Supplementary Methods:

Subjects and Recruitment

The study was approved by the respective local Ethics Committees, and all subjects provided written informed consent.

The study sample comprised 1,038 schizophrenia patients recruited from consecutive admissions to psychiatric hospitals in Germany. All individuals were of self-reported German ancestry and fulfilled DSM-IV criteria for schizophrenia. Multiple sources of information were used for the assessment of phenotypes. The primary source was the operational criteria checklist (OPCRIT (1)).

For polygenic score based analyses, genome wide data from a previously published study were used (2) that had been enhanced by including further patients and controls originating from the same geographic regions and finally comprised n=2,172 controls and n=1,540 schizophrenia cases. This study included 767 patients of the abovementioned 1,038 individuals, for whom data on both clozapine use and the OPCRIT items of interest were available. Of these 767 patients, data on clozapine response were available for a subset (3) of n=123 (responders: n=78; non-responders: n=45).

The definition of extreme treatment resistant schizophrenia (ETRS) was based on response to clozapine and a 4-level ordinal, physician-rated scale with possible values of: 0="no improvement of symptoms or deterioration of symptoms"; 1="only small and insufficient improvement of symptoms"; 2="good response with adequate improvement of symptoms"; 3="almost complete remission of symptoms". Individuals rated under categories 0 and 1 were classified as being extremely treatment resistant.

Genotyping

Genotyping and quality control have been described in detail elsewhere (2). Briefly, DNA was extracted from whole blood using standard methods. Genotyping was performed using Illumina HumanHap550v3, Illumina Human610, and Illumina Human 660w quad bead chips (Illumina San Diego, CA, USA). Stringent quality control (QC) filtering criteria were applied. These accounted for call rates, heterozygosity, cross-contamination, population stratification, relatedness, non-random-missingness, Hardy–Weinberg equilibrium, minor allele frequency, and others. After QC, the data set contained genome wide data for n = 453,557 markers.

Statistical Analysis

Data preparation and statistical analysis were conducted using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) and R version 2.15.3 (http://www.r-project.org/) software packages.

Polygenic risk scores were calculated according to the method introduced by Purcell (4). Marker weights were based on association results from the Psychiatric Genomics Consortium (5), after removing individuals from the discovery sample who were included in the present study (n_{final}=20,078). For a second analysis, we extended the PGC1 sample with data from a very recently published Swedish schizophrenia GWAS sample, which just became available, as discovery set (6) again after exclusion of individuals contained in the present study (n_{final}=27,199).

Marker weights were calculated according to (4) using the natural logarithm of odds ratios provided in the results file from PGC SCZ. LD-pruning was done by using only SNPs present in a "clumped" version of the file which was filtered to contain only independent SNPs (pairwise $r^2<0.25$ within 500kb window) present in quality controlled genotype data of our sample. For the inclusion of p-value-based-filtering of SNPs in polygenic score analysis, a number of p-value cut-offs have been considered ($p<0.01$, $p<0.05$, $p<0.1$, $p<0.2$, $p<0.3$, $p<0.4$, $p<0.5$). According to Purcell et al. (4), the target R^2 for the score is dependent on the genetic model and the selected p-value cut-off for the considered loci. For smaller samples ($n<=3000$), as used in the present study, use of a less stringent p-value cut-off appears to increase power almost uniformly across the models considered in the simulation approach of (4), as well as in the data reported by (4). Our own results (see supplementary table 5) are consistent with the finding that less stringent cut-offs provide most power. Thus a single marker p-value cut-off of $p < 0.5$ was applied for primary analysis. This resulted in a set of 58,663 independent autosomal loci for consideration in the score analysis. In the second analysis using the extended discovery sample, the original SNP set was used (with the exception of SNPs that were filtered out during QC in the extended discovery sample) in order to render the analyses as comparable as possible. A set of 40,082 independent markers with $p < 0.5$ in the extended sample were considered in the score analysis.

Association testing of (extreme) treatment resistance with quantitative clinical features was performed using one-tailed t-tests with correction for heterogeneous variance. For binary variables, chi^2-tests were used. For ordinal features with more than two levels, results from the Armitage-trend-test are reported. Association tests of polygenic scores with clinical outcomes were performed using a logistic regression approach throughout. This included adjustment for population stratification by including the first two principal components from resulting from principal component analysis in the model as covariates. To test whether association between polygenic score and (extreme) treatment resistance was confounded or mediated by the premorbid clinical variables significantly associated with the outcome, these were included in the regression model in a 2nd step.

Supplementary Results and Discussion

Premorbid and clinical characteristics

Supplementary Table 1: Association between treatment resistance and premorbid clinical features (significant items are highlighted in grey):

opcrit Item	p-value	Adjusted p-value	Effect size[a]
3 Sex	0.56	1	1.08 (0.84 , 1.39)
4 Age of onset	**3.0e-6[b]**	**3.0e-5[b]**	**0.29 (0.16 , 0.41) [c]**
5 Mode of onset	**4.4e-3**	**0.04**	**-0.20 (-0.33 , -0.06) [c]**
9 Poor premorbid work adjustment	0.83	1	1.04 (0.74 , 1.45)
10 Poor premorbid social adjustment	**8.1e-5**	**8.1e-4**	**1.85 (1.35 , 2.55)**
11 Premorbid personality disorder	0.60	1	1.13 (0.69 , 1.86)

12 Alcohol / Drug abuse within year of onset	0.28	1	0.84 (0.6 , 1.17)
13 Family history of schizophrenia	0.95	1	1.01 (0.74 , 1.38)
14 Family history of other psychiatric disorder	0.59	1	1.07 (0.83 , 1.39)
16 Definite psychosocial stressor prior to onset	0.48	1	1.11 (0.82 , 1.52)

[a]Effect sizes are provided as Odds ratios if not specified otherwise; [b]t-test results; [c]Cohen's d

In a second step, differences in lifetime clinical symptoms between clozapine treated and non-clozapine treated patients were analysed (supplementary tables 2 and 3). Patients treated with clozapine showed significantly more negative symptoms. However, as patients with negative symptoms are those who are most likely to receive clozapine treatment, this finding was expected and indicates the consistency of the retrospectively assessed clinical data.

Supplementary Table 2: Association between treatment resistance and further OCPRIT items (nominally significant items are highlighted in bold, items remaining significant after Bonferroni adjustment in grey):

opcrit Item	**p-value**	**Adjusted p-value**	**Effect size[a]**
6 Family (married-0 / single-1)	**4.7e-4**	**4.1e-2**	**1.56 (1.21 , 2.02)**
7 Employment	0.32	1	0.85 (0.61 , 1.18)
8 Duration of illness in weeks	0.21[b]	1[b]	-0.05 (-0.18 , 0.07)[c]
15 Coarse brain disease prior to onset	0.87	1	1.07 (0.45 , 2.55)
17 Bizarre behavior	0.58	1	0.93 (0.72 , 1.21)
18 Catatonia	**2.0e-5**	**1.7e-3**	**2.17 (1.49 , 3.18)**
19 Excessive activity	0.68	1	0.03 (-0.10 , 0.15)[c]
20 Reckless activity	0.21	1	0.08 (-0.05 , 0.2)[c]
21 Distractibility	**0.01**	**1**	**0.15 (0.03 , 0.28)**[c]
22 Reduced need for sleep	0.65	1	0.03 (-0.1 , 0.15)[c]
23 Agitated activity	0.99	1	-9.4e-4 (-0.13 , 0.12)[c]
24 Slowed activity	**6.3e-5**	**5.5e-3**	**-0.25 (-0.38 , -0.13)**[c]
25 Loss of energy / tiredness	**7.1e-3**	**0.63**	**-0.17 (-0.3 , -0.05)**[c]
26 Speech difficult to understand	**3.7e-5**	**3.3e-3**	**0.59 (0.45 , 0.76)**
27 Incoherent	**1.3e-3**	**0.12**	**0.64 (0.49 , 0.85)**
28 Positive formal thought disorder	**3.4e-3**	**0.30**	**0.68 (0.53 , 0.89)**
29 Negative formal thought disorder	**1.5e-13**	**1.3e-11**	**2.73 (2.07 , 3.62)**
30 Pressured speech	0.48	1	0.04 (-0.08 , 0.17)[c]
31 Thoughts racing	0.30	1	0.07 (-0.06 , 0.19)[c]
32 Restricted affect	**1.7e-7**	**1.5e-5**	**2.15 (1.59 , 2.91)**

opcrit Item	p-value	Adjusted p-value	Effect size[a]
33 Blunted affect	0.32	1	1.14 (0.87 , 1.49)
34 Inappropriate affect	0.43	1	1.11 (0.85 , 1.45)
35 Elevated mood	0.61	1	0.03 (-0.09 , 0.16) [c]
36 Irritable mood	**0.03**	**1**	**-0.14 (-0.26 , -0.02)** [c]
37 Dysphoria	0.95	1	-4.1e-3 (-0.13 , 0.12) [c]
38 Diurnal variation	0.94	1	1.01 (0.76 , 1.34)
39 Loss of pleasure	0.67	1	-0.03 (-0.15 , 0.10) [c]
40 Diminished libido	0.57	1	1.1 (0.79 , 1.53)
41 Poor concentration	**1.1e-5**	**9.7e-4**	**-0.28 (-0.4 , -0.15)** [c]
42 Excessive self reproach	0.20	1	0.08 (-0.04 , 0.21) [c]
43 Suicidal ideation	**6.9e-3**	**0.61**	**-0.17 (-0.3 , -0.05)** [c]
44 Initial insomnia	0.50	1	-0.04 (-0.17 , 0.08) [c]
45 Middle insomnia	0.72	1	1.05 (0.81 , 1.35)
46 Early morning waking	0.13	1	0.10 (-0.03 , 0.22) [c]
47 Excessive sleep	0.29	1	-0.07 (-0.19 , 0.06) [c]
48 Poor appetite	0.09	1	0.11 (-0.02 , 0.23) [c]
49 Weight loss	**0.02**	**1**	**0.14 (0.02 , 0.27)** [c]
50 Increased appetite	0.92	1	0.01 (-0.12 , 0.13) [c]
51 Weight gain	0.30	1	0.07 (-0.06 , 0.19) [c]
52 Relationship psychotic / affective symptoms	0.50	1	0.04 (-0.08 , 0.17) [c]
53 Increased sociability	0.19	1	0.08 (-0.04 , 0.21) [c]
54 Persecutory delusions	0.63	1	0.92 (0.65 , 1.3)
55 Well organised delusions	**8.1e-6**	**7.1e-4**	**1.78 (1.37 , 2.31)**
56 Increased self esteem	0.14	1	0.09 (-0.03 , 0.22) [c]
57 Grandiose delusions	0.22	1	0.08 (-0.05 , 0.20) [c]
58 Delusions of influence	**0.03**	**1**	**0.72 (0.54 , 0.97)**
59 Bizarre delusions	0.15	1	0.83 (0.63 , 1.08)
60 Widespread delusions	**4.9e-3**	**0.43**	**1.46 (1.11 , 1.91)**
61 Delusions of passivity	0.55	1	0.93 (0.71 , 1.2)
62 Primary delusional perception	**6.1e-4**	**5.3e-2**	**1.59 (1.21 , 2.1)**
63 Other primary delusions	**1.4e-7**	**1.2e-5**	**2.17 (1.61 , 2.95)**
64 Delusions & Hallucinations last for one week	0.69	1	1.06 (0.79 , 1.41)
65 Persecutory / jealous delusions & hallucinations	**0.03**	**1**	**1.33 (1.01 , 1.73)**
66 Thought insertion	0.92	1	0.99 (0.75 , 1.3)
67 Thought withdrawal	0.93	1	1.01 (0.73 , 1.42)

opcrit Item	p-value	Adjusted p-value	Effect size[a]
68 Thought broadcast	0.80	1	1.03 (0.79 , 1.35)
69 Delusions of guilt	0.09	1	1.36 (0.94 , 1.97)
70 Delusions of poverty	**0.02**	**1**	**2.52 (1.07 , 6.61)**
71 Nihilistic delusions	**8.7e-3**	**0.76**	**1.79 (1.13 , 2.88)**
72 Thought echo	0.51	1	0.86 (0.52 , 1.41)
73 Third person auditory hallucinations	**0.01**	**0.97**	**1.41 (1.07 , 1.87)**
74 Running commentary voices	**0.02**	**1**	**1.35 (1.03 , 1.77)**
75 Abusive / accusatory / persecutory voices	**0.03**	**1**	**1.35 (1.02 , 1.8)**
76 Other auditory hallucinations	**0.03**	**1**	**1.33 (1.02 , 1.73)**
77 Non-affective hallucination in any modality	**0.02**	**1**	**1.37 (1.05 , 1.8)**
78 Life time diagnosis of alcohol abuse / depend	0.32	1	0.85 (0.62 , 1.18)
79 Life time diagnosis of cannabis abuse / depend	**1.4e-3**	**0.13**	**0.59 (0.42 , 0.83)**
80 Life time diagnosis of other abuse / depend	0.20	1	0.75 (0.48 , 1.18)
81 Alcohol abuse / dependence with psychopathology	**0.02**	**1**	**0.6 (0.39 , 0.93)**
82 Cannabis abuse / dependence with psychopathology	**3.3e-3**	**0.29**	**0.54 (0.35 , 0.83)**
83 Other abuse / dependence with psychopathology	0.24	1	0.71 (0.39 , 1.3)
84 Information non credible	**0.04**	**1**	**0.52 (0.26 , 1)**
85 Lack of insight	**0.02**	**1**	**0.68 (0.49 , 0.95)**
86 Rapport difficult	0.78	1	1.06 (0.7 , 1.59)
87 Impairment / incapacity during disorder	0.55	1	0.04 (-0.09 , 0.16)[c]
88 Deterioration from premorbid level of function	**0.04**	**1**	**1.49 (1 , 2.23)**
89 Psychotic symptoms respond to neuroleptics	0.40	1	0.69 (0.24 , 1.81)
90 Course of disorder	**1.0e-15**	**8.9e-14**	**-0.54 (-0.67 , -0.41)[c]**

[a]Effect sizes are provided as odds ratios if not specified otherwise; [b]t-test results; [c]Cohen's d

Supplementary Table 3: Pairwise correlations of OPCRIT items significantly associated with clozapine treatment after correction for multiple testing in 2nd step

	26 Speech difficult to understand	10 Poor premorbid social adjustment	6 Family (married-0 / single-1)	4 Age of onset	5 Mode of onset	55 Well organised delusions	63 Other primary delusions	18 Catatonia	24 Slowed activity	41 Poor concentration	90 Course of disorder	32 Restricted affect	29 Negative formal thought disorder
26 Speech difficult to understand	1,00	-0,06	-0,13	-0,08	0,07	0,01	-0,03	0,04	-0,05	-0,10	-0,01	0,01	0,07
10 Poor premorbid social adjustment	-0,06	1,00	0,25	-0,23	0,20	-0,06	0,10	0,08	0,08	0,04	0,21	0,12	0,10
6 Family (married-0 / single-1)	-0,13	0,25	1,00	-0,27	0,01	-0,05	0,02	0,05	-0,01	0,11	0,13	0,00	-0,01
4 Age of onset	-0,08	-0,23	-0,27	1,00	-0,22	0,03	-0,01	-0,04	-0,01	-0,02	-0,09	-0,10	-0,09
5 Mode of onset	0,07	0,20	0,01	-0,22	1,00	0,12	0,18	0,03	0,10	0,04	0,18	0,11	0,19
55 Well organised delusions	0,01	-0,06	-0,05	0,03	0,12	1,00	0,23	0,01	0,04	0,11	0,14	-0,07	0,08
63 Other primary delusions	-0,03	0,10	0,02	-0,01	0,18	0,23	1,00	0,05	0,09	0,17	0,19	0,10	0,14
18 Catatonia	0,04	0,08	0,05	-0,04	0,03	0,01	0,05	1,00	0,19	0,11	0,14	0,10	0,18
24 Slowed activity	-0,05	0,08	-0,01	-0,01	0,10	0,04	0,09	0,19	1,00	0,44	0,16	0,11	0,28
41 Poor concentration	-0,10	0,04	0,11	-0,02	0,04	0,11	0,17	0,11	0,44	1,00	0,20	0,07	0,25
90 Course of disorder	-0,01	0,21	0,13	-0,09	0,18	0,14	0,19	0,14	0,16	0,20	1,00	0,19	0,26
32 Restricted affect	0,01	0,12	0,00	-0,10	0,11	-0,07	0,10	0,10	0,11	0,07	0,19	1,00	0,26
29 Negative formal thought disorder	0,07	0,10	-0,01	-0,09	0,19	0,08	0,14	0,18	0,28	0,25	0,26	0,26	1,00

Association between premorbid clinical characteristics and polygenic scores

Association between premorbid clinical features and polygenic scores was tested in different subgroups (all patients with GWAS data, Cloz$^+$ patients, and clozapine non-responders) in order to determine whether these attributes represent the underlying genetic features.

Supplementary table 4: Association test p-values between premorbid clinical features and polygenic scores

Group	Poor premorbid social adjustment[a]	Age at onset (quantitative) [b]	Insidious disease onset[a,c]	Family history[a]
All patients with GWAS data	0.08	0.005	0.95	0.01
Cloz$^+$ patients	0.02	0.002	0.15	0.15
Clozapine non-responders	0.10	0.06	0.13	0.61

[a]logistic regression results; [b]linear regression results; [c]insidious disease onset was based on dichotomizing OPCRIT variable "mode of onset" (onset period > 6 months vs. < 6 months)

Explained variance of outcome

Several different cut-off values for including markers based on the single marker association test results in the discovery sample were applied and Nagelkerke R^2-values for the observed primary outcomes were calculated (supplementary table 5).

Supplementary Table 5: Nagelkerke R^2-values and right-tailed p-values resulting from logistic regression modelling without adjustment for additional clinical covariates

p-value cut-off	SCZ diagnosis (n = 1540 cases, 2172 controls)	TRS[a] (n = 434 w/ TRS, 370 w/o TRS	ETRS[b] (n = 45 w/ ETRS, 78 w/o ETRS
<0.01	R^2=0.043 (p=1.5e-28)	R^2=0 (p=0.58)	R^2=0.008 (p=0.81)
<0.05	R^2=0.049 (p=1.7e-32)	R^2=0.005 (p=0.04)	R^2=0.001 (p=0.36)
<0.1	R^2=0.052 (p=3.5e-34)	R^2=0.005 (p=0.04)	R^2=0.006 (p=0.23)
<0.2	R^2=0.06 (p=3.4e-39)	R^2=0.008 (p=0.02)	R^2=0.021 (p=0.07)
<0.3	R^2=0.062 (p=1.8e-40)	R^2=0.007 (p=0.02)	R^2=0.019 (p=0.08)
<0.4	R^2=0.063 (p=3.5e-41)	R^2=0.006 (p=0.02)	R^2=0.019 (p=0.09)
<0.5	R^2=0.064 (p=1.1e-41)	R^2=0.007 (p=0.02)	R^2=0.024 (p=0.06)

[a]Treatment resistant schizophrenia; [b]extreme treatment resistant schizophrenia

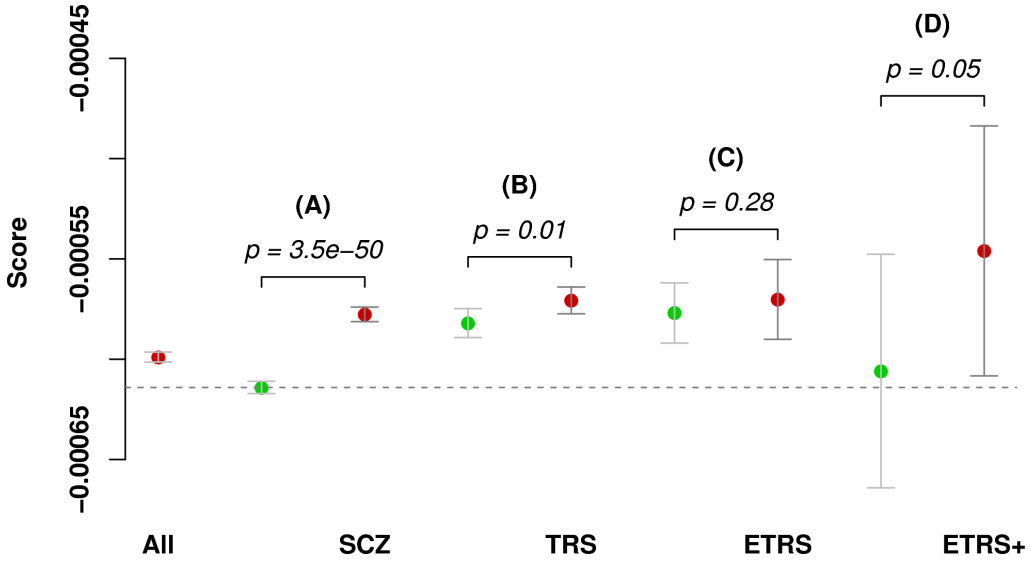
This approach clearly shows that the association between polygenic score and treatment resistance becomes non-significant, when the variables that were initially shown to be significantly associated with the outcome (i.e. poor premorbid social adjustment, age at onset, and insidious onset) are taken into account (supplementary table 6). However, this finding – in conjunction with the observation that the covariates in question were correlated with both polygenic scores and treatment resistance – suggests that these features may mediate the genetic effect on outcome.

Supplementary Table 6: right-tailed p-values from logistic regression with adjustment for clinical covariates

p-value cut-off	TRS[a]	ETRS[b]
<0.01	0.58 (0.46)[c]	0.80
<0.05	0.05 (0.01)	0.35
<0.1	0.10 (0.03)	0.20
<0.2	0.04 (0.01)	0.06
<0.3	0.05 (0.02)	0.07
<0.4	0.07 (0.02)	0.07
<0.5	0.06 (0.02)	0.05

[a]Treatment resistant schizophrenia; [b]extreme treatment resistant schizophrenia; [c]in parentheses are p-values for model without adjustment for covariates based on reduced sample size after excluding individuals with missing data on any of the considered covariates

Full interpretation of the results may depend on the proportion of treatment resistant individuals in the discovery sample. However, this is unknown. Furthermore, the significance of the findings changed when the discovery sample was extended: while significance for treatment resistance increased, the trend for extreme treatment resistance disappeared (supplementary figure 1 and tables 7 and 8).



Supplementary Figure 1: Comparison of polygenetic risk scores between groups (p-values derived from right-tailed logistic regression models) after inclusion of Swedish Sample: A) Population based controls vs. all schizophrenia patients (SCZ); B) patients responding to standard medication vs. patients with treatment resistant schizophrenia (TRS) requiring clozapine treatment; C) patients responding to clozapine vs. patients with extreme treatment resistant schizophrenia (ETRS) not even responding to clozapine; D) patients with ETRS only vs. patients with ETRS and additional poor premorbid social adjustment and early and insidious disease onset (ETRS+).

Supplementary Table 7: Nagelkerke R^2-values and right-tailed p-values resulting from logistic regression modelling without adjustment for additional clinical covariates after inclusion of Swedish sample in discovery set

p-value cut-off	SCZ diagnosis (n = 1540 cases, 2172 controls)	TRS[a] (n = 434 w/ TRS, 370 w/o TRS	ETRS[b] (n = 45 w/ ETRS, 78 w/o ETRS
< 0.01	R^2=0.044 (p=2.2e-29)	R^2=0.007 (p=0.02)	R^2=0.009 (p=0.83)
< 0.05	R^2=0.062 (p=8.6e-41)	R^2=0.01 (p=7.8e-03)	R^2=0.005 (p=0.75)
< 0.1	R^2=0.069 (p=5.9e-45)	R^2=0.008 (p=0.02)	R^2=0 (p=0.56)
< 0.2	R^2=0.071 (p=9.5e-47)	R^2=0.008 (p=0.01)	R^2=0.002 (p=0.35)
< 0.3	R^2=0.073 (p=1.7e-47)	R^2=0.009 (p=0.01)	R^2=0.003 (p=0.30)
< 0.4	R^2=0.074 (p=2.1e-48)	R^2=0.009 (p=9.6e-03)	R^2=0.004 (p=0.27)
< 0.5	R^2=0.077 (p=3.5e-50)	R^2=0.008 (p=0.01)	R^2=0.004 (p=0.28

[a]Treatment resistant schizophrenia; [b]extreme treatment resistant schizophrenia

Supplementary Table 8: right-tailed p-values from logistic regression with adjustment for clinical covariates after inclusion of Swedish sample

p-value cut-off	TRS[a]	ETRS[b]
<0.01	0.03 (0.01)[c]	0.81
<0.05	0.04 (0.01)	0.70
<0.1	0.05 (0.02)	0.49
<0.2	0.07 (0.03)	0.29
<0.3	0.05 (0.02)	0.25
<0.4	0.04 (0.02)	0.22
<0.5	0.05 (0.02)	0.22

[a]Treatment resistant schizophrenia; [b]extreme treatment resistant schizophrenia; [c]in parentheses are p-values for model without adjustment for covariates based on reduced sample size after excluding individuals with missing data on any of the considered covariates

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