	MetS Mean ± SD	No MetS Mean ± SD	ANOVA
BACS			
Verbal memory	34.44 ± 12.44	35.71 ± 10.60	$F = 0.38$ $p = 0.53$
Working memory	16.18 ± 4.59	16.16 ± 4.31	$F = 0.0003$ $p = 0.98$
Psychomotor speed/coordination	67.23 ± 13.72	63.43 ± 18.29	$F = 1.36$ $p = 0.25$
Processing speed	35.97 ± 11.07	36.87 ± 11.43	$F = 0.18$ $p = 0.66$
Verbal fluency	36.32 ± 11.26	35.24 ± 11.65	$F = 0.25$ $p = 0.61$
Executive functions	21.03 ± 6.33	22.77 ± 5.61	$F = 2.57$ $p = 0.11$
Cognitive index	1.08 ± 0.83	1.14 ± 0.81	$F = 0.13$ $p = 0.71$

clozapine treatment have a higher prevalence of Metabolic Syndrome than those treated with other antipsychotics (32.69 vs. 17.5%, $p = 0.02$).

Cognitive Performance

The ANOVA showed no significant differences between patients with or without metabolic syndrome on the cognitive domains assessed by each BACS subtest, nor on the Cognitive Index. **Table 2** shows mean BACS scores in patients with or without metabolic syndrome.

Relationship Between the Cognitive Deficit and Psychopathological Status

The one-way ANOVA with Cognitive Index (poor vs. normal performance) as independent variable and PANSS as dependent variables, showed significant difference on PANSS Total, Positive and Negative scales, with higher scores in patients with cognitive deficit, while no significant differences were detected for General symptomatology. Detailed results are reported in **Table 3**.

TABLE 3 | Symptoms' severity (Positive and Negative Syndrome Scale- PANSS scores) in patients stratified according to presence or absence of global cognitive impairment.

	Cognitive deficit Mean ± SD	No cognitive deficit Mean ± SD	ANOVA
PANSS			
Positive scale	16.37 ± 5.07	18.55 ± 5.68	$F = 4.95$ $p = 0.02$*
Negative scale	20.83 ± 6.06	25.02 ± 6.80	$F = 12.78$ $p < 0.001$*
General scale	36.84 ± 8.25	38.82 ± 7.91	$F = 1.72$ $p = 0.19$
Total score	74.04 ± 14.90	82.40 ± 15.18	$F = 9.04$ $p = 0.003$*

**Significant p-value.*

Effect of Metabolic Syndrome on Cognitive Remediation Therapy Outcome

In the sample of patients treated with 3-months CRT (138 subjects, of which 86 males and 52 females), the Repeated Measures ANCOVA showed a significant effect of MetS for executive functions and global cognition. Detailed results are reported in **Table 4**, while **Figures 1**, **2** show trajectories, respectively, of executive functions and global cognition from pre- to post-CRT in patients with and without MetS. *Post-hoc* analyses showed a significant improvement from baseline after CRT for both executive functions ($p = 0.0009$) and global cognition ($p = 0.0001$) only among patients without a diagnosis of Metabolic Syndrome.

Effect of SREBF1 Polymorphism on Metabolic Syndrome and Cognitive Measures

In the subsample of 51 patients, genotyped for SREBF1 rs11868035, the relative frequencies of the genotypes were 6% A/A, 53% GA, and 41% GG. The genotype distribution is in Hardy-Weinberg equilibrium ($Chi^2 = 2.24$).

Significant differences between genotype groups emerged both in metabolic indices and cognition. As reported in **Table 5**, we found a significantly higher prevalence of Metabolic Syndrome, as well as significantly higher triglycerides values among G/G homozygous compared to A carriers.

Regarding cognition, the ANOVA showed a significant difference between genotypes only for processing speed, with poorer performance among G/G homozygous compared to A carriers. Detailed results are provided in **Table 6.**

DISCUSSION

Cognitive dysfunction is a core feature of schizophrenia, with a significant negative impact on long-term functional outcome (34), thus representing a critical treatment issue. Currently available antipsychotic drugs seem to have only limited effects

TABLE 4 | Effects of metabolic syndrome on cognitive improvement after cognitive remediation therapy (Repeated Measures ANCOVA).

BACS	*F*	Degrees of freedom	*p*-value
VERBAL MEMORY			
Duration of illness	7.8	1	0.009*
MetS diagnosis	2.36	1	0.12
Time	16.13	1	<0.001*
Time*Duration of illness	1.46	1	0.23
Time*MetS diagnosis	0.67	1	0.41
WORKING MEMORY			
Duration of illness	4	1	0.048*
MetS diagnosis	0.003	1	0.95
Time	2.85	1	0.09
Time*Duration of illness	0.68	1	0.40
Time*MetS diagnosis	0.82	1	0.36
PSYCHOMOTOR SPEED AND COORDINATION			
Duration of illness	0.002	1	0.96
MetS diagnosis	0.50	1	0.47
Time	0.39	1	0.53
Time*Duration of illness	0.10	1	0.74
Time*MetS diagnosis	4.27	1	0.04*
VERBAL FLUENCY			
Duration of illness	0.46	1	0.49
MetS diagnosis	1.32	1	0.25
Time	7.2	1	0.008*
Time*Duration of illness	1.25	1	0.26
Time*MetS diagnosis	1.55	1	0.21
PROCESSING SPEED			
Duration of illness	6.33	1	0.01*
MetS diagnosis	0.73	1	0.39
Time	8.91	1	0.003*
Time*Duration of illness	1.11	1	0.29
Time*MetS diagnosis	1.08	1	0.29
EXECUTIVE FUNCTIONS			
Duration of illness	0.21	1	0.64
MetS diagnosis	4.50	1	0.03*
Time	7.89	1	0.006*
Time*Duration of illness	1.09	1	0.29
Time*MetS diagnosis	0.38	1	0.53
COGNITIVE INDEX			
Duration of illness	0.29	1	0.59
MetS diagnosis	4.35	1	0.04*
Time	13.59	1	<0.001*
Time*Duration of illness	1.31	1	0.25
Time*MetS diagnosis	2.08	1	0.15

*Significant of p-value.

on cognition (35, 36) and, while some antipsychotics may outperform others in certain domains, no antipsychotic shows a global uniform cognitive profile (37). So far, cognitive remediation strategies are considered the best tool to improve cognition in schizophrenia, but there is still a high variability in results, pointing to the need of identifying predictors. Moreover, cognitive deficits are not only the main determinants of functional disability in treatment responders, but they are also associated with treatment resistance and a better clarification of the individual's factors associated with cognitive function may thus impact on global outcome. Among such factors, metabolic syndrome has gained increasing attention as a possible cause of more pronounced cognitive deterioration.

Based on these premises, innovatively with respect to previous literature, we investigated the relationship between MetS, cognition and outcome after CRT, in a sample of patients with chronic schizophrenia with the hypothesis that metabolic alterations may hamper the capacity to restore cognitive deficits. Secondary aims include the analysis of the association between the cognitive impairment and psychopathological status and, in a subgroup of patients, the evaluation of the effect of SREBF1 rs11868035 genetic polymorphism on both cognitive functioning and MetS.

Concerning the relationship between cognitive abilities and psychopathology, our results showed that patients with global cognitive deficit had more severe positive and negative symptoms, as assessed with the PANSS. These data add to the still open debate on the relationship between cognitive measures and symptoms' domains and are in line with previous evidence of an association between poorer cognitive performance and both positive and negative symptoms (38, 39).

Addressing metabolic syndrome, the prevalence in the sample was 22%, in line with the meta-analysis of Malhotra et al. reporting that the prevalence in patients treated with antipsychotics varies across studies from 11–69% (19). However, more recent meta-analyses indicate an overall rate of MetS of over 30% in patients with schizophrenia, with increased risk related to duration of illness and specific antipsychotic treatments (40, 41). The relatively low prevalence recorded in our sample may depend on the younger mean age (under 35) compared to other studies, as well as on the different antipsychotic treatments.

When analyzing the relationship between metabolic status and cognitive measures at baseline, no significant differences emerged between patients with or without MetS. The lack of a significant association between the diagnosis of Mets and poorer baseline cognitive abilities is in line with results of CATIE, reporting no relationship between MetS and cognitive deficit (23). However, several studies have found associations between some cognitive domains and both Metabolic Syndrome, as well as its components (21, 42).

The mechanisms by which MetS may influence cognition have been investigated in non-psychiatric populations. Several reviews (17, 43, 44) show how MetS and its components not only affect the peripheral circulation, but also induce structural and functional alterations in the cerebral vessels, including resistance, stiffening, and remodeling. For instance, changes in cerebral microcirculation also contribute to the development of cerebral small vessel disease possibly leading to white matter lesions, changes in gray matter microstructure, cerebral microbleeds, and neuronal atrophy. However, this association may be less detectable in subjects with schizophrenia, as these patients already present a pronounced cognitive impairment. The biological processes contributing to the core

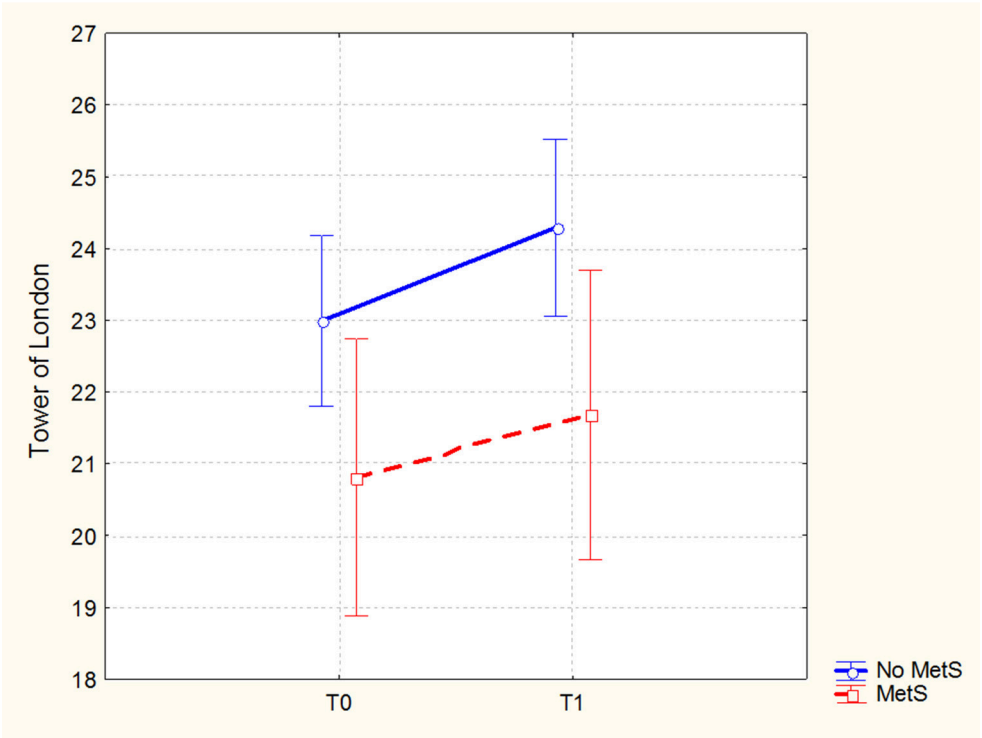

FIGURE 1 | Executive functions (Brief Assessment of Cognition in Schizophrenia-BACS, Tower of London adjusted mean scores and 0.95 confidence intervals) from pre- to post-cognitive remediation therapy in patients stratified by metabolic syndrome.

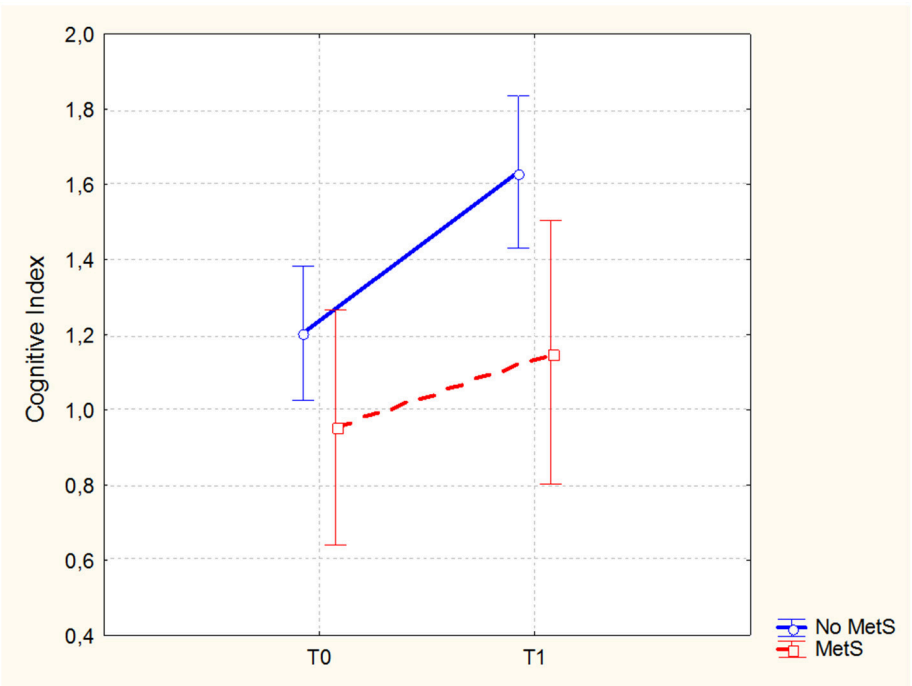

FIGURE 2 | Global cognitive performance (Brief Assessment of Cognition in Schizophrenia-BACS, Cognitive Index mean scores and 0.95 confidence intervals) from pre- to post-cognitive remediation therapy in patients stratified by metabolic syndrome.

TABLE 5 | Mean and standard deviation of metabolic indices in patients stratified by SREBF-1 genotype.

	SREBF-1 G/G	SREBF-1A Carriers	Chi^2/ANOVA
Metabolic syndrome	52.38%	16.67%	$p = 0.007$*
Waist circumference	97.21 ± 13.93	93.00 ± 14.67	$p = 0.41$
Triglycerides	195.05 ± 101.94	135.43 ± 87.67	$p = 0.03$*
HDL cholesterol	42.9 ± 11.43	48.24 ± 14.29	$p = 0.171$
BLOOD PRESSURE			
Systolic	115.95 ± 14.02	115 ± 8.9	$p = 0.775$
Diastolic	74.29 ± 6.76	75 ± 6.93	$p = 0.722$
Fasting plasma Glycaemiae	96.35 ± 32.26	89.6 ± 31.74	$p = 0.468$

Significant of p-value.

TABLE 6 | Cognitive measures (Brief Assessment of Cognition in Schizophrenia-BACS adjusted scores) in patients stratified by SREBF-1 genotype.

	SREBF-1 G/G Mean ± SD	SREBF-1A carriers Mean ± SD	ANOVA
BACS			
Verbal memory	3.33 ± 12.80	38.16 ± 9.25	$F = 2.45$ $p = 0.12$
Working memory	15.19 ± 4.81	16.86 ± 3.77	$F = 1.93$ $p = 0.16$
Psychomotor speed/coordination	68.01 ± 20.40	69.65 ± 15.58	$F = 0.10$ $p = 0.74$
Processing speed	33.31 ± 12.78	41.36 ± 9.20	$F = 6.85$ $p = 0.01$*
Verbal fluency	33.73 ± 10.38	38.86 ± 12.49	$F = 2.37$ $p = 0.12$
Executive functions	20.60 ± 7.37	22.86 ± 5.58	$F = 1.51$ $p = 0.22$
Cognitive index	1.02 ± 0.86	1.33 ± 0.84	$F = 0.56$ $p = 0.21$

Significant of p-value.

cognitive impairment are extremely relevant also in a therapeutic perspective. Interestingly CRT, which is the current gold standard for treatment of cognitive deficit has been proved to induce several structural and functional brain changes, probably through modulation of neuroplasticity (45, 46).

With the hypothesis that metabolic status could influence the patient's response to CRT, we analyzed the effects of MetS diagnosis on cognitive outcome in a sample of patients who completed a 3-months CRT protocol. In particular, we observed significant effects of the diagnosis of metabolic syndrome on executive functions, in its subcomponent of planning tested with the Tower of London, and on the Cognitive Index, a measure of global cognitive efficiency. Planning is a core domain of cognition, typically impaired in schizophrenia. It can be defined as a higher cognitive function, relying on a broad neural network that involves also other specific and lower level cognitive functions (i.e., memory and attention), also directly trained through CRT (47). Our results, showing a significant improvement in executive functions, as well as in the cognitive index only among patients without MetS, suggest that the standard CRT protocol may not be sufficient for patients with MetS. It is important to remind that, as explained above, MetS creates changes in brain microcirculation and in gray matter microstructure. Even though the mechanisms underlying CRT are not yet clarified, it can be hypothesized that the molecular processes and subsequent brain changes associated with MetS may provide a disadvantageous environment, negatively affecting CRT.

Although CRT currently represents the best available tool to treat cognitive deficits, effect sizes of improvements fall into a low-to-medium range, outlining the need to look for individual predictors of response, also as possible target for further potentiation. Our results suggest that future studies should address strategies for treatment of MetS, in order to make CRT much more effective in people affected by schizophrenia. For instance, recent evidence supports the effect of simple aerobic exercise (AE) on cognition, as AE also induces a cascade of molecular processes and brain volume changes (48). These data provide a biological rationale for a synergy of AE and CRT, with the hypothesis that a combined intervention will produce greater improvements that may be particularly relevant in patients affected by MetS, with additional benefits on physical health.

Further supporting a convergence in the biological mechanisms underlying both MetS and cognition, our results also revealed significant effect of SREBF-1 genetic polymorphism. In details, concerning MetS, a significant difference between the two genotypes emerged, showing that G/G homozygous subjects have higher triglycerides, as well as an increased prevalence of MetS, compared to carriers of the A allele. It is known that SREBPs are implicated in the synthesis of fatty acids, triglycerides, and cholesterol in all organs (49) and our finding supports previous literature showing an association between the G allele and the risk to develop Type II Diabetes, as well as other metabolic disturbances in both non-psychiatric populations and patients with schizophrenia (50, 51). Regarding cognitive functions, the role of SREBF on cognition is less explored and far from being clear. Our results evidenced significantly lower scores among patients G/G homozygous, compared to A carriers in processing speed. This domain is particularly relevant in schizophrenia, as it correlates with poor prognosis and functional disability (52). Moreover, processing speed usually shows only modest improvement after CRT (53). Interestingly, in a neuroimaging study, Bollettini et al. observed that the rs11868035 G/G genotype is associated with increased fractional anisotropy in several white matter tracts mainly located in the left hemisphere of patients affected by schizophrenia (54). Although they did not explore neuropsychological correlates, increases in fractional anisotropy have been associated with poorer cognitive performance in visuo-spatial abilities in neurological conditions (55). Our finding is also in line with a recent study of Chen et al. which showed, in an animal model of schizophrenia, that SREBP1 modulates an immediate early gene, known to influence cognitive functioning (26). This evidence thus points out to a role of SREBP1 modulation in brain development and cognitive function, with possible implications for schizophrenia.

This study presents some limits that need to be acknowledged. First, this is a retrospective study and the association between MetS and cognitive outcome after CRT still needs more exhaustive investigation through prospective clinical trials, also including a control group. Moreover, patients were not stratified

according to different pharmacological treatments and age groups, relevant aspects that need to be taken into account in future studies. Furthermore, it is to notice that all patients were taking chronic pharmacological treatment and we cannot exclude that medications may cause both disturbances in cognition and metabolic syndrome, rather than there being any direct link. Finally, the analysis on the genotype is conducted on a very limited sample size, which hampers the statistical power and therefore no conclusions can be drawn.

Despite these limitations, results suggest that MetS influences CRT-induced dynamic modulation of cognitive functions. The data also point out possible effects of SREBF-1 polymorphism on both cognition and metabolic syndrome, highlighting the need to further explore putative converging mechanisms underlying both conditions. At the clinical level, results emphasize the importance to comprehensively assess the metabolic status of patients in rehabilitation settings in order to implement individualized strategies to reach a better global outcome in patients with schizophrenia. In this view, the evaluation of MetS before starting CRT may allow to identify a sub-population of "difficult-to-treat" patients, requiring an integrated and more intensive approach to improve cognition. On the one hand, these patients may benefit from a concomitant intervention specifically addressing MetS, such as aerobic exercise, on the other they may also obtain further gains with a longer duration of CRT.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Good Clinical Practice with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Commitee of ASL Città di Milano and prorogated by the Ethics Commitee of Ospedale San Raffaele di Milano.

AUTHOR CONTRIBUTIONS

MBo: Undertook the data analysis. MBo, MBu, and LS: Drafted the manuscript. MBu, MBe, FC, LB, CG, MS, FB, and SB: Collected the data and contributed to data interpretation. FB and SB: Performed bibliographic search. MBo, MBu, MBe, and MS: Were engaged in forming the concept and designing the study, supervised by RC. All authors critically revised the manuscript, contributed to and have approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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Antipsychotic Drug Responsiveness and Dopamine Receptor Signaling; Old Players and New Prospects

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Antipsychotic drugs targeting dopamine neurotransmission are still the principal mean of therapeutic intervention for schizophrenia. However, about one third of people do not respond to dopaminergic antipsychotics. Genome wide association studies (GWAS), have shown that multiple genetic factors play a role in schizophrenia pathophysiology. Most of these schizophrenia risk variants are not related to dopamine or antipsychotic drugs mechanism of action. Genetic factors have also been implicated in defining response to antipsychotic medication. In contrast to disease risk, variation of genes coding for molecular targets of antipsychotics have been associated with treatment response. Among genes implicated, those involved in dopamine signaling mediated by D2-class dopamine receptor, including *DRD2* itself and its molecular effectors, have been implicated as key genetic predictors of response to treatments. Studies have also reported that genetic variation in genes coding for proteins that cross-talk with DRD2 at the molecular level, such as *AKT1*, *GSK3B*, *Beta-catenin,* and *PPP2R2B* are associated with response to antipsychotics. In this review we discuss the relative contribution to antipsychotic drug responsiveness of candidate genes and GWAS identified genes encoding proteins involved in dopamine responses. We also suggest that in addition of these older players, a deeper investigation of new GWAS identified schizophrenia risk genes such as *FXR1* can provide new prospects that are not clearly engaged in dopamine function while being targeted by dopamine-associated signaling molecules. Overall, further examination of genes proximally or distally related to signaling mechanisms engaged by medications and associated with disease risk and/or treatment responsiveness may uncover an interface between genes involved in disease causation with those affecting disease remediation. Such a nexus would provide realistic targets for therapy and further the development of genetically personalized approaches for schizophrenia.

Keywords: dopamine, risk factors, antipsychotic agents, genetic variants, schizophrenia therapy

INTRODUCTION

Whole genome association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with enhanced risk for schizophrenia (1). These studies underscore the extraordinary polygenicity of schizophrenia and raises the hope that understanding the developmental causes of the disease may lead to new therapies (2, 3). Yet, most of these SNPs were not found in genes traditionally associated to the mechanism of action of existing antipsychotic drugs and it remains unclear whether it will be practically possible to intervene on pathways involved in disease causation.

Alternatively, several genetic factors have been shown to modulate the severity of schizophrenia symptoms and treatment responsiveness. Twins studies have demonstrated that, like pathophysiology and risk for schizophrenia, response to antipsychotic medication is a heritable trait (4, 5). Furthermore, reports have highlighted that schizophrenia risk, pathophysiology and response to treatments likely share a common genetic background (6). Based on such evidence, GWAS are currently considered a powerful tool to identify new molecular targets for antipsychotic treatments, while confirming the involvement of the already established ones. Genes and related proteins belonging to dopaminergic signaling have consistently been indicated as key elements for antipsychotics treatment responsiveness by both hypothesis- and data-driven pharmacogenetic studies.

The neurotransmitter dopamine is involved in the regulation of several cerebral functions including, reward, mood, sensory motor gating, affect and locomotor functions, learning and motivation (7, 8). Importantly, dopamine receptors, especially the D2 dopamine receptor (DRD2), are major pharmacological targets of all existing antipsychotic drugs (9–11) (**Table 1**). Several dopamine producing neuron populations exist in the adult brain. Cells from the nigrostriatal pathway project from the *substantia nigra pars compacta* to the caudate nucleus and the *putamen* in the striatum (12, 13). Dopamine neurons from the mesolimbic pathway (14) project from the ventral tegmental area to the ventral striatum, amygdala, and several cortical areas (e.g., pre-frontal cortex) expressing dopamine receptors (15). Dopamine neurons from the infundibular nucleus of the hypothalamus are also involved in the dopamine-mediated regulation of the pituitary gland (16). Among these neuronal networks, the mesolimbic pathway has received the most attention in the context of schizophrenia.

Here, we will provide a brief overview of the biological regulation of dopamine and of the cellular mechanisms engaged by its receptors; for readers that would want a more complete description of the biology of dopamine receptor we would suggest more exhaustive reviews (7, 17, 18). We will then examine how molecular pathways involved in dopamine function intersect with genetic factors for antipsychotic drug responsiveness and, if applicable, disease causation. To evaluate this, two investigators independently conducted a systematic PubMed search (June 2018) for studies of antipsychotic medication responsiveness involving dopamine signaling cascade and related genetics. Combinations of the following keywords were used: Antipsychotics (APs), first-generation APs, second-generation APs, AP response, AP pharmaco-genetics, AP pharmaco-genomics, schizophrenia risk, schizophrenia genetics, schizophrenia genomics, schizophrenia polygenic risk score, dopamine, dopamine receptors, dopamine signaling. Finally, we will underscore how a newly discovered pathway may provide new avenues to investigate the genetic of antipsychotic drug responsiveness.

TABLE 1 | List of main First-Generation (FGA) and Second-Generation (SGA) Antipsychotics with respective target Dopamine (DA) Receptors (D1–D5).

Antipsychotic		Target DA receptor				
		DRD1	**DRD2**	**DRD3**	**DRD4**	**DRD5**
FGA	Haloperidol	–	+++	++	+	–
	Chlorpromazine	+	+++	++	–	?
	Amisulpiride	–	++++	++++	–	?
	Perphenazine	?	++++	–	–	?
SGA	Aripiprazole	+	++++ PA	+++ PA	+ PA	–
	Clozapine	++	++	++	+++	+
	Olanzapine	+++	+++	+++	+++	+
	Lurasidone	?	+++	?	?	?
	Paliperidone	++	+++	+++	++	?
	Quetiapine	+	++	++	+	–
	Risperidone	+	+++	++	+++	+
	Ziprasidone	+++	+++	+++	+++	+
	Asenapine	++	++	+++	++	?
	Iloperidone	+	+++	+++	++	?

+ Low Affinity.
++ Medium Affinity.
+++ High Affinity.
++++ Very High Affinity;—No Affinity; ? Uncertain data.
PA, Partial Agonist.

DOPAMINE HOMEOSTASIS

During dopamine synthesis, the amino acid tyrosine undergoes hydroxylation to levodopa that is catalyzed by the enzyme tyrosine hydroxylase (TH) (**Figure 1**). This is followed by a decarboxylation of levodopa to dopamine by the enzyme dopa-decarboxylase (DDC) (19). In adult neurons, dopamine is loaded into synaptic vesicles by the vesicular monoamine transporter 2/solute carrier family 18 member A2 (VMAT2/SCL18A2) (20). Following synaptic release, dopamine can either be preferentially up-taken or degraded, depending upon neuronal circuit. The major transporter in charge of dopamine reuptake is the dopamine transporter/solute carrier family 6 member 3 (DAT1/SLC6A3) (21). This transporter is the key element in the mechanism removing extracellular dopamine in striatal structures. However, dopamine has also been shown to be reuptaken by other transporters such as the norepinephrine transporter/solute carrier family 6 member 2 (DAT/SLC6A2) in brain regions expressing low levels of the SLC6A3 gene product (22, 23). The major enzyme in charge of dopamine degradation

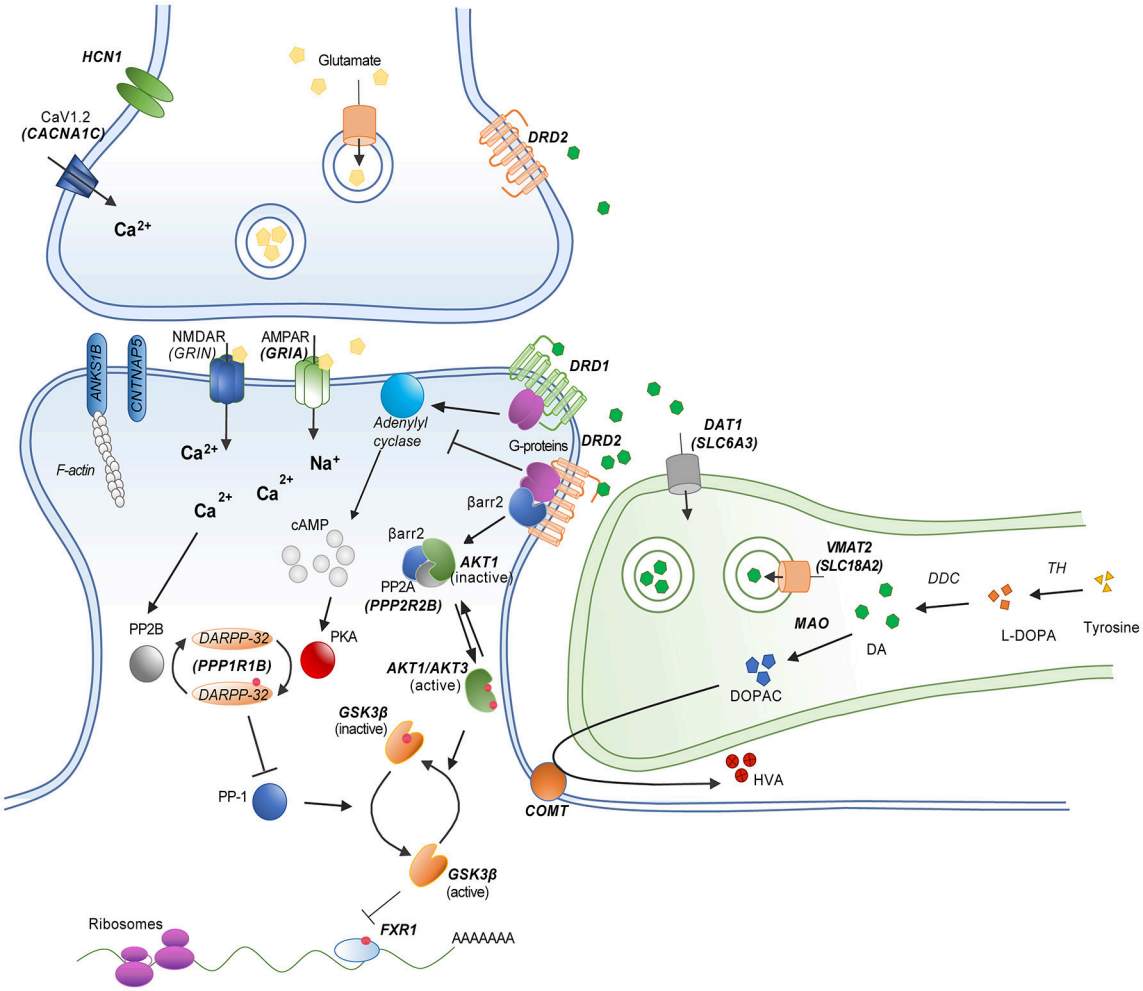

FIGURE 1 | Schematic model of a striatal dopamine/glutamate synapse showing the functional relationship between different dopamine related gene products. Genes implicated in candidate gene studies and/or GWAS are indicated in bold. Red dots represent phosphorylation events.

is the Catechol-O-methyltransferase (COMT), which methylates dopamine into 3-metoxytyramine (3-MT). Degradation by COMT is the principal mechanism of dopamine clearance in the pre-frontal cortex. Dopamine can also be oxidized into 3,4-Dihydroxyphenyl-acetic acid (DOPAC) by the monoamine oxidases (MAOA and MAOB). The final step of dopamine metabolism involves the production of homovanillic acid (HVA) from 3-MT by monoamine oxidases or from DOPAC by COMT (24).

DOPAMINE RECEPTORS SIGNALING MECHANISMS

Dopamine exerts its effects via the stimulation of five different G-protein coupled dopamine receptors (**Figure 1**). These receptors are divided in two groups based on their coupling and gene structures (17). D1-class dopamine receptors DRD1 and DRD5 are mostly coupled to $G_{\alpha s}$ G-proteins and encoded by genes that are devoid of introns. D2-class dopamine receptors DRD2, DRD3, and DRD4, are encoded by genes that comprise introns and are generally coupled to $G_{\alpha i/o}$ G-proteins. D1-class receptors mediate post-synaptic responses to dopamine. In contrast D2-class receptors can both mediate post-synaptic responses and act as presynaptic auto-receptors to limit dopamine synthesis and release (19). Of note, the *DRD2* gene encodes two splice variants of the receptor. The long isoform (D2L) is mostly expressed on post-synaptic neurons while the short isoform (D2S) is preferentially expressed by pre-synaptic dopamine neurons (25).

Activation of D1-class receptors results in an increased production of the second messenger cyclic Adenosine Monophosphate (cAMP) by class 3 adenylyl cyclases (ADCY) (26). Activation of D2-class receptor results in a reduction of cAMP by inhibiting this same mechanism (27). Major downstream effectors of dopamine receptors are cAMP-dependent protein kinases A (PKA) (7). PKA are holoenzymes comprised of a catalytic subunit and different regulatory subunits. Catalytic subunits are encoded by the genes *PRKACA*,

PRKACB, and *PRKACG*. Regulatory subunits are encoded by the *PRKAR1A, PRKAR1B, PRKAR2A,* and *PRKAR2B* genes in humans. Regulation of PKA activity by dopamine receptors is involved in several cellular processes including, among others, the regulation of gene expression by transcription factors and the regulation of ionotropic receptors for various neurotransmitters. Among several targets of interest, the activity of cAMP response elements binding proteins family of leucine zipper transcription factors (i.e., CREB1) can be modulated by dopamine. Subunits of AMPA and NMDA ionotropic glutamate receptors (i.e., GRIN1, GRIA1, GRIA4) are also regulated by PKA downstream of dopamine receptors (7, 18). Finally, the protein phosphatase 1 regulatory subunit 1B (PPP1R1B/DARPP-32) has been shown to be regulated by dopamine and cAMP and to play a role in the balance of phosphorylation/dephosphorylation of several PKA substrates involved in dopamine receptor signaling and the integration of metabotropic (slow) and ionotropic (fast) neurotransmission (28, 29).

The signaling of dopamine receptors is not restricted to the regulation of cAMP production. Some receptors have been reported to have the possibility to couple to $G_{\alpha Q}$ G-proteins to regulate intracellular inositol and calcium signaling (30, 31). Furthermore, activation of $G_{\beta\gamma}$ G-protein subunits by DRD2 results in neuronal hyperpolarization by regulating the activity of L and N-Type calcium channels (LTCC and NTCC) and G-protein gated inwardly rectifying potassium channels (e.g., GIRK2/KCNJ6) (7). Furthermore, DRD2 modulates neuronal function by acting on G-protein independent mechanisms. Following their activation, dopamine receptors are phosphorylated by G-protein receptor kinases (e.g., GRK2, GRK6) (32). This leads to the recruitment of beta-arrestins (ARBB1 and ARBB2), which inactivate G-protein coupling, stimulate receptor internalization and mediate additional cell signaling functions (33, 34). In the case DRD2, the recruitment of ARBB2 results in the formation of a protein complex that favors the inactivation of protein kinases from the Akt family (AKT1, AKT2, AKT3) by protein phosphatase 2 holoenzymes (i.e., PPP2R2B, PPP2CA, PPP2CB). The inactivation of AKT kinases downstream of DRD2 releases the inhibition of glycogen kinase 3 family proteins (GSK3A, GSK3B) thus increasing their activity (35, 36). The nature of the GSK3 substrates involved in DRD2 signaling is still unclear. One frequently investigated potential target is the canonical wingless (WNT) signaling transcription factor beta-catenin (CTNNB1) (37). However, beta-catenin does not appear to be a major determinant of antipsychotic drug action downstream of DRD2 in mice (38). The RNA binding protein fragile-X-mental-retardation-autosomal-homolog-1 (FXR1) is a recently identified GSK3B substrate (39) that has been shown to modulate ionotropic glutamate receptor functions *in vivo* (40, 41). It is thus possible that FXR1 may contribute to the regulation of neuronal functions by GSK3-mediated DRD2 signaling (42).

CANDIDATE GENE STUDIES

Candidate gene studies have often been focused on the role of dopamine receptors and related genes (namely *DRD1, DRD2, DRD3, DRD4,* and *DRD5*) as modulators of antipsychotic drug responsiveness and schizophrenia symptoms. In particular, genetics studies on schizophrenia endo-phenotypes, i.e., behavioral and/or neuroimaging phenotypes associated with the disorder, have suggested that variations in genes coding for this cluster of receptors, especially those coding for DRD2, DRD3, and DRD4, are associated with cognitive deficits and related brain pre-frontal cortex malfunction, which are typical phenotypes of schizophrenia. Candidate gene investigations have identified allelic variation in SNPs of functional relevance to D2, D3, and D4 receptors to be associated with response to antipsychotic medication (**Table 2**).

Genetic studies on the role of *DRD2* genetic variation in the pathophysiology and risk for schizophrenia and response to antipsychotics have been motivated by the evidence that DRD2 antagonism is a key mechanism of action of antipsychotic agents to the extent that occupancy of this receptor subtype is correlated with antipsychotic potency (**Table 1**). The *DRD2* locus is the only dopamine receptor gene for which common variants have been associated to schizophrenia risk by GWAS (1). Candidate gene studies on functional polymorphisms of *DRD2* gene, involving SNPs that affect *DRD2* gene expression and/or *DRD2* Long/Short transcription isoform ratio, along with DRD2 membrane density, have also pointed to a role of such genetic variation in schizophrenia. For example, rs1800497 (also known as Taq1A) is a missense variant determining a Glu-to-Lys substitution at position 713 of *DRD2* gene and associated with the gene mRNA stability and expression (68), striatal dopamine receptor density (69), and modification in DRD2 binding in human striatum (70). Interestingly, rs1800497 has been associated with performance in Working Memory, a prototypical endophenotypes of schizophrenia, mainly subtended by pre-frontal cortex activity (71). Similarly, rs1801028, a SNP occurring in *DRD2* gene and determining a Ser-to-Cys substitution at position 311 of *DRD2* gene (Ser311Cys) has been shown to alter the physiology and function of DRD2 receptor (72) and has consistently been associated with risk for schizophrenia in a number of studies since 1994 (73)—for a meta-analysis see: (74). Another example has been provided by rs1076560, a SNP located in intron 6 of the *DRD2* gene, whose T allele has been reported to shift the splicing of *DRD2* transcript from D2S to D2L isoforms (75). Rs1076560 has been associated with risk for schizophrenia (76), behavior and brain activity during cognitive and emotion processing in healthy controls and patients with schizophrenia (77), with efficiency of pre-fronto-striatal activity during Working Memory (78) and levels of striatal dopamine (79).

Since DRD2 neuronal signaling is mediated by a number of partner molecular elements downstream of the receptor, including proteins belonging to cAMP-dependent and cAMP-independent pathways, such as AKT1, GSK3B, PPP2R2B, and CTNNB1, not surprisingly also genes coding for such proteins have been associated with phenotypes that are relevant to schizophrenia pathology. For example, rs1130233, a *cis*-eQTL of *AKT1* gene that interacts with rs1076560 in affecting pre-frontal *AKT1* mRNA expression levels along with level of phosphorylation of the AKT1 kinase target GSK3beta, interacts with rs1076560 also on cingulate cortex activity during attentional control, another cognitive function typically impaired

TABLE 2 | Genes and corresponding mutation implicated in antipsychotic response/resistance by Candidate gene studies or GWAS.

	Gene	Mutation	Biological function/effect	Study	Pharmacogenetic effect
Candidate genes	*DRD2*	rs1800497 or Taq1A	Missense, Glu-to-Lys substitution at position 713; affects *DRD2* mRNA stability and expression along with striatal DRD2 density and binding	(43)	Nemonapride response
				(44)	Haloperidol response
				(45)	Risperidone response
				(46)	Aripiprazole response
		rs1801028	Ser-to-Cys substitution at position 311; affects the physiology and function of DRD2 receptor	(47)	Risperidone response
		rs1079597 or Taq1B	B1 allele associated with reduced DRD2 density in striatum	(48)	B2 allele associated with response to Clozapine
		rs1799732 or −141C Ins/Del	Deletion (vs. insertion) of cytosine at position-−141, located in the 5′ promoter region	(49)	Chlorpromazine response
		rs2514218	Genome Wide Association with SCZ	(50)	Risperidone and Aripiprazole response in first episode psychosis
	DRD3	rs6280	Serine to glycine substitution at amino acid position 9; associated with altered DRD3 dopamine affinity and density in some brain areas	(51)	Clozapine response
				(52)	Positive symptom response to Olanzapine
				(53)	Aripiprazole response
	DRD4	rs4646984 or 48bp-VNTR	48 bp Variable Number Tandem Repeat (48.bp VNTR) falling within the third exon of the gene	(54)	Response to multiple neuroleptics
				(55)	Clozapine response as compared to other APs
				(56)	Clozapine response
				(57)	Response to different APs
	COMT	rs4680 or Val108Met	Met/Met subjects have lower enzyme activity (hence, reduced prefrontal dopamine clearance) as compared to Val/Val individuals	(58)	Met/Met associated with reduced response to FGA
				(59)	Val/Val associated with reduced response of Negative Symptoms to Olanzapine
				(60)	Met/Met associated with better response of Cognitive Symptoms to Clozapine
				(61, 62)	Interaction with *NOTCH4* SNP2 polymorphism on response to FGA
				(62)	Response to multuiple-AP treatment
				(63)	Risperidone response
				(64)	Risperidone response
	SLC6A3	3'VNTR	SLC6A3 expression levels	(65)	AP resistance
GWAS genes	Unknown	rs17390445	Genome Wide Association with SCZ; located on Chromosome 4 in intergenic position; unknown function	(66)	Ziprasidone response
	ANKS1B	rs7968606	Unknowkn	(66)	Olanzapine response
	CNTNAP5	rs17727261	Unknown	(66)	Risperidone response
	AKT1	rs1130233	*AKT1* mRNA expression levels along with level of phosphorylation of the AKT1 kinase	(67)	Interaction with DRD2 rs1076560 on Olanzapine response

Putative biological impacts along with pharmacogenetics results are also reported.

by schizophrenia (67). Similarly, rs12630592 (80) and rs609412 (81), two eQTLs of *GSK3beta* and *PPP2R2B* gene, respectively, have been associated with cognitive performance and related brain activity critically implicated in the disorder.

Functional variation of *DRD2* gene has also been studied as predictor of antipsychotic response, (for a review see: (82). In general, dopamine receptors variants associated with reduced expression of the receptor protein or altered functioning were also associated with poorer response to antipsychotic drugs, confirming that these receptors mediate antipsychotic activity. Rs1800497 has been associated with response to nemonapride (43), haloperidol (44), risperidone (45), and clozapine (48). Similarly, rs1801028 has been associated with response to risperidone (47), while interaction between rs1076560 and rs1130233 has been implicated in response to olanzapine as measured by variation in Positive and Negative Symptoms Scale total scores after 56 days of stable dosage treatment (67). Other SNPs in *DRD2* have been associated with antipsychotics response, including rs1079597, also known as Taq1B, whose B1 allele has been associated with reduced DRD2 protein density in the striatum, and B2 allele has been implicated in response to clozapine (48). Further mutations have been associated with clinical response to chlorpromazine (49) and aripiprazole (46).

DRD3 has systematically been studied by pharmacogenetics since many antipsychotic drugs show high affinity for the D3 dopamine receptor (83). A missense polymorphism in exon 1 of *DRD3* leading to a serine to glycine substitution at amino acid position 9 (rs6280) has been associated with altered DRD3 dopamine affinity and density in some brain areas (84) along with response to first-generation antipsychotics and lack of response to clozapine (51). Some studies (52) found rs6280 to be associated with greater acute positive symptom remission after olanzapine treatment. The same effects were observed for different SNPs in linkage disequilibrium with rs6280. Further studies have also implicated rs6280 in response to the second-generation antipsychotic aripiprazole (53).

Evidence has also established a correlation between response to antipsychotics and functional genetic variation of the *DRD4* gene. In particular, since DRD4 is targeted by clozapine, the most effective antipsychotic drug currently available (85), studies have particularly focused on the effect of genetic variability of corresponding gene on response to such a drug. One of the numerous polymorphisms occurring in *DRD4* gene (namely, rs4646984), is a 48 bp Variable Number Tandem Repeat (48.bp VNTR) falling within the third exon of the gene and resulting in a different length of the third cytoplasmatic loop of DRD4 protein. Importantly, the longer repeat version of the protein has been associated with reduced clozapine binding, thus suggesting the 48 bp VNTR to be an interesting candidate polymorphism to study as predictor of clinical response to this antipsychotic. However, while some studies have reported an effect of rs4646984 on responsiveness to various antipsychotics including clozapine (54–57) several other studies also reported no associations, as reviewed in Zhang and Malhotra (83).

Along with genes coding for dopamine receptors, other genes belonging to the dopaminergic system have also been implicated in pathophysiology and risk for schizophrenia, hence becoming candidate genes for as regulators of response to antipsychotics (**Table 2**). The *COMT* and *SLC6A3* genes, have been particularly relevant within this framework.

Studies have consistently demonstrated that a common polymorphism in *COMT*, rs4680 or Val108Met, is responsible for a functional variation in enzymatic activity with the Met/Met condition characterized by lower activity (hence, reduced pre-frontal dopamine clearance) as compared to the Val/Val condition. A large number of studies have investigated the genetic association between rs4680 and the diagnosis of schizophrenia with mixed results—for a meta-analysis see: (86). In line with the role of COMT in dopamine turnover, pharmacogenetic investigations have reported an association between rs4680 allelic variations and the clinical response to antipsychotics acting on dopamine receptors. An early study found that individuals with schizophrenia carrying a met/met genotype were less responsive to first-generation antipsychotics (58). The val/val genotype has also been associated with reduced response of negative symptoms to olanzapine (59) while the met/met genotype has been associated with increased response of cognitive symptoms to clozapine (60). A number of additional studies, then, confirmed the impact of the COMT Val158Met polymorphism on the efficacy of first-generation (61, 62) and second-generation antipsychotics (63, 64, 87).

Studies have also implicated *SLC6A3,* coding for the dopamine transporter, in schizophrenia phenotypes and response to medication. A functional Variable Number of Repeats polymorphism at 3' end of this gene (3'-VNTR) has been described (88, 89). Alleles of this polymorphism range from 3 to 11 repeats, with the 9- and 10-repeat alleles by far the most common (88, 89). As compared with the 9-repeat allele, the 10-repeat allele has been associated with increased *SLC6A3* gene expression both *in vitro* (90, 91) and in human striatum (92). Furthermore, studies have reported that the 10-repeat allele is associated with more focused cortical activity during memory and attention, two critical endophenotypes of schizophrenia (93–98). Evidence has also documented an interaction between *DRD2* rs1076560 and *SLC6A3* 3'-VNTR on further imaging phenotypes associated with the disorder, such as pre-fronto-striatal activity and volume in humans (77).

Although not directly targeted by antipsychotic medications, the dopamine transporter, may influence dopamine-signaling intensity by virtue of its function of dopamine clearance at the synaptic level and contribute to treatment outcome. Consistently, a number of studies have reported association between polymorphisms in the gene coding for this transporter, including 3'-VNTR and level of response to first and second-generation antipsychotics (65, 99).

GENOME WIDE ASSOCIATION STUDIES

GWAS have allowed for detection of genetic variants associated with a specific disease without any *a priori* hypothesis on its pathophysiology (hypothesis-free or data-driven studies). Such an approach has confirmed the polygenic nature of risk for

schizophrenia since it has identified more than one hundred genetic variants associated with the disorder at genome wide statistical level of significance (i.e., $p < 10^{-8}$) (1). Furthermore, studies attempting at collapsing GWAS multigenic risk in so called Polygenic Risk Scores (PRSs) have probed these scores to predict behavioral and neuroimaging phenotypes of schizophrenia (81). Nevertheless, since GWAS and PRSs do not provide any specific insights into the biological role of genetic variation associated with a disease, different approaches have been developed in order to dissect risk into biologically meaningful pathways and probe the impact of pathway-specific risk variants and PRSs onto pathophysiology of schizophrenia and related phenotypes (81).

Several genes, including *DRD2*, have been associated with schizophrenia by GWAS, even though, most findings have implicated different variants from those detected by candidate gene approaches (1). Nonetheless, as for candidate genes investigation, risk variants identified by GWAS have been implicated in response to antipsychotic medication, confirming the hypothesis that schizophrenia pathophysiology and response to treatment share genetic bases that are partially involving dopamine signaling. A prototypical example is provided by rs2514218, a SNP in *DRD2* gene that was associated with schizophrenia in a large GWAS published by the Psychiatric Genomics Consortium (1). Zhang and collaborators (50) examined whether genotype at this SNP could predict response to 12 weeks of risperidone or aripiprazole treatment in a cohort of patients with first episode of psychosis and found that homozygotes for the risk (C) allele at this SNP had significantly greater reduction in positive symptoms after treatment with either risperidone or aripiprazole as compared to the T allele carriers.

From a pharmacogenomics perspective, a pivotal role in the study of antipsychotics response has been played by the Clinical Antipsychotics Trials of Intervention Effectiveness (CATIE) (100), a multicenter research project promoted by the USA National Institute of Health and investigating the effectiveness of first and second-generation antipsychotics. Within the CATIE, a number of GWAS have been conducted on both treatment response and adverse reaction (**Table 2**).

The first GWAS (66) found a SNP (rs17390445) located on Chromosome 4 in an intergenic position, to be associated with response of schizophrenia positive symptoms to the second-generation antipsychotic ziprasidone, while another SNP in the same intergenic region approached, but did not reach, genome wide significance. Both rs17390445 and this second SNP functions remain unknown, but involvement in dopamine signaling cannot be excluded. Interestingly, in a different study, the same group looked at neurocognition as an outcome of antipsychotics response (101) and found a weak association with SNPs in *DRD2* gene. This same study identified SNPs associated with olanzapine and risperidone response in Ankyrin Repeat and Sterile Alpha Motif Domain-Containing Protein 1B (*ANKS1B*) and in the Contactin-Associated protein-Like 5 genes (*CNTNAP5*) which, are not directly connected with dopamine signaling, but are involved in interneuron communication.

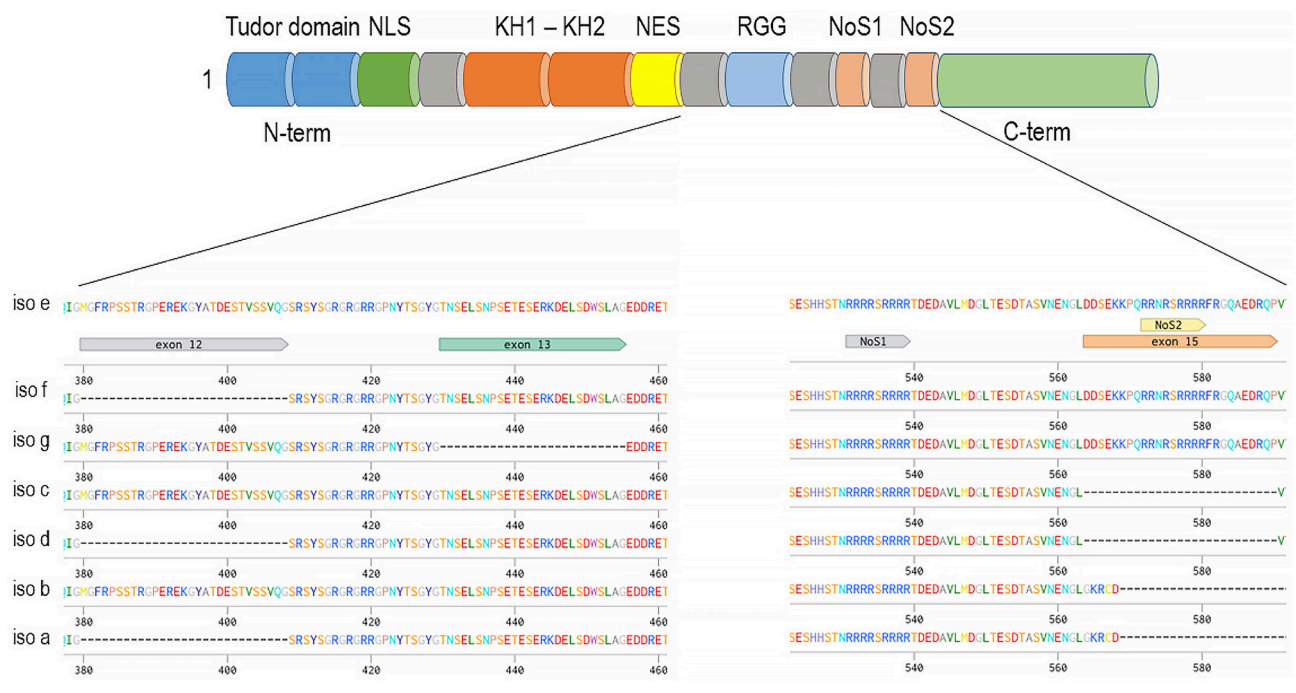

FIGURE 2 | Fxr1 gene (mouse gene depicted as an example) alternatively spliced mRNAs and common domain structure on example of the longest isoform—e. The presence of exon 15 provides an additional nucleolar targeting signal.

GWAS were also used to explore the clinical response to new antipsychotics partially exerting their pharmacological action by blocking dopamine transmission. For example, two of these studies (102, 103) investigated genome wide association with response to Iloperidone, an antagonist of D2 and D3 dopamine receptors also blocking noradrenergic and serotonergic neuronal signaling. None of the SNPs reaching genome wide significance were directly involved in dopaminergic system, even though one of the genes associated, *GRIA4*, codes for AMPA 4 glutamate receptor, that may impact on dopamine neurotransmission by mediation of glutamate signaling. In fact, evidence suggests that aberrant glutamatergic function may alter dopamine system function in psychotic disorders (104, 105) and dopamine exert several of its biological actions by modulating ionotropic AMPA and NMDA glutamate receptor functions (7, 29). Furthermore, dopamine D2/D3 receptor availability is linked to the severity of psychotic symptoms induced by glutamatergic antagonism (106) suggesting that factors influencing glutamate signaling may contribute to dopamine dysregulation and symptoms or response to dopamine-mediated treatments (107). Interestingly, a study (108) looking at drugs targeting proteins encoded by genes GWAS associated with schizophrenia, found that antipsychotics are the only medication surviving enrichment procedures and that, two of the four genes associated with antipsychotic response were either directly implicated in glutamatergic signaling (namely, *GRIN2A*) or indirectly related to dopaminergic system (i.e., *AKT3*). The remaining two genes, *CACNA1C* and *HCN1*, respectively coding for LTCC Ca_v 1.2 and Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, have also been implicated in dopamine signaling modulation (109, 110).

GWAS-based pharmacogenomics studies on antipsychotic response have also used PRS strategies in order to explore the cumulative role of genetic risk on pharmacological effects. For example, one study (111) showed a PRS based on schizophrenia-associated SNPs reported by the Psychiatric Genomics Consortium (1) to predict clozapine response, in that clozapine responders had higher PRS as compared to non-responders. Furthermore, a study using a multistage GWAS-PRS-Pathway Analysis approach (6) to detect genetic variation associated with response to the second-generation antipsychotic lurasidone, suggested associated variants to belong to functional categories of relevance to neuronal transmission and being, at least partially, involved in dopamine signaling by impacting on glutamate an serotonin systems modulation.

AN INTERESTING NEW CANDIDATE GENE, THE FRAGILE X AUTOSOMAL HOMOLOG 1

At first glance, most schizophrenia risk SNPs identified by GWAS are not associated to genes encoding products generally related to dopamine transmission or cellular responses to antipsychotic drugs (1). However, some of these loci may still encode proteins regulated by therapeutic agents. Investigating such genes in the context of known mechanisms of antipsychotic actions may thus be a fruitful avenue for future studies.

For example, the RNA binding protein FXR1 encode on chromosome 3q26.33 represents an interesting new candidate among the genetic risks factors for schizophrenia identified by GWAS (1). FXR1 belongs to the fragile X proteins family, which comprises three RNA-binding proteins members—Fragile X mental retardation protein (FMR1/FMRP), and two Fragile X related proteins—FXR1 and FXR2 (112, 113). All three proteins play a role in regulating the development of several tissues including the brain. Furthermore, these proteins are also implicated in the regulation of neuronal functions (114–116).

The Fragile X mental retardation syndrome is a primary monogenic cause of autistic spectrum disorders (ASDs) and is caused by mutations in FMR1 gene, which is highly expressed in neurons and plays a crucial role in regulating synaptic plasticity (117–119). Both FXR1 and FXR2 have also been recently shown to participate in synaptic plasticity (40, 41, 120). Proteins from this family comprise homologous amino-terminal regions containing a tandem Tudor domain which can mediate binding to methylated lysine on histones (121). The amino-terminal region of these proteins also includes a nuclear localization and export signals (NLS and NES) that mediate shuttling between nucleus and cytoplasm (122). The medial regions of FMR1, FXR1, and FXR2 comprise RNA-binding KH domain and RGG box that allow RNA-binding (123). Finally, the three proteins sequences are most divergent at the carboxyl-terminal domain, which seem to functionally distinguish members of the family (124). All three fragile X family proteins participate to the regulation of mRNA translation, degradation, and are associated with ribosomes (116, 123, 125).

FMR1 is by far the most studied of these three proteins. Interestingly, an exome study of rare genetic variants in schizophrenia identified an enrichment for gene encoding mRNA that are associated to FMR1 (126). Furthermore, a postmortem study has shown altered protein levels of FMR1 targets in the frontal cortex of subjects with schizophrenia or bipolar disorder (127). However, SNPs in *FMR1* are not associated to schizophrenia as defined by a GWAS studies of common genetic risk variants (1). In contrast to FMR1, FXR1 came out of the shadow after its identification as one of the top 30 potential genetic risk factor for schizophrenia (1). For a long time FXR1 was considered either a protein functionally redundant to FMR1 (125) or as its muscle specific homolog (115). Both FXR1 and FMR1 have been shown to interact together but can also have mutually exclusive functions and cellular localization (128). It would thus be premature to conclude that changes in FMR1 targets identified in schizophrenia results from altered FXR1 functions since these two proteins are not biologically equivalent.

The *FXR1* gene encodes seven major alternatively spliced mRNA variants (**Figure 2**, mouse gene used as an example), three of which are expressed specifically in muscles and testis (115, 129). Characterization of the schizophrenia risk allele in the *FXR1* locus (rs34796896) has shown it to be in linkage disequilibrium with splicing quantitative trait loci (sQTL) SNP

(rs1805564) identified in *postmortem* human dorsolateral pre-frontal cortex (DLPFC). Altered splicing in the locus of exon 15 is associated and functionally changed in presence of the rs34796896 schizophrenia risk allele (130). The expression of exon 15 containing isoforms of *FXR1* has only been demonstrated in muscle and testicular tissues by means of western blot. However, the presence of exon 15 RNAs has been detected by sequencing in other tissues, thus suggesting that minor amounts of the protein carrying this exon could be expressed outside of the muscle and testis (130). Similar non-canonical expression of intronic sequence possessing novel isoform of FXR1 in CD4+ T cells was also shown by means of mass spectrometry as a confirmation of RNA-sequencing data (131).

Exon 15 provides an additional nucleolar-targeting signal (NoS2), which is necessary for efficient shuttling between cytoplasm and nucleoli. This shuttling was demonstrated in Cos-7 cell line for the isoform *e* of FXR1 as well as for FXR2 but not for FXR1 isoform *d* or FMR1 (132). Lately, nucleolar localization was also demonstrated for FMRP in Hela cells where FMRP expression is high (133). Interestingly, unlike other family members that exhibit a similar cytoplasmic localization between fetal and adult tissues, FXR1 has been reported to have nuclear localization only during brain and muscle development (114, 115). Thus, it is possible that alteration in the nuclear or nucleolar localization of FXR1, regulated by the NoS2 of exon 15, may contribute to increase the risk to develop schizophrenia.

FXR1 may also contribute to DRD2 and GSK3β mediated signaling (42). Indeed, GSK3β, which is activated following DRD2 stimulation and inactivated by several antipsychotics (134), is able to directly phosphorylate Fxr1 and regulate its expression in a negative manner (39). Furthermore, chronic treatment with lithium and valproate resulting in an inhibition of GSK3β activity also increased Fxr1 expression in the mouse striatum and pre-frontal cortex (39). Interestingly, GSK3β and FXR1 are not affected by chronic lithium or valproate treatment in mice lacking ARBB2 (39). Furthermore, lithium also fails to engage AKT-GSK3 mediated signaling in mice lacking either ARBB2 of DRD2 (135, 136), which suggests an engagement of the DRD2-ARBB2-AKT-GSK3 pathway in the regulation of Fxr1 levels.

A functional interaction has been reported between SNPs affecting the relative expression of the *FXR1* (rs496250) and *GSK3B* (rs12630592) genes (39). In healthy humans, this interaction has been shown to affect amygdala response to emotional faces, as measured by functional Magnetic Resonance Imaging (fMRI). The same interaction was observed on measures of trait Emotional Stability as conceived within the Big Five Model of Personality (39). Finally, interaction between these functional SNPs has been replicated and showed to affect symptoms severity and, putatively, treatment responsiveness in bipolar patients (137). Evidence for a contribution of *FXR1* to dopamine signaling and responsiveness to psychiatric disorders remains preliminary. However, the existing observations would support that *FXR1* is one example of a gene for which further investigation of contribution to schizophrenia, DRD2 signaling, and antipsychotics drug responsiveness is warranted. Further examination of other GWAS identified loci may allow finding additional candidate genes of interest with more distal relations to dopamine neurotransmission.

CONCLUSION

Genetic investigations of risk factors for schizophrenia and determinants of drug responsiveness revealed a very multi-genic landscape for both indications. This suggests that schizophrenia arises from a combination of multiple genetic and socio-environmental hits occurring during development. This is also in line with the variable profile of drug responsiveness observed at the clinical level. This picture can be discouraging as several risk factors may only participate to disease at a pre-onset stage or, contribute to ubiquitous functions across different organs, thus preventing their practical or ethical use as therapeutic targets. Nonetheless, the overlap of schizophrenia risk and genes affecting responsiveness to existing drugs also points toward a nexus of biological mechanisms engaged by medication and those contributing to disease etiology. This supports the idea that disease remediation can intersect with disease causation and help compensate for developmental insults, even during adulthood. The prevalence of genes involved in dopamine transmission among those associated with treatment responsiveness is flagrant. However, dopamine is an "old player" in schizophrenia therapy and the prevalence of dopamine-associated genes probably results from a bias induced by the mechanism of action of therapeutic agents. The further examination of genes that are more distal to dopamine signaling and are nonetheless associated with schizophrenia risk and drug responsiveness may provide a new roster of "prospects" that can be realistically targeted for therapies whether alone, or in conjunction with older dopamine-targeting therapeutics. Furthermore, the integrated investigation of new and old variants associated both to genetic risk and treatment responsiveness can provides the bases for the development of personalized treatment protocols for schizophrenia.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. JB also provided funds for publication.

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REVIEW
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Factors Associated With Response and Resistance to Cognitive Remediation in Schizophrenia: A Critical Review

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Cognitive impairment is a central feature of schizophrenia and has shown to play a crucial role in the psychosocial function of the disorder. Over the past few years, several cognitive remediation (CR) interventions have been developed for schizophrenia, whose effectiveness has also been widely demonstrated by systematic reviews and meta-analysis studies. Despite these evidences, many questions remain open. In particular, the identification of CR response predictors in patients with schizophrenia is still a topic with equivocal findings and only a few studies have looked for the relationship between CR response or resistance and the biological, socio-demographic, clinical and cognitive features in schizophrenia. The current knowledge on positive or negative response predictors to CR treatment in schizophrenia include: age, duration of illness, premorbid adjustment, baseline cognitive performance, intrinsic motivation, hostility, disorganized symptoms, neurobiological reserve, genetic polymorphisms, the amounts of antipsychotics, the type of CR, etc. The aim of this review is to identify neurobiological, psychopathological, cognitive, and functional predictors of CR response or resistance in schizophrenia, taking into account both cognitive and functional outcome measures. The information obtained could be very useful in planning integrated and personalized interventions, also with a better use of the available resources.

Keywords: schizophrenia, cognitive remediation, predictors, cognitive improvement, functional improvement, treatment personalization

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INTRODUCTION

Cognitive impairment is a central feature of schizophrenia (Green et al., 2004; Keefe et al., 2006) and has shown to play a crucial role in the psychosocial function of the disorder (Bowie et al., 2006, 2008). A few years ago, the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative identified the presence of seven distinct cognitive domains compromised in schizophrenia (Nuechterlein et al., 2004). Numerous studies have shown that cognitive deficits are one of the main causes of severe functional disabilities associated with schizophrenia and are also related to a worse outcome of the disorder (Green, 1996; Green et al., 2000; Bowie et al., 2006). More in detail, recent findings linked functional outcome to the seven cognitive domains identified by the NIMH's Measurement and Treatment Research to Improve

Cognition in Schizophrenia (MATRICS) initiative: verbal learning and memory, visual learning and memory, working memory, speed of processing, reasoning and problem solving, attention, and social cognition (Nuechterlein et al., 2004). Cognitive impairment predicts functional outcome at the same level or even better than positive and negative symptoms and is associated with disability even in phases of clinical remission (Galderisi et al., 2014, 2016). In a comprehensive literature review, Green et al. (2000) highlighted that different cognitive deficits might have an impact on specific areas of psychosocial functioning. Cognitive deficits explain 20–60% of the variance of real-life functioning (Green et al., 2004; Fett et al., 2011). From the greater knowledge of the role of cognitive impairment in schizophrenia, its improvement became an essential goal in the treatment of this disorder (Medalia and Choi, 2009). Despite effectiveness of antipsychotic drug treatment in reducing positive symptoms of schizophrenia, cognitive symptoms have proven to be poorly responsive to such treatments (Nielsen et al., 2015). For this reason, new non-pharmacological interventions to improve cognitive symptoms in schizophrenia are under study, with the ultimate goal to also obtain a better functional outcome (Vita and Barlati, 2018). According to the modern neuroscientific knowledge, the brain would be able of changing and developing throughout lifespan (Kaneko and Keshavan, 2012). In this perspective, CR bases its theoretical principles on the concept of cerebral plasticity and neurogenesis (Eack et al., 2010). Moreover, learning that occurs within a CR intervention, if carried out in a stimulating context, would seem to facilitate the development of the brain plasticity, also improving patient functioning (Bowie et al., 2012). In this context, CR aims to recover cognitive functioning through a series of specific methods and techniques (Barlati et al., 2013). CR strategies can be distinguished into two main models: "compensatory" and "restorative." The "compensatory" treatments try to eliminate or to bypass the specific cognitive deficit, using the subject's residual cognitive abilities. On the other hand, the "restorative" methods are based on knowledge deriving from neurosciences, in particular neuronal plasticity, and have the objective to correct a specific deficit trying to repair the specific underlying compromised function using the capacity of the brain to develop and repair itself throughout the whole life. Restorative remediation strategies utilize two different approaches: bottom-up or top-down. Bottom-up approaches start with remediation of basic neurocognitive skills, such as attention, and advance to more complex skills, such as problem solving. In contrast, top-down approaches use more complex skills with the aim of improving single and specific neurocognitive domains. Thus, some restorative techniques take into account the use of drill and practice exercises, in order to restore cognitive functions and, possibly, improve neuronal plasticity, while others are based on the implementation of new strategies and tend to favor the generalization in different contexts through the execution of different tasks that involve the use of similar strategies (Barlati et al., 2013).

Over the past few years, several CR interventions have been developed for schizophrenia, whose effectiveness has also been widely demonstrated by systematic reviews and meta-analysis studies (McGurk et al., 2007; Grynszpan et al., 2011; Wykes et al., 2011; Medalia and Saperstein, 2013; Vita et al., 2014). Despite the evidence of CR effectiveness in schizophrenia, many questions remain open. In particular, the identification of CR response predictors in patients with schizophrenia is still a topic with equivocal findings and only a few studies have looked for the relationship between CR response or resistance and the biological, socio-demographic, clinical and cognitive features in schizophrenia (Medalia and Richardson, 2005; Kontis et al., 2013; Medalia and Saperstein, 2013; Bowie et al., 2014). The current knowledge on positive or negative response predictors to CR treatment in schizophrenia include: age, duration of illness, premorbid adjustment, baseline cognitive performance, intrinsic motivation, hostility, disorganized symptoms, neurobiological reserve, genetic polymorphisms, the amounts of antipsychotics, the type of CR, etc.

The aim of this review is to identify neurobiological, psychopathological, cognitive, and functional predictors of CR response or resistance in schizophrenia, taking into account both cognitive and functional outcome measures. The information obtained could be very useful in planning integrated and personalized interventions, with a better use of the available resources.

MATERIALS AND METHODS

Search Strategy

Electronic searches were performed using MEDLINE/PubMed, PsycINFO and EMBASE databases combining the following search terms: "schizophrenia," "cognitive remediation," "cognitive rehabilitation," "cognitive training," "functional outcome," "response," "resistance," "predictors," "cognitive improvement," "functional improvement." Detailed combinations of the above search terms are available from the authors on request. Two of the authors (SB, GD) independently reviewed the database in order to avoid errors in the selection of articles. In addition, the reference lists of the included articles were carefully hand-searched to further identify other studies of possible interest.

Selection Criteria

All the studies, meta-analyses, and review articles on cognitive remediation in schizophrenia published until June 2018 have been included. Studies were included according to the following criteria: (a) being an original paper published in a peer-reviewed journal, (b) being an English language paper, and (c) having performed experiments using a CR technique in schizophrenia. Studies on psychological, psychosocial, or psychoeducational interventions only, without any cognitive remediation approach or technique, were not considered.

RESULTS

Cognitive and Functional Improvement After CR in Schizophrenia: Preliminary Considerations

First of all, it is crucial to define what does it mean with the improvement and normalization concepts and what the

scientific literature affirms about them. A number of studies have computed the minimally important difference (MID) for assessment tools, determining that the discrimination threshold for changes in chronic diseases appears to be approximately half (0.5) a standard deviation (SD) (Norman et al., 2003). Harvey et al. (2006) consider as "improved" those patients with a cognitive amelioration of 0.5 SD and as "normalized" those with an improvement of more than −1 SD. Vita et al. (2013) defined as "improved" those patients with a global cognitive improvement greater than or equal to $Z = 0.5$ and as "not-improved" those with a global cognitive change lower than $Z = 0.5$ from baseline to post-treatment. Furthermore, the same authors defined as "normalized" those patients with a global cognitive change from $Z < -0.5$ at baseline to $Z \geq -0.5$ at post-treatment and as "non-normalized" those with a cognitive change at post-treatment lower than $Z = -0.5$. The definition of Vita et al. is similar, but more restrictive than the previous Harvey's definition.

Overall, scientific literature reports that the rate of improvement and normalization after CR in at least one cognitive domain is around 50 and 40% respectively, but some factors predicted a positive outcome up to 70% in the improvement possibility after CR (Kurtz, 2012). In particular, CR in schizophrenia produces 0.5 SD improvements in measures of cognition and also leads nearly 0.5 SD improvements on measures of function (Kurtz, 2012). In the study performed by our group, 46.2 and 41.8% of patients respectively showed a cognitive and functional improvement and 32.4 and 23.6% respectively achieved a cognitive and functional normalization after CR (Vita et al., 2013). Similar findings are also reported by Medalia and Richardson (2005), showing how 49.5% of patients reached a significant cognitive improvement in at least one cognitive domain after CR (NEAR-Neuropsychological Educational Approach to Remediation). Although with a wider definition of the normalization concept, higher percentages of cognitive normalization have been found by Fiszdon et al. (2005), highlighting that 43% of schizophrenia patients achieved cognitive normalization after CR (NET-Neurocognitive Enhancement Therapy). In the study by Vita et al. (2013), 26 patients received the first 2 subprograms (cognitive differentiation and social perception) of the Integrated Psychological Treatment (IPTcog), and 30 patients received a CACR intervention. The IPT is a group-based structured cognitive behavioral program for schizophrenia in which neurocognitive remediation and social cognitive remediation are integrated with psychosocial rehabilitation (Brenner et al., 1994). The IPT-cog groups, composed of 8–10 patients, attended therapy sessions twice a week, 45 min each session, for 24 weeks. The CACR used the Cogpack (Marker Software®) program. The Cogpack includes different neurocognitive exercises that can be divided into domain specific exercises, aimed at training specific cognitive areas among those known to be impaired in schizophrenia (verbal memory, verbal fluency, psychomotor speed and coordination, executive function, working memory, attention) and non-domain-specific exercises that require the use of various functions at the same time and engage culture, language, and calculation skills. The CACR was administered individually twice a week, in 45-min sessions, for 24 weeks. NEAR approach consists in an individual/group patient (3–10) sessions, integrated with a computer-assisted sessions and noncomputer-assisted sessions. Sessions are of 60 minutes, twice a week (about 4 months) (Medalia et al., 2002). NET approach consists in individual / group sessions, integrated with computer-assisted sessions and noncomputer-assisted sessions. Sessions are of 45 minutes at least 5 times a week (about 6 months) (Bell et al., 2001). In another study, Kurtz et al. (2007) observed that 61% of the participants in the CR condition showed evidence of at least a small (≥0.2 SD) Z score improvement and only 22% showed a large (≥0.8 SD or greater) Z score improvement for the working memory domain. In this study, the CR intervention consisted in a 12-month (100 h) standardized computer cognitive exercises designed to improve attention, verbal and non-verbal memory. and language processing through repeated drill-and-practice (Bracy, O. PSS CogRehab, Version 95. Indianapolis, IN: Psychological Software Services, Inc; 1995). In a recent study performed by Bosia et al. (2017), 70% of schizophrenia patients improved in at least one cognitive domain and over 50% obtained a normalized score after CR (Cogpack), consisting in 36 sessions of domain-specific computer-aided exercises, three 1-h sessions a week for 3 months. Presently, one of the challenges facing clinicians and CR developers is a limited understanding of who responds to CR. A number of studies investigated positive and negative response predictors to CR (Choi and Medalia, 2005; Fiszdon et al., 2005, 2006; Medalia and Richardson, 2005; Kurtz et al., 2009; Twamley et al., 2011). These studies found that there are several patient and treatment characteristics, influencing a positive or a negative response to CR. In particular, patient variables include baseline cognitive profile (Fiszdon et al., 2005, 2006; Medalia and Richardson, 2005; Kurtz et al., 2009; Lindenmayer et al., 2017), psychological variables such as motivation (Choi and Medalia, 2005; Twamley et al., 2011) and biological features such as age (Wykes et al., 2009; Kontis et al., 2013), phase of the illness (Bowie et al., 2014), catechol-O-methyltransferase (COMT) polymorphisms (Bosia et al., 2007; Panizzutti et al., 2013), and antipsychotic drugs and genetic polymorphisms (Bosia et al., 2014a). Taken together, these studies identified three broad patient variables that could be useful in tailoring CR: cognitive, psychological, and biological. As for other types of psychosocial interventions, variability in response has been observed among recipients. A better understanding of who is able to benefit from CR would enable clinicians to more effectively refer patients to CR or tailor the intervention to the individual. Finally, treatment variables associated to CR response include the administration methods, such as treatment intensity and frequency, the use of drill and practice and/or strategy learning techniques, the integration of CR with other psychiatric rehabilitation interventions (Medalia and Richardson, 2005; McGurk et al., 2007; Wykes et al., 2011). **Table 1** summarizes the most investigated patient and treatment characteristics predicting cognitive and functional response to CR in schizophrenia. **Table 2** summarizes the literature main findings about predictive factors influencing CR response in schizophrenia.

TABLE 1 | The most investigated predictive factors influencing CR response in schizophrenia.

Patient predictive factors
Age
DOI
Phase of illness
Chronicity (number of hospitalizations)
Diagnosis
Premorbid functioning (adjustment)
Pretreatment symptoms severity (positive, negative, disorganized symptoms and hostility)
Pretreatment cognition
Pretreatment functioning
Psychological characteristics (depressed mood, anxiety, cooperative attitude, intrinsic motivation)
Cognitive change after CR and functional outcome
Neurobiological predictive factors (cognitive reserve, genetic variability)
Treatment predictive factors
Presence of the therapist
The role of the therapist
Therapeutic alliance
CR characteristics (type of CR treatment)
CACR
Use of strategic CR
Use of drill and practice CR
Use of massed practice
Pharmacological treatment (amounts of antipsychotics intake)
Presence of integrated treatment

CACR, Computer-Assisted Cognitive Remediation; CR, Cognitive Remediation; DOI, Duration of Illness.

TABLE 2 | Predictive factors influencing CR response in schizophrenia: literature main findings.

Patient positive response predictors	**Treatment positive response predictors**
Younger age	Presence of a highly trained therapist
Shorter DOI	The active role of the therapist
Early phase of illness	Therapeutic alliance
Lower pretreatment disorganized symptoms	Use of strategic CR
Lower pretreatment hostility	Use of massed practice
Lower pretreatment negative symptoms	Presence of integrated treatment
Greater intrinsic motivation	Lower amounts of antipsychotics intake at baseline
Greater cognitive improvement after CR	Lower anticholinergic burden at baseline
Greater pretreatment cognitive reserve	
Patient controversial predictors	**Treatment controversial predictors**
Chronicity (number of hospitalizations)	Type of CR treatment (CACR vs. non-CACR)
Diagnosis	Use of drill and practice CR
Premorbid functioning (adjustment)	
Cognitive impairment at baseline	
Functional impairment at baseline	
Genetic variability	

CACR, Computer-Assisted Cognitive Remediation; CR, Cognitive Remediation; DOI, Duration of Illness.

Patient Characteristics Predicting Cognitive Response to CR in Schizophrenia

Several studies examined whether patient demographics, illness, or cognitive characteristics are predictive of the amount of change in cognitive performance after CR. Few relationships have been reported consistently.

Age and Phase of Illness

With regard to demographics, the impact of age has been of greatest interest. Although in some studies age has been unrelated to cognitive improvement (Fiszdon et al., 2005; Medalia and Richardson, 2005; Wykes et al., 2011) and others have reported mixed results (Thomas et al., 2017), a number of studies confirmed that younger patients are more likely to achieve cognitive improvement after CR, showing that patients over the age of 40 have a lower response to CR than patients under 40 (Wykes et al., 2009; Kontis et al., 2013; Vita et al., 2013; Corbera et al., 2017; Lindenmayer et al., 2017).

There is some evidence that stage of illness—an issue closely related to age—might affect cognitive improvement after CR. A meta-analysis that investigated CR effect in patients at their first psychotic episode (Revell et al., 2015) identified a similar modality in cognitive improvement, but with a lower degree, compared to the results of a previous meta-analysis on chronic patients (Wykes et al., 2011). However, other studies achieved different results. Specifically, in the study by Corbera et al. (2017) the early-stage (25 years or younger; mean duration of illness—DOI = 3.4 years) and early-chronic (26–39 years; mean DOI = 7.6 years) patients receiving CR showed larger improvements in working memory, compared to the late-chronic group (40 years and over; mean DOI = 18.2 years). Furthermore, a study performed by Bowie et al. (2014) demonstrated that early course patients (less than 5 years from the psychotic onset) showed a greater improvement in processing speed and executive functions, compared to chronic patients (more than 15 years of disease) after CR. Authors concluded that DOI was inversely associated with improvement in cognition after a CR intervention. If these results will be confirmed, they could support the full inclusion of CR techniques among those tools to be taken into account in the early intervention programs of schizophrenia (McGurk et al., 2007; Wykes et al., 2009).

For these reasons, there is great interest in determining whether CR interventions make a greater impact on cognitive and functioning outcomes for individuals in the prodromal or early phase of illness and, despite more research is needed in this area, preliminary results seem to be encouraging (Barlati et al., 2012, 2015, 2016; Fisher et al., 2013; Revell et al., 2015; Glenthøj et al., 2017).

Illness Characteristics and Psychopathological Status

Studies examining the impact of illness characteristics on the efficacy of CR have focused on diagnosis, chronicity, and symptoms severity. Diagnosis (schizophrenia vs. schizoaffective disorder) and indicators of illness chronicity (number of previous hospitalizations) have not been predictive of treatment response (Medalia and Richardson, 2005; Scheu et al., 2013). Symptoms severity has been found to relate to CR- induced cognitive change in some (Fiszdon et al., 2005; Wykes et al., 2011; Vita et al., 2013) but not all studies (Medalia and Richardson, 2005; Scheu et al., 2013). When a relationship was found, lower baseline symptom severity in conceptual disorganization and hostility (Fiszdon et al., 2005; Vita et al., 2013; Lindenmayer et al., 2017) and lower baseline negative symptoms severity (Lindenmayer et al., 2017) were associated with a greater response to CR. Moreover, CR therapy was more effective when patients were clinically stable (Wykes et al., 2011). Lastly, preliminary data of our group showed a negative correlation between autistic traits in patients with schizophrenia and cognitive change (processing speed, verbal memory, and global cognitive score) after CR (Vita et al., 2018; Abstract presented at Cognition in Schizophrenia 2018: A Satellite Meeting of the Schizophrenia International Research Society. Florence, 4 April 2018).

Other patient characteristics, that have been found to be important in predicting CR efficacy, include some psychological characteristics, such as: anxiety, depression, a cooperative attitude, intrinsic motivation to complete treatment and low self-esteem (Fiszdon et al., 2005; Medalia and Richardson, 2005; Ventura et al., 2014). Poor intrinsic motivation—a central feature of schizophrenia with prevalent negative symptoms—has been associated with a low cognitive performance in patients with schizophrenia and has also been identified as a negative predictor of CR efficacy (Saperstein and Medalia, 2016). In this regard, several structured intervention programs on intrinsic motivation have been investigated, with the aim of optimizing CR effectiveness (Choi and Medalia, 2010; Medalia et al., 2018).

Pretreatment Cognitive Profile

Research has also looked at whether baseline cognitive characteristics are predictive of response to CR. Baseline cognitive performance measured by neuropsychological test performance has been found to relate to CR- induced cognitive change in some (Fiszdon et al., 2005; Medalia and Richardson, 2005; Kurtz et al., 2009; Vita et al., 2013) but not all studies (Scheu et al., 2013). Fiszdon et al. (2005) have shown how a better baseline cognitive profile in vigilance and verbal memory, was associated with greater CR efficacy, also predicting a 70% of improvement possibilities in memory tasks (digit span) after NET. Vita et al. (2013) have shown how better baseline performances in executive functions and in verbal memory predicted a greater chance of cognitive normalization after CR. This is in line with previous research findings, in which a good baseline performance in executive functions was associated with a better learning of specific strategies during CR (Velligan et al., 2006). A recent study by Davidson et al. (2016) found that baseline learning potential (LP)—the ability to quickly learn and apply a new skill under testing conditions—significantly predicted skill acquisition in verbal and visuo-spatial memory domains after a 8-week CR intervention in patients with schizophrenia. Furthermore, Benoit et al. (2016) demonstrated that higher initial cognitive insight was significantly correlated with greater improvement in speed of processing and visual memory after CR. Burton and Twamley (2015) found no evidence that unawareness of cognitive impairment is a barrier to participation in or ability to benefit from cognitive training, demonstrating no differences in patients with or without neurocognitive insight in terms of treatment utilization and good treatment outcomes in verbal memory and functional capacity (measured by the UCSD Performance-Based Skills Assessment—UPSA).

Other studies, using a clinician assessment of cognitive functions, have been predictive of treatment response. People with schizophrenia who were rated as less impaired on the Positive and Negative Syndrome Scale (PANSS) Cognitive Factor, an index reflecting difficulties with disorientation, poor attention and abstract thinking, and conceptual disorganization, were found to be more likely to improve after CR (Medalia and Richardson, 2005). Conversely, Pillet et al. (2015) observed numerous negative correlations between cognitive performance at baseline and patients improvements following CR, with a lower cognitive performance predicting greater cognitive improvements. Overall, although better baseline cognitive performance may be associated with greater effectiveness of CR, intervention- induced cognitive improvement is not fully related to baseline neuropsychological performance, and patients can benefit from CR regardless of their level of illness- related cognitive impairment (Twamley et al., 2011; Scheu et al., 2013; Rodewald et al., 2014).

Patient Characteristics Predicting Functional Response to CR in Schizophrenia

In recent years, a few studies have begun to examine whether patient characteristics at the onset of CR are predictive of post intervention functional change. Age, symptoms, baseline cognitive performance, and pre- intervention functioning have been found to be predictive of change in functional status, including progress in psychosocial rehabilitation programs, community function and role-play measures of social and everyday life skills (Kurtz, 2012).

Age and Phase of Illness

Similar to the research on cognitive change, Vita et al. (2013) reported that patient age was predictive of functional improvement (measured by the Global Assessment of Functioning and the Health of the Nation Outcome Scale), with younger patients making greater gains after CR. In line with these findings, Bowie et al. (2014) showed that early course patients had larger improvements in adaptive competence and real-world work skills, than patients in the chronic course of schizophrenia after CR, demonstrating that DOI was inversely associated with improvement in real-world work skills after a CR intervention. Revell et al. (2015) in their meta-analysis on CR in

first episode schizophrenia patients identified a similar modality in functional improvement, but with a lower degree, compared to the results of a previous meta-analysis on chronic patients (Wykes et al., 2011). Conversely, other studies reported mixed results on functioning, suggesting that CR may differentially benefit persons with severe mental illnesses depending on age (Thomas et al., 2017).

Illness Characteristics and Psychopathological Status

Lower baseline conceptual disorganization and lower positive symptoms severity predicted better social function improvement after CR (Vita et al., 2013; Farreny et al., 2016; Lindenmayer et al., 2017).

Pretreatment Cognitive Profile

Baseline attention, working memory and verbal learning ability have been found to be predictive of change in functional capacity after Computer-Assisted Cognitive Remediation (CACR) (Kurtz et al., 2007, 2009), and a trend has been noted for executive functioning to predict post- CR functioning in the community (Vita et al., 2013). In both studies higher baseline performance predicted greater functional gains with CR. Lindenmayer et al. (2017) found a significant association between faster speed of processing, better visual and verbal learning at baseline and greater functional improvement after a systematic cognitive intervention within a rehabilitative setting. Conversely, Bosia et al. (2017) showed no significant effect of baseline cognitive function on functional outcome after CR. This topic is still a matter of debate in literature, as some studies suggested a relation between specific cognitive functions and improvement in psychosocial functioning after CR (Medalia and Richardson, 2005; Kurtz et al., 2009; Vita et al., 2013; Farreny et al., 2016), while others didn't support any relation (Roder et al., 2002; Bosia et al., 2017), or have supported a negative correlation (Twamley et al., 2011; Scheu et al., 2013; Rodewald et al., 2014).

Pretreatment Level of Functioning

The term premorbid adjustment refers to a broad set of abilities, including premorbid intelligence quotient (IQ), overall individual's social, interpersonal, academic and occupational functioning prior to the onset of psychotic symptoms (Addington and Addington, 2005). There are few and conflicting data demonstrating a correlation between pre- intervention level of functioning and CR responsiveness. In a study by Bell et al. (2014) participants were assigned to receive either supported employment or supported employment with CR. The lower functioning people with schizophrenia who received supported employment and CR achieved significantly higher employment rates than individuals in supported employment only. In contrast, among higher- functioning participants, the addition of CR to supported employment did not enhance outcomes. Conversely, a recent study by Buonocore et al. (2018a), investigating the effect of premorbid adjustment (measured by the Premorbid Adjustment Scale and proposed as an index of cognitive reserve) on cognitive improvements after CR in schizophrenia patients, confirmed the association between premorbid adjustment and cognitive impairment and the possible role of premorbid adjustment on the capacity to recover from cognitive deficits through CR.

Cognitive Change After CR and Functional Outcome

The current findings link specific cognitive change features after CR to changes in psychosocial functioning and several studies in investigating cognitive change after CR found a significant improvement in cognitive and in psychosocial functioning (Fiszdon et al., 2008; Eack et al., 2011; Wykes et al., 2012; Rispaud et al., 2016). In particular, Wykes et al. (2012) emphasized that only one cognitive variable—planning/executive functioning—was a predictor of work improvement, despite moderately sized. Furthermore, Rispaud et al. (2016) provided evidence that not all cognitive domains improvement was the same with respect to functioning improvements. While several cognitive domains improved after CR, only working memory and processing speed improvement predicted a better functioning over 1-year. More in detail, this association was specific to patients with at least a moderate cognitive improvement (0.5 SD) and when authors included patients with a smaller degree of cognitive improvement—small (0.2 SD) or median (0.37 SD)—only an improvement in working memory led to a better functioning. Consistent with these findings, a recent study performed by Bosia et al. (2017) showed that the proportion of normalized cognitive performance–i.e., the number of cognitive domains in which patients achieved a "normal" score after CR with respect to baseline deficits—was the only significant predictor of functional outcome. Overall, together these studies support a relationship between cognitive change after CR and functional outcome, although the importance of improving a single cognitive domain versus the number of domains improved, is still uncertain.

Neurobiological Factors Predicting Response to CR

Recent international literature identified a number of biological factors that appear to influence CR response. Knowledge of these indicators could have important implications in optimizing and customizing CR intervention, even for patients resistant to standard treatments (Medalia et al., 2018). Among these factors, cognitive reserve and some genetic variables seem to play an important role.

Cognitive Reserve

In schizophrenia, there is evidence suggesting that a greater cognitive reserve could modulate and counteract some neurodegenerative processes. Cognitive reserve is a term that describes brain resilience against brain damage. At a neural level, cognitive reserve consists for example in a higher synaptic density, in a greater number of neurons, in the ability to use alternative neural networks or different cognitive strategies (Kaneko and Keshavan, 2012). Thus, pretreatment cognitive reserve—cortical surface area and gray matter volume—has been investigated as a predictor of CR efficacy. In this regard, Keshavan et al. (2011) investigated if cortical pre-treatment brain volume influenced Cognitive Enhancement Therapy (CET) effectiveness on cognition and social cognition in a group of

early stages schizophrenia patients. Authors demonstrated that greater basal temporal and frontal cortical surface area and higher gray matter volume broadly predicted a more rapid social cognitive response after CET. These findings were confirmed in a recent study by Penadés et al. (2016) in which a greater basal frontal and temporal cortical thickness was correlated with a higher CR post-treatment improvement in non-verbal memory cognitive domain in schizophrenia patients. Conversely, Kontis et al. (2013) showed a limited impact of cognitive reserve on neurocognitive outcome after CR, highlighting the impact of age on CR outcome.

Genetic Variability

COMT gene variability has been associated with cognitive performance in schizophrenia (Diaz-Asper et al., 2008) and with CR response, although results have been equivocal (Bosia et al., 2007, 2014a; Greenwood et al., 2011; Pieramico et al., 2012; Panizzutti et al., 2013; Lindenmayer et al., 2015). In a controlled study on CACR intervention in schizophrenia patients, Bosia et al. (2007) found how COMT polymorphism might predict cognitive gain after 3-months follow-up, with a higher improvement in the Quality of Life Scale (QLS) in Met polymorphism after CR in comparison to Val/Val polymorphism after standard treatment. In line with these findings, a more recent study showed how COMT Val/Met and Met/Met genotype was linked to CR efficacy, with a greater improvement in three MATRICS cognitive domains—verbal learning, visual learning, and attention/vigilance—compared to Val/Val genotype (Lindenmayer et al., 2015). In contrast to these positive findings other studies did not find such a great association (or no association) between COMT Met/Met genotype and greater improvement after CR (Greenwood et al., 2011; Twamley et al., 2014). Moreover, further investigation provided new awareness of the role of COMT in CR response, pointing out a potential interaction between COMT polymorphism and antipsychotic drugs (Bosia et al., 2014a) and serotonin 1A receptor (5-HT1A-R) (Bosia et al., 2014b). Overall, these data suggest how COMT genotype could provide useful information in the selection of an appropriate and personalized pharmacological and rehabilitative treatment in schizophrenia (Medalia et al., 2018).

Treatment Characteristics Predicting Response to CR

Investigators have examined the impact of treatment factors on response to CR, including CR characteristics and medication.

CR Characteristics and the Role of Therapist

Regarding CR treatment characteristics that appear to influence outcome, Medalia and Richardson (2005) found that patients were more likely to respond to CR when a highly trained, doctoral- level therapist provided it, as compared to a clinician with less training. They also found intensity of CR training to be a significant moderator of treatment response. Individuals who completed the training more efficiently, attending at least two sessions a week, benefited significantly more than individuals who attended sporadically. Surprisingly, the literature currently offers very little guidance regarding the amount and intensity of cognitive training needed for CR to be effective. In some clinical trials, intervention "dose" has ranged from 24 to 100 sessions (Fisher et al., 2009; McGurk et al., 2009). In a recent study, Buonocore et al. (2017) investigated whether a longer treatment might further increase CACR efficacy in cognition and functioning. Results supported 3-months CACR efficacy both in cognition and in functioning, suggesting that a longer CR intervention could lead to further advantages in executive functions and daily functioning.

Recently, a panel of experts met to develop recommendations for future studies. They suggested that, at a minimum, programs offer at least 2 h of training weekly for a total of 30 to 40 sessions over a 3-month period (Keefe et al., 2011). Although this guideline is helpful, several CR developers have noted that intervention "dose" is likely to be dependent on treatment goals, with different "doses" needed to produce short- vs. long- term change in cognition or impact other potential treatment targets such as psychosocial functioning or neural systems (Wykes and Spaulding, 2011; Vinogradov et al., 2012). Furthermore, a much larger effect of CR on functioning was found when a strategic approach was adopted (McGurk et al., 2007; Wykes et al., 2011). CR methods based on strategy are very different from those based on training (drill and practice), and these two types of CR techniques almost certainly have different effects on brain activity. In their recent review, Bon and Franck (2018) showed an increase brain activity for the training-based method, but a broader network activation for the strategy-based one. Vita et al. (2013) showed that the type of CR treatment—cognitive subprograms of Integrated Psychological Therapy (IPT-cog) vs. CACR (Cogpack)—was not associated with cognitive gain in schizophrenia patients, but predicted functional improvement (CACR > IPT-cog) in this population.

Moreover, significantly stronger effects on functioning were found when CR is offered as part of broader psychosocial rehabilitation interventions (McGurk et al., 2007; Wykes et al., 2011; Bowie et al., 2012). In their meta-analysis on CR in early schizophrenia, Revell et al. (2015) found that CR's effect on functioning was larger in trials with adjunctive psychiatric rehabilitation and small group interventions. In line with these findings, Buonocore et al. (2018b) showed that a longer standard rehabilitation following CR may lead to a significant and stable (5 years) benefit in terms of daily functioning and QoL in patients with schizophrenia.

In a recent study, Cella and Wykes (2017) found that cognitive improvement after CR was associated with massed practice, number of useful strategies and therapeutic alliance, but improvement in functioning was associated only with therapeutic alliance. Authors highlighted that, as for other psychological therapies, it appears that therapeutic alliance may be an important factor for CR outcomes, particularly functioning, in people with schizophrenia, emphasizing the crucial role of the therapist and his impact on patient motivation. Future research should investigate whether the therapist-patient relationship is a useful variable to be taken into account in choosing a type of CR intervention. In view of a greater treatment personalization, some patients could benefit from a CR intervention mediated by

the therapist, others from a computer-based intervention, while others from a home-delivered modality (Medalia et al., 2018).

Pharmacological Treatment

In people with schizophrenia, CR is intended to be an adjunct to pharmacotherapy. Few studies have examined the impact of medication- related factors on response to CR. Vita et al. (2013) reported that a lower antipsychotic dose at baseline was the strongest predictor of cognitive and functional improvement after CR. The predictive role of a lower antipsychotic dosage associated with a better cognitive and functional outcome after CR in subjects with schizophrenia may suggest both that patients with a more severe illness could have less benefit from CR, both that high antipsychotic dosages could limit CR effectiveness. Similar findings were obtained from other studies (Rodewald et al., 2014), while others showed the lack of influence of antipsychotic dose on the efficacy of CR (Bosia et al., 2017). A medication- related factor that has been found to influence CR response is anticholinergic burden. Many medications, including a few of the antipsychotics prescribed to treat schizophrenia and some of the medications prescribed to control antipsychotic—related side effects have anticholinergic properties. Vinogradov et al. (2009) examined the level of serum anticholinergic activity at baseline and found that it negatively predicted CR- induced improvement on a global measure of cognition, indicating that this medication factor is adversely impacting response to CR.

DISCUSSION

CR is a behavioral training technique designed to address cognitive and functional impairments associated with schizophrenia. Metanalytic studies offer a good support for CR efficacy on cognitive and functional outcomes in patients with schizophrenia (McGurk et al., 2007; Wykes et al., 2011). Despite these evidences, many questions remain open. Overall, scientific literature reports that the rate of improvement and normalization after CR in at least one cognitive domain is around 50 and 40%, respectively, but some factors predicted a positive outcome up to 70% in the improvement possibility after CR (Kurtz, 2012). In this perspective, a better understanding of who is able to benefit from CR would enable clinicians to more effectively refer patients to CR or tailor the intervention to the individual (Farreny et al., 2016). In particular, the identification of CR response predictors in patients with schizophrenia is still a topic with equivocal findings and only a few studies have looked for the relationship between CR response or resistance and the biological, socio-demographic, clinical, and cognitive features in schizophrenia. The current knowledge on positive or negative predictors of CR efficacy in schizophrenia include: age, DOI, premorbid adjustment, baseline cognitive performance, intrinsic motivation, hostility, disorganized symptoms, neurobiological reserve, genetic polymorphisms, the amounts of antipsychotics, the type of CR, etc (Choi and Medalia, 2005; Fiszdon et al., 2005, 2006; Medalia and Richardson, 2005; Kurtz et al., 2009; Twamley et al., 2011). These studies found that there are several patient and treatment variables, influencing a positive or a negative response to CR. Research in this field identified three broad patient characteristics probably useful to personalize CR: cognitive, psychological, and biological. In particular, patient variables include baseline cognitive profile (Fiszdon et al., 2005, 2006; Medalia and Richardson, 2005; Kurtz et al., 2009; Lindenmayer et al., 2017), psychological variables such as motivation (Choi and Medalia, 2005; Twamley et al., 2011) and biological variables such as age (Wykes et al., 2009; Kontis et al., 2013), stage of illness (Bowie et al., 2014), COMT polymorphisms (Bosia et al., 2007; Panizzutti et al., 2013), and antipsychotic drugs and genetic interactions (Bosia et al., 2014a). Regarding treatment variables associated to CR response, they include the administration methods, such as treatment intensity and frequency, the use of drill and practice and/or strategy learning techniques and the integration of CR with other psychiatric rehabilitation interventions (Medalia and Richardson, 2005; McGurk et al., 2007; Wykes et al., 2011). Overall, CR seems to be more effective in schizophrenia patients with the following features: younger age, shorter DOI, few disorganized symptoms, greater pretreatment cognitive reserve, greater improvement after CR, and lower dosages antipsychotics in their current treatment. About CR characteristics, a much larger effect of CR on functioning was found when a strategic approach was adopted and when CR was offered as part of broader psychosocial rehabilitation interventions. On the other hand, international scientific literature is controversial on the following predictive factors: genetic variability, cognitive, and functional impairment at baseline (see also **Table 2**).

Despite these findings, there is a further limit to be taken into account, represented by the numerous correlations and interconnections among the different predictors, which makes very difficult to understand the individual weight of each factor. Furthermore, although CR is described as a single intervention, there are multiple dimensions that may distinguish one approach from another, making it difficult to compare results of one study with another. Currently, one barrier to treatment development is our lack of understanding of the critical elements of the intervention and the relative effectiveness of training techniques and approaches (Saperstein and Kurtz, 2013). Direct comparisons studies of CR techniques, identifying the active components of an intervention approach are needed (Kaneko and Keshavan, 2012). Overall, there are some limits in data interpretation. One meaningful difficulty is that there are many standardized CR programs—computerized and non-computerized, individualized or provided in group sessions—but very few studies compared the efficacy of these interventions with each other. Similarly, many studies didn't quantify cognitive and functional improvements in the same way, making the literature very difficult to compare directly. We have to identify the potential predictors of outcome, allowing us to develop a set of variables for tailoring treatments. Accomplishment of this task will require carefully designed studies that control for potential confounding factors to the study's validity. Currently there are still few data suggesting which type of CR intervention is most effective for a specific patient and there is little rigorous evidence to make decisions on which patient should be excluded from this treatment.

CONCLUSIONS

To date, personalization of CR interventions still depend on a clear case formulation where individual goals are set and an appropriate integrated treatment programme is provided (Wykes, 2018). It is therefore critical to identify clinical, neurobiological and genetic predictors of positive or negative response to CR and future research should identify predictors of CR efficacy and effectiveness, not only at an individual level, but also at a community level for a rational resources allocation (Cella et al., 2015). Moderator analyses should be employed to examine how therapeutic response varies across personal, cognitive and biological factors (Genevsky et al., 2010; Wykes and Spaulding, 2011; Cella et al., 2016). Such studies would require the considerable expansion of the traditional clinical trials framework in psychosocial treatment studies to include neuroimaging and genomics assessments (Eack, 2016). Research findings should then be used to personalize CR intervention, improving its delivery and maximizing its efficacy. The final challenge is to begin transitioning CR from an experimental intervention to one incorporated into standard clinical care and to determine how to make the intervention most effective and accessible for patients and their families.

AUTHOR CONTRIBUTIONS

SB, GD, AG, AP, PV, CT, and AV participated in the writing process of the first draft of the manuscript. SB and GD made literature search and independently reviewed electronic databases. SB, GD, and AV revised the final version of the manuscript. All authors contributed to reading and approving the final version of the manuscript.

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Predictive Factors of Treatment Resistance in First Episode of Psychosis: A Systematic Review

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Background: Clinical and functional outcome improvement in psychotic disorders is a challenge for the investigators. Recent advances offered opportunities for ameliorating the course of the illness during its early stages and for identifying treatment-resistant patients. Patients who had not response to two different antipsychotics, administered at correct doses for a sufficient period, can be operationally considered treatment-resistant. Available evidence suggested that the response's trajectory to the antipsychotic treatment revealed that a small proportion of subjects are poor responders (8.2%), the majority of patients have a moderate response (76.4%), and only 15.4% can be considered rapid responders with the greatest magnitude of response. Patients with first episode of psychosis generally obtain a more favorable response profile. Nevertheless, in around 25% of these patients symptoms of psychosis persist with a worse long-term course of illness.

Objectives: The aim of this review is to report current evidences on the main predictors of treatment non-response in patients at early stage of psychosis.

Methods: We used a specific string that guaranteed a high sensitive search in pubmed. We included the following types of publications: randomized-controlled trials, observational studies, longitudinal studies, retrospective studies, case-control studies, open-label investigations, cohort studies, and reviews. Publications must concern predictors of treatment resistance in early psychosis.

Results: Forty-seven records were included: 5 reviews, 3 meta-analyses, 22 longitudinal studies, 2 retrospective studies, 1 naturalistic study, 6 randomized controlled trials, 2 open-label studies, 2 case-control studies, 4 cohort studies, 2 retrospective studies. Several factors were identified as predictors of treatment resistance: lower premorbid functioning; lower level of education; negative symptoms from first psychotic episode; comorbid substance use; younger age at onset; lack of early response; non-adherence to treatment; and longer duration of untreated psychosis. The role of gender and marital status is still controversial. More evidences are needed about neurobiological, genetic, and neuroimaging factors.

Conclusions: The identification of specific predictive factors of treatment resistance in patients with first episode of psychosis ameliorates the quality of clinical management of these patients in the critical early phase of schizophrenia.

Keywords: schizophrenia, first episode of psychosis, treatment resistance, non-response, predictors of response, clinical factors, biological factors

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INTRODUCTION

The point prevalence estimated for schizophrenia is around 0.6–0.8% and the lifetime prevalence is about 1%. In general, first psychotic episode starts in young adulthood, but the onset of the disorder is preceded by a variety of prodromal symptoms (1–4). There is a general accordance among investigators that treatment response in schizophrenia is very heterogeneous (5, 6). "Treatment outcome has been extensively studied in first-episode schizophrenia. However, the majority of investigations have mainly focused on favorable outcome measures such as response, remission and recovery" (7). Although a good number of patients obtain the remission of symptoms, a significant percentage of cases remains "actively and persistently psychotic despite correct pharmacological treatments" (8). Nevertheless, whether the resistance to treatments is present from the onset of illness (first episode of psychosis-FEP) or whether patients gradually become resistant due to the disease progress is still little known today (8). Some authors state that "patients with FEP may show long-term incomplete remission or treatment resistance in a percentage ranged between 10 to 50%" (9–11). Outcomes in first-episode psychosis (FEP) vary on a continuum from complete remission and full recovery to complete failure of response or treatment resistance. A possible reason of this variability is the intrinsic diagnostic instability of patients at first episode of psychosis.

Resistance to treatments represent a critical topic in schizophrenia spectrum disorders as it is linked with an higher risk of a clinical deterioration, hospitalizations, chronicity, neurotoxic effects of relapses, suicide, aggressive conducts, poor quality of life, and low level of real-world functioning (7, 8, 12–16). Clinical, social, and vocational recovery failure increases the economic cost and enhances burden for family members and stigma for patients (11). Available evidences suggest that the trajectory of response to the antipsychotics treatment reveals that a small proportion of subjects are poor responders (8.2%), the majority of patients have a moderate response (76.4%), and only 15.4% can be considered rapid responders with the greatest magnitude of response (17). Patients with FEP generally obtain a more favorable response profile than patients after multiple episodes. Nevertheless, in around 25% of these patients symptoms of psychosis persist with a worse long-term course of illness (17–22). The precocious identification of individuals who fail to respond to initial interventions may ameliorate the treatment approach at an earlier phase of illness to avoid multiple, unnecessary switches or repeated medication trials and to prevent accruing morbidity. Specialized integrated early interventions, including antispychotics, individual psychological treatment, family, and vocational support are shown to be effective to improve treatment response (23). Unfortunately, as regards the predictive factors of treatment resistance in early phase of illness, the literature to date is still sparse and inconclusive. The present review is aimed to provide an updated overview of current evidences on the main predictive factors of non-response and treatment resistance in patients at early stage of psychosis.

TREATMENT NON-RESPONSE AND TREATMENT-RESISTANCE: DEFINITIONS

Investigations indicated that response to antipsychotic treatments begins in the first weeks of treatment with the largest effect in reducing symptoms in the first 2 weeks (17, 24). Remission was defined as "a state, of at least 6 months' duration, in which no symptoms or only mild symptoms, not interfering with daily functioning, were experienced" (25). Early non-response was operationally defined as "<20% improvement on Positive and Negative Symptoms Scale (PANSS) or Brief Psychiatric Rating Scale (BPRS) total score at 2 weeks" (26). Some authors suggested that "patients who have not a minimal improvement after 2 weeks of treatment are unlikely to respond at a later phase and may benefit from a drug change" (26, 27).

Kane et al. (28) defined treatment resistance with three criteria. "First, the patient fails to respond to three or more adequate trials of antipsychotic treatment within the last 5 years, including antipsychotics of two distinct classes at dose greater than or equal to the equivalent of 1,000 mg/day of chlorpromazine." Moreover, it is widely accepted that three or more second generation antipsychotics failures define treatment resistance (29). "Second, at least two of the symptoms of conceptual disorganization, suspiciousness, hallucinations, and unusual thought content persist with a score at least moderate in severity. Lastly, patient has evidence of substantial symptoms despite current optimized treatment to which the patient is adherent, defined as a score ≥45 on the BPRS or ≥90 on the PANSS" (28).

In line with the National Institute for Health and Care Excellence (NICE) (2) criteria, "patients who had received two sequential antipsychotic trials, each of at least 4 weeks at a daily dose of 400–600 mg of chlorpromazine equivalents, but continued to have persistent psychotic symptoms, which was defined as having a rating of at least moderate severity on one or more positive symptoms, and despite recorded adherence to medication, were classified as treatment resistant. Patients were classified as treatment resistant at onset if they met criteria for treatment resistance following the first two trials with antipsychotics" (2).

Although the definition of the treatment resistance is mainly centered on clinical symptoms' relief, several authors suggested to include the evaluation of psychosocial elements, such as adherence to medications, and daily functional outcome in the context of resistance definition. In this view, patients can be considered resistant to therapy only if both clinical and functional outcome are compromised (30–32).

A recent review performed by the treatment-resistant schizophrenia working group consensus guidelines (33) concluded that there was a relative consensus among authors in defining subjects treatment-resistant: a confirmed diagnosis of schizophrenia based on validated criteria, an adequate pharmacological treatment, and the persistence of significant symptoms despite adequate treatment (33). Although significant differences exist among the main guidelines for the treatment of schizophrenia in terms of operationalized definition of

resistance, some commonalities can be observed. In particular, the shared criteria concern the requirements for at least two failed treatment trials, each of a minimum of 6 weeks, and the use of standardized rating scales (2, 34–38). In addition, the working group consensus guidelines (33) suggested to incorporate into criteria for defining treatment-resistance two further elements: patients adherence and functional impairment.

METHODS

In April 2018, we performed an electronic search in PubMed on predictive factors of treatment resistance in FEP, with no filter or MESH restriction, using the following search string: "schizophrenia" OR "psychosis" OR "first-episode of psychosis" OR "early psychosis" AND "predictive factors" OR "predictors of response" AND "resistant patients" OR "treatment resistance" OR "treatment non-response." This string provided a high specific search, obtaining an accurate selection of article indexed in PubMed. We included the following types of publications: randomized-controlled trials, observational studies, longitudinal studies, retrospective studies, case-control studies, open-label investigations, cohort studies, and reviews until July 2018. Publications must concern predictors of treatment resistance in early psychosis as the principal issue (all definitions of resistance described in the previous paragraph are included in our revision). We excluded publications written in languages different from English.

RESULTS

The search described in the previous section provided 1208 records. Eighty additional records were identified from another research platform (Google scholar). We removed the duplicates records (246). Eligibility status for all retrieved articles was determined in two stages. First, all studies were screened basing upon title and abstract. Second, papers passing the initial title and abstract screen were reviewed basing upon the full manuscript. Nine hundred seventy-seven records were excluded because they did not fit the objective of the review, 11 as the full text was not available. Full text articles selected for eligibility were 54; seven of them were excluded as were not written in English. This review included 47 records: 5 reviews, 3 meta-analyses, 22 longitudinal studies, 2 retrospective studies, 1 naturalistic study, 6 randomized controlled trials, 2 open-label studies, 2 case-control studies, 4 cohort studies.

Number of studies participants was ranged between 56 and 1,175. All studies included both genders; the majority of studies had an equal distribution of males and females. The predominant ethnicity was the Caucasian. Duration of the longitudinal studies was ranged between 1 and 10 years, while the duration of controlled-trials was ranged between 6 weeks (acute phase) and 1 year. Ninety percent of studies enrolled participants at early stage of illness (first episode of psychosis, first hospital contact, recent onset of psychosis). All subjects presented a schizophrenia spectrum disorder. The PRISMA flow chart of this review is presented in **Figure 1**.

Patient-Related Predictors of Treatment Resistance

Predictors of treatment resistance that are linked to patients characteristics were investigated in recent years. In particular age, gender, premorbid functioning, level of education, and marital status are the main individual-related factors that were studied in the context of resistance to treatment.

Crespo-Facorro et al. (40) performed a randomized controlled study, in which 172 patients with a first episode of non-affective psychosis were assigned to haloperidol, olanzapine, and risperidone in a random way. Results concerned the "6-weeks acute phase of a large epidemiological and longitudinal (3 years) intervention program of first-episode psychosis" (40). Among the patient-related variables, authors found that lower premorbid functioning was one of the most important factors in distinguishing antipsychotic non-responders from responders. This result was confirmed in a second study conducted by the same authors (41) on 375 FEP subjects. Similar findings were also reported by Addington and Addington (42) in 240 schizophrenia spectrum disorder patients with FEP in a period of follow-up of 36 months, and by Albert et al. (43) in a 5-years study including 255 FEP patients. Several investigations adequate for sample characteristics and duration (44–47) suggested that a good premorbid adjustment and social environment may predict a better response to treatments. Recently, Lasalvia et al. (48) confirmed this result stating that "premorbid adjustment and insight predicted outcome regardless of the kind of treatment" (48).

Wimberley et al. (49) performed a 9-years cohort study (population-based) in 8624 patients with a diagnosis of schizophrenia to identify predictive factors of treatment resistance at first hospital contact. Results showed that "a younger age, living in a less urban area, and primary education level were all significantly associated with treatment-resistant schizophrenia" (49). The relationship between lower educational level and treatment resistance was previously found by other three studies performed by Verma et al. (50), Díaz et al. (51), and Lasalvia et al. (48) in samples of respectively, 1,175 subjects with schizophrenia spectrum disorders, 174 patients with FEP, and 444 patients with schizophrenia. In a recent study published by Di Capite et al. (52) evaluating predictors of relapse in 63 patients with first-episode psychosis who have discontinued antipsychotic medications in a period on 1 year, authors concluded that "to be engaged in education or training was not predictive of relapse" (52).

Only few studies found a relationship between age and treatment-response, also due to the fact that many investigations have not considered age but age at illness onset. Nevertheless, two studies (43, 53) reported an association between older age and remission. Study published by Zhang et al. lasted 1 year and included 398 patients never medicated with FEP in schizophrenia spectrum disorders. The role of gender, in particular male gender, as predictor of worse response to treatments since the FEP is still controversial, although male gender is traditionally retained an indicator of poor outcome in schizophrenia. Some studies actually suggested that male gender may be considered a predictor of treatment non-response in

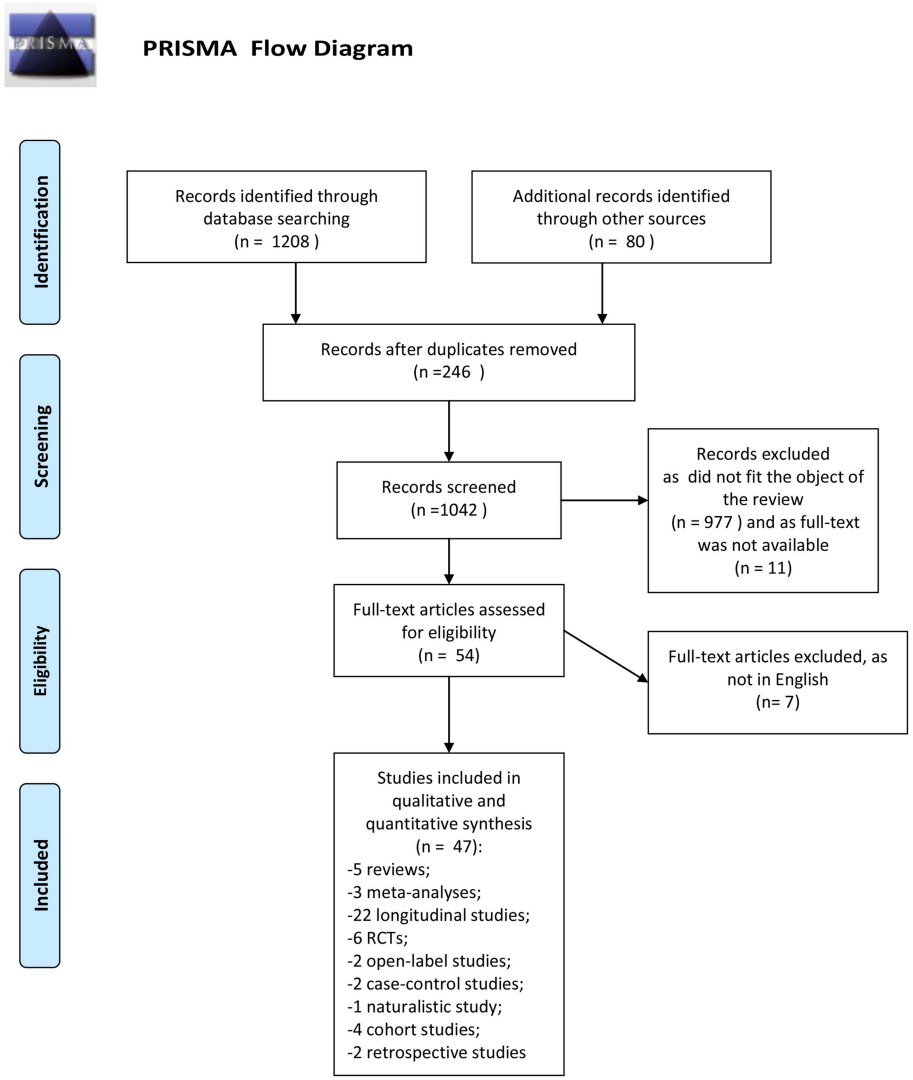

FIGURE 1 | PRISMA flow diagram. Adapted from Moher et al. (39).

FEP and schizophrenia spectrum disorders. In particular, Selten et al. (54) performed a study with a follow-up phase lasting 30 months in 125 subjects with FEP and schizophrenia spectrum disorders diagnosis. They found that the predominant predictor of poor outcome was male gender (together with substance abuse). Similar findings were reported by Derks et al. (55) and Díaz et al. (51). The two study had a similar design, the same duration of 1 year and the same criteria of inclusion: patients with FEP in schizophrenia spectrum disorders, including brief reactive psychosis and schizoaffective disorder. The only significant difference concerned the sample size, as in the first study were included 498 patients, while in the second they were only 174. Lower odds for remission were found in male patients in both studies. These findings were confirmed by the study performed by Di Capite et al. (52) that suggested that males had a higher risk of relapse after antipsychotic discontinuation then females and in the study conducted by Lally et al. (56) in 246 FEP patients with schizophrenia spectrum disorders concluding that treatment resistance was strictly connected with male sex. Other investigations considered the female gender as one of predictive factors of response to treatment. There was a broad accordance among authors in retaining that the female sex represents a strong predictor of remission and recovery (43, 50, 57). However, it must be noticed that many other studies have not confirmed the effect of gender on response to treatments (15, 19, 21, 44, 47, 58–64).

Data concerning the role of marital status in predicting treatment resistance are scarce and heterogeneous. Emsley et al. (45) found in a study of 1 year on 57 patients with FEP and schizophrenia spectrum disorders a significant relationship between single status and resistance to treatments. In a similar way Díaz et al. (51) reported that single status predicted

non-response and non-remission. On the contrary, Teferra et al. (65) reported that single status may predict better outcome in a 5-years study performed in Ethiopia in 312 patients with schizophrenia.

Disorder-Related Predictors of Treatment Resistance

With respect to clinical predictors of treatment resistance in FEP several symptomatic factors have been discussed about treatment resistance and poor long-term outcome. Positive symptoms were thought for long time to be the most important outcome measure and were the standard parameters for treatment resistance assessment. This was due to the fact that other symptoms were not correctly recognized or undervalued, or symptoms such as negative symptoms were considered unresponsive to treatment.

Considering the clinical response only in terms of the positive symptoms decrease is clearly reductive. In fact, schizophrenia since its early stages includes a wider spectrum of symptoms, involving negative, cognitive, and/or disorganized symptoms, as well as functional deficits. Several authors have shown that residual positive symptoms and global psychopathology, cognitive impairment, and enduring negative symptoms constituted the indicators of the severity of schizophrenia and were associated with non-response to antipsychotics (66–68).

In an epidemiological cohort study lasting 18 months and including 367 FEP patients "treated with olanzapine or risperidone" (69), authors found that 33% of patients with schizophrenia had continuous positive symptoms and another 22% presented positive symptoms following relapse. Overall, 35% of patients were found to be in symptomatic remission at 18 months but 20% had persistent psychoses with an unchanged severity of illness. Crespo-Facorro et al. (41) found that the presence of positive and disorganized symptoms at baseline predicted resistance to treatment. Addington and Addington (42) stated that "the high level of both positive and negative symptoms may predict poor outcome in schizophrenia spectrum disorders."

Investigations focused on the evaluation of positive symptoms of schizophrenia as poor response and resistance predictors remain rather sparse. However, on this topic, an interesting 10-year follow-up study has been performed to investigate "long-term trajectories of positive and negative symptoms in FEP" (70). Four-hundred-ninety-six patients with diagnosis of schizophrenia spectrum disorders were assessed with several evaluation tools, such as the Scales for the Assessment of Positive (SAPS) and Negative Symptoms (SANS) (71). Results indicated that around 60% of subjects experienced a reduction followed by a stabilization of positive symptoms during a period ranged between one and 5 years, while changes in negative symptoms did not reach the same degree. Moreover, in patients who responded to treatment the trend of positive symptoms continued to improve across 10 years. On the other hand, 50% of the cohort did not obtain a reduction of negative symptoms over the 10 years (70). Individuals who persistently suffer from negative symptoms may present impaired functioning and psychological outcomes with a higher rates of treatment resistance in comparison with people who show a decrease of negative symptoms over time (72, 73). As negative symptoms are already present and prominent at the early phase of the illness, in a minority of patients the full syndrome of treatment resistance is present since the FEP. Milev et al. (67) performed a longitudinal first-episode study with a 7-year follow-up on 99 subjects who were in their first episode of illness. Authors found a significant influence of both cognitive and negative symptoms on response to treatments. Similar findings were observed by Siegel et al. (74) in 208 patients with schizophrenia monitored for 3 years.

Higher severity of negative symptoms at the beginning of the trials was recognized to be a powerful predictor of resistance to treatments also in more recent investigations. In particular, Ventura et al. (75) showed that the degree of negative symptoms in 149 recent-onset (1 year) subjects with schizophrenia was associated with impaired everyday functioning 7 years later. Negative symptoms in early psychosis did not change in the first year and predicted social functioning after 12 months. In addition, negative symptoms at onset of schizophrenia were related to the persistence of negative symptoms after 8 years. These results suggested that "negative symptoms may be an important early course target for interventions to promote the recovery" (75). Demjaha et al. (8) performed a longitudinal study in a large cohort of 323 FEP patients that were studied for 10 years of follow-up. Findings showed that the strongest effect on treatment resistance was exercised by the negative symptoms at onset of illness. Other predictors of non-response and resistance in this study were the younger age of onset and the diagnosis of schizophrenia. Yoshimura et al. (76) confirmed the previous results about the influence of negative symptoms on resistance in 131 patients with schizophrenia. Investigation conducted by Downs et al. (77) in 638 subjects with early-onset psychosis highlighted the importance of the negative symptomatology in predicting response also in particular populations such as children and adolescents. In fact, authors concluded that early psychosis is characterized by negative symptoms that significantly contributed to the unsuccessful response or resistance to treatment. Other investigators evaluated the predictors of remission in schizophrenia spectrum disorders. Some of them (78) observed that a lower degree of positive, negative, and general symptoms was linked with remission, while other (62) found that only a lower degree of negative symptoms at baseline was responsible of a better response to treatment. Cognitive performances and disorganized symptoms obtained less attention among investigators and few studies have been performed on this issue. Chiliza et al. (7) concluded that both cognitive and disorganized symptomatology predicted resistance in 126 patients with schizophrenia spectrum disorders. Other Levine and Rabinowitz (47) identified only cognitive impairment at baseline as predictor of non-response to treatment in 49 FEP patients with schizophrenia spectrum disorders. This finding is in accordance a more recent study (16) in which authors compared resistant and responder schizophrenic patients and observed that resistant subjects had more severe cognitive impairment than responders, in particular in verbal memory tasks.

Two illness-related factors that received particular attention in the context of resistance to treatment are the diagnosis of schizophrenia and the age at onset of disease. Some

studies considered both these factors as predictors of non-response (8, 41, 47). Other investigations found a significant association only between diagnosis of schizophrenia and treatment resistance (49, 70, 79).

Comorbidity is another clinical factor that we need to consider in this context. In particular, substance use disorders had a significant impact in terms of clinical manifestations and treatment outcome. Around 40% of individuals with schizophrenia spectrum disorders meet criteria for alcohol use, and about 30% for substance use disorders (80). To our knowledge only 5 studies evaluated how substance use may predict outcome in schizophrenia spectrum disorders. The first was a 30 months follow-up study (54) and involved 125 FEP patients with schizophrenia spectrum disorders. Results showed that the conjunction of male gender and substance abuse (cannabis) was a predominant predictor of non-response to treatment in this population. Pelayo-Terán et al. (81) confirmed these findings in a 6-weeks study on 161 FEP patients with schizophreniform and schizoaffective disorders, specifying that misuse of cannabis predicted non-response of both positive and negative symptoms. Studies performed by Austin et al. (70) and Wimberley et al. (49) confirmed the role of substance use disorders in treatment resistance in patients with FEP. The design of these investigations was previously described in this review.

Boter et al. (82) in a 12-months follow-up study considered this topic from another point of view and investigated predictors of remission in 498 FEP patients with schizophreniform or schizoaffective disorder. They identified the absence of substance use disorder as predictor of remission.

Neurobiological Predictors of Treatment Resistance

One of the controversies in literature concerned whether psychosis onset derives by some neurobiological abnormalities or whether it exerts a long-term toxic effect on the brain *per se*. We have limited knowledge to identify which neurobiological factors allow to separate from the FEP responders and resistant patients. Anyway, some neurobiological and neuroimaging factors may be detected as potentially involved in the mechanisms of response/non-response to therapies. Some experts have suggested that patients with FEP present a variability in response to antipsychotics that is induced by different neurobiological correlates. One of the hypotheses concerns the relationship between the activity of dopamine system and treatment response. Some findings support this hypothesis as they found high levels of synthesis and release of dopamine in schizophrenic patients with a good response, in comparison with resistant subjects (83).

Some studies examined in plasma the level of dopamine metabolites and observed that a lower concentration before treatment is related to a less favorable response to first-line medications (84, 85). Moreover, a study conducted post-mortem compared two groups with positive and negative response to treatment and identified a lower number of dopaminergic synapses in patients with poor response.

Kim et al. (86) performed a small study including 12 patients with schizophrenia who received clozapine and were considered resistant, 12 patients who had considered responders, and 12 controls with no psychiatric diagnosis. Authors found that the subgroup of resistant patients were distinguished by reduced level of dopamine synthesis in striatum. This findings may suggest that some neurobiological factors may be responsible for treatment resistance in schizophrenia and a candidate biomarker of response is the level of that dopamine synthesis.

In another recent study (87) authors reported that a greater decrease of myelination in substantia nigra was observed in cases of schizophrenia with a poor response to treatment in comparison with responders and healthy controls. This finding does not allow to conclude that substantia nigra aberrations may be considered as predictors of treatment resistance. In fact, these aberrations could be explained at least in part as a consequence of toxicity of relapses and non-response to therapies. Nevertheless, this investigation indicates the effort of investigators to better understand, also in terms of neurobiological abnormalities the treatment resistance phenomena.

Alterations in the levels of cortisol and other markers of inflammations have been registered at the onset of psychosis. Mondelli et al. (88) performed a 12 weeks follow-up study on 68 FEP patients and 57 controls. Authors collected saliva and blood samples to measure the level of cortisol and serum markers of inflammations before and after antipsychotic administration. Results showed that blunted cortisol awakening response and increased proinflammatory cytokines were predictors of resistance in the early phases of psychosis. These factors are potentially considered as strong predictive factors of non-response in this phase of the illness.

Regulatory system of cortisol was already found implicated in schizophrenia treatment resistance in previous studies. For example, pituitary volume measured at the onset of psychotic disorders was investigated as a factor that can predict response in FEP (89). Authors evaluated if baseline pituitary volume was significantly related to treatment response in 42 FEP patients treated with quetiapine for 12 weeks. Results indicated that pituitary volume had an inverse relation with decrease of symptom severity. This association highlighted the relevance of hypothalamic-pituitary-adrenal axis in the early stages of psychosis.

In recent years, neuroimaging techniques have allowed to study if there is a relationship in psychotic disorders between refractoriness to therapies and structures of brain. Some investigators sustained that poor treatment response is significantly related to the diminished volume of gray matter (90). In one-hundred-twenty-six patients, including 80 subjects with a diagnosis of first-episode psychosis, gyrification was measured in multiple areas of brain and a significant hypogyria was found in comparison with 46 healthy subjects. In particular, subjects who did not respond to treatment showed hypogyria in bilateral insular regions, left frontal area, and right temporal area when compared with patients who responded. So, authors concluded that at first stages of illness non-responders had significant alterations of cortical folding compared with responders and with healthy subjects. Due to the scarcity of investigations on this field it is not possible to draw any conclusion. Nevertheless, it seems that the pituitary volume measured with structural magnetic

TABLE 1 | Summary of studies on predictive factors of treatment resistance.

	Study design	Patients (*n*)/type	Trial duration	Predictors of
PATIENT-RELATED FACTORS				
Malla et al. (44)	Longitudinal study	107 FEP SZ	2 years	**Response/remission** Better premorbid adjustment
Emsley et al. (45)	Longitudinal study	57 FEP SSD	2 years	**Non-response/resistance** Single status, lower premorbid functioning
Crespo-Facorro et al. (40)	RCT olanzapine vs. haloperidol vs. risperidone	172 FEP SSD	6 weeks acute phase (in the context of 3 years longitudinal intervention)	**Non-response/resistance** lower premorbid functioning
Selten et al. (54)	Longitudinal study	125 FEP SZ	30 months	**Non-response/resistance** Male gender (plus substance use)
Albert et al. (43)	Longitudinal study	255 FEP	5 years	**Response/remission** Female gender, higher age, good premorbid function
Addington and Addington (42)	Longitudinal study	240 FEP SSD	36 months	**Non-response/resistance** Reduced social functioning and lower premorbid functioning
Levine et al. (46)	Longitudinal study	263 SSD at recent onset	2 years	**Response/remission** Good premorbid functioning
Derks et al. (55)	Randomized, open-label ,prospective study olanzapine vs. haloperidol vs. risperidone	498 FEP SSD	1 year	**Non-response/resistance** Male gender
Verma et al. (50)	Naturalistic study	1,175 FEP SSD	2 years	**Response/remission** Female gender, tertiary education
Teferra et al. (65)	Longitudinal study	312 FEP SZ	5 years	**Response/remission** Single status
Crespo-Facorro et al. (41)	RCT olanzapine vs. haloperidol vs. risperidone	375 FEP SSD	6 weeks	**Non-response/resistance** Poorer premorbid adjustment
Díaz et al. (51)	Randomized, open-label ,prospective study olanzapine vs. haloperidol vs. risperidone	174 FEP SSD	1 year	**Non-response/resistance** Male gender, single status, and low education level
Zhang et al. (53)	Prospective cohort study	398 FEP SZ	1 year	**Response/remission** Higher age
Di Capite et al. (52)	Longitudinal study	63 FEP SSD antipsychotic discontinuation	1 year	**Non-response/resistance** Male gender Not related with educational level
Wimberley et al. (49)	Cohort study	8,624 SZ at first hospital contact	9 years	**Non-response/resistance** Younger age, living in less urban area, low education level
Lally et al. (56)	Longitudinal study	246 FEP SSD	5 years	**Non-response/resistance** Male gender
Lasalvia et al. (48)	Retrospective study	444 FEP SSD	9 months	**Response/remission** Good premorbid adjustment and insight regardless of treatment, higher educational level
Friis et al. (79)	Longitudinal study	301 FEP SSD	10 years	**Non-response/resistance** Lower premorbid functioning
DISEASE-RELATED FACTORS				
Lambert et al. (69)	Retrospective study	367 FEP SSD	18 months	**Non-response/resistance** High positive symptoms
Milev et al. (67)	Longitudinal study	99 FEP SSD	7 years	**Non-response/resistance** Cognitive and negative symptoms
Siegel et al. (74)	longitudinal study	208 FEP SZ	2–8 years (mean 3 years)	**Non-response/resistance** High positive, negative, and depressive symptoms
Selten et al. (54)	Longitudinal study	125 FEP SZ	30 months	**Non-response/resistance** Substance use
Addington and Addinton (42)	Longitudinal study	240 FEP SSD	36 months	**Non-response/resistance** High positive and negative symptoms

(Continued)

TABLE 1 | Continued

	Study design	Patients (*n*)/type	Trial duration	Predictors of
Boter et al. (82)	Longitudinal study	498 FEP SSD	1 year	**Response/remission** Absence of use disorder
Strauss et al. (72)	Longitudinal study	56 FEP SZ	20 years	**Non-response/resistance** Deficit syndrome
Levine and Rabinowitz (47)	Longitudinal study	49 FEP SSD	2 years	**Non-response/resistance** Cognitive impairment Diagnosis of schizophrenia Early age at onset
Üçok et al. (62)	Longitudinal study	93 FEP SZ	2 years	**Response/remission** High positive and low negative symptoms at onset
Galderisi et al. (73)	RCT olanzapine vs. amisulpride vs. ziprasidone vs. quetiapina	345 FEP SSD	1 year	**Non-response/resistance** Persistent negative symptoms
Verma et al. (50)	Naturalistic study	1,175 FEP SSD	2 years	**Non-response/resistance** Diagnosis of schizophrenia
Crespo-Facorro et al. (41)	RCT olanzapine vs. haloperidol vs. risperidone	375 FEP SSD	6 weeks	**Non-response/resistance** High positive and disorganized symptoms, diagnosis of schizophrenia, and early age at onset
Gaebel et al. (78)	RCT risperidone vs. haloperidol	166 FEP SZ	1 year	**Response/remission** Low positive and negative symptoms
Pelayo-Terán et al. (81)	RCT risperidone vs. haloperidol	161 FEP SSD	6 weeks	**Non-response/resistance** Cannabis use
Austin et al. (70)	longitudinal study	496 FEP SSD	10 years	**Non-response/resistance** Negative symptoms Substance use
Chiliza et al. (7)	Longitudinal study	126 FEP SSD	1 year	**Non-response/resistance** High negative symptoms
Ventura et al. (75)	Longitudinal study	146 SZ recent onset	1 year + 7 years of follow-up	**Non-response/resistance** Early negative symptoms
Friis et al. (79)	Longitudinal study	301 FEP SSD	10 years	**Non-response/resistance** Diagnosis of schizophrenia
Wimberley et al. (49)	Cohort study	8,624 SZ at first hospital contact	9 years	**Non-response/resistance** Diagnosis of schizophrenia Substance use
Demjaha et al. (8)	Longitudinal study	323 FEP SSD	10 years	**Non-response/resistance** Negative symptoms at onset diagnosis of schizophrenia and early age at onset
Yoshimura et al. (76)	Retrospective study	131 FEP SZ	Not reported	**Non-response/resistance** High negative symptoms
Downs et al. (77)	Cohort study	638 early-onset psychosis (10–17 years)	5 years	**Non-response/resistance** High negative symptoms
NEUROBIOLOGICAL FACTORS				
Garner et al. (89)	Controlled dose-finding study	42 FEP SZ with quetiapine	12 weeks	**Non-response/resistance** Larger pituitary volume
Palaniyappan et al. (90)	Case-control study	126 (80 FEP) SSD	12 weeks	**Non-response/resistance** Hypogyria at bilateral insular, left frontal, and right temporal regions
Mondelli et al. (88)	Longitudinal study	68 FEP 57 controls	12 weeks	**Non-response/resistance** Blunted cortisol awakening response and increased proinflammatory cytokines
Kim et al. (86)	RCT	12 SZ—TR with clozapine vs. 12 SZ responders vs. 12 healthy controls	12 weeks	**Non-response/resistance** Lower dopamine synthesis capacity in striatum

(Continued)

TABLE 1 | Continued

	Study design	Patients (*n*)/type	Trial duration	Predictors of
Walker et al. (87)	RCT (post-mortem)	14 SZ (6 TR) 9 healthy controls	Not available	**Non-response/resistance** Reduction of myelination in substantia nigra
TREATMENT-RELATED FACTORS				
Bottlender et al. (101)	Longitudinal study	58 FEP SZ	15 years	**Non-response/resistance** Longer DUP
Correll et al. (93)	Open-label study	131 acute SSD with fluphenazine	4 weeks	**Non-response/resistance** Poor response at week 1
Malla et al. (44)	Longitudinal study	107 FEP SZ	2 years	**Response/remission** Higher level of adherence
Leucht et al. (94, 95)	Data analysis from 7 RCTs	1,708 SSD	Not available	**Non-response/resistance** Poor response at week 1 and 2
Kinon et al. (26)	Data analysis from 5 RCTs	1,077 SSD	6 months	**Non-response/resistance** Poor early response (but early response does not predict subsequent response)
Boter et al. (82)	Longitudinal study	498 FEP SSD	1 year	**Response/remission** Higher level of adherence
Kinon et al. (96)	RCT	628 SSD with risperidone. If non response switch to olanzapine	12 weeks + 10 weeks if non early response	**Non-response/resistance** Poor response within 2 weeks
Üçok et al. (62)	Longitudinal study	93 FEP SZ	2 years	**Response/remission** Higher level of adherence
Zhang et al. (53)	Prospective cohort study	398 FEP SZ	1 year	**Response/remission** Higher level of adherence
Austin et al. (70)	Longitudinal study	496 FEP SSD	10 years	**Non-response/resistance** Longer DUP
Friis et al. (79)	Longitudinal study	301 FEP SSD	10 years	**Non-response/resistance** Longer DUP
Demjaha et al. (8)	Longitudinal study	323 FEP SSD	10 years	**Non-response/resistance** Longer DUP
Yoshimura et al. (76)	Retrospective study	131 FEP SZ	Not reported	**Response/remission** Shorter DUP

SZ, schizophrenia; SSD, schizophrenia spectrum disorders; FEP, first episode of psychosis; TR, treatment resistant; RCT, randomized-controlled study; DUP, duration of untreated psychosis.

resonance may represent a potential predictor of response/non-response in psychosis at onset.

Treatment-Related Predictors of Resistance

In our opinion three factors related to treatment are particularly relevant in predicting resistance in schizophrenia and require to be mentioned and discussed: adherence, early response, and duration of untreated psychosis. Concerning adherence to therapies, antipsychotic treatment non-adherence was found tightly linked to low odds for response and remission, in particular in the first stages of illness (20, 22, 52, 91). Some Pelayo-Terán et al. (81) suggested that adherence was one of the most robust predictors of the first relapse. The main studies on this topic were in accordance to conclude that an higher level of adherence since FEP predicted response and remission of the illness (44, 53, 62, 82). In light of these considerations, prescription of long-acting antipsychotics in patients with several risk factors for relapse (for example diagnosis of schizophrenia, comorbid substances abuse, prominent negative symptoms, and non-adherence to oral antispychotics) also at first-episode of psychosis may significantly improve outcome of FEP (22). Response to antipsychotic treatments should begin within first weeks of therapy, with the greatest effect in the first 2 weeks (24, 92). Correll et al. (93) performed an open-label 4-weeks study in 131 patients with schizophrenia who received fluphenazine. They showed that poor response in the first week of treatment with a typical antipsychotic may predict non-response also at fourth week of the trial. The same results were obtained by Leucht et al. (94, 95) who analyzed data from 1708 patients with schizophrenia or schizophreniform disorder enrolled in 7 randomized controlled trials (RCT) on antipsychotics. In addition, these authors stated that minimal symptoms reduction at week 2 had high specificity and sensitivity in identifying responders at week 4. This datum was also confirmed by Samara et al. (27) in a meta-analysis of 34 studies aimed to evaluate the association between lack of symptoms

improvement at week 2 and later non-response. Kinon and collaborators conducted two investigations (26, 96). In the first one, authors analyzed data from 5 double-blind RCTs including 1,077 patients with schizophrenia spectrum disorders who received second-generation antipsychotics. Authors considered a period of observation of 6 months and assessed at different time points medication discontinuation rates. Results showed that early non-response predicted subsequent lack of response, but early response could not be considered as a predictor of following response. The same Kinon et al. (26) performed a 12-weeks RCT aimed to investigate in 628 patients diagnosed with schizophrenia or schizoaffective disorder who initially received risperidone whether the early response (within 2 weeks) to an antipsychotic medication may predict the following response. Subjects who responded proceeded with risperidone, while patients who did not respond in the first 2 weeks were randomized to continue with risperidone or to receive olanzapine for other 10 weeks. Findings reported that early non-responders may require more than 4–6 weeks to respond to antipsychotics.

Another important variable associated with non-response and resistance is the duration of untreated psychosis (DUP). DUP is "the period between the time psychosis begins to the time adequate treatment is sought and secured. The mean duration of untreated psychosis is ranged between 1 and 2 years and the median is about 6 months" (97). A more prolonged DUP has been related to a longer time of response to treatment in patients who presented a first-episode of psychosis and to an impaired course of the disorder (21, 98–100). Bottlender et al. (101) conducted a long-term study lasting 15 years in 58 patients with schizophrenia followed-up since their first psychiatric admission. Authors observed that a higher level of negative, positive and general psychopathological symptoms and a lower global functioning 15 years after the first psychiatric admission were associated with a prolonged DUP. In accordance with these results, another 10-years follow-up study (70) concluded that a longer DUP predicted worse trajectories of positive and negative symptoms in time, with a poor response to medications. Friis et al. (79) stated that first-episode psychosis patients that have not begun an adequate antipsychotic treatment at least within 6 months (having a longer DUP) presented an higher risk to become resistant patients. In line with previous findings, Demjaha et al. (8) reported that longer DUP predicted treatment resistance. Moreover, Yoshimura et al. (76) found that a shorter DUP predicted favorable response and remission in FEP patients with schizophrenia.

All literature findings highlighted the importance to detect psychosis at onset and to early consider treatment-related factors because they are modifiable risk variables.

DISCUSSIONS

There is a general consensus among authors in retaining that the identification of specific factors predicting treatment response in patients with FEP significantly ameliorates the quality of clinical management of these patients in the critical early phase of pathology. Adequate early interventions produce a positive effect on long-term illness outcome, in terms of remission and recovery.

Main findings of our review show that among patient-related predictors of resistance to treatment lower premorbid functioning is an important factor in distinguishing antipsychotic non-responders from responders. Lower educational level can be also considered as a robust predictor of resistance, while the role of age and marital status is still controversial. Several studies suggest the male gender as a potential risk factor for treatment non-response, but we have to consider that many other studies have not confirmed the effect of gender.

Regarding disease-related predictors of treatment resistance, the higher level of negative symptoms from the FEP and their persistency over time induces a worse impairment of social functioning, more serious psychopathological phenomena, and a higher degree of refractoriness to treatment then controls who present a progressive decrease of negative symptoms. Positive, disorganized, and cognitive symptoms seem to be less significant in predicting treatment response. According to our review, the two main predictors of resistance related to the disorder are the diagnosis of schizophrenia and the younger age at onset. Among comorbidity conditions substance use disorder is the most studied predictive factor of treatment resistance and poor outcome.

Some neurobiological and neuroimaging factors may be identified as potentially involved in the mechanisms of response/non-response to therapies, but none of these factors has been identified as reliable predictor that can allow to separate responders and resistant patients in the course of the FEP. Some literature data support the hypothesis that the level of the dopamine synthesis is a potential biomarker of responsiveness to treatment. In addition, blunted cortisol awakening response and higher concentrations of pro-inflammatory cytokines are biological predictive factors of treatment resistance in early stages of psychotic disorders. Some neuroimaging studies show that at first stages of illness patients who do not respond have a significant reduction of pituitary volume and defects of cortical folding. Few innovative investigations have explored potential genetic predictors of treatment resistance, but initial data do not allow to draw any conclusion. Genetic studies about response to medications in early phases of psychosis are required, considering that only few initial investigations with inconclusive results are available.

TABLE 2 | Clinical predictive factors of treatment resistance.

Stable factors	Changeable
Poor premorbid functioning	Lower educational level
Male gender	Single marital status
Younger age at onset	Negative symptoms
Diagnosis of schizophrenia	Substance use disorder
Neurobiological factors	Non-adherence
	Early non-response (within week 2)
	Duration of untreated psychosis

Finally, lack of adherence to prescriptions, no early response (within 2 weeks) to antipsychotics, and prolonged of duration of untreated psychosis are the most important treatment-related factors that predict resistance.

Data provided from cited studies are displayed in **Tables 1**, **2**.

CONCLUSIONS AND REMARKS FROM THE SYSTEMATIC REVIEWS

In literature we have examined five systematic reviews that focus on the topics of our investigation and can contribute to support our conclusions (20, 21, 32, 102, 103). In summary, these studies demonstrate that outcomes for patients with schizophrenia spectrum disorders can be significantly improved ameliorating early treatments and shortening the period of time that divides the beginning of symptoms from adequate specific interventions. Authors highlight that available trials are affected by some criticalities that are at least partly responsible for the heterogeneity of findings. For example, studies vary considerably in defining diagnosis of patients who can be enrolled (some authors include subjects with unspecified psychosis or brief psychotic disorder that can present a completely different course and outcome from schizophrenia). The diagnostic instability intrinsic to first episode has to be considered as potential bias for the results of investigations. In addition, few studies are designed with a sufficient statistical power to measure the predictive effect of several clinical factors with respect to treatment response. Studies have different duration and frequency of assessments during follow-up. Another very important limitation is that, as we have explained in the introduction of this review, criteria for determining resistance are different among studies, sometimes limited to severity of symptoms, in other cases extended to cognitive performances and social functioning. Despite these limitations and persisting uncertainty on the actual role of most clinical and biological factors, there is no doubt that developing research and refining knowledge on predictors of response in the early stage of psychotic disorders can produce noticeable results in terms of improvement of long-term clinical and functional outcome. In particular, some key predictive factors, like duration of untreated psychosis, or non-adherence to medications, can be modified by early intervention with significant effects on long-term outcome.

AUTHOR CONTRIBUTIONS

PB and SB equally contributed to revise studies in literature and to write the manuscript. PR contributed to project the review and to organize the structure of manuscript and tables.

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REVIEW
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Treatment-Resistant Schizophrenia: Genetic and Neuroimaging Correlates

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Schizophrenia is a severe neuropsychiatric disorder that affects approximately 0.5–1% of the population. Response to antipsychotic therapy is highly variable, and it is not currently possible to predict those patients who will or will not respond to antipsychotic medication. Furthermore, a high percentage of patients, approximately 30%, are classified as treatment-resistant (treatment-resistant schizophrenia; TRS). TRS is defined as a non-response to at least two trials of antipsychotic medication of adequate dose and duration. These patients are usually treated with clozapine, the only evidence-based pharmacotherapy for TRS. However, clozapine is associated with severe adverse events. For these reasons, there is an increasing interest to identify better targets for drug development of new compounds and to establish better biomarkers for existing medications. The ability of antipsychotics to improve psychotic symptoms is dependent on their antagonist and reverse agonist activities at different neuroreceptors, and some genetic association studies of TRS have focused on different pharmacodynamic factors. Some genetic studies have shown an association between antipsychotic response or TRS and neurodevelopment candidate genes, antipsychotic mechanisms of action (such as dopaminergic, serotonergic, GABAergic, and glutamatergic) or pharmacokinetic factors (i.e., differences in the cytochrome families). Moreover, there is a growing body of literature on the structural and functional neuroimaging research into TRS. Neuroimaging studies can help to uncover the underlying neurobiological reasons for such resistance and identify resistant patients earlier. Studies examining the neuropharmacological mechanisms of antipsychotics, including clozapine, can help to improve our knowledge of their action on the central nervous system, with further implications for the discovery of biomarkers and the development of new treatments. The identification of the underlying mechanisms of TRS is a major challenge for developing personalized medicine in the psychiatric field for schizophrenia treatment. The main goal of precision medicine is to use genetic and brain-imaging information to improve the safety, effectiveness, and health outcomes

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of patients via more efficiently targeted risk stratification, prevention, and tailored medication and treatment management approaches. The aim of this review is to summarize the state of art of pharmacogenetic, pharmacogenomic and neuroimaging studies in TRS.

Keywords: treatment resistant schizophrenia (TRS), genetic, pharmacogenetic, pharmacogenomic, neuroimaging, precision medicine

INTRODUCTION

Schizophrenia is a disabling disease and many patients who are affected will not be able to achieve their goals in most areas of life. Schizophrenia outcome is quite heterogeneous, with a course of illness characterized by different trajectories (Van Eck et al., 2018). Antipsychotic medication has revolutionized schizophrenia treatment, but approximately one-third of patients show scarce or no response to these drugs (Kane, 2012). The efficacy of antipsychotics for the initial treatment of psychosis is now well established and early antipsychotics introduction in first episode psychosis seems also to improve the long-term course of schizophrenia. Moreover, the effectiveness of antipsychotics maintenance treatment in schizophrenia management and in the relapse prevention represents a therapeutic practice supported by strong data (Goff et al., 2017). The Remission in Schizophrenia Working Group (RSWG) established schizophrenia clinical remission criteria, through the cut-off severity of some characterizing symptoms of the disease (Andreasen et al., 2005). However, clinical remission criteria, while necessary, are not sufficient to explain schizophrenia full functional remission, not taking into account other essential elements of recovery, such as: cognitive performance, depressive symptoms, experiences and daily functioning, quality of life and personal satisfaction (Zipursky and Agid, 2015). Indeed, in the past, more attention was focused on positive symptoms, giving less weight to negative symptoms, cognitive and psychosocial functioning (Vita and Barlati, 2018). It is now well established that positive symptoms explain only a small part of the variance of psychosocial functioning and that the greatest contribution to the functional outcome of schizophrenia is given by negative symptoms, cognitive and social cognitive impairment, as well as anxiety and depression (Galderisi et al., 2014, 2016). Despite the presence of effective antipsychotic drugs and the introduction of evidence-based psychosocial interventions, the course of schizophrenia is characterized by the alternation of remissions and relapses and only a few patients are classified as meeting recovery criteria (Zipursky and Agid, 2015). All this evidence leads to the conclusion that, regardless of the crucial role of antipsychotics, some patients who don't achieve clinical and functional recovery are defined as treatment-resistant schizophrenia (TRS) patients. Epidemiological data from the scientific literature report that approximately 30% of schizophrenic patients will develop TRS during the course of their disease (Kane et al., 1988; Elkis and Buckley, 2016). The first definitions of TRS were mostly based on the persistence of positive symptoms, despite an adequate antipsychotic treatment for doses and duration (Itil et al., 1966). However, the most commonly used TRS definition in clinical and research fields remains that of Kane's clozapine study (Kane et al., 1988). However, it has become clear that there is a need to revise Kane's resistance criteria, or some their variants, giving more attention to psychosocial functioning and not only to positive symptoms. Suzuki et al. (2012) proposed a broad definition of TRS and suggested the criteria include a failure to respond to two adequate doses and durations of antipsychotic treatment. Furthermore, the authors also recommended a comprehensive functional assessment. Recently, the National Institute for Health and Clinical Excellence (NICE) has defined the criteria for the TRS as an insufficient response to at least two different sequential antipsychotic drugs at appropriate doses and taken for an appropriate period of time (Nice Guideline, 2014).

Despite some efforts to standardize the resistance criteria of schizophrenia, there is considerable discrepancy in current clinical approaches. In addition to the NICE criteria, TRS definitions have been proposed by other relevant treatment guidelines, such as: the American Psychiatric Association (APA) (Lehman et al., 2004), the Texas Medication Algorithm Project (Moore et al., 2007), the Schizophrenia Patient Outcome Research Team (PORT) (Buchanan et al., 2010), the World Federation of Societies of Biological Psychiatry Guidelines (Hasan et al., 2012), and the International Psychopharmacology Algorithm Project (IPAP)[1]. All these TRS definitions are different and exposed to a wide range of interpretations, potentially leading to inconsistent clinical management and inaccurate treatment (Howes et al., 2017). Furthermore, even in research field a wide variety of TRS criteria have been applied in different studies. Variation in criteria limits studies comparison, complicates finding interpretation and their replication. Heterogeneity of study designs and populations, including less restrictive definitions of treatment resistance, may contribute to these inconsistencies (Howes et al., 2017). To address this issue, the Treatment Response and Resistance in Psychosis (TRRIP) Working Group has developed consensus criteria and guidelines on TRS, providing a fixed point for research and clinical translation (Howes et al., 2017).

Aim of the Review

Currently, it is not possible to predict those patients will or will not respond to antipsychotic treatment, and there is a growing interest in identifying new targets for drug development projects and better response biomarkers for current medications. Various levels of evidence have shown that treatment response and resistance in schizophrenia may be associated with certain genetic factors and brain abnormalities

[1] http://www.ipap.org

(Lally et al., 2016; Mouchlianitis et al., 2016). It is plausible that both neurodevelopmental and neurodegenerative factors may contribute to TRS, in terms of structural, functional brain abnormalities, neurochemical abnormalities or dysregulated gene expression (Elkis and Buckley, 2016). From this perspective, the aim of this review is to summarize the genetic and neuroimaging correlates associated with TRS, to uncover the underlying neurobiological mechanisms of such resistance and to find methods or markers for the early detection of this group of patients. In particular, this review aimed to provide an integrated point of view between genetics and neuroimaging regarding the possible causes of TRS.

MATERIALS AND METHODS

Search Strategy

Electronic searches were performed using MEDLINE/PubMed, PsycINFO, and EMBASE databases combining the following search terms: "schizophrenia," "pharmacogenetics," "pharmacogenomic," "candidate gene study," "genome wide association study," "GWAS," "neuroimaging," "Positron Emission Tomography – PET," "Single Photon Emission Computed Tomography – SPECT," "functional Magnetic Resonance Imaging – fMRI," "Magnetic Resonance Spectroscopy – MRS," "typical or first-generation antipsychotics – FGAs," "atypical or second-generation antipsychotics – SGAs," "response," "resistance," and "refractory." Detailed combinations of the above search terms are available from the authors on request. Two of the authors (SB, AM) independently reviewed the database to avoid mistakes in the selection of articles. In addition, the reference lists of the included articles were carefully hand-searched to identify other studies of possible interest.

Selection Criteria

All the studies, meta-analyses, and review articles on pharmacogenetics, pharmacogenomic, structural and functional neuroimaging related to TRS published until June 2018 were included. Studies were included if they met the following criteria: (a) being an original paper published in a peer-reviewed journal, (b) being an English language paper, and (c) involving subjects with TRS, defined according to established international criteria. When the inclusion criteria for TRS were not clearly defined, the study was excluded. Pharmacogenetics or neuroimaging studies on pharmacokinetics or on antipsychotic side effects were not considered.

RESULTS

Brain Structural and Functional Abnormalities in Schizophrenia

Since the first MRI study of schizophrenia, the use of this technique allowed the quantification of gray (GM) and white matter (WM) and the measurement of discrete, cortical and subcortical brain structures (Smith et al., 1984). Early morphological studies of schizophrenia primarily assessed specific brain regions of interest (ROIs) (Wible et al., 2001). More recently, functional neuroimaging has provided a direct way of investigating regional brain activity and the pathophysiology of schizophrenia *in vivo*. The presence of multiple small structural brain abnormalities in schizophrenia is now well established (Vita et al., 2015). Results about the progressive brain changes over time in schizophrenia are controversial, and the potential confounding effects of antipsychotics on brain structure is still under discussion. The presence of multiple structural brain abnormalities has been demonstrated by a large number of computed tomography (CT) and MRI studies in the past 40 years and confirmed by several meta-analytic reviews (Olabi et al., 2011; Fusar-Poli et al., 2013; Haijma et al., 2013; Vita et al., 2015). These are predominantly evident in some cerebral regions, such as the ventricular system, cortical GM and subcortical regions (Shenton et al., 2001). Reductions in whole brain measures (3%) and GM volume (2%), primarily in the frontal and temporal lobes, and enlargement of the lateral ventricles (16%) are among the most replicated findings. A small but significant reduction was also found in the WM (1%) (Haijma et al., 2013). A more exhaustive examination of regional brain structural abnormalities has been accomplished by voxel-based morphometry (VBM) studies, which confirmed earlier observed patterns of distributed GM reductions in the bilateral medial frontal and temporal regions, inferior parietal lobe, limbic and striatal regions, insula, thalamus, and basal ganglia (Bora et al., 2011; Palaniyappan et al., 2012). In their VBM meta-analysis, Bora et al. (2011) indicated a reduction in GM density in the dorsal and rostral anterior cingulate cortex (ACC), left lateral prefrontal areas, superior frontal gyrus, and orbitofrontal and fusiform regions.

Additionally, studies of WM tracts showed evidence of disorganization and an absence of alignment in white fiber bundles in frontal and temporoparietal brain regions and a reduction in WM diffusion anisotropy in schizophrenia subjects (Burns et al., 2003; Davis et al., 2003). More recently, diffusion tensor imaging (DTI) studies have identified several regions with decreased fractional anisotropy, reflecting altered WM connections and supporting the "disconnection model of schizophrenia" (Ellison-Wright and Bullmore, 2009; Crossley et al., 2017).

Regarding functional neuroimaging, this technique has been used to study patterns of increased or decreased activity within the brains of subjects with and without schizophrenia during rest and various assigned behavioral tasks; these studies have revealed that the affected parts of the central nervous system (CNS) are not contained within a single brain region but rather lie within neural networks that include numerous brain regions (Gur and Gur, 2010). Functional brain abnormalities in schizophrenia include alterations in information storage and retrieval by the dorsolateral prefrontal cortex (dlPFC), alterations in inhibitory responses to sensory stimuli by the ACC, deficits in memory encoding and retrieval by the hippocampus, alterations in sensory information reception and integration by the thalamic nuclei, primary sensory cortices and multimodal cortices and impairments in performance of cognitive tasks associated with the basal ganglia, thalamus, and cerebellum (Wright et al., 2000; Davis et al., 2003; Glahn et al., 2005). fMRI studies showed

patterns of widespread alterations in task-induced activity, which overlap with patterns of GM findings leading to one consistent results that is a decreased activation of frontal regions during cognitive tasks (Glahn et al., 2005; Minzenberg et al., 2009). However, this finding surprisingly was not consistently replicated when SPECT semiquantitative assessments were replaced by fMRI (Callicott et al., 2003). Furthermore, functional studies of social cognition and emotional processing suggested altered responses of the amygdala and hippocampus, potentially with respect to aversive stimuli (Li et al., 2010). The pathogenesis of structural and functional alterations in schizophrenia is still poorly understood, and only an ongoing integration of structural data with functional imaging may offer more insight in this field (Gur and Gur, 2010). Several longitudinal and cross-sectional MRI studies examined the meaning of such brain abnormalities, their static or progressive nature and their time of occurrence (van Haren et al., 2008). Finally, some recent studies have been reported that brain changes appear to be especially relevant in the first years of illness (Schnack et al., 2016; van Haren et al., 2016), although other studies have not confirmed these findings (Roiz-Santiañez et al., 2015).

Brain Abnormalities in Schizophrenia: Are They Reversible or Not?

In the last two decades, several studies have been conducted for understanding if reported abnormalities could reversible or not with some interventions. Among the first investigations, Keshavan and collaborators have shown an ameriolation of GM volume deficits in the superior temporal cortex and hippocampus in schizophrenia patients (Keshavan et al., 1998). In a more recent longitudinal study in a subgroup of first-episode psychosis patients who presented a remitting course after approximately 18 months, has been obtained a reversal of temporal lobe GM deficits (Schaufelberger et al., 2011). These results are consistent with other findings about the brain volume deficits reversibility in association with schizophrenia symptom improvement (de Castro-Manglano et al., 2011; Roiz-Santiáñez et al., 2015; Torres et al., 2016).

The longitudinal MRI studies further suggest that the degree of progression of brain structural abnormalities over the course of schizophrenia partially occurs with of the chronic antipsychotic usage. However, according to Vita et al. (2015) the class of antipsychotic is a key variable, because of more progressive GM loss correlates with higher mean daily antipsychotic intake in patients treated with at least one FGA, whereas less progressive GM loss correlates with higher mean daily antipsychotic intake in patients treated with SGAs only.

In addition, several neuroimaging studies on non-pharmacological intervertions in schizophrenia, indicated that cognitive remediation improves brain activation in two main areas: the prefrontal and thalamic regions. Accordingly, it has been suggested a positive effect of cognitive remediation on brain functioning in terms of the functional reorganization of neural networks, and structural changes were described both in GM and WM, confirming a neuroprotective effect of cognitive remediation (Penadés et al., 2017, 2019). Promising results have been also obtained with cognitive behavioral therapy (Mason et al., 2016, 2017) and physical aerobic exercise (Svatkova et al., 2015; Malchow et al., 2016).

On the other hand, several well-conducted MRI investigations have provided evidence that structural brain abnormalities associated with the diagnosis of schizophrenia may progress from the first psychotic episode to chronic disease stages, particularly during the initial few years after illness onset, even if these irreversible brain changes are restricted to subgroups of patients with an unremitting disease course and poorer outcome (Andreasen et al., 2013; Cannon et al., 2015).

Overall, literature data are controversial and further studies will be needed to better understand if brain abnormalities are reversible and which are not, at which stage of illness and with which type of intervention. Despite these limitations, most robust results demonstrate a reversibility of some brain abnormalities, particularly in the early stages of the illness, in relation to schizophrenia outcome.

Brain Structural Abnormalities in Treatment-Resistant Schizophrenia

Ventricular enlargement is one of the variables most studied in TRS. Early CT studies showed an inverse relationship between degree of ventricular enlargement and antipsychotics treatment response (Weinberger et al., 1979; Friedman et al., 1992; Mitelman and Buchsbaum, 2007). These findings were confirmed by subsequent CT studies, using also morphometric techniques, such as ventricular brain ratio (VBR). Over the last three decades, CT and then MRI cross sectional studies including chronic patients have found an association between ventricular enlargement and poor outcome (Friedman et al., 1992; Mitelman and Buchsbaum, 2007). In particular, studies in patients whose illness is progressive and resistant to treatment have shown abnormalities such as ventricular enlargement and decrease in GM (Mitelman and Buchsbaum, 2007; Mitelman et al., 2010). Many subsequent studies tried to replicate these findings, but a first meta-analysis of these early studies as well as a critical review of this subject found no relationship between ventricular enlargement and treatment response in schizophrenia patients (Borgio et al., 2010). Several longitudinal studies conducted on chronic patients (Davis et al., 1998) or first psychotic episode patients (Mitelman and Buchsbaum, 2007) confirmed these structural changes in the brain and found that they were progressive over the course of illness. In particular, in the first study just mentioned, it has been shown that "Kraepelinian patients" manifested left-sided ventricular enlargement compared to treatment responsive patients followed over the same 5-year follow-up period (Davis et al., 1998).

In an early ROI MRI study, Lawrie et al. (1995) found that poorly responsive patients had lower volumes of most brain structures than treatment responders, but no brain-imaging variables were statistically related to the outcome. In a later MRI study performed by the same research group, TRS patients showed a tendency to greater atrophy than those were treatment responsive (Lawrie et al., 1997). In this study, patients were selected as dichotomous groups (matched for age, sex, and

illness duration) of treatment-responsive and TRS patients using a descriptive criteria: responsive patients showing a marked reduction of symptoms and being able to return to the same social situation; resistant cases showing severe residual symptoms and requiring long-term institutional care.

In addition, in the MRI study performed by Buchsbaum et al. (2003), schizophrenia patients with a good outcome had larger relative mean putamen size, most marked for the dorsal putamen and right hemisphere, than poor outcome patients or normal controls. The authors suggested that the expansion of putamen size may be a physiological correlate of antipsychotic responsiveness and that small putamen size at disease onset may be a predictor of poor outcome (Buchsbaum et al., 2003).

The GM decrease in total volume or localized reductions in certain regions, such as the frontal, temporal and occipital cortexes and ventral thalamus were identified in very poor outcome schizophrenia patients (Mitelman and Buchsbaum, 2007). Overall, TRS showed a GM reduction particularly in frontal, temporal, and occipital regions (Molina et al., 2008; Quarantelli et al., 2014; Ahmed et al., 2015; Anderson et al., 2015) compared with healthy subjects and a GM reduction particularly in frontal regions (Lawrie et al., 1995; Mitelman et al., 2005; Zugman et al., 2013; Quarantelli et al., 2014; Anderson et al., 2015) compared with responders.

Recent studies using the VBM technique found significant differences between TRS patients and non-TRS patients. Zugman et al. (2013) showed that TRS patients showed a decrease in cortical thickness in all brain regions in comparison to healthy controls, with a marked decrease in dlPFC thickness when compared to responder patients. Quarantelli et al. (2014) showed more pronounced degrees of GM atrophy in TRS patients, both compared to healthy controls and to responders schizophrenic patients. Moreover, in an MRI cross-sectional study, Anderson et al. (2015) found GM reductions both in TRS and in clozapine-resistant schizophrenia ("ultra-TRS") patients (Anderson et al., 2015). In a longitudinal MRI study of TRS patients switched to clozapine, Ahmed and colleagues found a progressive regional brain volume loss in the prefrontal cortex (PFC) and in the periventricular area and a global cortical thinning, compared with healthy controls (Ahmed et al., 2015). However, due to the heterogeneity of these studies, two recent systematic reviews showed contrasting results concerning reductions in GM in TRS patients (Nakajima et al., 2015; Mouchlianitis et al., 2016).

Abnormalities of WM have been reported in the frontal, parietal and temporal regions and have been associated with poor outcomes (Mitelman and Buchsbaum, 2007; Molina et al., 2008). Moreover, in a DTI study, TRS patients showed an enlargement of the posterior corpus callosum, particularly the splenium, and widespread disruptions to WM tract integrity compared with healthy subjects (Holleran et al., 2014) and enlarged WM volumes compared with treatment-responsive patients (Molina et al., 2008; Anderson et al., 2015). In addition, connectivity in TRS patients, compared to non-TRS patients, showed a reduction in ventral striatum and substantia nigra connections, and an alteration in the distribution of corticostriatal connections (White et al., 2016). A recent systematic review (Mouchlianitis et al., 2016) showed an increase in basal ganglia WM in TRS, compared to schizophrenia patients who were responsive to treatment.

In summary, TRS patients show greater GM reduction, especially in frontal regions, and an increase in WM volume. Despite these findings have been replicated, more research is need to identify a neuroimaging profile able to recognize subject with higher risk to not respond to antipsychotics and consequently with higher vulnerability to develop TRS.

Brain Functional Abnormalities in Treatment-Resistant Schizophrenia

Functional neuroimaging techniques offer indirect ways of investigating brain activity *in vivo*. Functional neuroimaging data showed that a lower striatal metabolism before antipsychotics treatment was a predictor of a good clinical response and that responders patients showed a greater increase in striatal metabolism after antipsychotics therapy (Buchsbaum et al., 1992a,b; Bartlett et al., 1998). A recent extensive review (Nakajima et al., 2015) pointed out a pattern of hypometabolism in the PFC and hypermetabolism in the basal ganglia. Similar results support these findings; for example another recent systematic review (Mouchlianitis et al., 2016) showed decreased metabolism in frontotemporal regions and increased perfusion in the basal ganglia in TRS. Moreover, some research groups investigated whether disruptions in resting-state functional connectivity were associated with TRS (Paul and Sharfman, 2016; McNabb et al., 2018). Recently, in a fMRI study, Ganella et al. (2017) assessed functional brain networks abnormalities in TRS patients in comparison with healthy subjects, showing a global brain functional connectivity reduction in patients. In particular, this study revealed a decrease in temporal, occipital, and frontal region (Ganella et al., 2017). Other studies focusing con brain connectivity performed with different paradigms lead to similar conclusion of a general functional connectivity decrease (Wang et al., 2015; White et al., 2016; Vanes et al., 2018).

Neurotransmission in Treatment-Resistant Schizophrenia: Findings From Molecular Neuroimaging Studies

Molecular neuroimaging provides a direct way of investigating brain activity. In addition, various levels of evidence have shown that treatment response and resistance in schizophrenia can be associated genetic factors influencing gene involved in the pharmacokinetics and pharmacodynamics of anti-psychotic drugs. Indeed, a single nucleotide polymorphism (SNP), can introduce a missense substitution, thus altering the encoded protein and its function, or can affect non-coding regulatory regions (promoter, 3′UTR, intronic regions), influencing RNA transcription and splicing. Additionally, alterations in the number of gene copies (CNV) can result in increased or decreased levels of active protein present in the cells. Thus, the combination of SNPs and/or CNVs in each individual determines a unique profile for the activity of genes with an impact on the response to different drugs.

To understand the possible causes of brain changes in TRS, genetics and neuroimaging, i.e., "imaging genetics," provides an integrated point of view. These studies suggest that TRS is related to a variety of alterations and pathophysiological mechanisms that implicate different neurotransmitter systems. In particular, dopaminergic, serotonergic, glutamatergic, and GABAergic dysregulation, as well as numerous other alterations affecting other neural systems, have been demonstrated to play a relevant role in treatment resistance.

Dopaminergic System

The dopaminergic system has been studied for a long time in schizophrenia, since the dopamine hypothesis was formulated in the 1960s after the discovery of the antipsychotic actions of chlorpromazine, and it was enormously useful as a heuristic principle for the interpretation of the phenomenology features of schizophrenia. The dopamine hypothesis assumes that hyperactivity of dopamine D2 receptor neurotransmission in subcortical and limbic brain regions contributes to the positive symptoms, while negative and cognitive symptoms can be attributed to hypofunctionality of dopamine D1 receptor neurotransmission in the prefrontal cortex (Nakata et al., 2017). Indeed, antipsychotic (D2 antagonistic) treatment reduces positive psychotic symptoms in most patients but there is considerable heterogeneity in treatment response with roughly one-third of patients showing insufficient clinical response (Lindenmayer, 2000). Furthermore, there is variability regarding the time to clinical response after antipsychotic treatment onset (Emsley et al., 2006) and variability regarding the re-emergence of symptoms despite sufficient D2-receptor blockade (Rubio and Kane, 2017). In this context, dopamine receptors are among the main targets of antipsychotic drugs and TRS patients have shown reduced striatal dopamine synthesis capacity compared who had responded to antipsychotic treatment (Demjaha et al., 2012). The same research group found that patients with high levels of glutamate in the ACC (as measured by MRS) and with normal presynaptic dopamine synthesis (as measured by PET) showed a poor antipsychotic treatment response (Demjaha et al., 2014). Taken together, these results allow to hypothesize a "non-dopaminergic" subtype of schizophrenia (Howes and Kapur, 2014). As compared to the "hyperdopaminergic" subtype, characterized by prominent striatal dopamine synthesis and release capacity, the "non-dopaminergic" subtype exhibited normal dopaminergic function, and the disorder symptoms were not related to dopaminergic transmission. This classification based on a neurobiological mechanism shows several advantages: it could lead to the identification of PET scanning tests that guide treatment choice at illness onset and could provide a basis for research in order to develop new treatment options (Howes and Kapur, 2014). Some studies suggested that glutamatergic alterations may underlie the "non-dopaminergic subtype" of schizophrenia. More specifically, treatment responders seem to have more marked dopaminergic aberrations, whereas treatment non-responders seem to have more marked glutamatergic abnormalities (Howes et al., 2015).

Moreover, Roberts et al. (2009) examined dopaminergic synapses at the electron microscopic level in postmortem caudate of non-TRS and TRS patients. Despite the results of this study should be confirmed by replication, because of the small sample size, a good treatment response has been correlated with higher density of dopaminergic synapses, which supports a biological basis for TRS (Roberts et al., 2009).

Given the central role of the dopaminergic neurotransmitter system in the antipsychotic response, related genes have been widely investigated in studies on treatment response/resistance in schizophrenia, focusing in particular on gene variations encoding the dopamine D2 (*DRD2*) and D3 (*DRD3*) receptors (Arranz et al., 2011; Reynolds, 2012a; Brandl et al., 2014). Among the single SNPs in the *DRD2* gene, the most investigated is rs1800497 (Taq1A). The A1 allele of the TaqIA polymorphism, has been shown to reduce gene expression and therefore has also been hypothesized to influence treatment response (Brandl et al., 2014). However, studies performed to date have reported inconsistent findings (Schäfer et al., 2001; Lencz et al., 2006; Kohlrausch et al., 2008). In addition, subsequent studies demonstrated that TaqIA is located in exon 8 of ankyrin repeat and kinase domain containing 1 (*ANKK1*) gene, located close to *DRD2*, where it causes a non-conservative amino acid substitution (Neville et al., 2004; Lucht and Rosskopf, 2008). It is not clear if ANKK1 gene plays any role in neuropsychiatric disorders and drug response variability previously associated with Taq1A or if this polymorphism is in linkage disequilibrium (LD) with some other variants in DRD2 gene actually responsible for the effects on the dopamine transporter. For other *DRD2* polymorphisms, such as TaqIB, Ser311Cys, or A-241G, few studies have described associations (Lane et al., 2004; Hwang et al., 2005; Lencz et al., 2006; Zhang et al., 2010), but contrasting data and an absence of replication make further investigations necessary.

In the *DRD3*, the Gly9 variant of the Ser9Gly polymorphism changes D3 receptor density (Jeanneteau et al., 2006; Prieto, 2017). Consequently, the impact of this variant concerning antipsychotics response has been widely investigated (Arranz et al., 2011; Reynolds, 2012a; Brandl et al., 2014), but as with other receptor genes, such as *DRD1*, *DRD4*, *DRD5*, inconclusive findings have been obtained (Hwang et al., 2010; Brandl et al., 2014; Lally et al., 2016).

With a more complex approach, Pergola et al. (2017) studied genetic variants in relation to the *DRD2* gene co-expression pathway in association with working memory behavior, the related brain activity and the response to treatment. This study showed that a *DRD2* co-expression gene set enriched for protein-coding genes associated with schizophrenia modulates PFC function during working memory and response to D2 antagonist antipsychotics. These data revealed important findings; *DRD2* co-expression can parse schizophrenia risk genes into biological pathways associated with intermediate phenotypes as well as with clinically meaningful information.

Another interesting and well-characterized *DRD2* polymorphism is the rs1076560 since it was associated with gene function and response to treatment. This SNP is a regulatory variant that decreases the expression ratio of *DRD2* short isoform relative to the long isoform (Zhang et al., 2007). Moreover, it has also been associated with response to antipsychotic

treatment, both alone and in interaction with another functional polymorphism rs1130233 within the serine/threonine kinase 1 (*AKT1*) gene pertaining to a cAMP independent D2 signaling pathway (Blasi et al., 2011). Furthermore, it has also been associated with several schizophrenia-related phenotypes in healthy individuals, such as increased activity of striatum and prefrontal cortex and reduced performance in working memory and attentional control tasks (Blasi et al., 2011; Colizzi et al., 2015).

In conclusion, pharmacogenetic studies carried out on genes involved in the dopaminergic system to date, have moderate sample sizes and examined single or few polymorphisms in selected candidate genes. Overall, most results remain conflicting, and most associations fail to be replicated in large Genome Wide Association Studies (GWAS) and meta-analyses (Liou et al., 2012; Gressier et al., 2016; Hettige et al., 2016; Terzić et al., 2016; Koga et al., 2017). The reported effect sizes for genetic variants associated with antipsychotics are modest, and none of them effectively predict the treatment response (Pouget et al., 2014). However, such modest effect sizes are not surprising, given the complexity and polygenicity of this endophenotype. Rare variants in dopamine-related genes also seem to influence the response to antipsychotics, as suggested by a recent analysis of whole exome sequencing data in a large cohort of TRS patients (Ruderfer et al., 2016).

Serotonergic System

A plethora of serotonin receptors, as well as transporter gene polymorphisms, have been suggested as being involved in the mechanism of action of antipsychotic responses in schizophrenia. Although several studies have reported significant associations, these results have not been consistently replicated. Of the 5-HT receptors, the 5-HT2A receptor has been the most studied in schizophrenia and relative treatments. The greatest number of studied were focused on two polymorphisms, that are the 102T/C (rs6313), a synonymous coding region SNP, and 1438A/G (rs6311), a promoter SNP that is in complete linkage with 102T/C and reportedly has functional effects on gene expression. Moreover, several studies have investigated a further functional non-synonymous coding region SNP, 452His/Tyr (rs6314). This SNP has been found associated with response to antipsychotic treatments, either alone (Arranz et al., 2011) or in combination with the *DRD2* polymorphism rs1076560 described above in the dopaminergic system section (Blasi et al., 2015). A better response to antipsychotics is reported for schizophrenia patients with the combination of rs1076560 T and rs6314 CC genotypes, in two small cohorts. This results suggest that the effect of 5-HT2A variants on treatment response could be influenced by a complex interaction with D2 receptor variants, given that both receptors share the same intraneuronal molecular pathway (de Bartolomeis et al., 2013).

In general, contrasting results have emerged; consequently, there are no clear findings regarding the pharmacogenetics of antipsychotics and 5-HT2A receptors. In detail, functional variants of the serotonin 5-HT2A receptor gene were associated with less amelioration in psychotic symptoms following the treatment with clozapine (Arranz et al., 2011), olanzapine (Ellingrod et al., 2002) and risperidone (Lane et al., 2002), but negative associations were also reported for the same drugs (Masellis et al., 1995; Malhotra et al., 1996a; Lin et al., 1999; Thomas et al., 2008).

In early studies, the 5-HT2C receptor has also been shown to have some associations of potentially functional SNPs with antipsychotic response with inconclusive findings (Masellis et al., 1995; Sodhi et al., 1995; Malhotra et al., 1996b; Rietschel et al., 1997; Ellingrod et al., 2002; Thomas et al., 2008; Liu et al., 2010). However, most part of pharmacogenetic studies of 5-HT2C gene have reported positive associations with the metabolic side effects. Concerning the remaining 5-HT receptor genes, sparse data and unsettled conclusions are available in relation to the clinical consequences of antipsychotic treatment (Yu et al., 1999; Masellis et al., 2001; Houston et al., 2007; Gu et al., 2008; Wei et al., 2009; Takekita et al., 2016).

The great majority of pharmacogenetics studies performed to date in psychiatric field, investigated the neuronal 5-HT transporter gene (HTT; *SLC6A4*). This mutation has a functional ins/del promoter polymorphism (HTTLPR) in which the short allele (del) leads to a reduction of transporter activity of the HTT protein due to lower expression. However, during the last decade, it has been critically noted that the analysis of 5-HTTLPR is incomplete because other polymorphisms have been identified in the proximity of the Ins/Del locus, such as rs25531, rs25532, rs2020933, and a 17-bp variable tandem repeat in the second intron (STin2) (Bonvicini et al., 2010). *SLC6A4* polymorphisms have been extensively examined in mood disorders and antidepressant treatment, while little work has been performed in relation to antipsychotic response, with few significant results (Arranz et al., 2000; Bozina et al., 2007; Wang et al., 2007; Dolzan et al., 2008; Kohlrausch et al., 2010).

Although there are inconsistent results concerning TRS/response to antipsychotics and serotonin system polymorphisms, there are some exciting data coming from neuroimaging genetic studies that have confirmed the crucial role of serotonergic signaling in the antipsychotic treatment response. A study by Blasi et al. (2013) showed that rs6314 of the 5-HT2A gene affects 5-HT2AR expression and functionally contributes to the genetic modulation of endophenotypes of schizophrenia, such as higher-level cognitive behaviors and related prefrontal activity, as well as to olanzapine response. In particular, this functional brain imaging study (Blasi et al., 2013) indicated that individuals carrying the T allele have overstated prefrontal responses during working memory and attentional control tasks and also impaired cognitive behavioral performance. Moreover, schizophrenia patients who carry the T allele, compared to those who do not, have an attenuated improvement in negative symptom scores after 8 weeks of olanzapine treatment.

Glutamatergic/GABA Systems

The contribution of glutamatergic/GABA systems to the development of schizophrenia has been hypothesized for many years. To date there has been a growing body of evidence showing alterations in glutamatergic neurotransmission in relation to several aspects of the disorders. This evidence led to several

studies investigating the role of these systems in antipsychotic treatment outcomes.

In a proton MRS (1H-MRS) study in first-episode psychosis, Egerton et al. (2012) found elevated glutamate levels in the ACC in patients who had persistent psychotic symptoms despite antipsychotic treatment, relative to responders (Egerton et al., 2012). In the same year, in a (18F-DOPA) PET study, Demjaha et al. (2012) showed that TRS patients were characterized by elevated ACC glutamate levels. In a later 1H-MRS study they also found that patients with high levels of glutamate in the ACC (as measured by MRS) and with normal presynaptic dopamine synthesis (as measured by PET) showed a poor antipsychotic treatment response (Demjaha et al., 2014). In authors opinion, these data suggest that treatment resistance in schizophrenia is associated with a combination of relatively normal striatal dopamine synthesis and elevated ACC glutamate levels (Demjaha et al., 2014).

Taken together, these studies suggest that neuroimaging measures of dopamine and glutamate function might provide a means of stratifying patients with psychosis according to their response to treatment. Therefore, it could be argued that in some patients with schizophrenia, antipsychotic treatment may be ineffective because they do not exhibit the elevation in dopamine synthesis capacity that is classically associated with the disorder.

A recent review summarized that TRS compared to responder patients have more regions with decreased GM and show glutamatergic but no dopaminergic abnormalities (Gillespie et al., 2017). A more recent systematic review has taken in consideration all longitudinal proton MRS studies investigating antipsychotic treatment effect on brain glutamate levels in schizophrenia patients (Egerton et al., 2017). The main finding reported from the authors is that most part of studies described a significant decrease in glutamate metabolites after antipsychotic treatment in at least one brain region. Because of schizophrenia is related with an increase in glutamate metabolites, this data provides some indications that antipsychotics can reduce glutamatergic levels. However, to date the results have shown that this effect are quite small and/or limited to subgroups of patients (Egerton et al., 2017).

Glutamatergic neurotransmission takes place through metabotropic and ionotropic glutamate receptors. The metabotropic receptor (mGluR) family is subdivided into 3 groups, with a total of eight identified subtypes, and the ionotropic receptor family is made of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptors (Nakanishi, 1992). While ionotropic receptors mediate fast excitatory transmission at the glutamatergic synapse, ligand binding at metabotropic receptors leads to conformational changes directly or indirectly influencing neurotransmission by second messenger pathways (Kew and Kemp, 2005).

On this basis, glutamate-related genes have been investigated in relation to antipsychotic treatment in schizophrenia. The glutamate metabotropic receptor 3 (*GRM3*) gene has been widely investigated since it modulates signaling through NMDA receptors which are a relevant contributor to the cognitive and negative symptoms of schizophrenia (Maj et al., 2016). Several studies have found an association between *GRM3* and antipsychotic response or treatment resistance (Bishop et al., 2005, 2011, 2015; Fijal et al., 2009; Kaur et al., 2014). The GRM3 gene was found associated to schizophrenia in large GWAS analysis (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) and it encodes for the mGluR3 receptor, with a prominent role in the glutamate signaling in the brain (Cartmell and Schoepp, 2000). Two SNPs in *GRM3* (rs1989796 and rs1476455) resulted associated to TRS in a cohort made mainly of Caucasian individuals with the rs1476455_CC and rs1989796_CC genotypes associated to higher BPRS scores (Bishop et al., 2011). Polymorphisms in this gene were also found associated to worsening after antipsychotic treatment (rs1468412) and improvement in negative symptoms (rs6465084) in first-episode schizophrenia patients (Bishop et al., 2015). Moreover, the SNP rs1468412 showed a synergistic effect with the SNP rs165854 within phosphatidylinositol 4-Kinase Alpha (*PI4KA*) gene influencing antipsychotic response in low-severity schizophrenia patients of Indian origin (Kaur et al., 2014).

Two recent studies have supported the glutamate system as a potential mechanism of the response to risperidone, showing interesting evidence concerning the glutamate metabotropic receptor 7 (*GRM7*) gene (Stevenson et al., 2016; Sacchetti et al., 2017). In particular, Stevenson et al. (2016) identified an association between two SNPs in *GRM7* (rs2069062 and rs2014195) and an antipsychotic treatment response by a candidate gene analysis in a sample of first episode psychosis patients. In contrast, our group (Sacchetti et al., 2017) has shown a relevant role of rs2133450 as a predictor of an early (2 weeks) response to risperidone in a sample of schizophrenia patients through an original GWAS and a confirmatory analysis carried out on the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) schizophrenia study sample (Stroup et al., 2003).

Spurious and contrasting results are available for other genes in the glutamatergic system, such as the glutamate ionotropic receptor delta type subunit 2 (*GRID2*) (Stevenson et al., 2016) and the glutamate ionotropic *N*-methyl-D-aspartate receptor 2B subunit (Hong et al., 2001; Taylor et al., 2016).

Interestingly, a recent analysis of whole exome sequencing data revealed an enrichment for singleton disruptive mutations in 347 gene targets of antipsychotics in a large cohort of TRS patients (Ruderfer et al., 2016). These genes also included genes of the GABAergic/glutamatergic system, such as gamma-aminobutyric acid (GABA) A receptor alpha 5 (*GABRA5*), gamma-aminobutyric acid receptor subunit beta 2 (*GABRB2*), and glutamate ionotropic receptor NMDA type subunit 2A (*GRIN2A*). Finally, NMDA receptor-mediated signaling genes, such as D-amino acid oxidase (*DAO*), protein phosphatase 3 catalytic subunit gamma isoform (*PPP3CC*), and dystrobrevin-binding protein 1 (*DTNBP1*) genes, were associated with both the pathogenic mechanisms of and antipsychotic treatment response in schizophrenia (Reynolds, 2012b; Sacchetti et al., 2013).

The future development of drugs capable of supporting the glutamatergic functions would be of great interest (Carlsson et al., 1999). Based on the *N*-Methyl-D-aspartate receptor (NMDAR) hypofunction hypothesis of schizophrenia (Coyle, 2006;

Moghaddam and Javitt, 2012), the setting of pharmacological agents that enhance NMDAR function could provide therapeutic benefits in patients with schizophrenia. Unfortunately, direct activation of NMDARs using traditional orthosteric agonists induces adverse effects such as excitotoxicity and seizures (Puddifoot et al., 2012). Furthermore, treatments with NMDAR obligate co-agonists such as glycine or serine failed to have consistent efficacy across multiple clinical trials (Iwata et al., 2015). More recently, selective NMDAR positive allosteric modulators (PAMs) that enhance receptor function in the presence of the endogenous agonists but are devoid of intrinsic activity have been reported (Hackos et al., 2016). It is possible that NMDAR PAMs could avoid the adverse effects associated with direct activation of NMDARs. The recent development of NMDAR PAMs such as GNE-6901 and GNE-8324 provide proof-of-principle for the development of allosteric modulators of NMDARs, however their poor pharmacokinetic properties and low CNS exposures hinder their uses for *in vivo* studies (Hackos et al., 2016).

In addition to NMDARs all three groups of mGlu receptors have been pursued as putative targets for novel antipsychotics due to their ability to directly alter NMDAR function or other aspects of glutamatergic signaling. The metabotropic glutamate receptors represent a large group of promising targets for novel therapeutics to treat all three symptom domains of schizophrenia (positive, negative, and cognitive symptoms). While many discovery efforts are still in preclinical phases of development, they have yielded several subtype-selective tool compounds with minimal adverse effect profiles and promising preclinical efficacy.

In conclusion, on one hand several data evidenced that glutamatergic drugs are effective for the treatment of schizophrenia, however on the other hand conclusions are somewhat mixed and, where supported by meta-analyses, the effect size is unfortunately modest.

Other Systems

Only a few studies have investigated candidate genes not belonging to the major neurotransmission systems and the relationship to antipsychotic responses. Some association studies have focused on genes involved in the transport of various drugs through the blood-brain barrier and multi-drug resistance (e.g., the ATP-binding cassette, ABC, transporter proteins). In particular, *ABCB1*, *ABCC1* and *ABCB11* were significantly associated with the efficacy of or response to different antipsychotic drugs, including clozapine (Gonzalez-Covarrubias et al., 2016; Mi et al., 2016; Piatkov et al., 2017). Going beyond the candidate gene approach, recent studies have used GWA approaches for hypothesis-free investigation of common genetic factors.

Several GWA studies on TRS failed to identify significant associations (Hettige et al., 2016; Martin and Mowry, 2016; Koga et al., 2017; Wimberley et al., 2017), probably due to small sample sizes. Indeed, when GWA studies were performed in larger cohorts, suggestive associations emerged for various genomic loci involving genes related to immune responses (Liou et al., 2012) or genes involved in neuronal transmission and neurodevelopment (Yu et al., 2018).

Another investigative approach is the use of polygenic risk scores (PRSs) that summarize genome-wide genotype data into a single variable that measures genetic vulnerability to a disorder or a specific trait. Currently, the PRS is also frequently used to follow up a GWAS, testing the prediction of a drug response. To date, no significant results have emerged for TRS (Hettige et al., 2016; Martin and Mowry, 2016; Wimberley et al., 2017). Based on these findings, the use of the PRS for schizophrenia to classify individuals with TRS to date is scarce to be of clinical utility.

Finally, recent advances in sequencing technologies have opened the way for GWA studies and TRS for rare variants. A large sequencing analysis on coding regions (exome) in TRS patients found an excess of disruptive mutations in 347 genes involved in antipsychotic mechanisms of action (Ruderfer et al., 2016). Interestingly, some of these genes, such as calcium voltage-gated channel subunit alpha1 C (*CACNA1C*), glutamate ionotropic receptor NMDA type subunit 2A (*GRIN2A*), AKT serine/threonine kinase 3 (*AKT3*), hyperpolarization activated cyclic nucleotide gated potassium channel 1 (*HCN1*), solute carrier family 1 member 1 (*SLC1A1*) were previously associated with schizophrenia pathogenesis or a specific antipsychotic response (Ryu et al., 2011; Liu et al., 2015; Pers et al., 2016; Kabir et al., 2017; Yu et al., 2018).

Finally, in addition to common genetic variants, rare variants indexed by deletion and duplication burden genomewide, can increase the understanding and clinical management of TRS patients; however, to date, little data are available (Martin and Mowry, 2016).

Table 1 summarizes the literature main findings about structural, functional, molecular and neurochemistry brain abnormalities in TRS.

Clozapine in Treatment-Resistant Schizophrenia

To date, clozapine is unique as it is the only evidence-based treatment for TRS with 60–70% of those treated showing a response and it appears superior to all antipsychotics, including other atypical antipsychotics, in treating this population (Chakos et al., 2001; Lally et al., 2016).

The pioneering CT study by Friedman et al. (1991) showed that the degree of prefrontal cortex reduction was inversely related to clozapine response. Subsequent CT and MRI studies replicated this finding, demonstrating that a lower level of prefrontal atrophy was associated with clozapine treatment response compared with clozapine non-responders (Honer et al., 1995; Konicki et al., 2001; Arango et al., 2003; Molina et al., 2003). However, others were unable to replicate these results (Bilder et al., 1994; Lauriello et al., 1998). Only the study performed by Molina et al. (2003) showed a correlation between psychotic symptoms improvement and temporal GM volume in TRS patients treated with clozapine, whereas disorganization symptoms improvement was inversely related to pretreatment hippocampal volume (Molina et al., 2003). Moreover, a longitudinal study (Chakos et al., 1995) showed that, over the course of 1-year, patients started on clozapine showed a reduction in caudate nucleus volume, whereas an

TABLE 1 | Brain abnormalities in TRS: literature main findings.

Structural abnormalities
Greater GM reduction, especially in frontal regions, compared to responders
Decrease in dlPFC thickness compared to responders
Greater GM reduction, particularly in frontal, temporal and occipital regions, compared to HC
Decrease in cortical thickness in all brain regions, compared to HC
Widespread increase in WM volume (frontal, parietal, occipital), compared to HC
Increase in basal ganglia WM volume, compared to responders
Enlargement in posterior sections of the corpus callosum (splenium), compared to HC
Widespread disruption in WM tract integrity, particularly in the corpus callosum, compared to HC
Functional abnormalities
Decreased metabolism and perfusion in frontal areas, compared to HC
Increased perfusion in the basal ganglia, compared to HC
Global brain functional connectivity reduction, particularly in frontal, temporal and occipital regions, compared to HC
Molecular and neurochemistry abnormalities
Reduced striatal dopamine synthesis compared to non-TRS, but no difference from HC
Elevated glutamate concentration in ACC, compared to responders
Increased glutamate and glutamine concentrations in the putamen and decreased in the dlPFC in TRS clozapine responders, compared to first-line antipsychotic responders

ACC, anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; GM, gray matter; HC, healthy controls; TRS, treatment-resistant schizophrenia; WM, white matter.

increase was showed in those remaining treated with typical antipsychotics. These findings were replicated by two studies showing that clozapine use led to caudate nucleus volume reductions over 24 weeks (Scheepers et al., 2001a) and 52 weeks (Scheepers et al., 2001b).

Concerning functional neuroimaging, SPECT or PET studies showed a correlation between prefrontal and thalamus metabolic activity reductions and clozapine treatment, but it is uncertain whether these findings were related to clinical response (Molina Rodríguez et al., 1996; Molina et al., 2003, 2005). Nakajima et al. (2015) extensively reviewed these studies, showing no association between brain changes and clozapine response. Furthermore, in an MRS study, clozapine-responsive TRS patients showed that glutamate and glutamine concentrations were increased in the putamen and decreased in the dlPFC (Goldstein et al., 2015).

Several studies have investigated the relationship between genetic variants and response to clozapine, and several significant associations were reported with genes that are mainly involved in the dopaminergic, serotonergic and inflammation/immune systems. However, even with all of these relevant results, only three genetic variants, the Ser9Gly polymorphism of the *DRD3* gene previously cited in the dopaminergic system section, the functional non-synonymous coding region SNP 452His/Tyr (rs6314) of the *5-HT2A* gene, and the C825T variant of the G protein subunit beta 3 (*GNB3*) gene, have had significant findings independently replicated (Samanaite et al., 2018).

Moreover, a recent study has suggested the existence of a more severe, genetically based schizophrenia subgroup, for whom early intervention with clozapine can be considered. If confirmed from further research this finding may have important implications for clinical practice (Frank et al., 2015).

Despite all these demonstrations and the efficacy of clozapine in TRS, it is underprescribed in most countries (Lally et al., 2016). The explanations for this include worries of side effects, the inconvenience of therapeutic blood monitoring, and all potential fatal outcomes associated to clozapine use (Li et al., 2018). This means that the levels of use are far less than the about 50–60% of TRS patients who could benefit from it, although several studies highlight that clozapine remains the gold-standard treatment for TRS (Taylor, 2017).

CONCLUSION AND FUTURE PERSPECTIVES

Although interesting data have come from pharmacogenetics, neuroimaging and the interaction of both fields of study, few converging findings are available that describe the antipsychotic treatment response and resistance mechanisms in schizophrenia (DeLisi and Fleischhacker, 2016). Based on the available evidence, the results from both neuroimaging and pharmacogenetic/pharmacogenomic studies point to an overlap in the neurobiological vulnerability risk factors influencing the antipsychotic drug response in schizophrenia and the risk factors underlying schizophrenia itself. Currently for TRS, not a single biological marker, both coming from neuroimaging or genetic studies, is available. Indeed, all researches carried out to date did not provide findings with strong requisites of reproducibility, specificity, robustness as well as clinical feasibility and cost-effectiveness. Consequently, it is difficult to delineate a model of pharmaco-resistance and a clear pathogenetic hypothesis. Several reasons are to address: (1) the definition of resistance for schizophrenia is still lack of a definitive consensus; (2) few studies are available on TRS and most are on clozapine. As in our review, we refereed mainly to non-response mechanisms that could be partly overlying the aetiopathogenesis of resistance; (3) most data come from *a priori* hypotheses studies focused on well-known pathways; (4) several methodological limitations in the existing literature, including lack of reliability data, clinical heterogeneity among studies, and inadequate study designs and statistics.

More investigations are necessary on this important topic, and future direction should be focused on GWAS on TRS that will permit to obtain results regarding the involvement of other pathways/systems rather than the usual to date investigated, allowing further targets of future neuroimaging studies.

We hope that technology development and the opportunity to carry out studies in clinically homogeneous patient samples could represent the opportunity to obtain predictive genetic testing for use in clinical practice. Moreover, drug repositioning associated to GWAS data and drug expression

profiling (So et al., 2017), could be applied to severe psychiatric disorders as TRS. Regarding neuroimaging results to be clinically translatable, upcoming investigations require to be adequately powered and integrated with other biological markers. Further studies with large cohorts are needed for a better evaluation of the genetic contribution to the mechanisms underlying antipsychotic treatment response and resistance, hopefully in combination with non-biological markers, such as childhood trauma, which represent a clinically relevant factor for the development of TRS (Koga et al., 2017).

AUTHOR CONTRIBUTIONS

AV, AM, SB, GD, EG, PV, CT, and MG participated in the writing process of the first draft of the manuscript. AM and SB made literature search and independently reviewed electronic databases. AM, EG, and MG revised the pharmacogenetics and pharmacogenomic correlates in TRS, while AV, SB, GD, PV, and CT revised neuroimaging correlates in TRS. AV, AM, SB, and MG revised the final version of the manuscript. All authors contributed to reading and approving the final version of the manuscript.

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Treatment-Resistant Schizophrenia: Insights From Genetic Studies and Machine Learning Approaches

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Schizophrenia (SCZ) is a severe psychiatric disorder affecting approximately 23 million people worldwide. It is considered the eighth leading cause of disability according to the World Health Organization and is associated with a significant reduction in life expectancy. Antipsychotics represent the first-choice treatment in SCZ, but approximately 30% of patients fail to respond to acute treatment. These patients are generally defined as treatment-resistant and are eligible for clozapine treatment. Treatment-resistant patients show a more severe course of the disease, but it has been suggested that treatment-resistant schizophrenia (TRS) may constitute a distinct phenotype that is more than just a more severe form of SCZ. TRS is heritable, and genetics has been shown to play an important role in modulating response to antipsychotics. Important efforts have been put into place in order to better understand the genetic architecture of TRS, with the main goal of identifying reliable predictive markers that might improve the management and quality of life of TRS patients. However, the number of candidate gene and genome-wide association studies specifically focused on TRS is limited, and to date, findings do not allow the disentanglement of its polygenic nature. More recent studies implemented polygenic risk score, gene-based and machine learning methods to explore the genetics of TRS, reporting promising findings. In this review, we present an overview on the genetics of TRS, particularly focusing our discussion on studies implementing polygenic approaches.

Keywords: schizophrenia, antipsychotics, response, clozapine, pharmacogenetics, polygenic risk score

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INTRODUCTION

Schizophrenia (SCZ) is a severe psychiatric disorder that affects approximately 1% of the general population and is associated with a significant socioeconomic burden (Kahn et al., 2015). Antipsychotics represent the mainstay treatment for SCZ, but around one-third of patients show no response (Gillespie et al., 2017). According to the American Psychiatric Association (APA) guidelines, treatment-resistant schizophrenia (TRS) patients are defined as those showing little or no response to at least two non-clozapine antipsychotic trials of adequate duration and dose range (Lehman et al., 2004). Clozapine is the only treatment with an indication for TRS. However, this drug is still underutilized due to monitoring requirement (Kelly et al., 2018) and potential adverse effects, some of which can be severe and life-threatening (Wheeler et al., 2009; Gillespie et al., 2017). Unfavorable response to first-line pharmacological treatments is generally associated with a more

severe course of disease in TRS patients (Gillespie et al., 2017; Nucifora et al., 2018). Moreover, TRS patients are highly exposed to the potential detrimental effect of inefficacious treatments, including risk for adverse reactions that could be obviated or reduced if treatment resistance was known in advance.

It has been suggested that differential treatment response to antipsychotics might underlie biologically distinct subphenotypes of SCZ (Farooq et al., 2013; Gillespie et al., 2017) and TRS might better constitute a distinct phenotype rather than just a more severe form of SCZ (Wimberley et al., 2017). In this scenario, it has become clear that TRS patients would significantly benefit from the identification of clinical and biological markers to possibly predict the risk for treatment resistance before starting pharmacological treatments. However, TRS is poorly understood and its neurobiological underpinnings have yet to be clarified.

Data from family studies suggest that TRS is a heritable trait and that heritability might be stronger than in responsive SCZ (Nucifora et al., 2018). Candidate gene and genome-wide association studies (GWAS) have investigated how genetic variation might explain the interindividual variability observed in response to antipsychotics, but only a limited number of them focused on TRS (Nucifora et al., 2018). The majority of these studies used clozapine prescription or treatment as a proxy for diagnosis of TRS, and the investigated genetic variants were selected mainly based on their previously association with SCZ. We can anticipate that findings from these studies have so far not allowed dissecting the genetic complexity underlining TRS. Indeed, it has become clear that TRS is characterized by a complex polygenic nature. Most recent investigations have applied novel approaches such as polygenic risk score (PRS) and kernel support vector machine (SVM) to aggregate the effects of multiple variants contributing to disease risk. These approaches might be better able to capture the polygenic architecture of psychiatric conditions as well as response to psychotropic medications. In light of these observations, in this article, we will review studies investigating the genetic bases of TRS with a focus on studies using polygenic analytical approaches. The articles described in this narrative review were retrieved through a search on PubMed using the following keywords: treatment-resistant schizophrenia, antipsychotic response, genome-wide association study, machine learning, polygenic risk score, and support vector machine.

OVERLAP BETWEEN PREDISPOSITION TO SCZ AND RESPONSE TO ANTIPSYCHOTICS

As in the case of other psychiatric disorders, pharmacogenetic studies of antipsychotics have explored the overlap between susceptibility to SCZ and probability to respond to pharmacological treatments. GWASs successfully identified a large number of underlying genetic loci involved in SCZ. Among the most significant efforts, a first mega-analysis of GWAS conducted by the Psychiatric Genomics Consortium (PGC), including 9,394 cases with SCZ and 12,462 controls of European origin, had identified seven genome-wide significant loci (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011). These results were replicated by a GWAS in which patients with SCZ treated with clozapine were compared with healthy controls (Hamshere et al., 2013). More recently, the second mega-analysis of GWAS conducted by the PGC (wave 2), including 36,989 cases and 113,075 controls, identified 108 loci associated with predisposition to SCZ (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Besides single-nucleotide polymorphisms (SNPs), rare disruptive mutations identified with exome sequencing have also been shown to increase liability for SCZ (Purcell et al., 2014). Based on the evidence that many of the variants identified by genome-wide studies on SCZ are located in genes playing a role in systems likely involved in its neurobiology, there is a rationale for investigating the association between genetic risk for SCZ and response to antipsychotics or TRS. Ruderfer et al. (2016) evaluated both common and rare SCZ-associated loci for enrichment in drug targets, providing interesting evidence that supports the role of some of these genes as drug targets. Authors used a gene-set analysis approach and found that 21% of 167 pharmacological subgroups were enriched for loci previously associated with SCZ. Dopamine receptor D2 (DRD2) was among the loci contributing the most to this finding. Indeed, *DRD2* encodes a known target not only of antipsychotics, but also of 46 different non-antipsychotic pharmacological subgroups out of the 167 evaluated in Ruderfer et al. (2016). The gene set including targets of antipsychotics was enriched for common and rare variants previously associated with SCZ. Authors also compared TRS (532 SCZ patients treated with clozapine) with SCZ patients treated with other antipsychotics (n = 2,002), showing a higher number of disruptive mutations in genes targeted by antipsychotics in the TRS group. Taken together, these results support the hypothesis that at least some of the genes identified as involved in the pathogenesis of SCZ might also explain part of the interindividual variability in response to antipsychotics as well as in susceptibility to TRS.

GENETIC BASES OF TRS

The number of studies exploring the correlation between genetic variants and TRS is relatively limited. There is still disagreement on the best approach to maximize the power and informativity of genetic studies on TRS, considering the likely complex genetic architecture of this phenotype and the discrepancies on the clinical definition of TRS. The most used operational criteria to define TRS were provided by Kane and colleagues in 1988 (Kane et al., 1988). These criteria include 1) three or more periods of treatment with at least two neuroleptic agents of different classes (1,000 mg/day of chlorpromazine equivalents) for at least 6 weeks, 2) no period of good functioning within the preceding 5 years, and 3) severe psychopathology according to the Brief Psychiatric Rating Scale (BPRS) and Clinical Global Impressions (CGI) scores (Kane et al., 1988). These criteria include more aspects related to functioning compared to the APA guidelines that, as mentioned in the Introduction section, define TRS as little or no response to at least two non-clozapine antipsychotic trials of adequate duration and dose range (Lehman et al., 2004).

Several studies that will be presented in this section used the APA guidelines or a modified version of the criteria defined by Kane et al. (1988), while others used clozapine use or prescription

and discontinuation from the prescribed antipsychotic to select TRS patients. It is therefore consequential that the comparison of findings from studies using different definitions may not be straightforward. However, taken together, findings in this field further highlight the importance of the genetic contribution in characterizing TRS.

Candidate Gene and GWAS

Candidate gene studies investigating the involvement of specific targets in TRS mostly focused on the dopaminergic and serotoninergic systems (Inada et al., 2003; Ji et al., 2008; Kohlrausch et al., 2008; Ota et al., 2012; Bilic et al., 2014; Terzić et al., 2015), as well as on systems involved in inflammation and oxidative stress (Jia et al., 2011; Pinheiro et al., 2017). Among the most interesting findings, Bilic et al. (2014) reported significant interactions between a dopamine transporter variable number tandem repeat (DAT–VNTR) and the serotonin transporter (SERT)-in2 polymorphism in a sample of 172 patients with SCZ, 92 of whom met the TRS definition based on the modified Kane criteria: 1) at least 5 years of inadequate social or occupational functioning, 2) current treatment with a chlorpromazine equivalent dose > 600 mg or score ≥3 on selected items of the Positive and Negative Schizophrenic Symptoms (PANSS) scale or Clinical Global Impression–Severity scale (CGI-S) score ≥4, and 3) history of previous treatment with at least two antipsychotics or history of at least one treatment with clozapine. Results from this study support the hypothesis that, besides the main effect of specific genetic variants, SNP–SNP interactions might also play a role in explaining the interindividual variance observed in response to antipsychotics.

The first large-scale study on the genetic bases underlying response to antipsychotics was conducted in a sample recruited within the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) trial (Need et al., 2009). Although this study (which tested 769 polymorphisms in 118 candidate genes) reported several nominal associations, no variant was significantly associated with response to antipsychotics after correction for multiple testing (Need et al., 2009). This study did not specifically include patients with TRS and used discontinuation from the prescribed antipsychotic as a proxy for non-response.

As for large-scale studies, only a small number were conducted in TRS patients. Zhang and coworkers (2013) selected SNPs nominally associated with non-response in the CATIE pharmacogenetic study (Need et al., 2009) and tested their association with TRS (using clozapine treatment as a proxy). Authors reported a significant association between TRS and several genetic variants in linkage disequilibrium (top hit: rs11030104) located in the brain-derived neurotrophic factor (BDNF) gene. Li and Meltzer (2014) conducted a GWAS on two cohorts of Caucasian patients with (n = 79 and n = 70) and without (n = 95 and n = 125) TRS. In this study, treatment resistance was defined as persistence of moderate to severe positive symptoms despite at least two trials of 4–6 weeks with typical or atypical antipsychotics other than clozapine. Although no SNP met the genome-wide significant threshold, interesting results were reported for the rs2237457 variant located in 7p12 (p value in the combined cohorts: 5.66×10^{-7}). This variant is located upstream of the gene encoding L-dopa decarboxylase, a rate-limiting enzyme in the synthesis of trace amines and neurotransmitters, including dopamine (Li and Meltzer, 2014). This result is particularly important based on the fact that a hyperdopaminergic state in the mesolimbic dopamine pathway is thought to play a crucial role in the development of psychotic symptoms according to the dopamine hypothesis of SCZ and that dopamine D2 receptors represent a main target for all antipsychotic drugs (Li et al., 2016).

Conversely, Teo and coworkers (2012) did not identify any significant association among 384 candidate gene loci in a study including 85 patients with TRS defined according to APA criteria and 155 non-resistant patients. Other studies focused on the association of genetic variants and clinical factors hypothesized to play a role in the development of TRS, such as childhood adversities. In a GWAS on a sample of 85 Caucasian patients with SCZ [31 of whom met the criteria for TRS defined according to the APA criteria (Lehman et al., 2004)], no SNP met the genome-wide significant threshold for association with TRS with or without taking into account history of childhood adversities (Koga et al., 2017).

Studies Using PRS

PRS analysis aggregates the effect sizes of several SNPs across the genome, thus providing a single estimate of the association with a specific trait or disease (Dudbridge, 2013). In the last few years, PRS analysis has been successfully applied to the study of different psychiatric disorders (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018; Kalman et al., 2018; Taylor et al., 2018; Richards et al., 2019). In the case of SCZ, PRS analysis has been used to evaluate how the polygenic burden can explain differences in specific symptoms (Wang et al., 2018; Anderson-Schmidt et al., 2019), functional and structural brain changes (Lieslehto et al., 2018; Ranlund et al., 2018; Velthorst et al., 2018), genetic overlap with other traits (International Consortium on Lithium Genetics (ConLi+Gen) et al., 2018), as well as gene co-expression networks in the brain (Radulescu et al., 2018). Studies investigating the potential value of PRS analysis to identify patients that are less likely to respond to treatment provided contrasting findings. In patients with first-episode psychosis (FEP), Zhang and colleagues (2018) found that participants with lower SCZ polygenic burden were more likely to respond to a 12-week antipsychotic treatment compared to patients with high SCZ PRS (odds ratio = 1.91). Conversely, although Santoro et al. (2018) reported a positive association between depressive symptoms and PRS at baseline in a sample of 60 FEP patients, after treatment with risperidone, patients with a higher SCZ PRS were more likely to show improvement in depressive and excitement symptoms. Of note, the study by Santoro and coworkers only included antipsychotic-naive patients.

Studies including patients with TRS also provided controversial findings (**Table 1**). Frank and coworkers (2015) reported that a PRS including SNPs associated with SCZ risk in the first meta-analysis from the PGC Schizophrenia group (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) was increased in patients with SCZ treated with clozapine compared to patients not treated with this drug. Moreover, the highest PRS was observed in patients who are non-responders to clozapine

TABLE 1 | Studies investigating the genetic bases of treatment-resistant schizophrenia (TRS) using a polygenic risk score (PRS).

Study	Discovery sample	Target sample	Treatment-resistance criteria	Results
Frank et al. (2015)	First meta-analysis from the PGC Schizophrenia group (Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011)	804 German patients with SCZ (434 with TRS)	History of clozapine treatment	Higher PRS in patients treated with clozapine compared to patients with no history of clozapine treatment. The highest PRS was observed in patients characterized by non-response to clozapine, early age at onset and poor premorbid social functioning
Martin and Mowry (2016)	Second meta-analysis from the PGC Schizophrenia group (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014)	612 Australian patients with SCZ (227 with TRS)	Poor functioning, continuous course of illness, and at least two among delusions, hallucinations, disorganization and negative symptoms during treatment with antipsychotics	No association between the PRS and non-response to antipsychotics. Association between TRS and total duplication burden genome-wide
Wimberley et al. (2017)	Second meta-analysis from the PGC Schizophrenia group (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), excluding the Danish cases	862 participants with SCZ (181 with TRS) included in the Danish Newborn Screening Biobank	Clozapine initiation or hospitalization during antipsychotic treatment after at least two periods of different antipsychotics monotherapy	No association between the PRS and TRS

PGC, psychiatric genomics consortium; PRS, polygenic risk score; SCZ, schizophrenia; TRS, treatment-resistant schizophrenia.

and characterized by early age at onset and poor premorbid social functioning (Frank et al., 2015).

On the other hand, using the population-based Danish register, Wimberley and coworkers found no association between a PRS for SCZ and TRS (Wimberley et al., 2017). In this study, the PRS was computed based on 24,755 SNPs from the PGC wave 2 paper, using a *p*-value threshold of 0.05 (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), and tested into an independent Danish cohort of 862 patients (181 of whom were considered to be treatment-resistant). In this study, treatment resistance was defined as either 1) clozapine initiation or 2) hospitalization during antipsychotic treatment after at least two periods of different antipsychotics monotherapy (Wimberley et al., 2017). Similarly, Martin and Mowry (2016) found no association between a PRS for SCZ and non-response to antipsychotics in a sample of 612 Australian patients with SCZ (227 of whom showed treatment resistance). In this study, patients were considered to be treatment-resistant in case they showed poor functioning, continuous course of illness, and at least two among delusions, hallucinations, disorganization, and negative symptoms during treatment with antipsychotics. Similarly to other works, the PRS was computed based on SNPs identified by the most recent PGC wave 2 GWAS mega-analysis (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), although a different *p*-value threshold was used ($p < 0.1$). Of note, the authors found a significant association between TRS and the total copy number duplication burden genome-wide (Martin and Mowry, 2016). A number of factors might explain the observed discrepancies between studies investigating genetic bases of TRS using PRS models. For instance, the different *p*-value thresholds chosen by the authors contribute to make these results difficult to compare. Moreover, the sample size of the available studies was generally limited, as the number of subjects with a diagnosis of TRS ranged from 181 to 434 (**Table 1**). To date, available studies only used PRS including SCZ risk variants. However, the development of a PRS specific for TRS might be of high relevance to better capture the contribution of loci underlying non-response to antipsychotics. Moreover, studies evaluating the contribution of a PRS together with clinical characteristics previously identified to be associated with TRS (e.g., earlier age at onset, family history of psychosis and history of substance abuse) would be of high interest.

COMPARISON BETWEEN PRS AND OTHER MACHINE LEARNING METHODS

PRS analysis aggregates the contribution of multiple SNPs assuming an additive effect. While this approach has proven to be extremely useful, it doesn't allow to take into account the potential interactions between different genetic variants. A recent study tried to address this gap using SVM algorithms (Vivian-Griffiths et al., 2019). SVM is a family of supervised learning methods that can be usefully applied to linear as well as non-linear and high-dimensional classification problems (Cai et al., 2001). Specifically, the SVM method allows one to find the optimum hyperplane that separates observations into different classes. Vivian-Griffiths and coworkers (2019) evaluated how this method (allowing to take into account pairwise and higher-order SNP interactions) might help to distinguish patients with TRS from healthy controls compared to a PRS. Models were based on 1) 125 genome-wide significant SNPs and 2) 4,998 independent top SNPs from the PGC wave 2 GWAS mega-analysis (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). The study was conducted in the CLOZUK sample (Hamshere et al., 2013), including 5,554 patients receiving clozapine and 6,299 healthy controls. In this study, two different typologies of SVM models were constructed:

SVM with linear and radial basis function kernels. Predictive performances were measured using the area under the receiver operating characteristics curve metric (AUC-ROC) and compared between these two models as well as against those of a PRS. The standardized reference allele counts for each polymorphism were used as input for the SVM model. While no evidence of interaction was found when analyzing the 125 top hits, findings from this study suggested the potential presence of interactions in the models including the more weakly SCZ-associated variants (Vivian-Griffiths et al., 2019). Although the SVM method might be more suitable to take into account these effects, the PRS model showed a higher accuracy in the classification of patients with SCZ treated with clozapine compared with controls. Nonetheless, the prediction accuracy shown by the PRS is still insufficient to support the implementation of the model into the clinical practice [best area under the receiver operating characteristics curve (AUC-ROC) = 0.697].

CONCLUSIONS

A growing number of studies provided intriguing hints on the complex polygenic architecture underlying TRS. However, to date, no genetic marker showed adequate prediction accuracy. Moreover, controversial findings were reported as regards the ability of available PRSs to discriminate individuals with or without TRS. The majority of available studies investigated the effect of variants previously associated with predisposition to SCZ. Indeed, it has been shown that antipsychotics' targets are enriched for variants previously associated with SCZ (Ruderfer et al., 2016), supporting the need of investigating a potential role for these genes in TRS development. However, in order to better capture the contribution of variants specifically implicated in response to antipsychotics, a more comprehensive approach might involve construction of a PRS specific for TRS (i.e., constructed using genetic data from patients characterized for response to antipsychotics) as such a score might show a higher predictive accuracy. The importance of this aspect is highlighted by the fact that the total duplication burden genome-wide has been associated with TRS (Martin and Mowry, 2016) but not with predisposition to SCZ (Buizer-Voskamp and Muntjewerff, 2011), supporting the hypothesis that only a part of TRS susceptibility might be explained by previously investigated targets.

Another aspect that limits our interpretation of available studies consists in the different criteria used by different researchers to define TRS. While some of the studies adopted the criteria suggested by APA (which define TRS as little or no response to at least two non-clozapine antipsychotic trials of adequate duration and dose range), other studies used different criteria [e.g., modified versions of the Kane criteria (Kane et al., 1988), which take into account different aspects, including global assessment of functioning] or simply considered history of clozapine treatment as a proxy of TRS. Although the latter approach is easier to apply (particularly in the case of studies performed using data from registers) and can therefore lead to studies with an increased sample size, it might lead to an underestimation of the number of patients with TRS. In fact, although clozapine is the only antipsychotic with an indication for TRS, this drug is currently underutilized due to possibly life-threatening adverse reactions as well as to the need of regular monitoring (Remington et al., 2016). In order to minimize this risk, some studies included not only patients treated with clozapine but also participants who would meet the criteria for initiating clozapine treatment based on the data extracted from population-based registers (Wimberley et al., 2017). Nevertheless, the use of standardized criteria to identify patients affected by TRS should be one of the main goals of future efforts, as this would allow evaluating the reproducibility and robustness of findings and the aggregation of available results in meta-analyses, ultimately leading us closer to the understanding of the genetic architecture of TRS.

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AS and CP searched the literature and compiled the review with equal contribution.

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REVIEW
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Glycine Signaling in the Framework of Dopamine-Glutamate Interaction and Postsynaptic Density. Implications for Treatment-Resistant Schizophrenia

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Treatment-resistant schizophrenia (TRS) or suboptimal response to antipsychotics affects almost 30% of schizophrenia (SCZ) patients, and it is a relevant clinical issue with significant impact on the functional outcome and on the global burden of disease. Among putative novel treatments, glycine-centered therapeutics (i.e. sarcosine, glycine itself, D-Serine, and bitopertin) have been proposed, based on a strong preclinical rationale with, however, mixed clinical results. Therefore, a better appraisal of glycine interaction with the other major players of SCZ pathophysiology and specifically in the framework of dopamine – glutamate interactions is warranted. New methodological approaches at cutting edge of technology and drug discovery have been applied to study the role of glycine in glutamate signaling, both at presynaptic and post-synaptic level and have been instrumental for unveiling the role of glycine in dopamine-glutamate interaction. Glycine is a non-essential amino acid that plays a critical role in both inhibitory and excitatory neurotransmission. In caudal areas of central nervous system (CNS), such as spinal cord and brainstem, glycine acts as a powerful inhibitory neurotransmitter through binding to its receptor, i.e. the Glycine Receptor (GlyR). However, glycine also works as a co-agonist of the N-Methyl-D-Aspartate receptor (NMDAR) in excitatory glutamatergic neurotransmission. Glycine concentration in the synaptic cleft is finely tuned by glycine transporters, i.e. GlyT1 and GlyT2, that regulate the neurotransmitter's reuptake, with the first considered a highly potential target for psychosis therapy. Reciprocal regulation of dopamine and glycine in forebrain, glycine modulation of glutamate, glycine signaling interaction with postsynaptic density proteins at glutamatergic synapse, and human genetics of glycinergic pathways in SCZ are tackled in order to highlight the exploitation of this neurotransmitters and related molecules in SCZ and TRS.

Keywords: N-methyl-d-aspartate, glutamate, dopamine, glycine transporter 1, PSD-95, Homer, disk-1, antipsychotics

INTRODUCTION: SCHIZOPHRENIA AND GLYCINE NEUROTRANSMISSION

Schizophrenia (SCZ) is a chronic and debilitating severe mental disorder affecting approximately 0.3–0.7% of the population worldwide (1). It is characterized by a pleomorphic symptomatology including hallucinations, delusions ("positive symptoms"), social withdrawal, avolition and anhedonia ("negative symptoms"), and deficits in multiple executive functions (cognitive symptoms). SCZ is nowadays conceptualized at molecular level as a disorder of the synaptic plasticity (2) and of abnormal cortical-subcortical connectivity (3–5). Most of the individuals affected by SCZ develop their illness in adolescence and early adulthood with about 15% showing a chronic and unremitting clinical course (6). The long-term, if not lifelong, illness trajectory, the associated high mortality, mostly determined by the elevated rates of medical comorbidities and suicide (7), and the low levels of recovery (8), make this disease a major psychiatric disorder with a great need of significant therapeutic innovation. Furthermore, the treatment response to antipsychotics, the mainstay of SCZ treatment, remains suboptimal (9). A recent study, in which analysis of 16 randomized controlled trials (RCT) were pooled together, showed that the percentage of short-term non-response ranged from 20 to 87% depending on the threshold applied, with a non-remission rate of 67% (9). In addition, a not-negligible proportion (up to 20%) of SCZ patients who are resistant to standard antipsychotic treatment, does not respond even to clozapine (10), which is the gold standard in this scenario. In this context, the identification of clinically novel effective and safe pharmacological treatments is crucial.

The interest for the role of glycine, a co-agonist with glutamate at N-methyl-D-aspartate receptor (NMDAR) in the framework of dopamine-glutamate interactions for SCZ pathophysiology and treatment, stems from the following evidence: 1) increased dopamine release in the striatum is one of the most replicated *in vivo* findings in SCZ pathophysiology (11–14); 2) all antipsychotics block or occupy dopamine D2 receptor (D2R) with no exception (15–18); 3) dopamine release is controlled, among other mechanisms, by NMDARs modulation (19); 4) NMDAR hypofunction is believed to be one of the putative pathogenetic mechanism of the disease (19, 20); 5) glutamatergic dysfunction, moreover, has been implicated even in those cases of SCZ that are not characterized by dopamine excess in subcortical regions and are not responsive to conventional antipsychotics (21–25); 6) multiple lines of evidence indicate a reciprocal modulation of both dopamine and glutamate by glycine (26); 7) over time different pharmacological glycine-centered approaches for treatment-resistant schizophrenia (TRS) have been proposed with strong preclinical rationale but mixed clinical results (27, 28).

New methodological approaches at the cutting edge of the technology such as long-timescale molecular dynamics simulations (29, 30) and single molecule fluorescence resonance energy transfer (smFRET) (31) have unveiled the very specific details of the structural and functional mutual interaction between glycine and glutamate system at preclinical level. At clinical level novel human genetic findings and imaging genetics studies link glycine signaling to SCZ. Given the extensive evidence that glycine is deeply involved in regulating glutamatergic neurotransmission, glycine reveals itself as promising potential candidate for drug discovery and safe novel pharmacological treatments. Glycine binding site on GluN1 and GluN3A NMDAR subunits is a major determinant in regulating NMDAR delivery on cell surface and furthermore influencing significantly the NMDAR activity (32).

Here, we aim to selectively review the preclinical and clinical evidence demonstrating that glycine, as well as the components of its signaling pathway, might be suitable targets for the identification of novel treatment strategies in severe psychiatric disorder and particularly in SCZ. The following research questions have led our dissertation:

1. Which is the role of glycine in the general framework of dopamine–glutamate interaction and SCZ pathophysiology?
2. How does glycine affect dynamics of post-synaptic proteins?
3. How and to what extent can glycine neurotransmission and its interaction with NMDAR be exploited to unveil novel treatments for TRS?

First, therein we describe glycine transmission, focusing on the characteristics of its components, namely receptors and transporters and their relevance in the brain circuits relevant for SCZ clinics and pathophysiology. Then, we detail the role of glycine in regulating dopamine-glutamate interaction, as well as its involvement in SCZ molecular pathophysiology. Furthermore, we review the evidence on the associations of genes encoding for elements of the glycine pathway with SCZ. Finally, a critical appraisal of potential role of glycinergic agents in treatment of psychiatric diseases is addressed.

DOPAMINE, GLUTAMATE, GLYCINE, AND THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

Traditional models of SCZ focused on dopaminergic dysfunction to explain key symptoms of the disorder. This hypothesis holds that hyperactivity of dopamine transmission is responsible for positive symptoms (1) and it was formulated in the 1960s, after the discovery of antipsychotic action of chlorpromazine (33), and further endorsed by the correlation between clinical response to antipsychotic drugs and their potency to block D_2 receptors (34, 35). Neuroimaging studies using positron emission (PET) or single photon emission (SPECT) demonstrated that, after acute amphetamine administration, patients with SCZ showed greater levels of dopamine release in subcortical regions (particularly in the striatum) compared to healthy subjects, and displayed a transient worsening of positive symptoms (11, 36), supporting the idea that hyperfunction of dopaminergic neurons is a relevant, albeit not unique, component in SCZ pathogenesis.

Administration of phencyclidine (PCP), ketamine, and other NMDAR antagonists has been known to reproduce those thought disorders observed in SCZ, such as poverty of speech, circumstantiality, and loss of goal (37). Furthermore, NMDAR antagonists may affect widespread neuropsychological domains: working memory, response inhibition, and executive processing, resulting in cognitive symptoms that are also described in SCZ, suggesting the involvement of glutamatergic neurotransmission in the pathogenetic mechanism underlying psychotic and cognitive abnormalities (38). Therefore, glutamatergic neurotransmission has been proposed as the major initial aberration in the pathophysiology of SCZ. Subcortical dopaminergic dysregulation itself might be a result of impairment in glutamatergic neurons projecting from prefrontal cortex (PFC) to midbrain dopaminergic neurons, therefore exerting control on their firing (19, 39). Indeed, in animals and humans, it has been demonstrated that NMDAR antagonist administration results in an increase of amphetamine-induced dopamine release (19, 40). These data support the hypothesis of a deficiency of glutamatergic control on dopamine neuronal activity that might underlie the increase in amphetamine-induced dopamine release.

The activation of GABAergic interneurons by glutamatergic projections is mediated by NMDAR, and NMDAR-hypofunction may specifically affect corticolimbic GABAergic parvalbumin-positive (PV+) interneurons, reducing their excitability and expression of specific molecular markers such as somatostatin and vasoactive intestinal peptide (VIP), as well as increasing oxidative stress (41). Transgenic mice with selective NMDAR deletion in cortical and hippocampal GABAergic interneurons showed specific SCZ-like phenotypes (42), supporting the so-called "GABAergic origin hypothesis" of SCZ (41).

Since NMDAR dysfunctions account for both dopaminergic and GABAergic dysregulation, it can be assumed that NMDAR dysfunction could represent the final common pathway leading from pathogenesis to symptoms (43). Glycine is deeply involved in regulating the glutamatergic transmission, acting as a co-agonist of NMDAR, allowing for its activation and enhancing excitatory glutamatergic tone (44, 45). Glycine is also involved in the regulation of dopamine transmission, exerting a multimodal action depending on its concentration and possibly inhibiting dopamine release in the striatum when administered at high doses (presumably by modulating the dopaminergic hyperfunction associated to SCZ) (46).

Potential implication of glycine signaling in the pathophysiology of SCZ is supported by a number of recent studies exploring genetic abnormalities within glycinergic system associated with SCZ as well as by the evidence of potential pro-cognitive and antipsychotic phenotype exhibited by animal models of Glycine Transporter type 1 (GlyT1) functional inhibition both by recombinant knock out (47) and pharmacological treatments (48, 49). Finally, the recent finding of elevated brain glycine and glutamate levels in patients with first-episode psychosis, measured *in vivo* by means of echo time–averaged proton magnetic resonance spectroscopy (MRS) at 4 Tesla, further confirm the relevant role of glycine in the framework of multiple interacting neurotransmitters in SCZ pathophysiology (50).

GLYCINE: FUNCTIONAL ANATOMY RELEVANT FOR DOPAMINE-GLUTAMATE INTERPLAY

Histological Distribution of Glycinergic Neurons and Glycine Receptor

Glycine is widely distributed in the mammalian central nervous system (CNS), functioning as an inhibitory or excitatory neurotransmitter, depending on its localization. Glycine is the main neurotransmitter in inhibitory interneurons of the spinal cord, brainstem, and in some other brain regions involved in the processing of sensorimotor information and locomotor behavior (51). In the CNS, glycine is synthesized through the catalysis of serine by the isoenzyme serine hydroxymethyltransferase (SHMT), and it is largely degraded by the glycine cleavage system, also known as glycine decarboxylase complex (GDC) (52). Glycine is released by Renshaw interneurons and regulates motoneurons' excitability, exerting negative feedback through recurrent inhibition (53). Glycinergic inhibitory interneurons are involved also in the spinal reflex coordination, mediating reciprocal inhibition in stretch reflex circuits and regulating the coordination of opposing muscles (54). The anatomical distribution of glycine immunoreactive (IR) cell bodies points to the cochlear nuclei, the superior olivary complex, the medial nuclei of the trapezoid body, the cerebellar cortex, the deep cerebellar nuclei, the area postrema, and the thalamus of adult rats as main localizations (55, 56). Moreover, glycine-IR fibers are localized in the hypothalamus and basal forebrain, distant from their glycine IR cell bodies (56). Glycine receptors (GlyR) have been found enriched in the spinal cord, in apical dendrites of pyramidal neurons in the cerebral cortex (57), in the limbic system, and in the hippocampus of humans and rats (58), where they are involved in synaptic plasticity (59) and in a variety of physiological processes, especially in mediating inhibitory neurotransmission.

Structure and Function of Glycine Receptors

Glycine can activate two classes of distinct ligand-gated ion channels: chloride-permeable inhibitory GlyRs, and cation selective excitatory NMDARs. GlyRs are ligand-gated anionic channels and belong to the pentameric Cys-loop receptor superfamily (60). Electrophysiological, immunocytochemical, and *in situ* hybridization studies have shown that GlyRs are prominent in the brainstem and spinal cord (61, 62) and detectable also in the following brain regions: prefrontal cortex, hippocampus, amygdala, hypothalamus, cerebellum, nucleus accumbens, ventral tegmental area, and substantia nigra (63–65). GlyRs exist either in homomeric or heteromeric forms and are composed by five subunits arranged symmetrically in a ring around a central Cl^- permeable pore. Heteromeric GlyRs are localized at the

synapses and consist of three α and two β subunits, forming a pentameric receptor complex. The homomeric forms are composed of five α subunits and are located extra-synaptically. The β subunits colocalize with receptor-associated protein gephyrin, that anchors the GlyR complex at the synaptic locus, thus providing a cluster of heteroligomeric GlyRs within synapses (66, 67). The expression of α subunits changes during neurodevelopment and it is regionally specific, whereas β subunits are transcribed in all developmental stages in several regions. Recent studies have detected functional GlyRs even in absence of the glycinergic terminals in dopaminergic neurons of the juvenile immature *substantia nigra pars compacta* and in developing cortical neurons, but the function of these non-synaptic GlyRs remains unclear (68, 69). Overall, a variety of functions may be performed by GlyR, depending on the major subunit of the receptor and its oligomerization. Moreover, the pattern of GlyR expression seems to be relevant during critical stages of brain development in cortical and subcortical brain regions that have attracted the attention for the animal modeling of SCZ pathophysiology.

As an excitatory neurotransmitter, glycine acts as a co-agonist of NMDAR, allowing for depolarization, removal of the magnesium blockade and $Na^+/Ca2^+$ passage through the channel, which ultimately enhances the glutamatergic excitatory tone that is critical for learning and neuronal plasticity (44, 45). While glutamate binds to a bi-lobulated cavity in NMDAR GluN2 subunit, glycine binds to a cavity located in GluN1 or GluN3, the so-called glycine-B site or strychnine-insensitive receptor (51). Glycine may be released at excitatory sites from at least two different sources: i.e., neuronal cells *via* alanine–serine–cysteine transporter-1 (Asc-1) (70) and astroglial cells *via* the functional reversal of GlyT1 (26, 71, 72). Moreover, since it colocalizes with NMDAR at post-synaptic level (73, 74), GlyT1 is believed to modulate the excitability of NMDAR by reducing glycine levels in the synaptic cleft, thus preventing saturation of the glycine-B site (75–78).

Noteworthy, the affinity of glycine for NMDARs is significantly higher than that of GlyRs (EC50 = 134 nM *vs.* EC_{50} = 270 μM) (63, 79), thus, under physiological conditions endogenous glycine may exert mainly an excitatory effect in the hippocampus, where both GlyRs and NMDARs are expressed. On the other hand, excessive glycine produced in pathological conditions, such as ischemia and epilepsy (80, 81), may spillover into extra-synaptic sites to activate inhibitory GlyRs in order to counteract the excitotoxic damage. In these conditions, GlyR-mediated inhibitory activity may be stronger than NMDAR-mediated excitatory one, resulting in a net effect of depression of excitatory post-synaptic currents (EPSCs) (82). Therefore, levels of glycine could be the major determinants in setting the polarity of glycine's role either in brain damage, either in correcting unwanted synaptic plasticity (82, 83)

Beyond Glycine: Other Agonists at the Glycine B-Site?

Multiple lines of evidence suggest a relevant crosstalk between glycine and D-amino acids during the neurodevelopmental stages that are critical to SCZ pathophysiology. D-Serine is synthetized in the neurons starting from astrocytic L-serine by serine racemase (SR), according to the "serine shuttle" hypothesis formulated by Wolosker, (84) and its levels in the synaptic cleft are controlled by Asc-1 transporter (70, 85, 86). Among D-amino acids, D-serine seems to be a crucial player in synaptic plasticity, such as long term potentiation (LTP) (87–91), and it has been considered the putative endogenous ligand at NMDAR glycine B-site (92, 93), since it appears to be functionally up to 100-fold more effective than glycine at potentiating NMDAR activity. The role of D-serine in the activation of NMDAR is confirmed by the reduction of synaptic transmission by treatment with D-amino acid oxidase (DAAO), which depletes endogenous D-serine but not glycine (93). Noteworthy, NMDAR responses do not seem to be fully reversed by DAAO and a "DAAO-insensitive fraction" has been shown in rat hippocampus, that accounts up to 30–50% of receptor activity (93), presumably because the remainder of the sites may be already occupied by glycine, which therefore may act in some parts of the brain and at certain stages of the neurodevelopment as the major ligand. Immunohistochemical studies comparing D-serine, glycine, and NMDARs pattern of distribution in rat brain, showed that D-serine and NMDARs overlap each other and have the highest concentration in telencephalon and developing cerebellum; conversely, glycine immunoreactivity does not correspond to NMDARs localization (except in the brainstem, where it parallels the distribution of NMDARs) but seems to prevail over D-serine in the adult cerebellum, hindbrain, and olfactory bulb (94). Papouin and colleagues performed an electrophysiological study, in order to assess the specific contribution of glycine and D-serine at synaptic and extra-synaptic NMDAR sites in CA1 region of hippocampus. Using specific enzymes that degrade either D-serine or glycine, they provide supporting evidence for assuming that D-serine may be the co-agonist at synaptic receptors, whereas glycine may act at extra-synaptic NMDARs, which have little or no role in synaptic plasticity (90). Nonetheless, Yan Li and colleagues proposed that the identity of the endogenous ligand might be determined by the level of synaptic activity, thus emphasizing the contribution of glycine in LTP induction process (91), and extending previous *in vitro* reports supporting the involvement of glycine in LTP enhancement (95). They showed that tonic activation of NMDARs in the amygdala under resting conditions may be achieved by D-serine, whereas glycine may be released from astrocytes in response to afferent impulses. Therefore, ambient D-serine may act as major ligand in absence of evoked synaptic events, while activity-dependent release of glycine may be involved in LTP-related NMDAR activation in the context of fear conditioning pathways (91). Rosenberg et al. also proposed that D-serine would not be the sole co-agonist at synaptic NMDAR sites: glycine and D-serine may have partial overlapping roles in regulating synaptic activity at NMDARs, and specific glycine effects may be revealed by deleting serine racemase (SR), the enzyme that synthesized D-serine (70). In fact, in an electrophysiological experiment, they demonstrated

that the synaptic NMDAR responses were essentially unaltered in adult SR-KO mice (70). Moreover, it has been demonstrated that even the GlyT1 inhibitor bitopertin increases the magnitude of LTP in rat hippocampal CA1 pyramidal cells, and this effect likely results from an increase in the extracellular levels of glycine (96). Direct application of glycine seems to exert the same effects on LTP induction, as well as increases the amplitude of NMDAR currents of approximately 50% (96). Nonetheless, it has been reported that application of high concentrations of glycine, exceeding the synaptic concentration of the endogenous Glycine B-site agonist, produce opposite effects on NMDAR currents amplitude and LTP, consistent with the internalization of a percentage of NMDARs primed by glycine (96). Glycine and glycine inhibitors may therefore display an inverted U-shape concentration-response profile on LTP induction, for whom higher glycine B-site occupancies may lead to a lack of efficacy (49). However, taken together, these findings suggest that two endogenous co-agonists, namely glycine and D-serine, may regulate distinct populations of NMDARs, with one or the other prevailing at a given synapse, finely tuning excitatory transmission in order to diversify a wide ranging repertoire of biological effects (97).

Interestingly, several lines of evidence disclose the role of D-serine for inflammation, excitotoxicity, and epileptogenesis. Inflammatory factors (such as amyloid β and lipopolysaccharide) stimulates the astrocytes and microglia to express SR (98, 99), thus these cells become the primary source of D-serine in inflammatory conditions (100). The amount of D-serine obtained by this way promotes excitotoxic damage and synaptic dysfunction through the activation of NR2 subunit at extra-synaptic sites (101). Transgenic mouse models for amyotrophic lateral sclerosis (ALS) exhibit several-fold higher levels of D-serine in spinal cord, and the elevation positively correlates with disease progression (102). Moreover, D-serine increase in spinal cord was observed even in sporadic postmortem human ALS cases or ALS relatives (102). Furthermore, it may be of interest that SR knockout mice were protected against cerebral ischemia and excitotoxic damage (103). These findings suggest that D-serine, rather than glycine, may be a key determinant for NMDAR-mediated neurotoxicity.

Correlation between D-serine levels and SCZ is demonstrated by multiple studies (104–107) that showed the decrease of its levels in CSF and serum of schizophrenic patients. Despite convergent lines of evidence pointing to the potential of D-serine in treating SCZ, it displays a low oral bioavailability, being largely metabolized by DAAO (104). Past clinical trials have demonstrated benefits of adding D-serine to the antipsychotic therapy in SCZ and bipolar disorder (108), but these results were not unequivocally replicated (109–111), leaving aside the fact that the high doses required may provoke peripheral neuropathies and nephrotoxic effects (112–114). Rather than therapeutic agent for SCZ symptoms, D-serine has recently been proposed as promising biomarker to antidepressant response to ketamine (112), with low plasma levels of D-serine predicting ketamine efficacy.

In summary, D-serine action at NMDAR may be more relevant than originally thought, and may have a pivotal role for synaptic plasticity and cognitive functions, as well as neurodegeneration and excitotoxicity.

Finally, even if it is not the topic of this review, the role of other D-amino acids in NMDAR modulation should be acknowledged, and among all the one of D-aspartate. This amino acid has been implicated in brain development (115, 116), a feature that is specifically appealing for SCZ, that is conceptualized as a pathology of the neurodevelopment with abnormal synaptic pathophysiology and altered brain connectivity. Moreover, multiple lines of evidence from animal modeling (117, 118) to postmortem brain abnormal gene expression and epigenetics (119) indicate a potential role of D-aspartate in SCZ pathophysiology, and lay the foundation for a potential use of D-aspartate as adjunctive therapy in those cases poorly responding to conventional antipsychotics (120).

Glycine and Neurodevelopment

Several recent studies have focused on changes in glycinergic signaling and expression pattern of glycinergic markers, such as glycine transporters and glycine receptors during the development (68, 121) making the neurotransmitter of interest for a disease believed to be of putative neurodevelopmental origin, such as SCZ. It can be hypothesized that the synaptic release of glycine is involved in the proper development of many motor and sensory circuits (i.e., auditory, visual, respiratory, and nociceptive) (122, 123). GlyRs expression is specifically regulated in terms of subunit composition during the development and throughout the CNS. Homomeric α_2 subunits are mainly expressed during the fetal period. Thus, a developmental switch from α_2 homomeric GlyRs to $\alpha_1\beta$ heteromeric GlyRs takes place between the birth and the third postnatal week in rats (69). Indeed, several studies demonstrated that α_1 is the most abundantly expressed subunit in adult rats and, since β subunit interaction with gephyrin is essential for the clustering of GlyRs in the synapses, it is plausible that the $\alpha_1\beta$ heteromeric form of GlyRs is the most common subtype within synapses (124).

Different subtypes of GlyRs might fulfill opposite roles during the development. Since intracellular chloride concentrations are high in embryonic neurons, homomeric α_2 GlyRs expressed during the fetal period might be excitatory, mediating the depolarizing chloride flux and the subsequent inward calcium flux. Therefore, GlyR activation may exert an excitatory action in immature neurons, whereas it mediates inhibitory neurotransmission in adult CNS, by increasing Cl^- permeability and leading to a membranal hyperpolarization (125). Precisely this latter subtype, α_2 GlyR, seems to be involved in pathophysiology of the autism spectrum disorder (ASD), condition that shares many clinical features and biomarkers with SCZ (126). In ASD is assumed to be an imbalance between glutamate and glycine in favor of an increased activity of glutamatergic neurotransmission early in neuromotor development. As highlighted by genetic and functional studies, suggesting the potential role of α_2 GlyRs in synaptic plasticity, as well as learning and memory, glycinergic

signaling might be linked to social and cognitive abnormalities in ASD (127). In summary, glycine neurotransmission seems to be highly modulated during brain development. This is in line with a potential crucial involvement of this neurotransmitter in pathophysiology of a disease, such as SCZ, strongly associated to a neurodevelopmental dysregulation.

GLYCINE RECIPROCAL REGULATION OF GLUTAMATE NEUROTRANSMISSION

NMDARs have unique functional characteristics: voltage dependence, calcium permeability, slow kinetics, and complex modulatory processes (128). NMDAR alone requires for an efficient gating the binding of both glutamate and a co-agonist, identified as glycine in the 1987 by Johnson and Asher (129). NMDARs are hetero-oligomeric proteins composed by a combination of different subunits called GluN1, GluN2, and GluN3. Notably, while GluN1 subunit is mandatory, different subunit composition leads to different receptor properties. The GluN1 subunit forms the glycine binding site, whereas the GluN2 subunit provides part of the glutamate binding site; moreover, the two sites appear to be allosterically coupled (130). Mice that express reduced levels of GluN1 subunits display a lowered glycine affinity and a variety of cognitive and learning defects, including hyperactivity, increased stereotyped behavior, disruptions of nest building activity, and poor performance in the Morris water maze, a measure of cued learning (131, 132). The behavioral phenotypes of these glycine-insensitive mutant mice may resemble in certain respects the positive and negative symptoms of SCZ, consistent with NMDAR hypofunction hypothesis. This evidence further emphasizes the role of glycinergic signaling in the pathophysiology of SCZ.

Glycine regulates glutamatergic neurotransmission at different levels; however, glutamate affects glycine concentration too. In fact, *in vitro* studies showed that elevated extracellular glutamate concentration reduces glycine release and high-frequency trains of stimulation decrease glycinergic inhibitory post-synaptic currents (IPSC) (133).

GLYCINE TRANSPORTERS 1 AND 2

Glycine transporters (GlyT) are membrane-bound proteins belonging to the Na^+/Cl^- dependent neurotransmitter transporters family involved in the reuptake of glycine from synaptic cleft. Two glycine transporters, encoded by different genes, are known: GlyT1 and GlyT2. They share an amino acid sequence identity of approximately 50%, but differ in their expression pattern, subcellular localization, and functional properties (125).

GlyT1 works in a bidirectional fashion with a stoichiometry of $2Na^+/Cl^-$/Gly, regulating glycine availability in the extracellular space, and terminates glycine signaling (134), significantly modulating the glutamatergic neurotransmission. GlyT1 has long been considered as exclusively expressed by glial cells, since early immunohistochemical studies did not recognize GlyT1 neuronal forms, presumably due to epitope occlusion of neuronal protein (135). However, there is an increasing evidence that GlyT1 is also expressed in neurons throughout the brain, where it is closely associated with the glutamatergic pathway (136). In glutamatergic neurons, GlyT1 is localized in both pre-synaptic membrane and postsynaptic density, where it interacts with the scaffold protein PSD-95 (136, 137).

GlyT1 plays a pivotal role in neurodevelopment as well as in cognitive processes of adult brain, as shown by the phenotype of GlyT1 mutant mice. Homozygous GlyT1−/− mutant mice appeared normal but unexpectedly died on the first day of birth, showing severe motor-sensory deficits, suggesting a vital role for GlyT1 that, even if dispensable for embryonic development, it is crucial for postnatal survival (138). Heterozygous GlyT1+/− mice, on the other hand, survive and show promnesic phenotypes, as well as a resistance to amphetamine disruptive effect on prepulse inhibition (PPI) (47, 139, 140). PPI of the acoustic startle reflex is an operational measure of the pre-attentive filtering process known as sensorimotor gating (141, 142) that is disrupted in SCZ as well as after stimulants administration even in healthy subjects, whereas antipsychotic drugs commonly are able to reverse PPI disruption (143–145). Therefore, it has been suggested that a radical reduction in expression of GlyT1 may be responsible for sensori-motor gating deficits due to the hyperactive inhibitory glycine-mediated signaling (146), whereas a mild reduction in expression of GlyT1 might enhance NMDAR function and memory retention, and might be protective against amphetamine-induced sensorimotor gating deficits, suggesting that drugs which inhibit GlyT1 might exert procognitive and antipsychotic effects (47).

Conversely to GlyT1, GlyT2 is exclusively expressed by glycinergic neurons and localized in presynaptic terminals adjacent to the active zones. Within the glycinergic bouton, GlyT2 appears associated with the plasma membrane or as discrete clumps and it seems to be excluded from the active site of the synapse and synaptic cleft (147). Unlike GlyT1, GlyT2 is coupled to electrochemical movement of $3Na^+$, maintaining the high concentration gradient on the presynaptic terminals and refilling presynaptic vesicles with glycine (148). Further, its expression is restricted to regions with glycinergic transmission, such as the cerebellum, brainstem, and the spinal cord (135). Homozygous GlyT2 −/− knockout mice also die in the second postnatal week, however, they show a phenotype entirely different from GlyT1 knockout mice, developing a lethal motor deficiency, reminiscent of severe forms of human hyperekplexia (hereditary startle disease), characterized by muscular spasticity, tremor, and impaired motor coordination (149).

GlyT1 function is thought to be closely regulated by several molecular mechanisms, e.g., inhibition by arachidonic acid, a second messenger released following phospholipase A2 activation (150). Moreover, intracellular pH value also modulates GlyT1 activity. Low doses of Zn^{++}, which is released with glutamate by different types of excitatory neurons, induce GlyT1 inhibition but have no effect on GlyT2 (151). Activation of

protein kinase C (PKC), induced by sustained intracellular Ca^{++} influx, decreases GlyT1 and GlyT2 expression on the neuron surface (152). Probably PKC does not affect directly GlyTs, but intermediate substrate proteins and additional kinases such as MEK1/2 kinases or PI3-kinase and CaMKII are involved in this mechanism (152). In addition, several proteins interacting with GlyTs regulate their trafficking and recycling at the pre-synaptic terminal. Particularly, Ca^{++} influx induced by depolarization promotes the GlyT2 expression on plasma membrane surrounding the active zone and this process is thought to be regulated by the interaction between GlyT2 and syntaxin-1 (153).

In summary, it has been hypothesized that GlyTs have a pivotal role in the regulation of neurotransmission both in glycinergic and in glutamatergic synapses and several lines of research suggest that changes in the activity, density, and localization of GlyTs in glial and nerve terminals are involved in synaptic efficacy and neuronal plasticity.

GLYCINE RECIPROCAL REGULATION OF DOPAMINE NEUROTRANSMISSION

Glutamate effects on dopamine regulation have been extensively recognized, since the evidence that NMDAR-antagonists increase dopamine release in the striatum dates back to 1998. It is also well known that glycine acts as a co-agonist of NMDAR, but less is known regarding the effect of glycine on dopamine release both in cortex and in striatum. Consistent with its ability to reverse PCP-induced hyperactivity and psychotic-like symptoms, it is conceivable that glycine may decrease NMDAR-mediated dopamine release. Nevertheless, evidence in this respect is controversial and glycine seems to be able to increase, decrease, or have no effect on dopamine release.

Earlier studies on rat striatal slices unexpectedly showed a net effect of glycine to potentiate dopamine release in the striatum (154), and these results were replicated in other studies (155, 156); later this phenomenon was observed even in freely moving rats, to whom glycine was administered *via* a microdialysis probe in the anterior striatum, and who exhibited increased local release of dopamine and its metabolites (157). A recent study also shows that glycine may potentiate the excitability of dopaminergic neurons in *substantia nigra pars compacta* (SNc) by amplifying NMDAR-dependent signals. In fact, exogenous applications of glycine on midbrain slices of juvenile rats may regulate dopaminergic firing, leading to a switch from tonic spontaneous firing to the bursting activity, and then increase dopamine release at post-synaptic sites (158). If that were true, glycine effect on dopamine in the striatum would not be advantageous for treating psychosis; nevertheless, glycine could rather be involved in nigral information processing and locomotor behavior. As shown by a recent study, glycine binding site stimulants might be helpful in alleviating antipsychotic-induced EPS in treated patients rather than their psychotic symptomatology, potentially by mitigating the reduced dopamine function in the nigrostriatal pathway (159).

On the other hand, according to an *in vitro* study on mouse striatal tissue, low-dose of glycine seems to potentiate basal dopamine release from presynaptic dopamine terminals, whereas high-dose glycine showed to significantly inhibit striatal dopamine release, which would be expected to be therapeutically beneficial in SCZ (160). The same authors have later demonstrated that a GlyT1 inhibitor, ALX5311, potentiates NMDA-dependent GABA-release, and that this effect leads to significant inhibition of striatal dopamine release, supporting a model in which NMDARs have dual excitatory/inhibitory function within striatum (46). Therefore, glycine might exert an excitatory effect by acting on NMDARs located on pre-synaptic dopamine terminal or conversely, an inhibitory effect by acting on NMDARs located on GABAergic interneurons, increasing or decreasing, respectively, striatal dopamine release (46).

In summary, glycine seems to exert a multimodal effect on regulation of dopamine release, depending on the brain region in which the action is considered, glycine concentration, pre-synaptic or post-synaptic action, as well as dopaminergic functional state. This multimodal action should be taken into account to explain some inconsistent clinical effects of glycinergic agents in SCZ therapy.

Another way in which glycine modulates dopamine neurotransmission is through GlyRs. GlyRs-mediated regulation of dopaminergic firing seems to involve other neurotransmitters such as GABA (161) and acetylcholine in ventral tegmental area (162), and glutamate in nucleus accumbens (163). The role of GlyRs in the regulation of mesolimbic dopaminergic neurotransmission is confirmed by a study in which accumbal perfusion of strychnine (the GlyRs antagonist) was found to decrease dopamine levels in rats, and this effect was reverted by glycine (164). Moreover, ethanol may produce its reinforcing and dopamine-elevating effects precisely *via* GlyRs: Molander and Söderpalm showed that accumbal perfusion of strychnine decreased dopamine levels *per se*, as well as prevented further dopamine increase after ethanol administration (165). Accumbal GlyRs seem to be involved not only in dopamine elevations induced by ethanol, but also may contribute also to dopamine elevations induced by cannabinoids and nicotine (166), thus showing important implications for mechanisms related to alcoholism, other addictions and dopamine-related psychiatric disorders such as psychosis.

Glycine may affect dopaminergic output indirectly, acting on presynaptic GlyRs expressed on GABAergic terminals. Noteworthy, at birth both GABA (167) and glycine (168, 169) are excitatory neurotransmitters, but during the development, they became inhibitory ones. Ye and coauthors demonstrate that, despite these differences during the neurodevelopment, the net effect of glycine on dopamine, through GABA, in ventral tegmental area consists of a strengthening of the dopamine firing (161). Furthermore, if glycine may affect dopamine release, it is also relevant the role of dopamine in regulation of glycine release. An intriguing modality by which dopamine could regulate glycine release has been recently proposed by Shibasaki et al. (26) The authors demonstrated that dopamine may

increase glycine release from cortical astrocytes by reversing the GlyT1. According to this view stimulation of dopamine receptors may change the intracellular metabolic *milieu* inducing glycolysis and oxidative phosphorylation (170), resulting in increased intracellular glycine levels. This increase in glycine concentration may reverse transport by GlyT1 (171).

Overall, in the framework of SCZ pathophysiology, a bidirectional regulation of glycine on dopamine should be conceived: in fact, changes in dopamine release could modulate glycine concentration, and in turn modify the response of NMDAR *via* Glyt1 potential reverse activity, especially at cortical level.

Therefore, an "inverse" and different mechanism from the canonical one, linking NMDAR and dopamine could be suggested in regulating dopaminergic balance, which is a crucial issue in SCZ.

GLYCINE TRANSPORTERS AND THE POSTSYNAPTIC DENSITY

Postsynaptic density (PSD) is an electron-dense structure composed of glutamate receptors (NMDARs, AMPARs, mGluRs), proteins involved in signal transduction (disrupted in schizophrenia 1, activity-regulated cytoskeleton-associated protein, calcium/calmodulin-dependent protein kinase II, Ras GTPase, and ion channels), scaffold proteins (post-synaptic density protein 95, Shank, Homers), and cytoskeletal structures (tubulin, septin, and others) localized at the distal tip of dendritic spines at excitatory synapses (172–174). The type and the number of the proteins highly influence PSD architecture, therefore significantly impacting the synaptic plasticity and dendritic shape (172–174). PSD has been implicated in pathophysiology of psychiatric disorders, including SCZ, and their treatment (175–178).

In several studies postsynaptic density protein 95 (PSD-95) has been found reduced in cortical and subcortical regions of postmortem brain samples from patients affected by SCZ (179–181). Considering that PSD-95 is physically and functionally linked to NMDARs and Homer proteins (182), all believed to be involved in SCZ, it will be of interest to understand how glycine may affect and interact with PSD-95. Immunohistochemical studies showed that in hippocampus and dentate gyrus GlyT1 is localized at the PSD of asymmetric glutamatergic synapses, belonging to a protein complex including NMDAR. Therefore, recent studies have highlighted that PSD-95 physically interacts with GlyT1 in the rat brain, stabilizing its localization at post-synaptic membrane and suppressing its internalization from cell surface, thereby increasing glycine uptake (136, 137). It has been hypothesized that PSD-95 could act as a link between GlyT1 and NMDAR, regulating glycine concentration in the micro-environment of NMDAR at glutamatergic synapses (**Figure 1**) (137). In heterozygote mutant GlyT1 +/− mice, who display increased concentrations of glycine, higher levels of GluN2 and increased expression of PSD-95 have been found, suggesting that PSD-95 may anchor GluN2-containing-NMDAR at synapses (that is the NMDAR subtype mostly involved in learning and synaptic plasticity) (183), preventing their internalization (184). Other studies examined how heterozygous GlyT1+/− mice display an enriched composition of PSD, showing concomitant increased levels of GluN1/2A NMDAR subunits and GluA1/2 AMPAR subunits, since an increase in NMDARs may probably cause an elevation of synaptic AMPARs (185). Nevertheless, other recent studies have found that the AMPAR/NMDAR ratio was decreased in mutants compared to wild-type mice displaying the complexity and variability of synaptic adaptation to altered GlyT1 function (140).

Calcium/calmodulin-dependent protein kinase II (CaMKII) is a core component of the PSD that may regulate GlyT1 activity including indirect phosphorylation mechanisms (e.g., activation of a signaling cascade or the phosphorylation of cytoskeletal protein involved in trafficking of GlyT1) and there is evidence that GlyT1 is inhibited by CaMKII inhibitors (**Figure 1**) (186). Sequence analysis of SLC6 family transporters, including GlyT1, revealed multiple consensus sites for phosphorylation by kinases. It is plausible that PKCα/β also could play a regulatory role in glycine transport by phosphorylating GlyT1 (152).

The role of glycine neurotransmission in the modulation of Homer gene expression has been explored in reference to the action of antipsychotics, alone or with the adjunction of glycine B-site agonists such as D-cycloserine (187). Multiple lines of evidence suggest an involvement of Homer (long and short forms, and their splicing variants) in antipsychotics action at level of PSD (188–191). Polese et al. have shown how add-on D-cycloserine to typical (haloperidol) and atypical (clozapine) antipsychotic treatment, modulate the expression of Homer 1a, in a negative trend in caudate-putamen (187). These data and the "dominant negative" function of Homer 1a can be predictive of a relative increase of mGluR surface clustering. It could be interpreted as a mechanism of mGluR activity enhancement, mediated by NMDAR glycine site agonists.

Among proteins located at PSD, disrupted in schizophrenia 1 (DISC-1) has attracted the attention of SCZ scholars in the last decade, since human genetic, imaging genetics, and preclinical models indicate a pivotal role of this protein in SCZ pathophysiology. Several antipsychotics, as well as compounds modulating the glutamate signaling, have been demonstrated to affect disk1 expression and function. Within the scope of this review, is noteworthy that rapastinel (formerly GLYX-13), an amidated tetrapeptide (threonine-proline-proline-threonine-amide) acting as allosteric partial agonist of glycine B-site (192) has been shown to counterbalance prepulse inhibition (PPI) disruption, hyperlocomotion, and memory deficits, induced by MK-801 administration in mice as well as to revert the associated decrease in disk-1 and GluN2B proteins. It is possible that this "antipsychotic-like" effect can be mediated by GluN2B expression, since GLYX-13 seems to be ineffective in GluN2B-knockdown mice (193).

Finally, although brain derived neurotrophic factor (BDNF) cannot be strictly considered a constitutive component of PSD, it has been isolated from this structure and directly affects its function. It has been demonstrated that in rat hippocampus BDNF reduces glycine reuptake by affecting the insertion of

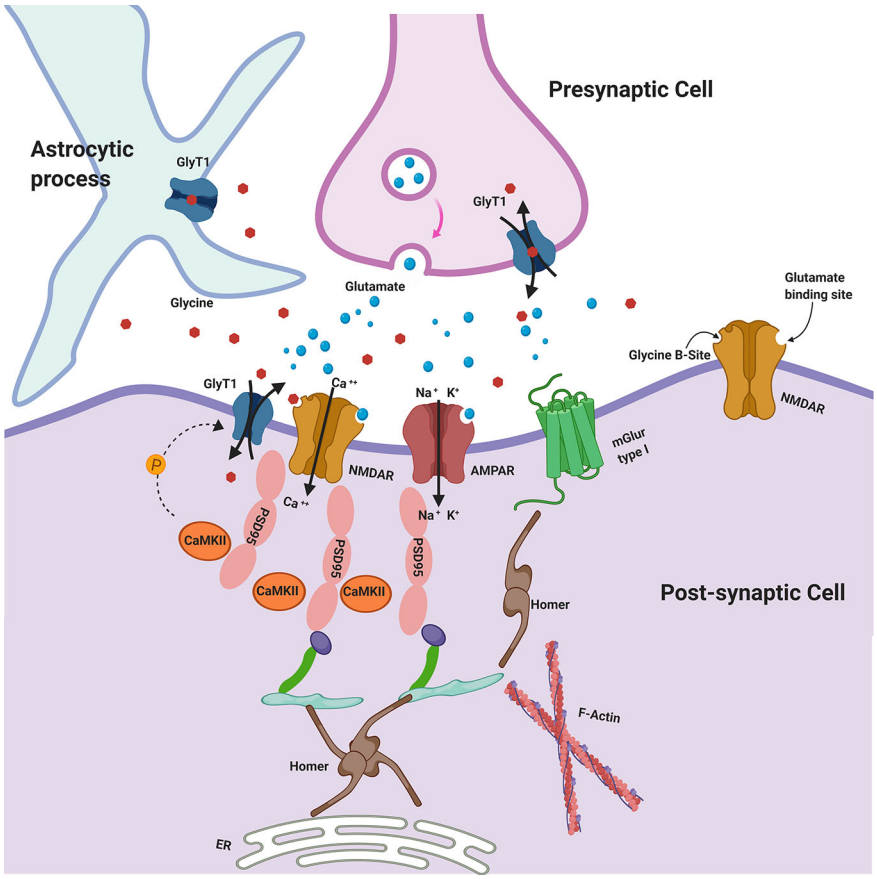

FIGURE 1 | Glycine Transporters and the Postsynaptic Density (PSD). GlyT1 is localized at the PSD of asymmetric glutamatergic synapses, belonging to a protein complex including NMDAR. PSD-95 physically interacts with GlyT1, stabilizing its localization at postsynaptic membrane. CaMKII may regulate GlyT1 activity via indirect phosphorylation mechanisms. Glycine may be released in the synaptic cleft also by astroglial cells via functional reversal of GlyT1. GlyT1: Glycine Receptor Transporter 1; NMDAR: N-Methyl-D-aspartate receptors; PSD-95: postsynaptic density protein 95; CaMKII: Ca2+/calmodulin-dependent protein kinase; AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; mGluR type I: metabotropic glutamate receptors type I.

GlyT2 in cell membrane (194). Despite the intriguing relationship between BDNF and glycine as underlined by the previous finding, the role of BDNF *in vivo* in regulating glycine pathways relevant for SCZ pathophysiology is still controversial. In fact, patients treated with sarcosine, an agonist at glycine B-site of NMDAR, display no changes in plasma levels of BDNF (195).

GENETICS OF GLYCINERGIC PATHWAY IN SCHIZOPHRENIA

A number of studies have explored the role of variation within genes encoding for elements of the glycinergic pathway in SCZ, with conflicting results in some cases. Deng et al. (196) found that a polymorphism within the SLC6A5 gene, encoding for GlyT2, was significantly associated with SCZ. Further, although indirect, support for a role of genetic variation within the glycinergic system in SCZ came from the study of Ohnuma et al. (197). These authors found that polymorphisms within the gene encoding for D-amino acid oxidase (DAO), which partially mediates the degradation of D-serine, a component of the glutamatergic transmission and an endogenous ligand for the glycine B-site on NMDAR (121), were statistically significantly associated with SCZ in a case-control study (197). More recently, a whole genome sequencing study in subjects with a high familial loading for psychotic disorders over three generations (198) found a frameshift mutation (rs10666583) in the GRIN3B gene, which codes for the GluN3B subunit of the NMDAR. This mutation was present in all family members with a psychotic disorder, but not in healthy relatives (198). The authors conclude that, given that this mutation induces an amino acid shift that degrades the S1/S2 glycine binding domain of the GluN3B subunit of the NMDAR, which subsequently affects the permeability of the channel pore to calcium ions, a decreased glycine affinity for the GluN3B subunit might cause impaired functional capability of the NMDAR (198). Of interest, a recent genetic association study in subjects with schizotypal traits showed statistically significant associations between the minor allele of three SNPs, rs2915885, rs11167557, and rs1428159, all positioned within the glycine receptor α1

subunit (GLRA1) gene, and dimensional schizotypy, specifically with the disorganized symptoms cluster (199). Further support for a role of the glycinergic system comes from a genetic-metabolomic study (200), which showed that 5-oxoproline, aspartate, and glutamate, known to affect NMDAR function, were significantly elevated in patients with rare variants in genes encoding for the glycine cleavage system. Finally, a groundbreaking study showed that genetically informed pharmacological treatment targeted at the glycinergic/glutamatergic signaling could improve significantly clinical response in patients with psychosis (201). These authors found several CNVs spanning 9p24.1 in a proband and his mother, who had diagnoses of schizoaffective disorder and bipolar disorder with psychotic features, respectively. Among the genes involved, the gene encoding for the glycine decarboxylase was of particular interest given its role in the catabolism of glycine (201). The authors performed two proof-of-principle clinical trials with glycine and d-cycloserine obtaining an additional 20 to 26% reduction in symptom severity with the former and 13 to 30% reduction with the latter (201)

Conversely, a series of negative studies do not appear to support a role for glycine transmission and signaling in SCZ molecular pathophysiology, at least on genetics ground. (202–206). The study of Feng et al. (202) explored the role of genetic mutations within the glycine receptor α2 subunit gene (GLRA2) in SCZ, using a sequencing approach. These authors detected three silent mutations in the coding region, C894T in exon 5, C1134T in exon 7, and C1476T in exon 9 (202), highlighting that a role of these variants in the pathogenesis of SCZ is unlikely. Similarly, subsequent case-controls studies confirmed these negative findings (203–206). Another negative finding derived from a gene expression analysis in *post-mortem* dorsolateral prefrontal cortex and cerebellum brain samples (207). Indeed, Burnet et al. did not find alterations of GlyT1 expression levels in these brain areas in 18 SCZ patients compared to 20 healthy controls (207).

Taken together, there is some discrepancy in the genetic findings of glycine and related signaling in SCZ. However, it should be noted that most of the early, negative, studies used case-control approaches often with inadequately powered sample sizes. The more recent translational evidence points to a role of genetic determinants in the pathophysiology of SCZ and contributes to the hypothesis that a subgroup of affected patients might take advantage of treatments targeted at the glycinergic/glutamatergic pathway.

CRITICAL APPRAISAL OF GLYCINE PHARMACOLOGY AND ITS POTENTIAL ROLE IN SCHIZOPHRENIA

Several studies indicate that cognitive processes may be regulated by glycine levels at glutamatergic synapses (47, 208, 209). Glycine concentration, in turn, is regulated by GlyT1, even if the glycine-B site would be tonically saturated (210). There is consensus, however, that GlyT1 prevents saturation of the glycine binding site on NMDARs and that further glycine increase can enhance NMDAR activation (155, 211), thus representing a potential target to modulate excitatory synapses.

Recent studies in GlyT1+/− mice showing NMDAR hyperfunction, highlighted the presence of an increased number of dendritic branching in the CA1 region of the hippocampus, an enhanced synaptogenesis (184), as well as higher density of excitatory glutamatergic synapses, and an increased expression of PSD-95 compared to wild type. In summary, these results suggest that glycine contributes to the regulation of synaptic plasticity, dendritic maturation, and glutamate-induced spinogenesis in the CNS (212).

Concerning behavioral phenotype, heterozygote GlyT1 +/− mice displayed improved memory retention during spatial learning task (47), and deletion of GlyT1 in the forebrain neurons resulted in a pro-cognitive profile characterized by facilitated associative learning, working memory, reference memory, and reversal learning (208, 209). Indeed, pharmacological blockade of GlyT1 exerted pro-cognitive effects in a preclinical model of SCZ, as also showed by a GlyT1 inhibitor, PF-3463275, that has been found to reverse ketamine-induced working memory deficits (213). Furthermore, SSR-504734, another GlyT1 inhibitor, facilitated cognitive flexibility, as assessed in the attentional set-shifting task in rats (214). These compounds probably may improve cognitive function and memory by increasing NMDAR signaling (214, 215); moreover, they may increase long term potentiation (LTP) that is one of the most studied manifestations of neuroplasticity. Therefore, it might be possible that GlyT1 inhibitors can reduce psychotic symptoms by improving neuroplasticity (216).

Deletion of the GlyT1 gene causes a divergent effect on PPI, depending on regional specificity. In fact, complete GlyT1 deletion in cortex confers resistance to PPI disruption induced by the NMDAR antagonist MK-801 (217), and may lead to a "psychosis-resistant" phenotype. On the contrary, deletion of GlyT1 in the striatum provokes a relevant PPI deficit, resembling a SCZ endophenotype, and the animals remain sensitive to the PPI-disruptive effect of MK-801 (217). Hence, there is no unequivocal support to the antipsychotic potential of GlyT1 inhibition and much more remains to be discovered.

Despite the complexity of the issue, in animal models of SCZ, such as neonatal lesion of the hippocampus (218) or acute NMDAR blockade (219, 220), GlyT1 inhibitors, such as ALX-5407, sarcosine, ORG 24598, SSR504734, and SSR103800 were effective in reverting PPI disruption, thus displaying an attractive antipsychotic-like activity (217).

The therapeutic strategies based on glycine neurotransmission have yielded contrasting results with significant improvement of SCZ symptoms in some clinical trials, as well as inconclusive results or no effect at all in other studies (221) (**Table 1**).

DIGGING INTO POTENTIAL MECHANISM RESPONSIBLE FOR GLYT1 INHIBITORS FAILURE IN CLINICAL PRACTICE

To date, more than 70 placebo-controlled clinical trials of agonists or partial agonists acting at NMDAR glycine modulatory site in SCZ (including glycine, D-serine, D-

TABLE 1 | Summary of the GlyT1 and GlyT2 inhibitors and their clinical and pre-clinical effects.

Type	Compound		Mechanism of action	Functional results	References
Sarcosine and some sarcosine-based GlyT-1 inhibitors	***Sarcosine***		GlyT-1 inhibitor	Improvement with positive symptoms, negative symptoms, and cognitive deficits	Lane et al. (109) Lane et al. (222) Lane et al. (223)
	NFPS/ALX5407		GlyT-1 inhibitor	[Gly] ↑ in rodent cerebral spinal fluid (CSF), pre-frontal cortex (PFC), and cerebellum *in vivo* induction of LTP Antipsychotic and pro-cognitive effects in rodent behavioral models Enhancement of pre-pulse inhibition (PPI)	Cioffi et al. (224)
	Org 25935		GlyT-1 inhibitor	Reduced ketamine-induced psychomimetic and perceptual alterations in measures of total positive and negative syndrome scale	Cioffi et al. (224)
	AM747		GlyT-1 inhibitor	Effective as adjunctive therapy for negative symptoms in schizophrenic patients concurrently maintained on an antipsychotic treatment	Amgen et al. (225)
	Org 24461		GlyT-1 inhibitor		Zafra et al. (54)
	Org 24598		GlyT-1 inhibitor		Zafra et al. (54)
Non-sarcosine based GlyT-1 inhibitors	***Benzoyl is indolines***	***Bitopertin***	Selective and non-competitive GlyT-1 inhibitor	Enhancement of LTP in Sprague-Dawley rat hippocampal CA1 pyramidal neurons [Gly] ↑ in rat CSF and striatal tissues upon oral administration. Dose-dependent [Gly] ↑ in CSF in humans	Pinard et al. (226) Hofmann et al. (227)
	Methylphenidate-derived	***SSR504734***	GlyT-1 inhibitor	Enhancement of working memory performance in wild-type mice with high retention demand Protection against depression in the chronic mild stress model of depression Dose-dependent anti-depressant effects in rats during the Porsolt forced swim test	Singer et al. (228) Depoortere et al. (229) Boulay et al. (230)
		SSR103800	GlyT-1 inhibitor	Reversion of short-term memory deficit induced by phencyclidine	Boulay et al. (230)
		GSK1018921	GlyT-1 inhibitor	Dose-limiting AEs including dizziness and visual disturbances in humans	Cioffi et al. (224)
	Alkyl and heteroaromatic substituted sulfonamides and sulfones	***ACPPB***	GlyT-1 inhibitor	Promotion of dopaminergic reinnervation of the dorsal striatum; reversion of 6-OHDA-induced lateralization of sensorimotor behavior in mice	Schmitz et al. (231)
		DCCCyB	GlyT-1 inhibitor	Reversion of PCP-induced cognitive deficits; reversion of ketamine-induced perceptual attentional set shifting in rat models	Blackaby et al. (232)
	Heteroaryl amides	***PF-03463275***	GlyT-1 inhibitor	Reversion of ketamine-induced working memory deficits in non-human primates	Roberts et al. (213)
GlyT-2 inhibitors	***Org-25543***		Irreversible GlyT-2 inhibitor	↑ Extracellular [Gly] in the lumbar dorsal spinal cord of rats.	Whitehead et al. (233)
	GT-0198		GlyT-2 inhibitor	Analgesic effect in a mouse model of neuropathic pain	Omori et al. (234)
	ALX1393		GlyT-2 inhibitor	Inhibition of pain transmission	Morita et al. (235)

cycloserine, and D-alanine) have been reported in medical literature, with controversial results (236). An alternative approach to increase glycine availability is to block glycine reuptake *via* GlyT1. However, even though several GlyT1 inhibitors seemed to be efficacious in animal models, clinical studies in humans have been disappointing at least for major endpoints. The most advanced compound tested with the highest accuracy in terms of sample size and duration of the trials, is the non-competitive GlyT1 antagonist bitopertin, which also failed to reach its endpoints in Phase III trials (236).

With respect to the failure of GlyT1 inhibitors in clinical trials, potential reasons are herein explored, in order to better understand why a promising pharmacological strategy should not be abandoned.

One aspect that could be critical in evaluating the effect of GlyT1 inhibitors, included bitopertin, is the possibility that this mechanism of action may lead to an increase in glycine levels merely at extra-synaptic sites, therefore being less effective in potentiating NMDAR synaptic function. Nonetheless, the experiments of Martina and colleagues (96), confirming the ability of bitopertin to increase the activity of synaptic NMDARs, as well as to induce LTP processes, headed in a different direction. Perhaps, bitopertin may correct a certain degree of NMDAR hypofunction, being still unable to restore completely the glutamatergic transmission. Beyond the degree of activity, NMDAR dysfunction of SCZ may lay in the decreased number of receptors (237), abnormal coupling with PSD proteins (238), altered phosphorylation status (239), and other non-neurotransmitter cues that can impact synaptic efficacy. Not all these alterations potentially occurring in SCZ can be reverted or counterbalanced merely by increasing NMDAR activity.

Moreover, we should observe that GlyT1 inhibitors display an inverted U-shape concentration-response profile of action and this element, taking into account wide inter-individual differences in drug metabolism and pharmacokinetics, may be responsible for conflicting clinical results. In fact, the inverted U-shape dose-response curve displayed by bitopertin in LTP induction processes, as well as the partial receptor occupancy needed for efficacy (<50%) (49), maybe made further complicated a successful translation from animals to patients in terms of dose-finding issues.

Another point that should be raised is that, despite the extensive preclinical evidence supporting the role of bitopertin in treating SCZ, chronic administration has never been tested in animal models, in order to exclude a potential loss of effect in prolonged treatments.

In the original protocol of the clinical study, bitopertin was co-administered with routine antipsychotic treatment (240), but the class of antipsychotic was variable within the sample. Therefore, a question remains to be answered about the possibility that a certain combination would be less effective than others, resulting in an overall lack of efficacy of bitopertin intervention. Noteworthy, preclinical studies showed that glycinergic agents, when combined with antipsychotics with different receptor profile, may exert a wide ranging molecular pattern of responses (187).

Placebo effects observed in bitopertin phase III trials were larger than in phase II, and placebo response rate was assessed at 56–61% both in DayLyte and FlashLyte studies, which could explain the failure to detect any significant difference between arms. The magnitude of symptoms improvement in the placebo groups of RCTs (even those testing antipsychotics) is considerably growing over time, as described by several reviews (241, 242), making it even more difficult for the active medication groups to separate in a statistically significant way from placebo.

Finally, it is also important to remember that bitopertin has been tested for negative symptoms of SCZ, that are not always easy to be assessed reliably, as well as are difficult to be distinguished between primary and secondary ones. Despite the validity of the assessment instruments, negative symptoms are often not a focus of assessment or treatment in clinical practice, because they are rarely responsible for acute crisis or hospitalizations (243). Noteworthy, to date, antipsychotic medications remarkably effective in treating negative symptoms are few. Trying to understand the reason of this granitic-like resistance, Velligan et al. proposed a negative symptom maintenance loop theory, wherein decreased initiation and withdrawal lead to a series of self-perpetuating outcomes (i.e., reduced responsiveness to social stimuli, low interest in relationships, and decreased reinforcements from the social context) (244). In this perspective, pharmacological treatment might struggle to break the cycle, and although they may motivate patients to increase their social drive, patients may still lack the ability to interact due to previous chronic isolation. Therefore, to achieve a tangible improvement of negative symptoms, adjunctive behavioral training may be required.

DISCUSSION

TRS is a major clinical and therapeutic challenge in the management of SCZ patients, representing also a crucial mental health issue for the social implications and for care costs (245, 246). Therefore, the search for new compounds alone or in combination with the available antipsychotics is warranted, especially when the gold standard (i.e., clozapine) therapy fails. Ongoing research suggests that the multidimensional symptoms of SCZ may arise from dysregulation in multiple signaling pathways that may revolve around glutamatergic neurotransmission. NMDAR may represent a converging point of environmental hits and genetic factors, leading to downstream neurochemical dysfunctions that may account for positive, negative, and cognitive symptoms. Therefore, it can be hypothesized that pharmacological augmentation of NMDAR transmission through glycine signaling enhancement might restore the function of prefrontal cortex to control dopamine release, offering a potentially useful strategy in SCZ treatment. Glycine-based treatments for SCZ have their rationale first of all for the potential of this amino acid to regulate glutamate signaling and to modulate in a reciprocal interplay dopamine release, interacting, indeed, with two

neurotransmitters shown to be among the major players in SCZ pathophysiology. Moreover, it should be remarked that TRS, at least for those cases that are not fully responsive to dopamine antagonists or partial agonists, is believed to be linked to aberrant glutamatergic signaling. Several lines of evidence suggest a glutamatergic mechanism of action even for clozapine that, coincidentally, is significantly effective in TRS. This superior efficacy is presumably due to an additional mechanism to D2 receptor occupancy and possibly to a pro-glutamatergic action. Indeed, it has been hypothesized that clozapine may have an intrinsic agonist or partial agonist activity at the glycine B-site, that may contribute to its unique clinical effects (24).

It is clear that, despite the relevance of the issue and the strong neurobiological rationale, glycine-based pharmacological interventions are still inconclusive but, at the same time, strongly suggestive of the high therapeutic potential, especially for the severe form of TRS.

How the utilization of glycine enhancers or modulators can be improved for SCZ therapy? A first level of analysis should clearly separate the strategies based on potentiation of transmission at NMDAR glycine B-site from the ones based on GlyT1 inhibition. Comparing the outcomes of the two types of strategies may be important to try to figure what kind of targets are respectively achieved in terms of clinical improvement, therefore a focused (meta?) analysis is needed.

From the perspective of clinical trial methodology, some trials with compounds active at NMDAR glycine B-site have shown positive results; however, larger sample size and more homogeneous subsets of patients, separating those with prevalent positive or negative symptoms and longer duration of treatment, should be required. A number of measures should be considered in order to minimize the placebo response, including reducing the number of collaborating study sites and recruiting patients preferably from academic ones (241). A better evaluation is needed to determine which patients should be treated only with glycine-based pharmacological intervention, and in which ones these agents should be administered in augmentation with canonical antipsychotics. Finally, clinical trials using glycinergic agents are not always designed specifically for TRS patients, therefore an effort for including this class of patients should be done.

Furthermore, for more TRS "tuned" treatment based on glycine signaling, a better knowledge of the major kinetic steps responsible for the activation of glutamate-bound NMDAR by glycine is paramount to elucidate the pharmacodynamics of glycinergic compounds (247). Therefore, how ambient glycine levels regulate NMDAR function under a pattern of multiple stimulations, how glycine transporters interact with multiple PSD proteins, and how glycine affect overall dopamine–glutamate interaction are key questions for the development of new compounds.

In conclusion, despite the mismatch between the significant advance of our knowledge of glycine signaling in the modeling of SCZ pathophysiology and the results of clinical trials, glycine–based pharmacological therapy, alone or in combination with available antipsychotics is still worth to be explored and refined.

AUTHOR CONTRIBUTIONS

AdB conceived the project of the manuscript. AdB, FM, AB, and LV conceived the strategy for literature retrieval and selection. AdB, AB, MM, LV, and FM participated in the writing process of the first draft of the manuscript. AdB, MM, and FI revised the final version of the manuscript. All authors have read and approved the final version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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