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Genome-wide analyses of smoking behaviors in schizophrenia: findings from the Psychiatric Genomics Consortium

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Disclosures

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Contributions

Authors R. Peterson, T. Bigdeli, and A. Fanous designed the study and wrote the first draft of the manuscript. R. Peterson, T. Bigdeli, and S. Ripke undertook the statistical analysis. All authors contributed to and have approved the final manuscript.

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Abstract

While 17% of US adults use tobacco regularly, smoking rates among persons with schizophrenia are upwards of 60%. Research supports a shared etiological basis for smoking and schizophrenia, including findings from genome-wide association studies (GWAS). However, few studies have directly tested whether the same or distinct genetic variants also influence smoking behavior among schizophrenia cases. Using data from the Psychiatric Genomics Consortium (PGC) study of schizophrenia (35476 cases, 46839 controls), we estimated genetic correlations between these traits and tested whether polygenic risk scores (PRS) constructed from the results of smoking behaviors GWAS were associated with schizophrenia risk or smoking behaviors among schizophrenia cases. Results indicated significant genetic correlations of schizophrenia with smoking initiation (r_g=0.159; P=5.05×10^{-10}), cigarettes-smoked-per-day (r_g=0.094; P=0.006), and age-of-onset of smoking (r_g=0.10; P=0.009). Comparing smoking behaviors among schizophrenia cases to the general population, we observe positive genetic correlations for smoking initiation (r_g=0.624, P=0.002) and cigarettes-smoked-per-day (r_g=0.689, P=0.120). Similarly, TAG-based PRS for smoking initiation and cigarettes-smoked-per-day were significantly associated with smoking initiation (P=3.49×10^{-5}) and cigarettes-smoked-per-day (P=0.007) among schizophrenia cases. We performed the first GWAS of smoking behavior among schizophrenia cases and identified a novel association with cigarettes-smoked-per-day upstream of the *TMEM106B* gene on chromosome 7p21.3 (rs148253479, P=3.18×10^{-8}, n=3520). Results provide evidence of a partially shared genetic basis for schizophrenia and smoking behaviors. Additionally, genetic risk factors for smoking behaviors were largely shared across schizophrenia and non-schizophrenia populations. Future research should address mechanisms underlying these associations to aid both schizophrenia and smoking treatment and prevention efforts.

Keywords

schizophrenia; genetics; GWAS; smoking initiation; cigarettes per day; pleiotropy

Introduction

Schizophrenia is a chronic mental illness affecting nearly 1% of the world's population and is associated with considerable morbidity and mortality(McGrath et al., 2008; Simeone et al., 2015). Affected persons are at markedly increased risk for substance use disorders, particularly nicotine dependence(Hartz et al., 2014; Volkow, 2009). Currently, 17% of US adults and upwards of 60% of schizophrenia spectrum cases smoke tobacco regularly(de Leon and Diaz, 2005; Jamal et al., 2015; Volkow, 2009). Furthermore, patients tend to smoke a greater number of cigarettes, extract more nicotine per cigarette, and experience greater withdrawal symptoms than smokers in the general population(Centers for Disease Control and Prevention (CDC), 2013; Strand and Nybäck, 2005; Tidey et al., 2014), thereby increasing their risk of nicotine dependence and associated adverse medical conditions including cardiovascular disease and cancers(Olfson et al., 2015).

Decades of twin and family studies have demonstrated that schizophrenia is highly heritable (~80%)(Sullivan et al., 2003). Common genetic variants captured by genome-wide single-nucleotide polymorphism (SNP) arrays account for at least one third of variance in risk(International Schizophrenia Consortium et al., 2009; Ripke et al., 2013). A landmark genome-wide association study (GWAS) meta-analysis of schizophrenia identified 108 robustly associated loci(Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), one of which resides in a gene cluster encoding neuronal nicotinic acetylcholine receptors (nAChR) on chromosome 15q25, which has previously been shown to be associated with heaviness of smoking in the general population(Tobacco and Genetics Consortium, 2010).

Similarly, twin and family studies have consistently shown a significant genetic component to the liability of smoking behavior, with estimated heritabilities on the order of 0.50–0.70 for smoking initiation and 0.60 for nicotine dependence among European ancestry populations(Maes et al., 2004; Vink et al., 2005). Large, population-based GWAS of smoking-related traits have yielded several putative risk variants, including an association between smoking initiation and *BDNF* on 11p14.1(Tobacco and Genetics Consortium, 2010) and several associations for smoking quantity, most notably the previously reported 15q25 locus harboring three genes encoding nAChR subunits *CHRNA5-CHRNA3-CHRNA4,* a second locus encoding nAChRs on 8p11 in and near *CHRNB3-CHRNA6*, and variants in and near *CYP2A6-CYP2B6* on 19q13 encoding nicotine metabolizing enzymes(Thorgeirsson et al., 2010; Tobacco and Genetics Consortium, 2010).

Mechanisms underlying the schizophrenia-smoking association are not completely understood. Several mechanisms have been proposed to explain elevated tobacco use in those with schizophrenia including: 1) the self-medication hypothesis, 2) that smoking causes schizophrenia, and 3) a shared liability underlying both traits. The self-medication hypothesis posits that smoking is used as a strategy to alleviate adverse positive or negative symptoms of schizophrenia, cognitive impairments, or medication side-effects(Kumari and Postma, 2005). The shared vulnerability hypothesis postulates that factors common to both disorders (i.e. genetic, environmental) drive their co-occurrence. For example, dysfunction in nAChRs represents a common substrate for various symptoms of schizophrenia and

comorbid nicotine use(Parikh et al., 2016; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Tobacco and Genetics Consortium, 2010). It has been suggested that reduced expression of the α7-nicotinic receptor in schizophrenia(Guillozet-Bongaarts et al., 2014; Severance and Yolken, 2008) results in reduced sensory gating inhibition as measured by paradigms such as P50 auditory-evoked potentials, prepulse inhibition, and mismatch negativity(Freedman, 2014). Such deficits could conceivably diminish an individual's ability to keep extraneous stimuli from awareness, possibly giving rise to hallucinations and delusions(Howes and Kapur, 2009). Additional research supports mechanisms 2 and 3(Chen et al., 2016; Gurillo et al., 2015; Kendler et al., 2015). For example, in a population-based Swedish cohort it was found that smoking prospectively predicted risk for schizophrenia in a dose-response relationship and shared familial/genetic factors accounted for a portion of the comorbidity between smoking and schizophrenia(Kendler et al., 2015).

Recent findings support a molecular genetic component underlying schizophrenia-smoking associations(Chen et al., 2016; Hartz et al., 2018, 2017) but has not been demonstrated conclusively(Brainstorm Consortium et al., 2018; B. Bulik-Sullivan et al., 2015; Gage et al., 2017; Gage and Munafò, 2015; Zheng et al., 2017). Therefore, in this study, we sought to advance the understanding of schizophrenia-smoking associations in the context of available smoking data in schizophrenia cases from the Psychiatric Genomics Consortium (PGC) study of schizophrenia (see Table 1 for study overview)(Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). First, we leverage summary statistics from genome-wide findings to estimate genetic correlations between smoking behaviors and schizophrenia. Next, using available phenotypic data on smoking initiation and smoking quantity for >5000 schizophrenia cases from 10 participating studies, we consider whether polygenic risk scores (PRS) constructed from results of the Tobacco and Genetics (TAG) consortium study of smoking behaviors(Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Tobacco and Genetics Consortium, 2010) can predict these same behaviors among schizophrenia cases. We perform the largest GWAS of smoking behaviors among schizophrenia cases to date. Finally, we consider whether smoking patterns among schizophrenia cases and genetic risk factors for smoking are related to the clinical presentation of schizophrenia including age-of-onset and symptom-based positive, negative, manic, and depressive factor scores.

Methods

Ascertainment and assessment

The subsamples included in this study comprise 10 constituent sites from Stage 2 of the PGC study of schizophrenia (Table 2). Ascertainment, diagnostic assessment, genotyping, and genotype quality control have been previously described(Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Briefly, 52 samples from the US, Europe, and Australia comprising 34,241 cases, 45,604 controls, and 1,235 parent affected-offspring trios were genotyped using a number of commercial SNP genotyping platforms. These data were processed using the stringent PGC quality control procedures, followed by imputation of SNPs and insertion-deletions using the 1000 Genomes Project reference panel (UCSC

hg19/NCBI 37)(1000 Genomes Project Consortium et al., 2012; Sachidanandam et al., 2010) using IMPUTE2(Howie et al., 2011, 2009), resulting in nearly 9.5*M* markers for GWAS analysis.

Smoking behavior and clinical phenotypes

Smoking behavior variables were harmonized across sites. Smoking initiation was coded as positive if any of the following were endorsed: ever smoked, ever regular smoker, smoked 100 cigarettes, current smoker, former smoker, smoke 1 or more cigarettes-per-day, or nicotine dependence. Since smoking quantity data varied by site, cigarettes-smoked-per-day was centered and scaled for each cohort. To account for initiation, only those who endorsed ever smoked were included in genetic analyses of cigarettes-smoked-per-day. A summary of individual sites, their sample sizes, and smoking measures available are presented in Table 2 and Supplementary Figures S1 and S2.

We assessed whether age-of-onset of schizophrenia or symptom-based factor scores representing dimensions of illness were associated with smoking behaviors among cases. Age-of-onset was determined retrospectively and defined the age at first diagnosis or hospitalization. Symptoms averaged over the course of illness were assessed using the Operational Criteria Checklist for Psychotic Illness and Affective Illness (OPCRIT), Positive and Negative Syndrome Scale (PANSS), Lifetime Dimensions of Psychosis Scale (LDPS), Schedules for Clinical Assessment in Neuropsychiatry (SCAN), Structured Clinical Interview for DSM (SCID), and Comprehensive Assessment of Symptoms and History (CASH). Factor analyses of constituent PGC studies identified positive, negative, manic, and depressive symptom dimensions and methodological details can be found in Ruderfer et al(Ruderfer et al., 2014). Association between smoking initiation or cigarettes-smoked-per-day and each clinical measure was assessed by logistic and linear regression, respectively, including sex, age-at-interview, and a study site indicator as covariates.

Estimation of SNP-based heritability and genetic correlation

We obtained estimates of SNP-based heritability (h^2) and genetic correlation (r_g) using the LD-score regression approach, as previously described(B. K. Bulik-Sullivan et al., 2015). Genome-wide summary statistics for schizophrenia and TAG smoking-related traits (ever-smoked, cigarettes-per-day, smoking cessation “former vs current”, log-transformed age-of-onset of smoking, logOnset) were filtered using default parameters (INFO>0.9, MAF>1%). Reference LD-scores estimated for European populations in the 1000 Genomes Project were used; regression weights were based on common SNPs present in Hapmap Phase 3, as suggested by the developers of this approach(B. K. Bulik-Sullivan et al., 2015). We reduced potential bias in heritability estimation by reanalyzing the PGC schizophrenia with overlapping TAG samples omitted, and constraining regression intercepts to one and zero when estimating univariate heritability and genetic correlation, respectively. For the schizophrenia case-only binary trait of smoking initiation, we assumed population prevalence estimates (K) equal to the observed sample prevalence (Supplemental Table S1).

Replication of the observed r_g between schizophrenia risk and TAG traits utilized meta-analysis summary statistics for three East-Asian studies from the PGC(Schizophrenia

Working Group of the Psychiatric Genomics Consortium, 2014) and the *popcorn* method for estimating cross-ancestry correlations(Brown et al., 2016). We compared estimates based on European and East-Asian schizophrenia samples by assuming an approximately normal distribution for r_g and obtaining a *Z*-score for the difference in values.

Polygenic scoring analyses

To test for polygenic effects on smoking behaviors or schizophrenia risk, we performed risk score profiling as previously described(Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). We constructed scores based on TAG results for smoking behaviors(Tobacco and Genetics Consortium, 2010). Given differences in the imputation reference panels between the TAG and PGC2 studies, we considered only overlapping SNPs with imputation INFO greater than 0.9 and minor allele frequency (MAF) greater than 1% in PGC2. Schizophrenia risk scores were generated for each study site in the PGC2 study of schizophrenia, using every other study as the training set in an iterative, “leave-one-out” procedure. This approach ensured no overlap in training and testing samples, while offering improved power compared to subdividing the full cohort into approximate halves. For both PGC-schizophrenia and TAG-based analyses, we computed several scores based on varying *P*-value threshold signifying the proportion of SNPs with smaller *P*-values in the training set; *P*-value thresholds (*Pt*) ranged between 0.0001 and 1.0. We tested for association between smoking behaviors and schizophrenia-PRS by linear regression, adjusting for sex, age, study-site and 10 associated ancestry principal components (PCs). Association between schizophrenia risk and TAG-based scores was assessed by logistic regression, adjusting for study-site and all covariates used in the primary PGC-schizophrenia association analysis. Because controls subjects from the Molecular Genetics of Schizophrenia (MGS) study were included in the TAG study, we excluded MGS from our case-control analyses of schizophrenia; in the context of genetic correlation estimation from summary statistics, this permitted us to constrain the intercept.

Genome-wide association and replication sample

For each trait, associated ancestry PCs were identified for the full cohort by backwards-stepwise regression ($P<0.159$), after adjusting for study site. We tested for association between SNPs and each trait by either linear or logistic regression, as implemented in PLINK v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/)(Purcell et al., 2007), using allelic dosages and adjusting for significant covariates including sex, age, and ancestry PCs. We performed GWAS of each trait separately for individual study sites, combining summary statistics in subsequent random-effects meta-analyses using METAL(Willer et al., 2010). We excluded all SNPs with MAF less than 0.01, average statistical imputation information (INFO) less than 0.6, absent from more than half of total number of sub-studies, or displayed evidence of excessive heterogeneity (Cochran’s test *P*-value < 0.05).

For replication efforts, a total of 1802 European-ancestry cases with complete phenotypic information from four independent “waves” were made available by Janssen Pharmaceuticals(Li et al., 2017; Metspalu et al., 2004). We identified independent (pairwise linkage disequilibrium $r^2 < 0.1$ within 500kb based on European 1000 Genomes Project samples), significant SNPs ($P < 10^{-5}$) from the random-effects meta-analyses of each

smoking behavior phenotype considered. We tested these SNPs for association by linear or logistic regression, using ancestry PCs, sex, age, and study site indicator as covariates. Subsequent, joint meta-analyses of the combined discovery and replication samples were performed using METAL.

Results

I. Genetic correlations between schizophrenia and smoking behaviors

We first estimated the genetic correlation (r_g) between schizophrenia (35,476 cases, 46,839 controls) and each smoking-related trait from TAG (Table 3). The estimated genetic correlation between schizophrenia and smoking initiation in the general population was positive and highly significant (r_g=0.159, 95% CI:[0.108,0.210]; P=5.05×10^{-10}); significant positive relationships between schizophrenia and both cigarettes-smoked-per-day (r_g=0.094, 95% CI:[0.027,0.161]; P=0.006) and age-of-onset of smoking (r_g=0.100, 95% CI: [0.026,0.174]; P=0.009) were also seen; a nominal association between schizophrenia and smoking cessation was found (r_g=−0.076, 95% CI:[−0.145,−0.007]; P= 0.032). We sought to replicate the observed genetic correlations using an independent cohort of East-Asian schizophrenia cases (n=1836) and controls (n=3383). No statistically significant cross-ancestry correlations were observed (Table 3) but confidence intervals overlapped with the results from the European ancestry cohorts. Results for the East-Asian cohort should be considered tentative until replication can be performed.

II. Association of smoking behavior polygenic risk scores with schizophrenia risk

We evaluated the predictive ability of polygenic scores based on TAG results for smoking behaviors as applied to the PGC study of schizophrenia (35,476 cases, 46,839 controls, Figure 1). Genome-wide scores for smoking initiation (“ever/never smoked”) were higher among cases (P_T< 0.3, β=0.014, 95%CI:[0.010, 0.017], P=4.94×10^{-15}), explaining 0.14% of the variance in schizophrenia risk. Scores based on independent SNPs significant at P_T< 10^{-5} in TAG for cigarettes-smoked-per-day were also significantly higher among schizophrenia cases compared to controls (β=0.026, 95%CI:[0.014, 0.038], P=3.58×10^{-5}), explaining 0.04% of the variance in schizophrenia risk. Although, this effect was attenuated at more inclusive P-value thresholds. Genome-wide scores based on TAG results for age-of-initiation of smoking were not associated with schizophrenia status (P>0.056). Scores based on TAG results for smoking cessation (“former vs current”) were significant, though only for P_T< 10^{-4} and were in the expected negative direction of effect (P_T< 10^{-5}, β=−0.130, 95%CI:[−0.222, −0.038], P=0.006).

We further investigated significant polygenic score associations in order to determine if they were driven by the chromosome 15q25 locus that has been independently associated with both schizophrenia risk and smoking quantity in the general population. SNPs from the 15q25 locus were removed from TAG polygenic scores (up to 171 SNPs depending on P_T) and were re-tested for association. Results remained robust for TAG-smoking initiation polygenic scores predicting schizophrenia (P_T< 0.3, β=0.014, 95%CI:[0.010, 0.017], P=1.13×10^{-13}) but associations with schizophrenia were attenuated for TAG-cigarettes-smoked-per-day (P_T< 10^{-5},β=0.015, 95%CI:[−0.025, 0.054], P=0.469), and smoking

cessation ($P_T < 10^{-5}$, β=−0.086, 95%CI:[−0.182, 0.011], P=0.081). These results suggest that the association seen between TAG-smoking initiation polygenic scores and schizophrenia were not due to confounding with the 15q25. However, associations of TAG-cigarettes-smoked-per-day and TAG-Cessation scores with schizophrenia were largely driven by this locus.

III. Smoking behavior among schizophrenia cases

The average smoking initiation rate across all cohorts was 72.9% and ranged from 52.6 to 77.3% (Table 2, Figure S1). Among schizophrenia cases that smoke 29.5% smoked more than a pack per day. Figure S2 displays prevalence of smoking quantity by cohort.

III.1 Heritability of smoking behavior among schizophrenia cases—

We applied the LD-score regression method to directly estimate SNP-based heritability (SNP-h^2) from GWAS summary statistics for smoking behaviors among schizophrenia cases. For neither smoking initiation nor cigarettes-smoked-per-day did observed inflation of genome-wide test statistics indicate confounding by population stratification, as indicated by regression intercept values close to one (0.998 and 0.999). Among schizophrenia cases, the SNP-based heritability of smoking initiation was estimated as 0.219 (95% CI:[−0.001,0.439]; P=0.051; n=5255); the corresponding estimate for cigarettes-smoked-per-day was 0.0917 (95% CI: [−0.096,0.280]; P=0.340; n=3370). That neither estimate was robustly statistically significant likely reflects the modest sample size. Although the SNP-based heritability point estimates for smoking behavior among schizophrenia cases were larger than the general population, their confidence intervals were overlapping (general population: smoking initiation SNP-h^2=0.075 (95% CI:[0.063,0.088], cigarettes-smoked-per-day SNP-h^2=0.056 (95% CI:[0.030,0.083]).[33]

III.2 Genetic correlations between smoking behaviors among schizophrenia cases and the general population—

We estimated the r_g to determine the magnitude of genetic overlap of smoking behaviors between schizophrenia cases ($n_{\text{smoking initiation}}$=5255, $n_{\text{cigarettes-smoked-per-day}}$=3370) and the general population (TAG). We observed a significant positive genetic correlation for smoking initiation (r_g=0.624, 95% CI: [0.228,1.020]; P=0.002). Though, a positive relationship for cigarettes-smoked-per-day was not statistically significant (r_g=0.689, 95% CI: [−0.179, 1.557]; P=0.120). Given the small sample size schizophrenia cases with data on smoking behaviors, these analyses are considered exploratory and require replication.

III.3 Association of smoking behavior polygenic risk scores with smoking behavior among schizophrenia cases—

We considered whether TAG scores for smoking initiation and cigarettes-smoked-per-day could predict smoking behaviors among schizophrenia subjects (Figure 1). TAG-based scores for smoking initiation significantly predicted initiation among schizophrenia cases ($P_T < 0.01$, β=0.087, 95%CI:[0.049, 0.126], P=9.57×10^{-6}, n=5255) accounting for 0.6% of the variance and results remained robust when SNPs from the 15q25 locus were removed from scores ($P_T < 0.01$, β=0.083, 95%CI: [0.042, 0.125], P=8.12×10^{-5}, Nagelkerke's pseudo-R^2=0.0046). The scores based on TAG results for cigarettes-smoked-per-day also significantly predicted cigarettes-smoked-per-day

among schizophrenia cases ($P_T < 0.01$, β=0.005, 95%CI:[0.002, 0.008], P=8.57×10^{-4}, n=3370) accounting for 0.35% of the variance and results remained robust when SNPs from the 15q25 locus were removed from scores ($P_T < 0.01$, β=0.006, 95%CI:[0.003, 0.009], P=4.42×10^{-4}, R^2=0.0039). For both smoking behaviors, the direction of the observed effect in schizophrenia was the same as that observed in the general population.

Next, we asked whether aggregate genetic risk of schizophrenia, as indexed by PGC2-based polygenic scores, was significantly associated with smoking behavior among schizophrenia cases (Figure S3). Neither smoking behaviors (smoking initiation, cigarettes-smoked-per-day) among schizophrenia-cases showed association with schizophrenia risk scores (P>0.1). Complete results for polygenic scoring analyses are reported in Tables S2-S7.

Finally, we estimated SNP-h^2 from GWAS summary statistics for schizophrenia stratified by smoking status. Among schizophrenia smokers (3832 schizophrenia-cases, 8518 controls), the heritability of schizophrenia was estimated as 0.237 (95% CI:[0.169,0.304]) and among schizophrenia non-smokers (1423 schizophrenia-cases, 8518 controls) was 0.133 (95% CI: [0.017,0.249]). The estimated genetic correlation between these groups (schizophrenia smokers and schizophrenia non-smokers) was 0.860 (95% CI: [0.497,1.224]) and was significantly different from 0 (P=3.52×10^{-6}) but not from 1. These results suggest that the genetic risk for schizophrenia is largely overlapping between smoking and non-smoking schizophrenia patients.

III.4 Genome-wide association of smoking behaviors among schizophrenia cases—Genomic inflation factors (λ) were 1.017 and 1.005 for smoking initiation (n=5255) and cigarettes-smoked-per-day (n=3370), respectively. The discovery GWAS did not yield SNP associations significant at established genome-wide criteria (5.0×10^{-8}). The strongest evidence of SNP-based association was observed for cigarettes-smoked-per-day, upstream of the *CBWD2* gene at chromosome 2q13 (rs1900325; P=1.01×10^{-7}). Subsequent follow-up of suggestively associated SNPs ($P < 10^{-6}$) in an independent European-ancestry cohort (n=1802) yielded a significant finding between cigarettes-smoked-per-day and rs148253479 upstream of the *TMEM106B* gene at 7p21.3 (Table 4, discovery P=1×10^{-6}, replication P=0.011, combined P=3.18×10^{-8}). Regional association and forest plots for top associations are provided in the accompanying supplemental information (Figures S7-8). Notably, none of the previously identified smoking behavior-associated SNPs were detected at genome-wide significant thresholds in our GWAS of smoking behaviors within schizophrenia cases, likely due in part to the limited power to detect small SNP effects in our modest sample size (Table S12).

IV. Phenotypic and polygenic associations between smoking behavior and schizophrenia symptom dimensions

We considered whether smoking patterns among schizophrenia cases and genetic risk factors for smoking were related to the clinical presentation of schizophrenia. For sex, age, and each clinical variable considered, Table 5 gives the estimated effect and significance from logistic or linear regression. Age-of-onset of schizophrenia was found to have a nominal association with smoking initiation (P=0.018) indicating higher rates of initiation in cases with earlier

onset. The positive symptom factor score showed a positive association with smoking initiation (P=3.21×10^{-5}) and cigarettes-smoked-per-day (P=0.015). Depressive symptoms were also nominally associated with cigarettes-smoked-per-day (P=0.014) indicating that those with higher depression scores endorsed smoking more cigarettes. No significant phenotypic associations were found between smoking behaviors and the negative and mania factor scores.

We followed-up phenotypic associations by examining the relationship between symptom dimensions and TAG-based polygenic scores for smoking behaviors (Tables S8-S11, Figure S4). Both the TAG-smoking initiation and TAG-cigarettes-smoked-per-day scores were associated with positive symptoms at nominal levels of significance (P=0.023, P=0.006 respectively).

Discussion

Despite conspicuous epidemiological and molecular genetic evidence supporting a link between smoking behavior and schizophrenia, the biological basis of this relationship is not well understood. Given the availability of subject-level clinical data from the PGC study of schizophrenia, we were able to characterize smoking patterns among >5000 schizophrenia cases. Using polygenic risk score methodology and genome-wide summary statistics, we not only provide confirmatory evidence of aggregate genetic effects contributing to both smoking initiation and risk of schizophrenia, but demonstrate also that risk factors influencing smoking initiation and quantity are at least partially shared between schizophrenia patients and the general population.

Of particular importance, we have successfully demonstrated shared genetic liabilities to schizophrenia and smoking behaviors in European populations. While polygenic scores based on TAG results for smoking initiation and cigarettes-smoked-per-day were both strongly associated with increased risk of schizophrenia, the association with cigarettes-smoked-per-day was largely driven by the 15q25 locus. Although the results support a polygenic overlap between smoking behavior in the general population and schizophrenia risk, we cannot definitively rule out the possibility that some identified schizophrenia genetic variants may be in fact indexing liability to smoking behavior (rather than having a pleiotropic effect on both traits) because of the high prevalence of nicotine use among affected persons. Future research is needed to disentangle this confounded relationship by collecting smoking behavior information for both schizophrenia cases *and* control subjects.

Polygenic scores for smoking initiation also significantly predicted initiation among schizophrenia cases. Taken together with an estimated genetic correlation of ~0.624, this suggests that genetic factors influencing smoking behavior are at least partially shared between schizophrenia and non-schizophrenia populations. We could rule out the possibility that they are entirely independent, but better powered studies are needed to more precisely estimate the degree of overlap. Similarly, polygenic scores for smoking quantity were also significantly predictive of smoking quantity among schizophrenia patients, albeit to a lesser degree of statistical significance.

By contrast, polygenic scores based on PGC results for schizophrenia were not predictive of smoking behavior among schizophrenia patients. Our results suggest a shared genetic liability to smoking behavior and schizophrenia, and that genetic liability to smoking is shared between the general population and schizophrenia patients, while liability to schizophrenia is *not* associated with smoking behavior among schizophrenia-affected individuals. The latter could be partially due to power and the restricted range of the smoking liability distribution among the selected schizophrenia population, as recent studies have found schizophrenia-PRS to be associated with smoking behavior in substance use enriched samples(Chen et al., 2016; Hartz et al., 2017).

Exploratory GWAS of smoking initiation and cigarettes-smoked-per-day among schizophrenia cases did not yield genome-wide significant evidence of association in the discovery stages. The top association was observed for cigarettes-smoked-per-day (rs1900325; $P=1.01\times10^{-7}$) was upstream of *CBWD2*, which has been previously implicated in sleep and metabolic traits(Doherty et al., 2018; Hammerschlag et al., 2017). In the replication phase, a single genome-wide significant association was observed between cigarettes-smoked-per-day and SNPs upstream of the *TMEM106B* gene, a much studied risk locus for frontotemporal lobar degeneration (FTLD)(Van Deerlin et al., 2010) that encodes a trans-membrane protein involved in lysosomal trafficking and dendritic branching(Brady et al., 2013; Schwenk et al., 2014). In addition to FTLD, the *TMEM106B* gene has demonstrated associations with the clinical presentation of Alzheimer disease(Rutherford et al., 2012), the volume of left-sided temporal lobe and interhemispheric structures(Adams et al., 2014), and amphetamine response(Hart et al., 2012). Although not genome-wide significant, our results support an association between the *CHRNA3/CHRNA5* locus (rs16969968) and cigarettes-smoked-per-day among schizophrenia cases (replication $P=0.0001$, Table S12). Interestingly, analysis of schizophrenia stratified by case smoking status revealed elevated odds ratio (and higher allele frequencies) for this SNP among schizophrenia cases that have ever smoked (Figure S9). Also notable is the lack of genome-wide significant associations between TAG-associated variants and smoking behaviors among schizophrenia cases, which could reflect our limited power to detect individual SNP effects in our current sample size (<35%; Table S12).

Consistent with the literature, schizophrenia cases had elevated smoking rates and smoked more cigarettes per day than smokers in the general population (>30 cigarettes: 16.5 versus 6.9% respectively)(de Leon and Diaz, 2005; Jamal et al., 2015). Earlier age of schizophrenia onset was associated with higher rates of smoking initiation. When examining clinical features of schizophrenia, the positive symptom factor score was associated with smoking behavior indicating that those endorsing hallucinations and delusions were more likely to initiate smoking and smoke more cigarettes. This is broadly consistent with the self-medication hypothesis, by itself does not tell against other etiological hypotheses, as it might represent a process by which symptoms might be reduced, irrespective of etiology. Pharmacological upregulation of nicotinic acetylcholinergic transmission using either acetylcholinesterase inhibitors or positive allosteric modulators (PAMs) have been shown to ameliorate symptoms of schizophrenia(Wallace and Bertrand, 2015). The clinical dimensions for which the literature most strongly supports a role for such treatments are negative and cognitive symptoms(Singh et al., 2012). However, animal models also support

a potential role in positive symptom-like features of ketamine-induced psychosis as well(Nikiforuk et al., 2016). Additionally, a nominal association was found between cigarettes-smoked-per-day and the depressive symptom dimension adding support for the role of nicotine on mood in schizophrenia patients. This might be consistent with an improvement in mood concomitant with an amelioration of symptoms of the illness overall. It might also represent an inherent antidepressant effect of agonizing nicotinic transmission, as has been suggested by studies using the forced swim test in rodents(Marcus et al., 2016; Onajole et al., 2016; Shang et al., 2016; Zhang et al., 2016).

The major limitation of this study was the number of available schizophrenia cases with detailed clinical and smoking-related data. Despite attaining a modest sample size for smoking analyses within schizophrenia cases, our power was limited to detect single-SNP associations with smoking initiation or cigarettes-smoked-per-day (Table S12). Another limitation was the use of self-report data to index smoking behavior among schizophrenia cases. Future research should incorporate objective nicotine metabolite biomarkers such as cotinine levels. The age of schizophrenia onset was determined retrospectively in some cases and since the duration of untreated psychosis can vary, the precision of the true onset is unknown. Also, because smoking data on control subjects was not available for the majority of participating studies, we were limited in our ability to relate findings from the smoking analyses within schizophrenia cases to variation in the general population. Recently, a large-scale GWAS from the GSCAN consortium reported 55, 378, and 99 associated genetic variants with cigarettes-smoked-per-day, smoking initiation, and alcohol drinks per week respectively(Liu et al., 2019). Forthcoming research needs to examine shared genetic risk of schizophrenia across substances as well as gender and diverse populations. As available sample sizes and phenotypic data grow(Pardiñas et al., 2018; Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020) it will be possible to apply alternative methods such as Mendelian Randomization to assess causal processes between these phenotypes. Nonetheless, findings suggest a portion of the schizophrenia-smoking association is due to shared genetic etiology, as we were able to demonstrate partial overlap between genetic liability to smoking behavior in the general population and (1) schizophrenia risk, and (2) smoking behavior among schizophrenia patients. In addition to supporting genome-wide pleiotropic effects, our smoking GWAS within schizophrenia cases highlighted a schizophrenia-specific genetic liability for smoking quantity. Future research needs to address mechanisms underlying associations between these traits (e.g., Mendelian randomization, pharmacogenetics) to aid both schizophrenia and smoking treatment and prevention efforts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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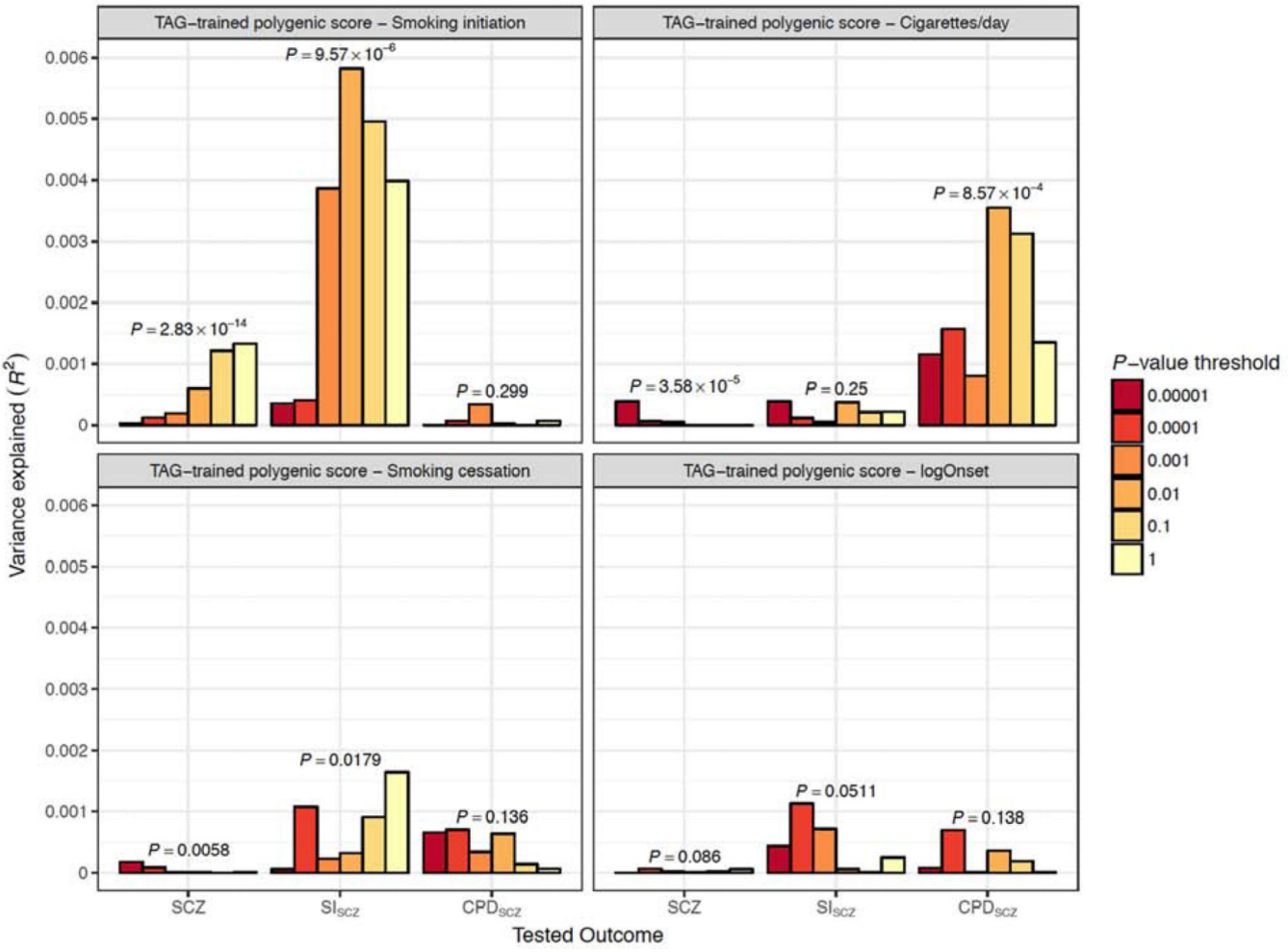

Figure 1. Association of TAG-based polygene scores with schizophrenia risk and smoking behaviors.

For polygenic scores based on analyses of smoking behaviors described by TAG, the variance explained for selected outcomes in PGC-schizophrenia is shown on the *y*-axis, in terms of Nagelkerke's pseudo-R^2(schizophrenia and smoking initiation) or R^2 (cigarettes-smoked-per-day); scores based on varying SNP *P*-value inclusion thresholds are displayed as colored bars. *logOnset* is log-transformed age-of-onset (see Methods).

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Table 1.

Conceptual overview of analyses of schizophrenia and smoking behaviors.

Research Question	Cohorts & Sample Sizes	Analysis
Question 1: Are there genetic correlations between schizophrenia and smoking behaviors?	*Primary* - PGC-Schizophrenia European ancestry: 35,476 cases, 46,839 controls; TAG-Smoking behaviors: smoking initiation 74,035, cigarettes-per-day 38,181, cessation 41,278, age of smoking onset 24,114	genetic correlation (LD score regression)
	Replication - PGC-Schizophrenia East-Asian ancestry: 1,836 cases, 3,383 controls	trans-ethnic genetic correlation (popcorn)
Question 2: Do polygenic risk scores for smoking behaviors also predict schizophrenia case status?	*Training Set* - TAG-Smoking behaviors: smoking initiation 74,035, cigarettes-per-day 38,181, cessation 41,278, age of smoking onset 24,114	polygenic risk scores (cross-trait association)
	Testing Set - PGC-Schizophrenia: 35,476 cases, 46,839 controls	
Question 3: What is the genetic architecture of smoking behavior among schizophrenia patients?		
3.1 What is the SNP-based heritability of smoking behaviors among schizophrenia cases?	PGC-Schizophrenia Phenotype Working Group - 10 study sites: smoking initiation 5,255, cigarettes-per-day 3,370	SNP-based heritability (LD score regression)
3.2 Are genetic factors for smoking behaviors shared between populations with and without schizophrenia?	PGC-Schizophrenia: smoking initiation 5,255, cigarettes-per-day 3,370; TAG-Smoking behaviors: smoking initiation 74,035, cigarettes-per-day 38,181	genetic correlation (LD score regression)
3.3 Do polygenic risk scores for smoking behaviors also predict these behaviors in schizophrenia patients?	*Training Set* - TAG-Smoking behaviors: smoking initiation 74,035, cigarettes-per-day 38,181	polygenic risk scores (within-trait across-cohort association)
	Testing Set - PGC-Schizophrenia: smoking initiation 5,255, cigarettes-per-day 3,370	
3.4 Are there schizophrenia-specific genetic risk variants for smoking behaviors?	*Primary* - PGC-Schizophrenia: smoking initiation 5,255, cigarettes-per-day 3,370	genome-wide association study meta-analysis of smoking behaviors among schizophrenia cases
	Replication - Janssen Pharmaceuticals: smoking initiation 1802, cigarettes-per-day 1802	
Question 4: Are there associations between smoking behaviors and clinical features of schizophrenia?		
4.1 Are smoking behaviors among schizophrenia cases associated with clinical presentation of schizophrenia?	PGC-Schizophrenia: smoking initiation 5,255, cigarettes-per-day 3,370, age-schizophrenia-onset 4,658; symptom dimension factor scores: positive 3,846, negative 3,845, manic 3,740, depression 3,740	phenotypic associations (linear/logistic regression)
4.2 Do polygenic risk scores for smoking behaviors predict schizophrenia symptom dimensions?	*Training Set* - TAG-Smoking behaviors: smoking initiation 74,035, cigarettes-per-day 38,181	polygenic risk scores (across-trait across-cohort association)
	Testing Set - PGC-Schizophrenia: age-schizophrenia-onset 11,600; symptom dimension factor scores: positive 8,330, negative 8,427, manic 6,965, depression 6,964	

PGC is Psychiatric Genomics Consortium, TAG is Tobacco and Genetics consortium, LD is linkage disequilibrium, SNP is single nucleotide polymorphism.

Table 2.

Sample characteristics for each PGC2-SCZ cohort.

Cohort	*N SCZ-cases*	*Sex % female*	Age (mean)	*N* % SI	*N CPD*
boco	756	43.5%	36.7	538 77.3%	185
cims	37	16.2%	33.3	33 66.7%	23
cou3	513	39.2%	44.2	501 75.0%	278
egcu	234	26.9%	46.5	234 52.6%	123
lie2	133	28.6%	36.9	128 60.2%	69
lie5	497	25.7%	36.6	479 63.3%	285
mgs2	2348	30.5%	43.5	2227 78.8%	1674
munc	420	36.4%	37.9	406 74.4%	301
top8	344	43.6%	33.0	320 57.5%	176
ucla	450	23.8%	34.7	389 70.7%	256
TOTAL	6183	6183	6132	5255	3370

Note: PGC = Psychiatric Genomics Consortium, SCZ - schizophrenia, Age = age at assessment, SI = smoking initiation, CPD = cigarettes per day.

Table 3.

Genetic correlations between TAG and PGC-SCZ phenotypes.

Trait 1	*Discovery*			*Replication*		
	Trait 2	***r_g* (se)**	***P***	***Trait 2***	***r_g* (se)**	***P'***
SI_{TAG}	SCZ_{EUR}	0.159 (0.026)	5.05×10^{-10}	SCZ_{EAS}	0.040 (0.124)	0.744
CPD_{TAG}	SCZ_{EUR}	0.094 (0.034)	0.006	SCZ_{EAS}	−0.080 (0.161)	0.619
$logOnset_{TAG}$	SCZ_{EUR}	0.100 (0.038)	0.009	SCZ_{EAS}	0.463 (0.251)	0.064
$Cessation_{TAG}$	SCZ_{EUR}	−0.076 (0.035)	0.032	SCZ_{EAS}	−0.051 (0.193)	0.793
SI_{TAG}	$SI_{SCZ,EUR}$	0.624 (0.202)	0.002	·	·	·
CPD_{TAG}	$CPD_{SCZ,EUR}$	0.689 (0.443)	0.120	·	·	·

For each pair of traits, r_g is the estimated genetic correlation; *P* is the significance of $r_g \neq 0$; *P'* is a 1-sided test of whether $r_g > 0$ or $r_g < 0$ in the replication sample. For comparisons of TAG phenotypes to SCZ risk, *EUR* and *EAS* denote European and East-Asian cohorts.

Table 4.

Association results for top SNP associations.

Trait	Chr	SNP	A1/ A2	*Discovery*			*Replication Phase*		Combined P	Gene (+/−Kb)
				Frq Info	Z	P *n*	*Z*	P *n*		
SI	1	rs58215884	T/G	0.162 0.975	4.53	5.9×10^{-6} 4991	2.09	0.037 1776	6.8×10^{-7}	*FCRL2* (+7.2)
	5	rs1592907	A/G	0.454 0.983	−4.44	9.2×10^{-6} 4991	−2.19	0.029 1760	8.5×10^{-7}	*FBXL17* (+91.2)
	9	rs117381175	T/C	0.031 0.792	−4.55	5.3×10^{-6} 4991	−2.29	0.022 1746	6.9×10^{-7}	intergenic
	13	rs754168	A/C	0.352 0.767	−4.85	1.2×10^{-6} 4991	−1.57	0.116 1277	4.6×10^{-7}	*LINC01044* (0)
CPD	1	rs3896119	A/G	0.018 0.832	4.77	1.9×10^{-6} 3321	1.45	0.148 1078	5.0×10^{-7}	intergenic
	1	rs1210	T/C	0.028 0.745	5.06	4.2×10^{-7} 3321	2.11	0.034 1052	5.2×10^{-8}	*RGS8* (+4.7)
	2	rs1900325	T/C	0.479 0.924	5.32	1.1×10^{-7} 3344	0.18	0.854 1011	2.0×10^{-6}	*CBWD2* (−39.4)
	3	rs833663	A/G	0.038 0.982	4.58	4.7×10^{-6} 3344	2.07	0.038 1097	4.7×10^{-7}	*CADPS* (0)
	7	rs148253479	A/C	0.988 0.694	−4.89	1.0×10^{-6} 2442	−2.54	0.011 1078	3.2×10^{-8}	*TMEM106B* (−22.6)

SI is smoking initiation, CPD is cigarettes-per day, SNP and Chr information for build hg19; INFO is the statistical imputation information; Freq is the frequency of the reference (first listed) allele, and *Z* is its estimated standardized effect; *P* is the *P*-value for association; *n* is the sample size. The nearest gene within 100Kb is shown; its position relative to a gene is given parenthetically and with respect to direction of transcription (negative and positive kb values indicate up- and downstream positions).

Table 5.

Association of smoking variables with clinical features in SCZ.

	Smoking initiation			Cigarettes per day		
	N	***β* (SE)**	***P***	***N***	***β* (SE)**	***P***
Sex	4991	−0.507 (0.069)	1.38×10^{-13}	3344	−0.210 (0.073)	0.004
Age	4991	−0.005 (0.003)	0.061	3344	0.018 (0.003)	5.53×10^{-10}
Age-of-onset	4658	−0.098 (0.038)	0.009	3168	−0.022 (0.021)	0.289
Positive	3846	0.157 (0.038)	3.23×10^{-5}	2796	0.071 (0.029)	0.015
Negative	3845	0.046 (0.038)	0.225	2794	−0.003 (0.029)	0.929
Mania	3740	0.034 (0.038)	0.367	2736	0.019 (0.019)	0.305
Depression	3740	−0.013 (0.038)	0.728	2735	0.047 (0.019)	0.014

For SCZ Age-of-onset and symptom factor scores, *N* is the number of subjects with non-missing data for both traits; *β* and SE are the beta regression coefficient and standard error from logistic or linear regression; *P* is the significance of the association between a given pair of traits.