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Schizophrenia polygenic risk scores, urbanicity and treatment-resistant schizophrenia

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ABSTRACT

Introduction: To investigate the impact of a polygenic risk score for schizophrenia (PRS-SZ) and urbanicity on the risk of treatment-resistant schizophrenia (TRS) in people diagnosed with schizophrenia and to evaluate the association between PRS-SZ and TRS across levels of urbanicity.
Methods: Cohort study of people born after 1981 with a first registered diagnosis of schizophrenia between 1996 and 2012 using Danish population registry data. Through linkage to genome-wide data, we calculated PRS-SZ based on a Psychiatric Genomics Consortium meta-analysis. We assessed urbanicity at birth (capital, provincial and rural areas). TRS was defined using prescription and hospital data. Performing Cox regression analysis, we calculated hazard rate ratios (HRs) and 95% confidence intervals (CI).
Results: Among 4475 people with schizophrenia, we identified 593 (13.3%) with TRS during 17,558 person years of follow-up. The adjusted HR for TRS associated with one standard deviation (SD) increase in the PRS-SZ was 1.11 (95% CI: 1.00–1.24). The adjusted HRs for TRS across levels of urbanicity were 1.20 (95% CI: 0.98–1.47) for provincial areas and 1.19 (95% CI 0.96–1.47) for rural areas compared with the capital area. Within strata of urbanicity, the adjusted HR for TRS was 1.39 (95% CI: 1.14–1.70) in the capital area with 1 SD increase in the PRS-SZ, 0.99 (95% CI 0.84–1.17) in provincial areas, and 1.03 (95% CI: 0.86–1.25) in rural areas.
Conclusion: The effect of genetic liability (i.e. PRS) on risk of TRS varied across urbanicity levels and was highest for people with schizophrenia born in the capital areas.

1. Introduction

Insufficient response to antipsychotic treatment, also termed treatment-resistant schizophrenia (TRS) may affect up to 30% of people with schizophrenia initiating antipsychotic treatment (Meltzer, 1997; Howes et al., 2017). The reasons for TRS are still not well understood (Gillespie et al., 2017; Wimberley et al., 2016b). Among a number of patient and treatment-related factors, we have previously shown that living in rural areas at birth was associated with increased risk of TRS (Wimberley et al., 2016b; Wimberley et al., 2016a). Specific genetic factors in relation to TRS have not been identified yet (Gillespie et al., 2017; Lally et al., 2016), though a few studies point towards a weak association between a polygenic risk score for schizophrenia (PRS-SZ) and TRS, indicating higher genetic

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liability for schizophrenia among people with TRS (Wimberley et al., 2017; Frank et al., 2015). The precision of these genetic studies was though potentially affected by low power of the sample sizes. Another study from Denmark showed that people with higher PRS-SZ tend to be born (and to live) more frequently in urban areas, thereby indicating that people in rural areas showing lower PRSs for schizophrenia (Paksarian et al., 2018). This led us to hypothesize that urbanicity and PRS-SZ may interact resulting in different estimates of the associations between PRS-SZ and TRS in different geographical areas.

Therefore, we investigated here: (1) whether we could replicate and estimate more precisely our previous findings of an association between both a PRS-SZ and urbanicity and TRS using the largest population-based sample of people with schizophrenia; (2) whether levels of PRS-SZ varied in people with and without TRS in different geographical areas.

2. Methods

2.1. Study design and data

We performed a prospective cohort study by linking Danish administrative registries that cover the entire Danish population using the unique civil registration number assigned to every person living in Denmark and thereby enabling linkage between registers. We obtained data from the following Danish national registers: The Danish Civil Registration System (Pedersen, 2011) containing records on gender, date of birth, change of address, date of emigration, vital status, and links to family members since 1968; The Danish Psychiatric Central Research Register containing all discharge diagnoses assigned at psychiatric hospitals in Denmark since 1969 (outpatient contacts included since 1995) (Mors et al., 2011). The diagnoses are coded according to International Classification of Disease (8th revision) (ICD-8) until the end of 1993, and 10th revision (ICD-10) thereafter. The Danish National Prescription Registry containing the date, kind of drug and volume of redeemed prescriptions at community pharmacies, registered since 1995 (Pottegard et al., 2017). The Danish Newborn Screening Biobank (Norgaard-Pedersen and Hougaard, 2007) containing genetic data extracted from dried blood spots collected in the first days after birth of nearly all infants born in Denmark since 1981.

2.2. Study population

Among individuals born after May 1, 1981 in Denmark, we identified people with a first diagnosis of schizophrenia as in- or out-patient or emergency room contact at a psychiatric hospital (ICD-10: F20) between January 1, 1996 and December 31, 2012. Thus, individuals with a ICD-8 diagnosis (295.x9 excluding 295.79) or a ICD-10 diagnosis of schizophrenia recorded before 1996 were not included. Furthermore, we included individuals only if they were 10 years or older at the first registered diagnosis of schizophrenia and alive at discharge, and had a known mother identified by linkage to the Danish Civil Registration System (Pedersen, 2011). To the identified individuals, we linked prescription data from the Danish National Prescription Registry and retrieved information on antipsychotic prescriptions (Anatomical Therapeutic Chemical (ATC) Classification system code N05A) (10). People with a clozapine prescription before their first registered diagnosis of schizophrenia were excluded. The final study population consisted of the individuals with a DNA sample available from the Danish Newborn Screening Biobank (Norgaard-Pedersen and Hougaard, 2007).

2.3. Outcome measure

2.3.1. Treatment-resistant schizophrenia

Our measure of TRS was based on hospital admission data from the Danish Psychiatric Central Research Register (Mors et al., 2011) and the Danish National Prescription Registry (Pottegard et al., 2017) regarding antipsychotic prescription redemptions. We defined TRS as the first occurrence of either clozapine initiation or hospitalization due to schizophrenia during antipsychotic treatment within 18 months after at least two periods of different antipsychotic monotherapies lasting at least 6 weeks each. This treatment-based definition, used in recent published studies, builds on international and Danish treatment guidelines and the Kane criteria (Wimberley et al., 2017; Wimberley et al., 2016b). We recently validated this algorithm against clinical records in UK and found a positive predictive value of 63.6%, and a sensitivity of 62.2% compared with a positive predictive value of 78.3% and a sensitivity of 40% for identifying TRS based on clozapine prescriptions only (Ajnakina et al., 2018).

2.4. Exposure measures

2.4.1. The polygenic risk score

DNA from the genetic material obtained from dried blood spot samples of the study population was whole-genome amplified in triplicate using the Qiagen REPLI-g mini kit [Qiagen, Hilden, Germany]. The three separate reactions were pooled (Hollegaard et al., 2009; Hollegaard et al., 2011) and genotyped with either Illumina Human 610-Quad BeadChip array, Illumina HumanCoreExome beadchip or Illumina Infinium PsychArray-24-v1.1 BeadChip (Illumina, San Diego, CA) (Pedersen et al., 2018; Agerbo et al., 2012; Meier et al., 2016).

PRS-SZ were calculated based on the summary statistics (effect allele, effect size) derived from the Psychiatric Genomics Consortium (PGC) discovery sample (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). The discovery sample comprised 34,600 schizophrenia cases and 45,986 controls from the PGC Genome-Wide Association Study (GWAS) meta-analysis for schizophrenia, excluding the Danish cases (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). We selected single-nucleotide polymorphisms (SNPs) associated at a p-value threshold of 0.05 or lower. This threshold was chosen in accordance with other studies including PRS-SZ (Wimberley et al., 2017; Agerbo et al., 2015) and is considered appropriate to achieve a balance between the number of false-positive and true-positive risk alleles (Wray et al., 2014). In our target sample, our independent study population, we calculated a PRS-SZ for each individual as the weighted sum of risk alleles at the selected SNPs with the weight being the effect estimates in the discovery sample. The calculated PRS-SZ, that was approximately normally distributed (Dudbridge, 2013), was standardized by subtracting the mean and dividing by the square root of the sample variance.

2.4.2. Urbanicity

We obtained information on geographical area at birth from the Civil Registration System (8). Geographical areas were categorized into three levels according to the degree of urbanization: capital area (capital and suburbs of the capital), provincial areas (provincial cities >10,000 inhabitants), and rural areas (provinvial town ≤10,000 inhabitants).

2.5. Covariates

We obtained information on age at first schizophrenia diagnosis and gender from the Civil Registration System (Pedersen, 2011). The year of diagnosis of schizophrenia was obtained from the date of the first diagnosis of schizophrenia recorded in the Danish Psychiatric Central Research Register (Mors et al., 2011). Because the samples were genotyped using three different types of arrays as described above, we accounted for this in the statistical analyses by adjusting for the different genotyping arrays. Ancestry was assessed by the first 10 genomic principal components (Price et al.,

2006). Pairs of related individuals were identified with pi-hat > 0.09 using PLINK's identity-by-descent analysis, and one individual of each such pair was excluded at random. Based on this relatedness-pruned set of individuals and a subset of SNPs (minor allele frequency > 0.001 and pruned for linkage disequilibrium (LD) ($r^2 < 0.05$)), we generated genomic principal components using smartPCA of the EIGENSOFT 6.1.4 package. All individuals were subsequently projected back into the principal component space based on their genotypes and SNP loadings.

2.6. Statistical analysis

We followed individuals from the date of their first diagnosis for schizophrenia until the time of meeting criteria for TRS, emigration, death or end of study period (June 30, 2013). The mean PRS-SZ for each level of urbanicity was estimated and adjusted for age at diagnosis, sex, year of diagnosis, the three types of genotyping arrays, and the 10 first genomic principal components. We performed Cox regression analysis and calculated hazard rate ratios (HRs) for TRS in relation to a PRS-SZ and urbanicity. The HRs were mutually adjusted for PRS-SZ and urbanicity and additionally for age at diagnosis, year of diagnosis, the three types of genotyping arrays, and the 10 first genomic principal components. Additionally, all analyses allowed different baseline hazards for males and females in the models. To evaluate whether the levels of PRS-SZ in relation to TRS were different in the different levels of urbanicity, we tested first for a statistical interaction between PRS-SZ and urbanicity. For this purpose we included an interaction term in the Cox regression model. In case of an interaction, we planned to evaluate further the association between PRS-SZ and TRS by investigating the association between PRS-SZ and TRS across the different levels of urbanicity (strata-specific analyses). To assess the robustness of our results we performed a sensitivity analysis where we restricted the TRS definition to people who redeemed at least one prescription for clozapine, which has been shown to have a better positive predictive value though lower sensitivity compared with the algorithm derived TRS status (Ajnakina et al., 2018).

To improve accuracy for the PRS-SZ, which is derived from summary statistics based on samples with European ancestry (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), we additionally performed a sensitivity analysis where we removed related individuals and genetic outliers as follows: Based on a LD-pruned set of SNPs, one of each related pair of individual (pi-hat > 0.2) were removed at random. A subsample of European ancestry was selected as an ellipsoid in the space of the first three principal components centered and scaled using the estimated mean and six standard deviation of a subsample with both parents and grandparents born in Denmark. Genomic principal components were then recalculated using the R-package SNPrelate.

All epidemiological statistical analyses were performed using STATA 13.1 and SAS 9.4.

The main analyses were performed between August 2017 and June 2018.

3. Results

We included 4475 people with a first registered diagnosis of schizophrenia between 1996 and 2012 and available genetic information. Characteristics of the study population are shown in Table 1. The median age at first schizophrenia diagnosis was 20.6 years (interquartile range 18.4 years to 23.1 years) and 43% were female. Slightly more than a third lived in the provincial areas at birth, and the third genotyping array contributed 61% of the total study population (Table 1). With regard to urbanicity, the groups showed significant differences regarding age, genotyping array, year of schizophrenia diagnosis, and PRS-SZ (all $p < 0.05$) (Table 1). With regard to TRS status, people fulfilling the criteria for TRS differed from those not meeting the TRS criteria with regard to age, gender, genotyping array, and year of schizophrenia diagnosis (all $p < 0.05$) (Table 1).

The study population was followed for 17,558 person years. During follow up, 61 people died, and 65 were censored due to emigration. 593 (13.3%) fulfilled the criteria for TRS during follow-up at a rate of 3.38 cases of TRS per 100 person years.

After removing genetic outliers from the study population for the sensitivity analysis, the distribution of characteristics remained basically unchanged (Supplementary material Table 1).

3.1. Associations between PRS, urbanicity and TRS

Crude and adjusted HRs of the associations between PRS-SZ, urbanicity and TRS are displayed in Table 2. The fully adjusted HR of the association between 1 SD increase in the PRS-SZ and TRS was 1.11 (95% CI: 1.00–1.24). The HRs increased with the PRS-SZ from the lowest to the highest quartile of the PRS-SZ with an upward trend. The adjusted HR for the highest versus the lowest quartile was 1.36 (95% CI: 1.06–1.73).

The fully adjusted HRs of the association between urbanicity at birth and TRS were 1.20 (95% CI: 0.98–1.47) for provincial areas and 1.19 (95% CI 0.96–1.47) for rural areas compared with the capital area.

We modeled the interaction between the continuous PRS-SZ and

Table 1
Baseline characteristics at first schizophrenia diagnosis, $n = 4475$.

Baseline characteristics	Total	Capital region	Provincial region	Rural region	TRS	No TRS
N (%)	4475 (100)	1391 (100)	1702 (100)	1382 (100)	593 (100)	3882 (100)
Age at diagnosis, years, median (inter-quartile range)	21 (18–23)	20 (18–23)	21 (19–23)	21 (19–23)	20 (18–22)	21 (19–23)
Females, n (%)	1944 (43.4)	580 (41.7)	755 (44.4)	609 (44.1)	316 (53.3)	1628 (41.9)
Urbanicity, n (%)						
Capital region	1391 (31.1)	n.a	n.a	n.a	166 (28.0)	1225 (31.6)
Provincial region	1702 (38.0)	n.a	n.a	n.a	239 (40.3)	1463 (37.7)
Rural region	1382 (30.9)	n.a	n.a	n.a	188 (31.7)	1194 (30.8)
Genotyping array[a], n (%)						
1	873 (19.5)	298 (21.4)	327 (19.2)	248 (18.0)	213 (35.9)	660 (17.0)
2	885 (19.8)	251 (18.0)	364 (21.4)	270 (19.5)	160 (27.0)	725 (18.7)
3	2717 (60.7)	842 (60.5)	1011 (59.4)	864 (62.5)	220 (37.1)	2497 (64.3)
Year of diagnosis, n (%)						
1996–2000	113 (2.5)	51 (3.7)	34 (2.0)	28 (2.0)	39 (6.6)	74 (1.9)
2001–2004	495 (11.1)	168 (12.1)	190 (11.2)	137 (9.9)	124 (20.9)	371 (9.6)
2005–2008	1345 (30.1)	393 (28.2)	511 (30.0)	441 (31.9)	251 (42.3)	1094 (28.2)
2009–2012	2522 (56.3)	779 (56.0)	967 (56.8)	776 (56.2)	179 (30.2)	2343 (60.3)
PRS, mean (sd)	0.00 (1.00)	0.19 (1.14)	0.00 (0.98)	−0.19 (0.83)	0.02 (1.01)	0.00 (1.00)

[a] Genotyping arrays used in different sampling frames: (1) Illumina Human 610-Quad BeadChip array; (2) Illumina HumanCoreExome beadchip; (3) Illumina Infinium PsychArray-24-v1.1 BeadChip (Illumina, San Diego, CA).

Table 2
Cox regression analysis of treatment-resistant schizophrenia during follow up.

	No. of individuals	No. of events	Time at risk in years	IR per 100 person-years	HRs and 95% CIs for TRS		
					Crude	Adjusted[a]	Adjusted[b]
Polygenic risk score							
Continuous	4475	593	17,558	3.38 (3.12–3.66)	1.02 (0.94–1.10)	1.10 (0.99–1.22)	1.11 (1.00–1.24)
Quartiles							
0 (lowest)	1118	143	4410	3.24 (2.75–3.82)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	1119	132	4386	3.01 (2.54–3.57)	0.92 (0.73–1.17)	0.92 (0.73–1.17)	0.96 (0.76–1.22)
2	1119	157	4419	3.55 (3.04–4.15)	1.09 (0.87–1.37)	1.11 (0.89–1.40)	1.19 (0.95–1.50)
3 (highest)	1119	161	4342	3.71 (3.18–4.33)	1.13 (0.91–1.42)	1.30 (1.02–1.65)	1.36 (1.06–1.73)
Urbanicity at birth							
Capital	1391	166	5664	2.93 (2.52–3.41)	1.0		1.0 (ref)
Provincial	1702	239	6574	3.64 (3.20–4.13)	1.20 (0.98–1.46)		1.20 (0.98–1.47)
Rural	1382	188	5320	3.53 (3.06–4.08)	1.17 (0.95–1.44)		1.19 (0.96–1.47)

IR: incidence rate.
HR: hazard ratio.
CI: confidence interval.
TRS: treatment resistant schizophrenia.
[a] Adjusted for 10 principal components.
[b] Different baseline hazards for males and females; mutually adjusted for polygenic risk score, age at diagnosis, year of diagnosis, geographical area at birth, type of genotyping array, 10 principal components.

urbanicity by including an interaction term, and found significant interactions for provincial ($p = 0.007$) and rural areas ($p = 0.068$). We therefore performed stratified analyses by urbanicity levels to quantify the associations between PRS-SZ and TRS within each level of urbanicity (Table 3). In the adjusted model, 1 SD increase in the PRS-SZ increased the risk of TRS by a HR of 1.39 (95% CI: 1.14–1.70) in the capital area. In provincial and rural areas, the adjusted HRs for the associations between PRS-SZ and TRS were around 1 for 1 SD increase in the PRS-SZ. When analyzing the association between the PRS-SZ and TRS in quartiles of the PRS-SZ, we found an increasing trend of the PRS-SZ associated risk of TRS from the second lowest quartile of the PRS-SZ (HR of 1.55 (95% CI: 0.92–2.62)) to the highest (HR of 2.44 (95% CI: 1.44–4.13)) within the capital area (Table 3).

For further illustration of the interaction between PRS-SZ and urbanicity on the risk of TRS, the adjusted mean PRS-SZ across levels of urbanicity are displayed in Supplementary Fig. 1. We found a trend towards higher PRS-SZ in the capital and provincial areas compared with rural areas, however not reaching statistical significance. Fig. 1 displays the adjusted mean PRS-SZ additionally stratified by TRS status in the three different levels of urbanicity. The adjusted mean PRS-SZ were significantly higher among people with TRS in the capital area (p-value < 0.01) compared with those with non-TRS, but not statistically different in the other geographical areas (provincial area: p-value = 0.93; rural area: p-value = 0.70).

3.2. Sensitivity analyses

In the sensitivity analyses, we compared people initiating clozapine, as an alternative definition of TRS, versus non-clozapine users, thereby not excluding people classified as TRS using the algorithm based TRS definition. We found similar estimates as for the broader TRS definition, though providing statistically insignificant

Table 3
Cox regression analysis of treatment-resistant schizophrenia during follow up, stratified by urbanicity at birth.

Urbanity at birth	No. of individuals	No. of events	Time at risk in years	IR per 100 person-years	HRs and 95% CIs for TRS		
					Crude	Adjusted[a]	Adjusted[b]
Capital							
Continuous	1391	166	5664	2.93 (2.52–3.41)	1.16 (1.03–1.31)	1.34 (1.10–1.63)	1.39 (1.14–1.70)
Quartiles							
0	299	23	1272	1.81 (1.20–2.72)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	321	36	1312	2.74 (1.98–3.80)	1.51 (0.89–2.55)	1.50 (0.89–2.54)	1.55 (0.92–2.62)
2	340	47	1448	3.25 (2.44–4.32)	1.78 (1.08–2.93)	1.77 (1.07–2.91)	1.92 (1.16–3.17)
3	431	60	1632	3.68 (2.85–4.74)	1.96 (1.21–3.16)	2.18 (1.29–3.67)	2.44 (1.44–4.13)
Provincial							
Continuous	1702	239	6574	3.64 (3.20–4.13)	0.91 (0.79–1.04)	1.00 (0.84–1.18)	0.99 (0.84–1.17)
Quartiles							
0	436	69	1649	4.18 (3.30–5.30)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	400	50	1559	3.21 (2.43–4.23)	0.77 (0.54–1.11)	0.77 (0.53–1.10)	0.77 (0.53–1.11)
2	436	55	1642	3.35 (2.57–4.36)	0.78 (0.55–1.12)	0.82 (0.57–1.17)	0.87 (0.61–1.25)
3	430	65	1724	3.77 (2.96–4.81)	0.92 (0.66–1.29)	1.13 (0.79–1.62)	1.08 (0.75–1.55)
Rural							
Continuous	1382	188	5320	3.53 (3.06–4.08)	1.00 (0.85–1.19)	1.02 (0.85–1.23)	1.03 (0.86–1.25)
Quartiles							
0	383	51	1489	3.43 (2.60–4.51)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	398	46	1515	3.04 (2.27–4.05)	0.86 (0.58–1.29)	0.89 (0.59–1.32)	0.94 (0.63–1.40)
2	343	55	1329	4.14 (3.18–5.39)	1.22 (0.83–1.78)	1.23 (0.84–1.80)	1.25 (0.85–1.84)
3	258	36	987	3.65 (2.63–5.06)	1.04 (0.68–1.59)	1.09 (0.70–1.70)	1.17 (0.75–1.83)

IR: incidence rate.
HR: hazard ratio.
CI: confidence interval.
TRS: treatment-resistant schizophrenia.
[a] Adjusted for 10 principal components.
[b] Different baseline hazards for males and females; mutually adjusted for polygenic risk score, age at diagnosis, year of diagnosis, geographical area at birth, type of genotyping array, 10 principal components.

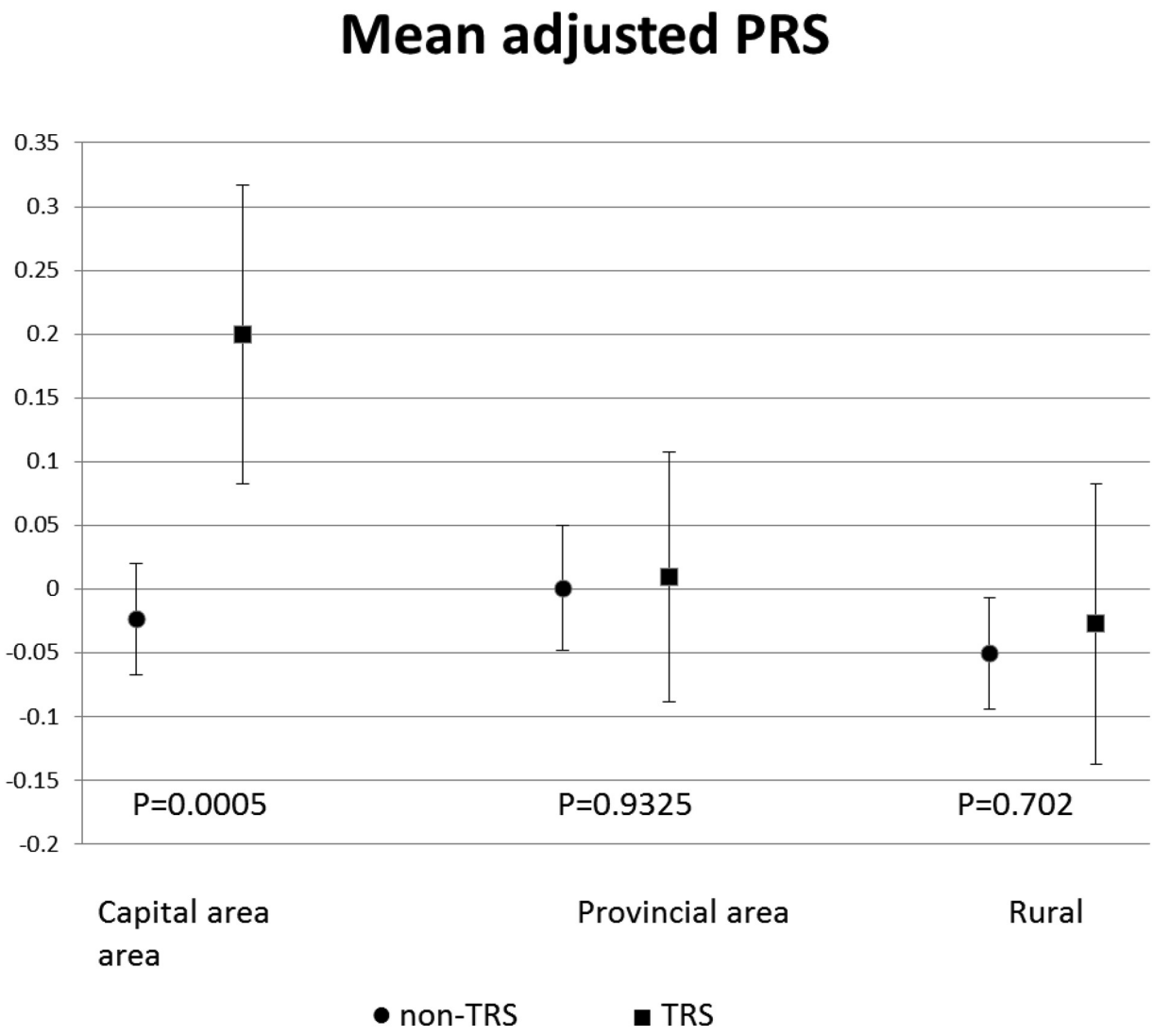

Fig. 1. Mean polygenic risk scores for schizophrenia in people with schizophrenia according to geographical area and by TRS status, adjusted for sex, age at diagnosis, year of diagnosis, type of genotyping array, 10 principal components. *p*-Values for the comparison of differences in means of polygenic risk scores.

results, potentially due to missing power and TRS case misclassification among controls of non-clozapine users (Supplementary Table 2). The HRs of the association between urbanicity at birth and clozapine were up to 38% increased in both provincial and rural areas compared with the capital area. The *p*-values for the interaction between the continuous PRS and urbanicity (3 levels) was $p = 0.08$ for provincial areas; and $p = 0.48$ for rural areas.

Removing genetic outliers and thereby focusing on individuals with mainly Danish ancestry, we found overall similar estimates, with a slightly attenuated fully adjusted HRs of 1.07 (95% CI: 0.99–1.17) for each SD increase in PRS-SZ (Supplementary material Table 3). At the same time the estimated fully adjusted HR for TRS in association with rural and provincial areas became significantly different from the capital area with an up to 29% increase in TRS risk. In the stratified analyses HR estimates were attenuated, but with remaining significantly increased HRs for the association between PRS-SZ and TRS in the capital region (1.28 (95%: 1.08–1.53)), also in a dose-response relationship comparing the highest with the lowest PRS quartile (2.01 (95%CI 1.20–3.37)) (Supplement Table 4).

4. Discussion

In this currently largest population-based sample of people with schizophrenia and TRS, we confirmed the previously reported weak overall effect of a 10% increased HR for each standard deviation increase of a PRS-SZ in association with TRS (Wimberley et al., 2017) (Frank et al., 2015). Comparing the highest PRS-SZ quartile with the lowest quartile of the PRS-SZ, we found an increased HR for TRS of 1.36 indicating the higher the load of genetic risk for schizophrenia the more likely the person was identified with TRS.

Thus, the impact of genetic liability for schizophrenia as measured by a PRS-SZ on risk of TRS appears on average low. This may be partly due to the fact that the ability of a PRS-SZ to discriminate between schizophrenia and TRS depends on which schizophrenia cases (ratio of non-TRS to TRS patients) went into the discovery study (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Additionally, our findings of a weak association between a PRS-SZ and TRS may indicate only little overlap in the genetic make up of schizophrenia and TRS in general. This may be interpreted that genetics involved in antipsychotic drug response are underrepresented in the PRS trained in samples of schizophrenia and more specific drug response GWAS are required to train genetic risk scores for drug response prediction purposes. Another study from Denmark found a significant correlation between a PRS-SZ with chronicity of schizophrenia as indicated by number and length of hospitalizations (Meier et al., 2016). As TRS is frequently detected only later in the course of treated schizophrenia, chronicity of the disorder may also play a role. As

discussed by Gillespie and colleagues, "antipsychotics response and resistance are not readily separable from group differences in illness symptomatology or severity." (Gillespie et al., 2017). A PRS-SZ based on GWAS conducted by the Psychiatric Genomics Consortium has been recently applied to assess treatment response in first episodes schizophrenia to different kinds of second-generation antipsychotics, such as olanzapine, risperidone, and aripiprazol (Zhang et al., 2019). The authors found that patients with a higher PRS-SZ showed less improvement with antipsychotic treatment.

Beside PRS-SZ, a few other studies have documented a potential role of genetics in TRS. Ruderfer and colleagues recently reported genetic overlap, including genes represented by our applied polygenic risk score in this study, between schizophrenia pathogenesis and the antipsychotic mechanism of action (Ruderfer et al., 2016). Furthermore, the authors found rare disruptive variants in gene targets of antipsychotics and in genes with evidence for a role in antipsychotic efficacy in people with TRS (Ruderfer et al., 2016). Martin and Mowry found increased rare copy number variants in people with TRS (Martin and Mowry, 2016). However, the role of these genetic markers has not been further investigated in pharmacoepidemiological/pharmacogenetic and population-based studies.

Regarding urbanicity, we found a HR of 1.2 for TRS in provincial and rural areas in line with our previous finding (Wimberley et al., 2016a). In the current study we were interested in investigating the interaction between genetic liability of schizophrenia and urbanicity, because of the previously reported inverse findings of lower rates of TRS in urban areas despite a tendency of a higher genetic liability of schizophrenia in urban areas (Wimberley et al., 2016a; Paksarian et al., 2018). We indeed detected an interaction between urbanicity and genetic liability for schizophrenia on the risk of TRS. Stratifying according to TRS status within geographical areas, we found a higher genetic liability for schizophrenia in people with TRS compared with those without TRS in the capital area only. Adjusted mean PRS-SZ levels were similar in people with or without TRS in the other geographical areas. Our results indicate that the otherwise small association between genetic liability and TRS was potentially driven by people with higher genetic liability for schizophrenia in the capital area. Thus, in the subgroups of patients with a higher PRS-SZ and born in capital areas, a PRS-SZ appears more predictive of TRS. This may indicate that our proxy for TRS reflects a higher severity of schizophrenia (as indicated by a higher polygenic risk score) in the capital area but not in rural areas. An alternative explanation beyond genetics may be regional differences in treatment practice, with more use of antipsychotic medication and earlier clozapine use in rural areas (Wimberley et al., 2016a). This indicates that our applied proxy for TRS may be less specific to detect actual TRS in rural areas.

Our study has several limitations. First, we investigated the association between the potential risk factors urbanicity and PRS-SZ within the group of people with a registered diagnosis of schizophrenia. Our findings may be partly explained by this selection, i.e. collider-stratification bias (Cole et al., 2010). The collider-stratification bias may occur when two independent risk factors (here PRS-SZ and urbanicity) for schizophrenia are conditioned on their common effect (schizophrenia), which can impact an association between the two independent risk factors even though the association may not exist. This potential bias may complicate comparisons between our results and those obtained from other studies not conditioning on the status of schizophrenia in people with a diagnosis of TRS.

Second, the predictive value of PRS-SZ based on the current known markers may be too unspecific to be of clinical utility, and other genetic markers of TRS are needed.

Third, the TRS definition was based on register information only, without clinical information on functioning or negative symptoms. Fourth, our definition of TRS may be cultural sensitive, or depending on differences in health care practice, such as availability of doctors, and threshold for admission. In this regard, we recently validated our algorithm against clinical records in a different clinical setting of psychiatric services in South-London, UK and found a PPV of 63.6%, and a sensitivity of 62.2% compared with a PPV of 78.3% and a sensitivity of 40% for identifying TRS based on clozapine prescriptions only (Ajnakina et al., 2018).

Last, in our main analysis, we did not exclude genetic outliers indicating different ethnicity other than Danish origin. A recent study from Canada found no association between ethnicity or migration and TRS (Bani-Fatemi et al., 2019), and we adjusted for the first ten principal components to account for a possible population stratification. Further, in a sensitivity analysis excluding genetic outliers, the estimates were attenuated but remained robust.

5. Conclusion

Among people with schizophrenia, we replicated previous findings of a weak genetic liability for schizophrenia and of place of birth in association with TRS. An interaction between urbanicity and PRS-SZ can be suspected with a higher PRS-SZ being predictive of TRS mainly in people with schizophrenia who resided in urban areas at birth. The reasons for this remain poorly understood.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2019.08.008.

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Contributors

CG wrote the protocol and first draft of the manuscript, HTH analyzed the data. YW is responsible for genetic information and polygenic risk score for schizophrenia. TDA performed the principal component analysis. All authors contributed to the design of the study. All authors contributed to and approved the final manuscript.

Declaration of competing interest

None.

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