Indicated association between polygenic risk score and treatment-resistance in a naturalistic sample of patients with schizophrenia spectrum disorders

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Abstract

Background: One third of people diagnosed with schizophrenia fail to respond adequately to antipsychotic medication, resulting in persisting disabling symptoms, higher rates of hospitalisation and higher costs for society. In an effort to better understand the mechanisms behind resistance to antipsychotic treatment in schizophrenia, we investigated its potential relationship to the genetic architecture of the disorder.

Methods: Patients diagnosed with a schizophrenia spectrum disorder (N=321) were classified as either being treatment-resistant (N=108) or non-treatment-resistant (N=213) to antipsychotic medication using defined consensus criteria. A schizophrenia polygenic risk score based on genome-wide association studies (GWAS) was calculated for each patient and binary logistic regression was performed to investigate the association between polygenetic risk and treatment resistance. We adjusted for principal components, batch number, age and sex. Additional analyses were performed to investigate associations with demographic and clinical variables.

Results: High levels of polygenic risk score for schizophrenia significantly predicted treatment resistance (p=0.003). The positive predictive value of the model was 61.5% and the negative predictive value was 71.7%. The association was significant for one (p=0.01) out of five tested SNP significance thresholds. Season of birth was able to predict treatment-resistance in the regression model (p=0.05).

Conclusions: The study indicates that treatment-resistance to antipsychotic medication is associated with higher polygenetic risk of schizophrenia, suggesting a link between antipsychotics mechanism of action and the genetic underpinnings of the disorder.

Key Words: Antipsychotic Agents; Psychotic Disorders; Precision Medicine; Genetic Research; Pharmacogenomics; Biological Psychiatry

1. Introduction

Schizophrenia is a severe mental disorder that has a profound impact on affected individuals and imposes large economic costs on the society (Chong et al., 2016; Knapp et al., 2004). Schizophrenia is ranked amongst the most disabling disorders globally (Charlson et al., 2018), with recovery rates as low as 13.5 % (Jaaskelainen et al., 2013). The disorder is associated with a significant loss of productivity (Knapp et al., 2004) and a 10-20 years shorter life expectancy (Chesney et al., 2014). Antipsychotic drugs have become a cornerstone in the treatment of schizophrenia (Leucht et al., 2012). Although varying in targeted receptors (Kusumi et al., 2015), all antipsychotics share the property of regulating dopamine signalling (Amato et al., 2018). The efficacy in reducing symptoms differ only but little between the drugs (Leucht et al., 2013).

Among individuals diagnosed with schizophrenia, about one third display treatment-resistance (TR) (Elkis, 2007), with persisting disabling symptoms after adequate trials of antipsychotic drugs. Recently, the Treatment Response and Resistance in Psychosis (TRRIP) working group was established to solve difficulties in comparing studies, interpreting meta-analyses and replicating research on TR, by standardizing the definition of TR (Howes et al., 2017). Clozapine is a well-documented antipsychotic drug against treatment-refractory symptoms (Siskind et al., 2016), but due to its severe adverse effects, clinicians often restrain from prescribing this drug (Warnez and Alessi-Severini, 2014). Thus, many patients are being subjected to a trial-and-error testing of drugs with various troublesome side effects (Iversen et al., 2018), which adds to the burden of the disorder itself (Charlson et al., 2018). Moreover, patients with TR have more impaired functioning, poorer psychosocial adjustment, higher rates of hospitalization and represent a higher cost to society relative to antipsychotic-responsive patients (Gillespie et al., 2017; Iasevoli et al., 2016; Kennedy et al., 2014).

Mapping predictors of antipsychotics response may take us closer to personalised medicine in schizophrenia (Lally et al., 2016; Lally and MacCabe, 2015).

It has been suggested that TR might be a categorically distinct subgroup (Gillespie et al., 2017) or represent the most severe cases on a continuum (Molent et al., 2019). MRI studies of the brain show that TR patients have lower grey matter volumes compared with both treatment responders and healthy volunteers (Anderson et al., 2015). Moreover, patients with TR seem to have lower levels of striatal dopamine synthesis capacity (Demjaha et al., 2012), as well as alterations in glutamate concentration (Demjaha et al., 2014) compared to treatment responders. In addition, patients with TR seem to have specific neurocognitive deficits (de Bartolomeis et al., 2013; Joober et al., 2002), and treatment response to their first antipsychotic trial, including lack of remission during the first three months, seems to predict long-term outcome of the disorder (Agid et al., 2011; Friis et al., 2016; Kolakowska et al., 1985). It has also been hypothesized that patients with TR may share genetic underpinnings (Nucifora et al., 2019). This is further supported by findings showing that family members of patients with TR are more likely to have a diagnosis of schizophrenia in comparison with family members of responsive patients with schizophrenia (Hajj et al., 2019; Silverman et al., 1987), indicating a common genetic component (Joober et al., 2005). Taken together, these findings indicate that TR might be at least partly determined by the burden of genetic risk for schizophrenia.

Recent GWAS in schizophrenia have established a large number of single nucleotide polymorphisms (SNPs) associated with the disorder (Pardinas et al., 2018). This has enabled the generation of a schizophrenia polygenic risk score (PRS-SZ) (Tesli et al., 2014) representing the weighed genetic predisposition of an individual to the disorder. PRS is a new and promising genetic measure in psychiatry (Pardinas et al., 2018; Purcell et al., 2009) as well as in medicine in general (Torkamani et al., 2018). There are some recent studies of PRS-

SZ and TR in schizophrenia, however with conflicting results. Associations between PRS-SZ and a history of clozapine treatment (Frank et al., 2015) and lack of response to antipsychotics (Zhang et al., 2019) have been reported, suggesting that the genetics of schizophrenia is also involved in TR. Three other studies found no association between PRS-SZ and TR (Legge et al., 2019; Martin and Mowry, 2016; Wimberley et al., 2017). There are several possible explanations for the different results. There is a variation of strategies for selecting SNP thresholds and number of thresholds tested (Wimberley et al. 2017; Zhang et al. 2019; Legge et al. 2019). Moreover, various definitions of TR are applied (Wimberley et al. 2017; Frank et al. 2015). In the study by Zhang et al. (2019), antipsychotic efficacy was assessed based on symptom scores as opposed to studies specifically investigating TR (e.g. Frank et al. 2015). Also, samples vary between first episode patients and more chronic conditions as well as in methods of determining diagnoses (Zhang et al. 2019; Wimberley et al. 2017). Hence, the genetic architecture of TR is mainly unresolved.

Efforts have been made to understand clinical correlates underlying biological mechanisms and predictive factors for TR (Nucifora et al., 2019; Wimberley et al., 2016). Clinical and demographic factors associated with TR include earlier age at onset (Legge et al., 2019; Wimberley et al., 2016), lifetime drug abuse (Wimberley et al., 2016), poorer premorbid social adjustment (Legge et al., 2019) and living in less urban area (Legge et al., 2019). Decreased plasma level of antipsychotic drugs have also been associated with TR (McCutcheon et al., 2018), suggesting that adherence and pharmacokinetic factors need to be addressed when investigating TR.

In the current study, we aimed to determine the potential of PRS-SZ to explain the heterogeneity in treatment response in a large, naturalistic sample of patients with schizophrenia spectrum disorders using the most recent consensus criteria for defining treatment resistance (Howes et al., 2017). We hypothesized that there is an increased risk of

TR associated with increasing PRS-SZ and thus overlapping mechanisms for schizophrenia and TR. Additionally we investigated previous suggested non-genetic predictors of TR such as earlier age at onset, lifetime drug abuse and family history of psychosis (Frank et al., 2015; Legge et al., 2019; Meltzer et al., 1997; Wimberley et al., 2016). We also investigated dose serum ratio of antipsychotic medication in relation to TR, serving to account for non-adherence as a possible confounder and drug turnover as a possible contributor in TR mechanism.

2. Methods

2.1 Participants

As part of the Thematically Organized Psychosis (TOP) study, participants (N=321) were included in the current study if they fulfilled diagnostic criteria for a schizophrenia spectrum disorder, defined as schizophrenia (N=195), schizophreniform disorder (N=5), schizoaffective disorder (N=66), psychosis not otherwise specified (N=39), brief psychotic disorder (N=2) or delusional disorder (N=14). Patients were recruited to the TOP study from the mental health clinics of the major hospitals in Oslo, currently covering a catchment area of close to 700 000 inhabitants. All patients were between 18-65 years of age and able to provide consent of participation. Patients were excluded if they had a history of severe somatic disease interfering with brain functioning including neurological disease, history of moderate or severe head trauma, or an IQ below 70. Participants recruited during their first psychotic episode were excluded from the current study as they were in the initial phase of antipsychotic drug trials. Demographic data, information about ancestry and information about current and past five years drug treatment were collected by interview and from medical records, as was information on duration, adherence and adverse effects of the treatment. See table 1 for details.

2.2 Clinical characteristics

2.2.1 Clinical assessment

Diagnostics were made by using the Structured Clinical Interview (SCID-1) (M.B. First, 1995) for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV (American Psychiatric Association, 2000). Symptoms of psychosis were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and function was assessed using the Global Assessment of Functioning (GAF-F) scale (Endicott et al., 1976). Previous psychiatric history was recorded based on interviews and medical records. Diagnostic interviews were performed by psychologists and physicians supervised by a senior professor in psychiatry. The research personnel are all comprehensively trained for the interviews based on a UCLA training program (Ventura et al., 1998).

2.2.2 Treatment Resistant Schizophrenia (TR) classification

Patients were classified as being TR or non-TR; the classification was adapted for retrospective data based on the Treatment Response and Resistance in Psychosis (TRRIP) working group's consensus criteria (Howes et al., 2017). A patient was defined as being TR based on (during the five years prior to inclusion) either 1) history of treatment with clozapine or 2) two or more failed trials of antipsychotic treatment, each of at least six weeks duration and with therapeutic dosage. At least one of the antipsychotics had to be a second generation antipsychotic. Previous trials were classified as failed if there were change in antipsychotic agents. The current trial was classified as failed if the patient had significant symptoms (a few patients had frequent psychotic episodes [five or more during the five years] and were thus classified as TR regardless of current symptoms). Current significant symptoms were defined as at least one score of at least moderate severity on the PANSS positive subscale together with a score of 60 or less on the GAF functioning scale (GAF-F), indicating at least moderately impaired functioning (Howes et al., 2017). The antipsychotic treatment was not counted as a failed trial if the medication was stopped due to adverse effects. Antipsychotic

drugs used for indications other than psychosis, typically as needed for sleep or anxiety, did not count as a trial of antipsychotic treatment (see supplementary table). Patients having used a third, or more, antipsychotic drugs during the last five years after the two initial trials, were classified as having treatment resistance regardless of ongoing symptoms. We did not apply any limitation on the maximum duration of an antipsychotic trial. Based on the criteria, both patients currently in remission and patients with psychotic symptoms could be classified as TR. Patients not fulfilling the criteria for TR were classified as non-TR. According to this definition, 108 patients (33.6 %) were classified as having treatment resistance in our sample. See table 1 for details.

2.3 Polygenic Risk Score for Schizophrenia

DNA was extracted from blood and saliva samples collected in the clinic. Genotyping was performed on Human Omni Express-24 v.1.1 (Illumina Inc., San Diego, CA, USA) at deCODE Genetics (Reykjavik, Iceland). Quality control was performed using PLINK 1.9 (Purcell et al., 2007). Briefly, variants were excluded if they had low coverage (<95%), had low minor allele frequency (MAF) (<0.01), deviated from Hardy-Weinberg equilibrium ($p<10^{-4}$), or occurred at significantly different frequencies in different genotyping batches (FDR<0.5). Whole individual genotypes were excluded if they had low coverage (less than 95%) or high likelihood of contamination (heterozygosity above mean + 5 standard deviations). MaCH software (Das et al., 2016; Li et al., 2010) was used to impute the genotypes of all participants onto reference haplotypes derived from samples of European ancestry in the 1000 Genome Project (genomic build GRCh37). The PRS-SZs were based on a meta-analysis of all Psychiatric Genomics Consortium Schizophrenia Working Group's genome-wide association sub-studies except TOP (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), which included patients with schizophrenia and schizoaffective disorder. The summary statistics were quality controlled by removing variants

that met any of the following conditions: MAF<0.05; imputation quality (ratio between observed and expected allelic variance) <0.8; not present in more than half of the sub-studies. Variants from the MHC region were also excluded. The remaining variants were clumped into independent regions on the basis of the linkage disequilibrium structure of the 1000 Genomes Phase III European population. PLINK v1.9 was used with the following parameters: --clump-p1 1.0 --clump-p2 1.0 --clump-r2 0.2 --clump-kb 500. The allelic dosage coefficients (or logarithms of the odds ratios) of the variants with minimum p-values from all independent regions were used in constructing the PRS-SZs. These were calculated for all individuals following Purcell et al's (2009) recipe of multiplying the number of effect alleles they carried by the allelic dosage coefficients calculated in the meta-analysis. Only European subjects were included in our sample to avoid confounding from population stratification.

2.4 Medication and serum levels

Blood was withdrawn from antecubital vein in the morning for assessments of antipsychotic drug serum level by methods previously described (Steen et al., 2017). Standardised relationships between dose and serum level of antipsychotics were calculated to enable the comparison of several antipsychotic drugs: First, the dose of each participant's primary antipsychotic drug was divided by the Defined Daily Dose (DDD) (Leucht et al., 2016) of the drug to obtain a standardised dose for each participant. Second, the measured serum level of this antipsychotic drug was divided by the median of the antipsychotic drug's reference range (Hiemke et al., 2018). Finally, the standardised dose was divided by the standardised serum level, to obtain a relationship between dose and serum level comparable across antipsychotics indicating the antipsychotic drug's turnover.

2.5 Ethics

All participants gave written consent of participation after a written and oral description of the study. The study was approved by The Regional Ethics Committee, The Norwegian Data Inspectorate, and the Norwegian Directorate of Health approved the biobank.

2.6 Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago II, version 24). To investigate differences in demographic and clinical variables we used chi-square tests for the categorical variables, independent sample t-test for normally distributed continuous variables and Mann Whitney U test for non-parametric distributions. Normality was assessed with Q-Q-plots, histograms and Kolmogorov-Smirnov statistics. Potential issues with multicollinearity and outliers in the regression analyses were ruled out. To assess the association of PRS-SZ with TR we used binary logistic regression. Patients were coded as either TR or non-TR and this binary variable was set as the dependent variable. The standardised PRS for schizophrenia (PRS-SZ) was set as the predictor variable with adjustments for ancestry (using the twelve first genetic principal components giving the highest classification correctness of TR and non-TR in the statistical model), and for genotyping batch (7 batches) in addition to age at inclusion and sex (main model). Adjustments for time since first episode of the disorder and for time since first drug treatment were also tested. Regression analyses were performed with PRS-SZs based on different SNP significance thresholds (Wimberley et al., 2017; Zhang et al., 2019), due to testing of five inclusion thresholds, we applied a Bonferroni-corrected significance level of $p<0.01$ for the effect of PRS-SZ. Secondary logistic regression analyses with the significantly associated PRS-SZ were performed by additionally adjusting for all the included diagnoses first, and then including season of birth (summer versus winter) and dose serum ratio, separately. Spearman's rho was applied to quantify the correlation between PRS-SZ and PANSS-total to assess the effect of PRS-SZ on current symptom severity, as well as the correlation between

PRS-SZ and GAF-F. Spearman's rho was also calculated to confirm the correlation between PRS-SZ at different thresholds. We also performed an additional logistic regression analysis after excluding all clozapine users to rule out a specific association with clozapine use, as well as a regression analysis without PRS-SZ to demonstrate the contribution of PRS-SZ to the model. Finally, we ran the same analysis including only the subgroup of patients with a schizophrenia diagnosis.

3. Results

3.1 Study sample, demographic and clinical variables

A diagnosis of schizophrenia was more frequent in the TR group than in the non-TR group ($p<0.05$) and the prevalence of delusional disorder was lower in the TR group than the non-TR group ($p<0.01$). Current use of anticonvulsants and lithium was significantly higher in the TR group ($p<0.05$). The positive ($p<0.001$), negative ($p<0.05$) general ($p<0.05$) and total ($p<0.01$) PANSS scores were higher and the GAF-functioning score was lower ($p<0.001$) in the TR group compared to the non-TR group. Winter birth was less common in TR ($p<0.05$), and the dose serum ratio was higher in TR ($p<0.05$). Drug abuse and family history of psychosis only showed a trend level difference ($p=0.07$ and $p=0.09$ respectively). See table 1 for details.

3.2 PRS-SZ and the relationship to TR

Higher PRS–SZ was significantly associated with TR-status ($p=0.003$, odds ratio 1.5[95% CI: 1.148-1.973], Omnibus test= (21, N=321, 35.77, $p=0.023$), figure 1 shows unadjusted data) in schizophrenia spectrum disorders when applying a significance threshold of $p=0.01$ for inclusion in PRS-SZ. The statistical model yielded a sensitivity of 29.6% and a specificity of 90.6%, corresponding to a correct classification of 70.1% of cases as TR or non-TR. The positive predictive value was 61.5% (correctly predicted TR) and the negative predictive value was 71.7% (correctly predicted non-TR). The total variance explained by the model

with PRS-SZ, age, sex, batch number and principal components 1-12 was 14.6% (Nagelkerke's pseudo R^2). The variance explained by the model without PRS-SZ, batch number and principal components was 1.7% (Nagelkerke's pseudo R^2). Table 2 contains the classification statistics for the main model. PRS-SZ was significantly associated to TR also after adjustments for time since first episode of the disorder and for time since first drug treatment, respectively. When excluding all clozapine users (N=21), the PRS-SZ effect on TR remained significant (p=0.007), with the model explaining 15.9% (Nagelkerke's pseudo R^2) of the variance and correctly classifying 71.7% of all cases. When adjusting for diagnosis, PRS-SZ was still associated with TR (p=0.005, odds ratio 1.5[95% CI: 1.128-1.963], with the model explaining 24.5% (Nagelkerke's pseudo R^2) of the variance and still correctly classifying 71.7%. Season of birth was statistically significant (p=0.05; PRS-SZ remained significant, p=0.004). Dose serum ratio did not have significant effect when added to the regression model (p=0.653). PRS-SZ based on other GWAS p-value thresholds were not significantly associated with TR (GWAS p-value thresholds of $p=5x10^{-8}$, p=0.05, p=0.1 and p=0.5 yielded p-values of 0.649, 0.074, 0.963 and 0.458, respectively, see table 3), the only exception being those based on p=0.05 in the secondary statistical model with adjustments for diagnosis and season of birth (p=0.047). There were no significant correlations between PRS-SZ and PANSS-total or GAF-F. By excluding age from the analyses, PRS-SZ was still significant, but the Omnibus test of the main model and season of birth in the secondary analysis became trend level significant (both p=0.063). The association between PRS-SZ and TR remained significant (p=0.018, OR 1.6 [95% CI: 1.079-2.253]) when analysing only patients with a schizophrenia diagnosis (N=195). There was a strong and significant ($p<0.01$) correlation between PRS-SZ at the different thresholds.

4. Discussion

In the current study, we found PRS-SZ at p=0.01 to be significantly associated with antipsychotic drug TR in patients with a schizophrenia spectrum disorder. This indicates PRS-SZ as a potential biological marker of TR and supports earlier assumptions of genetic factors involved in TR. The findings indicate genetic mechanisms in common for schizophrenia and TR. Season of birth was significantly associated with TR, also when adjusting for PRS-SZ and diagnosis. Associations to PRS-SZs based on other GWAS significance thresholds were non-significant, suggesting the current threshold as the most suitable for TR prediction (Zhang et al., 2019).

The current main finding is in line with previous findings of a higher likelihood of responding to treatment among first-episode psychosis patients with a low PRS-SZ (Zhang et al., 2019), indicating PRS-SZ as a biological marker regardless of duration of illness. An important strength of our sample is the inclusion of patients with a well-established diagnosis and with a higher likelihood to have received several trials of antipsychotics beyond the initial antipsychotic treatment (Haddad and Correll, 2018), assuring the validity of the effect of PRS-SZ on TR in schizophrenia spectrum disorders. Our finding is also in line with the increased PRS-SZ observed in patients with a history of clozapine treatment compared to patients with no such history (Frank et al., 2015).

TR status in our sample was based on the most recent consensus definition criteria (Howes et al., 2017), yielding a TR rate of 33.6%. Contrary to the current study, several other studies have restricted the definition of TR to involving clozapine use, which may limit the representativeness of the TR sample (Howes et al., 2017; Kelly et al., 2010). The three studies that did not find significant association between PRS-SZ and TR (Legge et al., 2019; Martin and Mowry, 2016; Wimberley et al., 2017) also used definitions of treatment-resistance somewhat different from those following the recent consensus-based criteria (Howes et al., 2017). Wimberley et al. (2017) defined TR as first occurrence of either clozapine initiation or

hospitalization during antipsychotic treatment within 18 months after at least two periods of different antipsychotic monotherapy, thus introducing hospitalization as a criterion. According to their definition, 21% of the patients were defined as TR during follow-up, which is below the general estimate of 30-40% (Elkis, 2007; Gillespie et al., 2017; Kane et al., 2019). The definition used by Martin and Mowry (2016), based on clinical features and course, resulted in a TR prevalence of 37.09%, more like ours. In the study by Legge et al. (2019), TR was defined as either rating negatively on the Operational Criteria Checklist (OPCRIT) item 89 or receiving clozapine treatment, resulting in a TR prevalence of 52.4%. The authors explain the high frequency of TR as due to recruitment from clozapine clinics. They also used a more general criterion from the Operational Criteria Checklist for Psychotic Illness and Affective Illness to decide TR classification. Their sample might thus be less representative of TR (Howes et al., 2017; Kelly et al., 2010) than the present naturalistic sample. The lack of consistence in TR definition criteria could contribute to the varying results with regard to a PRS-TR association. The inconsistent findings could also be due to the use of GWAS of smaller samples (Frank et al., 2015).

The current study comprises a naturalistic sample with TR based on the most recent consensus criteria and a TR rate comparable with the generally reported prevalence (Elkis, 2007; Gillespie et al., 2017; Kane et al., 2019). It is however possible that some of the current associations could be due to overlap between TR characteristics and illness severity. If the cohorts analysed in the GWAS included mostly chronic patients with more severe illness, one could speculate that the PRS-SZ association exists because individuals with TR tends to be more severely ill. Attempting to test this possibility, we performed a sub-analysis using the same model but excluding all clozapine users. In this analysis, we found effects similar to those found in the main analysis. Moreover, there were no significant correlations between PRS-SZ and symptom severity.

Season of birth was significantly associated with TR, in line with one previous study showing a lower prevalence of winter births in TR (Wimberley et al., 2016). The effect remained significant after adjustment for PRS-SZ, suggesting the existence of genuine effects unrelated to the genetics of the disorder, indicating the complexity of TR. Considering the increased prevalence of winter or spring births in schizophrenia (Davies et al., 2003), the finding supports the hypothesis of TR being a categorically distinct subgroup (Gillespie et al., 2017).

It has been suggested that the TR group represents a more "genuine schizophrenia" with differences in response potentially representing genetic and sociocultural factors (Itil et al., 1966). Several subsequent studies have supported this hypothesis (Frank et al., 2015; Gillespie et al., 2017; Joober et al., 1999; Wolkin et al., 1989). Studies exploring the mechanisms of TR have reported a lower level of striatal dopamine synthesis capacity (Demjaha et al., 2012) as well as higher levels of glutamate in the anterior cingulate cortex (Demjaha et al., 2014). Significant interactions between dopamine transporter variable number tandem repeats (DAT-VNTR) and the serotonin transporter (SERT)-in2 polymorphism are also reported in TR (Bilic et al., 2014). Further, a strong association was found between TR and the variants for brain derived neurotrophic factor (BDNF), which is associated with schizophrenia (Di Carlo et al., 2019) and interacts with monoaminergic neurotransmitters (Zhang et al., 2013). Moreover, an increased number of rare copy number variants (CNV) are associated with TR (Martin and Mowry, 2016). This is in line with the current findings of TR linked to the genetics of schizophrenia (Owen et al., 2016). Furthermore, the current association between TR and PRS-SZ might indicate underlying factors in TR, as recent studies have revealed genetic pleiotropy between schizophrenia and phenotypes such as brain structure volumes (Terwisscha van Scheltinga et al., 2013; Smeland et al., 2018; Chen et al., 2019), immune related conditions (Andreassen et al., 2015) body mass index (Bahrami et al. 2020) and lipids (Andreassen et al., 2013), all of which potentially

related to antipsychotic drug treatment response (Barry et al., 2019; Hutcheson et al., 2014; Noto et al., 2015; Pillinger et al., 2020).

The current study has several strengths. Our large, well-characterized sample enabled the application of criteria for TR well matching the most recent consensus-criteria, resulting in a TR rate in accordance with the literature (Elkis, 2007; Gillespie et al., 2017; Kane et al., 2019) and demonstrating associations to PRS-SZ and demographic data. We did not find any mediating effect of diagnosis. The naturalistic design assures relevance to clinical samples, with the current results being of special interest for treatment response in long-term and thus the long-term prognosis, as opposed to studies of first-episode patients with a different response patterns to antipsychotic medication (Haddad and Correll, 2018). The limitations include the cross-sectional design, which made us unable to assess actual reduction of symptoms during antipsychotic medication and therefore to implement the criterion of lack of 20% reduction of symptoms (Howes et al., 2017). This could lead to classification bias as some patients being classified as TR due to fulfilling symptom criteria might have experienced a 20% reduction of symptoms during the present trial and hence should have been classified as non-TR. Moreover, although previous antipsychotic administrations were adequate in dosage and duration, we lack serum concentration data, and cannot exclude issues with adherence during these previous trials. However, thanks to our detailed protocol, we were still able to adapt the prospective based criteria to match the TR rates shown previously (Elkis, 2007; Gillespie et al., 2017; Lally et al., 2016), and confirm the PRS-SZ effect in the clozapine-free subsample. By excluding first-episode patients and missing out on individuals that did not survive long enough to be included in the study, we might have failed to include patients with a fast recovery or severe illness, decreasing the power and variability of the data. Moreover, we cannot fully exclude this as a source of bias, although there were no significant correlation between PRS-SZ and PANSS-total or GAF-F, suggesting that variation in

symptoms would not have affected the results. Various strategies may be applied for choosing PRS-SZ threshold values (Wimberley et al., 2017; Wray et al., 2014) and there exists no well-founded procedure; however, the thresholds selected in the current study have been used previously (Zhang et al., 2019) and results for all of them were reported. Moreover, the results were strictly adjusted for the number of levels analysed. The reason why only threshold p=0.01 results in a significant association with PRS-SZ is matter for speculation. It might be that the PRS-SZ obtained with less stringent thresholds are too unspecific and therefore unable to detect the association, while more stringent thresholds exclude variants of importance.

In conclusion, we found an association between PRS-SZ at SNP significance threshold p=0.01 and TR in patients with a schizophrenia spectrum disorder, as well as an association between season of birth and TR. This indicates an association between PRS-SZ and treatment response, although the failure to replicate the findings with other SNP significance thresholds makes the association less robust. The findings suggest that TR is related to genetic factors that also drive core pathophysiological processes in schizophrenia spectrum disorders, as well as to factors independent of schizophrenia genetics. The positive and negative predictive values of the current model are not sufficient for clinical use. Still, the study confirms the utility of PRS-SZ as a predictive supplement based on forthcoming expansions of GWAS data.

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Figure legends

Fig. 1. Standardized polygenic risk score (p=0.01) for schizophrenia (y-axis) in patients with treatment-resistance (TR) and non-treatment resistance (non-TR), grouped by schizophrenia only and all patients (unadjusted mean). Error bars represents one standard error.

Tables

Table 1. Demographics, clinical characteristics and medication in TR and non-TR.

	TR, N (%)=108 (33.6)	Non-TR, N (%)=213 (66.4)
Sex female, N (%)	51 (47.2)	103 (48.4)
Age, median (IQR)	31 (12)	34 (18)
BMI, median (IQR)[ı]	25 (6.4)	24.5 (4.7)
Age at onset, median (IQR)	22.5 (9)	23 (13)
Season of birth (Des-March), N (%)	26 (24.1)*	76 (35.7)
Family history of psychosis, N (%)	30 (21.4)	44 (30.3)
PANSS-total, median (IQR)	60 (19)**	56 (23)
PANSS-positive, median (IQR)	15 (7)***	12 (7)
PANSS-negative, median (IQR)	14 (7)*	12 (9)
PANSS-general, median (IQR)	31 (10)*	29 (11)
GAF-F, median (IQR)	41 (13)***	45 (13)
Diagnosis, N (%)		
Schizophrenia	74 (68.5)*	121 (56.8)
Schizophreniform disorder	0 (0)	5 (2.3)
Schizoaffective disorder	26 (24.1)	40 (18.8)
Psychosis NOS	8 (7.4)	31 (14.6)
Psychosis, brief	0 (0)	2 (0.9)
Delusional disorder	0 (0)**	14 (6.6)
Medication, N (%)		
Antipsychotics	102 (94.4)	188 (88.3)
Antidepressants	28 (25.9)	71 (33.3)
Anticonvulsants and Lithium	28 (25.9)*	33 (15.5)
Time since first episode, median years (IQR)[ıı]	9 (8.8)	9 (11)
Time since first medical treatment, median years (IQR)[ıı]	6 (7)	5 (9)
Lifetime drug abuse, N (%)	27 (25)	35 (16.4)
Lifetime alcohol abuse, N (%)	14 (13)	25 (11.7)
Inpatient when first diagnosed, N (%)	48 (51.6)	80 (50)
Dose/serum ratio, median (IQR)[ıı]	0,531250 (0.5)*	0,434227 (0.6)

*p≤0.05, *p ≤0.05, **p<0.01, ***p< 0.001

Missing data for [ı]58, [ıı]16, [ıı]21, [ıı]65 participants.

Abbreviations: BMI = Body Mass Index; GAF-F = Global Assessment of Functioning, functioning scale; IQR = Interquartile range; N = Number; NOS = Not otherwise specified; PANSS: Positive and Negative Syndrome Scale; SD = Standard deviation; TR = Treatment-resistance;

Table 2. Classification table of the logistic regression model[a].

		Observed	
	TR (N)	Non-TR (N)	
Predicted TR (N)	32	20	61.5%[d]
Non-TR (N)	76	193	71.7%[e]
Percentage correct	29.6%[b]	90.6%[c]	70.1%

[a]The model included polygenic risk score, sex, age, principal components 1-12, batch number (independent variables) and Treatment-resistance and non-Treatment resistance (dependent variable), [b]Sensitivity, [c]Specificity, [d]Positive predictive value, [e]Negative predictive value

Table 3. Association between PRS-SZ and treatment resistance with different SNP significance thresholds. Unadjusted and adjusted for batch number, principal components, age and sex.

PRS-SZ	[a]Unadjusted	[a]Adjusted	[b]Model R^2
GWAS	p=0.541, OR=0.9 (0.737-1.173)	p=0.649, OR=0.9 (0.741-1.205)	0.2/10.7
0.01	p=0.007, OR=1.4 (1.092-1.768)	p=0.003, OR=1.5 (1.148-1.973)	3.2/14.6
0.05	p=0.094, OR=1.3 (0.962-1.645)	p=0.074, OR=1.3 (0.974-1.788)	1.2/11.9
0.1	p=0.977, OR= 1.0 (0.780-1.291)	p=0.963, OR=1.0 (0.748-1.320)	0.0/10.6
0.5	p=0.566, OR=1.1 (0.824-1.423)	p=0.458, OR=1.1 (0.823-1.542)	0.1/10.8

[a]Odds ratio (OR) given with 95% confidence interval
[b]Explained variance (Nagelkerke pseudo R^2) of the unadjusted/adjusted model
Abbreviations: GWAS= Genome-Wide Association Study, PRS-SZ= Polygenic Risk Score for Schizophrenia, SNP= Single Nucleotide Polymorphism

Figures

Fig.1