Mendelian Randomization Causal Analysis

**Tobacco smoking is causally associated with antipsychotic medication use and schizophrenia, but not with antidepressant medication use or depression**

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Abstract

**Background:** Tobacco smoking is more common among patients with schizophrenia and depression than among healthy individuals. We tested the hypothesis that high tobacco smoking intensity is causally associated with antipsychotic medication use, schizophrenia, antidepressant medication use and/or depression in the general population, and compared results with those for chronic obstructive pulmonary disease.

**Methods:** We used self-reported smoking intensity in cigarettes/day and a polymorphism in the *CHRNA3* gene cluster (*rs1051730*) associated with smoking intensity, on 63,296 20–100-year-old individuals from the Danish general population; 23,282 were never-smokers and 40,014 ever-smokers. For schizophrenia, we compared our results with those in the Psychiatric Genomics Consortium.

**Results:** In smokers, heterozygotes (CT) and homozygotes (TT) for *rs1051730* genotype had higher smoking intensity compared with non-carriers (CC). Furthermore, in ever-smokers homozygotes had increased risk of antipsychotic medication with an odds ratio (OR) of 1.16 [95% confidence interval (CI) 1.02–1.31] compared with non-carriers, whereas in never-smokers the corresponding OR was 1.07 (0.87–1.31) (P-interaction: 0.60). Correspondingly, ORs were 1.60 (0.74–3.47) and 1.02 (0.11–9.10) for schizophrenia (P-interaction: 0.85), 1.02 (0.93–1.13) and 0.99 (0.85–1.15) for antidepressant medication (P-interaction: 0.87), 0.85 (0.66–1.10) and 1.26 (0.87–1.83) for depression (P-interaction: 0.30) and 1.31 (1.16–1.47) and 0.89 (0.58–1.36) for chronic obstructive pulmonary disease (P-interaction: 0.16). Odds ratios per *rs1051730* allele for schizophrenia and antipsychotic medication use in ever-smokers in the general population were 1.22 (95% CI: 0.84–1.79) and 1.06 (1.00–1.12). In the Psychiatric Genomics Consortium, the
corresponding OR for schizophrenia was 1.06 (1.04–1.08) in ever- and never-smokers combined.

**Conclusion:** Our data suggest that tobacco smoking could influence the development of psychotic conditions causally, whereas an influence on depression seems unlikely.

**Key words:** CHRNA3, schizophrenia, Mendelian randomization, general population, genetic variant, rs1051730

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**Key Messages**

- Increased smoking intensity per day and number of pack-years were associated with use of antipsychotic medication, schizophrenia, use of antidepressant medication and depression in observational analyses.
- The rs1051730 genotype in the nicotinic acetylcholine receptor gene cluster was associated with increased smoking and with use of antipsychotic medication in ever-smokers, but not in never-smokers. The effect sizes were similar for schizophrenia.
- The rs1051730 smoking genotype was not associated with use of antidepressant medication or depression in neither ever- nor never-smokers.
- These data suggest that tobacco smoking could influence the development of psychotic conditions causally, whereas an influence on depression seems unlikely.

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**Introduction**

Tobacco smoking is a well-established causal risk factor of several somatic diseases including chronic obstructive pulmonary disease, cardiovascular disease and lung cancer. Smoking has also been associated with major psychiatric diseases in large observational studies showing that tobacco smoking is much more common in patients with schizophrenia and depression than in individuals without psychiatric disease. A possible explanation is that patients with psychotic/depressive symptoms increase their smoking to relieve symptoms (reverse causation), but it has also been argued that smoking per se could contribute causally to high risk of psychiatric symptoms, maybe by affecting neurotransmitter activity in the brain. Thus, the directionality of these associations remains unclear.

A recent genome-wide association study of more than 150,000 individuals identified more than 100 genetic variants associated with schizophrenia, including a variant in the nicotinic acetylcholine receptor gene cluster CHRNA5-A3-B4 which is associated with smoking intensity but not with smoking initiation. One potential explanation for this finding is that the association between the genetic variant and schizophrenia is caused by the effect the genetic variant has on smoking intensity, that is, if smoking per se contributes causally to risk of schizophrenia.

We tested the hypothesis that high tobacco smoking is causally associated with antipsychotic medication use, schizophrenia, antidepressant medication use and depression in 63,296 individuals from the Danish general population, using a Mendelian randomization approach. The Mendelian randomization approach enables testing of causality between an exposure (e.g. tobacco smoking) and an outcome (e.g. schizophrenia and depression) by the use of genetic variants. Mendelian randomization exploits the random allocation of genetic variants during gamete formation, which subsequently results in different phenotypes (i.e. increased smoking intensity in individuals who choose to smoke). As such, it can be argued that the principle of a Mendelian randomization study is analogous to the principle of a randomized controlled trial where participants are randomized to either intervention or placebo, and the randomization at baseline ensures equal distribution of confounders and excludes the possibility of reverse causation. Consequently, if tobacco smoking is in fact causally related to schizophrenia, we would expect smokers with genetic variants associated with increased smoking to have a higher risk of schizophrenia or depression. Conversely, in never-smokers there is no behavioural difference between participants with different alleles of the variant and we would therefore not expect to see a difference in the schizophrenia risk.

In order to fully apply the Mendelian randomization approach, we conducted four different analyses. First, we tested whether high-intensity tobacco smoking was associated with each of the endpoints in observational analyses. Second, we tested whether a single nucleotide polymorphism close to the CHRNA3 gene, rs1051730, was associated with self-reported smoking intensity. Third, we tested...
whether the rs1051730 genotype was directly associated with each of the endpoints, stratified on smoking status. We included chronic obstructive pulmonary disease as a positive control. Finally, for genetic association with the endpoint schizophrenia, we compared our results with those from the Psychiatric Genomics Consortium.9

Methods
Participants
We examined 63,296 individuals from two independent general population studies, the Copenhagen General Population Study (CGPS) 2003–09 (n = 54,379) and the Copenhagen City Heart Study (CCHS) 1991–94 and/or 2001–03 examinations (n = 8917).16,17 Participants were 20–100 years old, White, of Danish descent and were randomly selected from the national Danish Civil Registration System18 to represent the Danish general population. Participants filled in a questionnaire which was reviewed together with an investigator on the day of attendance. Furthermore, all participants had a physical examination performed and had blood samples drawn for biochemical measurements and DNA extraction. If a participant appeared in more than one study (n = 329), only data from the first study were included (Supplementary Figure 1, available as Supplementary data at IJE online). Based on an individually unique identification number, we used the national Danish Civil Registration System to register emigration or death for all participants. Herlev Hospital and a Danish ethical committee (KF-100.2039/91 and H-KF-01-144/01) approved the studies. All participants gave written informed consent.

Tobacco smoking
Based on questionnaire information, participants were divided into current, former and never-smokers. Current or former smokers were asked about tobacco type (cigarettes, cheroots, cigars and/or pipe tobacco), amount smoked per day and duration of smoking in years. Based on this information, the number of cigarettes or equivalent smoked per day was calculated. Finally, cumulative cigarette consumption was calculated in pack-years (defined as 20 cigarettes or equivalents smoked per day for a year) for those participants who reported smoking duration.

Genotyping
DNA was isolated from leukocytes and stored at −45°C. We used the TaqMan® method (Applied Biosystems, Foster City, CA, USA)11,19 to genotype rs1051730 close to the CHRNA3 gene at the Department of Clinical Biochemistry, Herlev Hospital. Due to re-runs, the genotyping call rate was 99.9%. The genotype was in Hardy–Weinberg equilibrium.

Endpoints
From the national Danish Register of Medicinal Product Statistics, we obtained information about every prescription of antipsychotic or antidepressant medication claimed by study participants from 1995 through 2012. For antipsychotic medication, we used Anatomical Therapeutic Chemical (ATC) codes N05A (excluding lithium, N05AN, primarily used for bipolar disorder) and included all participants with a lifetime purchase of antipsychotic medication. For antidepressant medication, we used ATC codes N06A and only included participants who at some point in their life had purchased antidepressant medication for a period of at least 6 continuous months with an average daily dose of at least 0.75 of a standard World Health Organization- (WHO)-defined daily dose.20

Diagnoses of schizophrenia, depression and chronic obstructive pulmonary disease were obtained from the national Danish Patient Registry which has information on all hospital discharge diagnoses from psychiatric and somatic hospitals since 1977 and from emergency rooms and outpatient clinics since 1995,21 and from the national Danish Causes of Death Registry which has information on causes of death on all individuals in Denmark since 1970, including diagnoses at time of death.22 Schizophrenia was classified according to the International Classification of Diseases Eighth edition (ICD-8) codes 295.0–9 until 1994, and 10th edition (ICD-10) codes F20.0–9 from 1994 through 2011. Depression was ICD-8 codes 296.0, 296.2, 298.0 and 300.4, and ICD-10 codes F32 and F33. Chronic obstructive pulmonary disease was ICD-8 codes 491 and 492, and ICD-10 codes J41–J44.

Covariates
Covariates for adjustment were chosen because they were associated with smoking intensity and/or the endpoints studied. Participants reported on alcohol intake (weekly consumption of drinks; 1 drink ~12 g alcohol), weekly physical activity (0–2 h light; 2–4 h light; > 4 h light activity/2–4 h vigorous; and > 4 h vigorous), level of education after lower secondary school (no education; shorter education (less than 3 years); basic vocational training (1–3 years); higher education (≥3 years); university education), level of income (lowest; middle; highest) and civil status (married; unmarried; separated; widow/widower). Body mass index (BMI) was measured weight in kilograms divided by measured height in meters squared. Plasma levels of C-reactive protein (CRP)
were measured with a high-sensitivity assay using latex-enhanced turbidimetry (Dako, Glostrup, Denmark) or nephelometry (Dade Behring, Deerfield, IL) at the Department of Clinical Biochemistry, Herlev University Hospital. Chronic disease was ascertained by collecting information on diagnoses from the national Danish Patient Registry, the national Danish Cancer Registry and the national Danish Causes of Death Registry on ischaemic heart disease, myocardial infarction, stroke, diabetes, hypertension, cancer, pneumonia, chronic obstructive pulmonary disease, asthma, deep venous thrombosis and pulmonary embolism.

Statistical analyses

Stata version 12.1 (StataCorp, College Station, TX) was used for all statistical analyses. To achieve maximal statistical power, data from the CGPS and the CCHS were combined. However, when the two studies were analysed separately, results were similar, and we adjusted all analyses for study. For all analyses we used two smoking variables including all current and former smokers combined (= ever-smokers, to maximize statistical power), that is, cigarettes/day and pack-years. However, we also analysed current and former smokers separately. We conducted five different analyses (Figure 1).

First, we tested whether smoking intensity as cigarettes/day (0; 1–10; 11–20; > 20) and number of pack-years (0; 0.1–20; 20.1–40; > 40) were associated with each of the endpoints, using logistic regression models to calculate odds ratios (ORs) with 95% confidence intervals (CIs); these groups each consisted of a large group of participants and were chosen to achieve sufficient statistical power for all analyses. We used two different models of adjustment: (i) age and gender and (ii) multifactorially including age, gender, alcohol, physical activity, education, income, civil status, BMI, plasma CRP and chronic disease. For trend tests, smoking intensity categories were assigned the values of 1, 2, 3 and 4. We had > 99% complete data on all covariates. Missing values were imputed based on age and gender before multifactorial adjustment. We used the Stata command MI to carry out five imputations of each missing value which were combined using Rubin’s rules.

Second, we tested whether rs1051730 genotype was associated with smoking intensity as cigarettes/day and pack-years using a non-parametric P-trend.

Third, we tested whether rs1051730 genotype was directly associated with all endpoints in ever-smokers and in never-smokers, using unadjusted logistic regression models (observed genetic risk). Interaction by smoking status was tested by introducing a two-factor interaction term in the model, and subsequently comparing the two models using a likelihood ratio test.

Finally, we calculated odds ratios per rs1051730 allele for schizophrenia and antipsychotic medication use in ever-smokers in the CGPS and the CCHS combined, as well as for schizophrenia in ever- and never-smokers.
combined in the publicly available genome-wide association study from the Psychiatric Genomics Consortium [http://www.broadinstitute.org/mpg/ricopili/].

Results

Baseline characteristics of the 63,296 participants by smoking intensity as cigarettes/day or equivalent are listed in Supplementary Table 1 and by pack-years in Supplementary Table 2 (available as Supplementary data at IJE online). In total, 23,282 participants (37%) were never-smokers and 40,014 (63%) were ever-smokers. For ever-smokers, there was a median tobacco smoking of 13 cigarettes/day (range: 0.5–100) and of 17 pack-years (range: 0.03–300). In total, 3866 (6%) had purchased antidepressant medication for at least 6 months and 1067 (0.1%) had a hospital diagnosis of chronic obstructive pulmonary disease (Supplementary Figure 2, available as Supplementary data at IJE online). Higher smoking intensity was associated with all potential confounders (Supplementary Tables 4 and 5, available as Supplementary data at IJE online). In observational analyses, smoking intensity as cigarettes/day was associated with all endpoints ($P$-trend $= 1 \times 10^{-13}$ to $<1 \times 10^{-300}$) (Figure 2). For antipsychotic medication use, the multifactorially adjusted OR was 1.53 (95% CI 1.36–1.72) for participants with > 40 pack-years vs never-smokers. Corresponding ORs were 4.13 (1.61–10.6) for schizophrenia, 1.83 (1.66–2.00) for antidepressant medication use, 1.46 (1.19–1.80) for depression and 12.5 (10.8–14.6) for chronic obstructive pulmonary disease.

Association between rs1051730 genotype and smoking intensity

In total, 11% and 44% of participants were homozygotes (TT) and heterozygotes (CT) for the rs1051730 genotype (Figure 3 and Supplementary Table 4). For ever-smokers, homozygotes and heterozygotes smoked 15.6 and 14.5 cigarettes/day compared with 13.6 cigarettes/day for non-carriers (CC) ($P$-trend $= 1 \times 10^{-37}$) and had smoked 24.3 and 22.3 pack-years vs 20.6 pack-years for non-carriers ($P$-trend $= 1 \times 10^{-32}$).

Association between rs1051730 genotype and endpoints in 40,014 ever-smokers and 23,282 never-smokers

Among ever-smokers, homozygotes (TT) had increased risk of antipsychotic medication use with an unadjusted OR of 1.16 (95% CI 1.02–1.31) ($P$-trend $= 0.05$ from CC to CT to TT genotype) compared with non-carriers (CC) (Figure 3). Correspondingly, ORs were 1.60 (0.74–3.47) for schizophrenia ($P$-trend $= 0.30$), 1.02 (0.93–1.13) for antidepressant medication use ($P$-trend $= 0.75$), 0.85 (0.66–1.10) for depression ($P$-trend $= 0.44$) and 1.31 (1.16–1.47) for chronic obstructive pulmonary disease ($P$-trend $= 3 \times 10^{-6}$). Corresponding analyses stratified on current and former smokers are shown in Supplementary Figure 3 (available as Supplementary data at IJE online).

When we examined never-smokers, rs1051730 genotype was not associated with antipsychotic medication ($P$-trend $= 0.73$), schizophrenia ($P$-trend $= 0.86$), antidepressant medication use ($P$-trend $= 0.89$), depression ($P$-trend $= 0.13$) or chronic obstructive pulmonary disease ($P$-trend $= 0.73$) (Figure 4). For antipsychotic medication use, the unadjusted OR was 1.07 (0.87–1.31) for homozygotes compared with non-carriers. Corresponding ORs were 1.02 (0.11–9.10) for schizophrenia, 0.99 (0.85–1.15) for antidepressant medication use, 1.26 (0.87–1.83) for depression, and 0.89 (0.58–1.36) for chronic obstructive pulmonary disease.
### Figure 2. Observational associations between smoking intensity, antipsychotic medication use, schizophrenia, antidepressant medication use, depression and chronic obstructive pulmonary disease. Based on 63,296 participants from the Copenhagen General Population Study and Copenhagen City Heart Study combined. Multifactorial adjustment was for age, gender, alcohol consumption, physical activity, education, income, civil status, body mass index, plasma C-reactive protein and chronic disease.
pulmonary disease. However, although these results differ for those among ever-smokers with respect to antipsychotic medication use and chronic obstructive pulmonary disease, there was no formal evidence of statistical interaction. $P$ for interaction was 0.60 for antipsychotic medication use, 0.85 for schizophrenia, 0.87 for antidepressant medication use, 0.30 for depression, and 0.16 for chronic obstructive pulmonary disease.

Comparing results from the Psychiatric Genomics Consortium and the CGPS and CCHS

Odds ratios per $rs1051730$ allele for schizophrenia and antipsychotic medication use in ever-smokers in the CGPS and the CCHS combined were 1.22 (95% CI: 0.84–1.79) and 1.06 (1.00–1.12), compared with 1.06 (1.04–1.08) for schizophrenia in ever- and never-smokers combined in the Psychiatric Genomics Consortium (Figure 5).

Sensitivity analysis

When we adjusted the genetic analyses for body mass index, results were similar (data not shown). Furthermore, there was no interaction of CRP and genotype on the endpoints in ever- and never-smokers (Supplementary Figures 4–5, available as Supplementary data at IJE online). Finally, when we performed observational analysis prospectively using Cox proportional hazards regression models, results were similar (Supplementary Figure 6, available as Supplementary data at IJE online).

Discussion

The principal finding of this study of 63 296 participants (including 40 014 ever-smokers and 23 282 never-smokers) from the general population is that the $rs1051730$ genotype in the nicotinic acetylcholine receptor gene cluster may be associated with higher use of antipsychotic medication in smokers and with schizophrenia overall, but not in never-smokers; however, there was no statistical evidence of an interaction between smoking status and genotype with any of the endpoints. These data suggest that tobacco smoking could influence the development of psychotic conditions causally, whereas an influence on depression seems unlikely. These findings are novel.

Mechanistically, the present findings could possibly be explained by an effect of smoking/nicotine on neurotransmitter activity in the brain; however, the precise underlying mechanism is currently unclear. Alternatively, the association could be mediated by increased inflammation, as smoking increases inflammation and as inflammation has been suggested to be a risk factor for schizophrenia. In the present study, the associations between smoking intensity and all endpoints were still present after adjusting for plasma C-reactive protein levels (a common marker of inflammation), $rs1051730$ genotype was not associated with plasma C-reactive protein levels and there was no interaction of C-reactive protein and genotype on the endpoints. Despite this, we naturally cannot fully exclude inflammation as a mediating link between smoking and schizophrenia. Another possible explanation for the
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### Antipsychotic medication use

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### Chronic obstructive pulmonary disease

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**Figure 4.** Associations between CHRNA3 rs1051730 genotype and antipsychotic medication use, schizophrenia, antidepressant medication use, depression and chronic obstructive pulmonary disease in 40,014 ever-smokers and in 23,282 never-smokers from the Copenhagen General Population Study and Copenhagen City Heart Study combined. Odds ratios were unadjusted, because genotypes were not associated with measured potential confounders (see Supplementary Tables 4–6, available at IJE online).
association is that the association between the rs1051730 genotype, smoking and psychosis may be mediated by increased cannabis use in smokers. Several studies including a systematic review and meta-analysis have consistently reported that cannabis increases risk of psychotic outcomes independently of confounding factors and transient intoxication effects. It is, however, also possible that tobacco smoking per se could be associated with psychosis risk through some of the same mechanisms by which cannabis is believed to cause psychosis, e.g. through modulated activity of dopaminergic, GABAergic and glutamatergic neurons.

Tobacco smoking has consistently been associated with schizophrenia and depression in previous studies, but whether smoking per se is a causal risk factor in the development of these diseases is unknown. This is contrary to chronic obstructive pulmonary disease where it is well-known that smoking is the most important pathogenic factor. The direction of the association between smoking intensity and depression has been examined in previous studies: one study including 53 601 participants found no association between the rs1051730 genotype and depression in smokers. Similarly, a study of 6294 pregnant women found that women carrying the T-allele were less likely to stop smoking but also less likely to report depressive symptoms. Finally, a recent Mendelian randomization meta-analysis of 128 000 participants similarly found no evidence of a causal association between smoking and depression. These results together with our results do not support a causal role of tobacco smoking on depression, even though a causal role has been suggested. For schizophrenia, genetic variants in the 15q25 gene cluster have been associated with schizophrenia variation in the rs1051730 genotype has been associated with negative symptoms (e.g. low energy, lack of emotional reactivity) in patients with schizophrenia and most recently with schizophrenia per se, which raises confidence in the present findings. However, these studies did not include information on smoking status, which means that whether this association is likely explained by smoking per se could not be evaluated.

Mendelian randomization is a variant of a more general method of instrumental variables that has been developed in econometrics. In general, it requires fitting a specific model in which the genetic factor acts as a proxy or instrumental variable for the exposure variable (here smoking). This instrumental variable is then used to predict the outcome (here psychosis/depression). There are, however, important limitations in this method which relate to the identifiability of the instrumental variable. In particular, genetic factors which are only weakly related to the exposure variable may lead to misleading conclusions, that is, if weak instrumental variables are used or if the variants relate to multiple aspects of the exposure (in this case if the genetic factor relates to both number of cigarettes smoked and how heavily they are smoked). Indeed, because of these potential limitations of an instrumental variable analysis, we on purpose chose not to perform such an instrumental variable analysis, but only looked at the direct association between genotype and the outcome.

An important strength of this study is the large sample size which is required because genetic effects are typically small. Furthermore, we had information on smoking intensity, rs1051730 genotype and register-based endpoints of all hospitalizations with schizophrenia and depression, and all prescriptions of antipsychotic or antidepressant medication use in a single study. We were also able to compare results for the psychiatric endpoints with the positive control of register-based hospitalization with chronic obstructive pulmonary disease, for which we know smoking is a causal risk factor. Also, because of the completeness of the Danish registers we had no losses to follow-up during a period of up to 20 years. Finally, we are not aware of other studies which have examined the association between genetically higher tobacco smoking

Figure 5. Odds ratios for schizophrenia per CHRNA3 rs1051730 allele in 40 014 ever-smokers in the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS) combined, and in 150 064 ever- and never-smokers in the Psychiatric Genomics Consortium (PGC) genome-wide association study (GWAS). Odds ratios were unadjusted in both studies.
intensity and schizophrenia and use of antipsychotic medication.

Potential limitations of this study include that we did not have validated diagnostic scoring scales for schizophrenia or depression. Instead, we used two independent registers of hospitalizations with schizophrenia or depression and purchase of antipsychotic and antidepressant medication from Danish pharmacies. All hospital diagnoses of schizophrenia and depression are clinical diagnoses made by physicians based on ICD-8 or ICD-10 criteria, and thereby are likely to have a higher specificity than medication use. However, using only hospital discharge diagnoses might have underestimated the number of individuals with schizophrenia or depression, as individuals treated in general practice or by private psychiatrists was not included. As a consequence, we included information on prescription antipsychotic and antidepressant medication in an attempt to include these participants. Potential limitations for these endpoints are that both antipsychotics and antidepressants are being prescribed for several conditions other than schizophrenia and depression, respectively. In Denmark, approved indications for use of antipsychotic medication include treatment of schizophrenia, psychoses, affective disorders with psychotic symptoms, delirium, organic diseases with psychotic symptoms, personality disorders, anxiety disorders and certain somatic diseases such as pain disorders, migraine and cancer. A study of 47,724 patients in the UK using prescription antipsychotic medication showed that less than 50% had a diagnosis of a psychotic disorder or bipolar disorder and other indications for antipsychotic medication use were anxiety, depression, dementia and sleep disorders. For antidepressants, approved indications include treatment of depression, anxiety disorders, pain disorders, obsessive compulsive disorder, bulimia nervosa and smoking cessation. Off-label use might include treatment of sexual disorders, sleeping disorders and incontinence. A Dutch study of 13,835 patients with prescription antidepressant medication showed that 46% received antidepressant medication for depression, and another 17% for anxiety disorders. Accordingly, in an attempt to exclude participants with symptoms that were not severe enough to reach the criteria for a diagnosis of depression or participants treated for conditions other than depression, we chose only to include participants who had purchased antidepressants for at least 6 months, i.e. the recommended duration of continued treatment after clinical recovery. However, when we included all participants who had ever received antidepressant medication (20%), results were similar (data not shown).

Another potential limitation of this study is selection bias, as individuals with psychiatric disease may not participate as often as healthy individuals in a study of the general population like the present study. This can also be seen in the relatively low point prevalences of schizophrenia and depression (0.1% and 2%, respectively) in our study, whereas the prevalences in Denmark are 0.5–1% for schizophrenia and 16–17% (lifetime prevalence), and 2–3% (point prevalence) for depression. In contrast, 6% of the individuals in our study had purchased antipsychotic medication. A recent report from the Danish Pharmacy Association showed that in 2013, 2.3% of the population was treated with antipsychotic medication, with higher proportions among older individuals. The higher proportion in our study may be because our population mainly included older individuals.

Another limitation to our endpoints is that the individuals with a diagnosis of schizophrenia or depression in our study are highly unrepresentative of individuals with schizophrenia or depression in Denmark in general, as they may have a milder/less debilitating condition. Furthermore, possibly due to selection bias the participants with schizophrenia and depression were quite old at the time of onset in our study, and studies suggest that participants with late-onset disease may differ from those with early-onset. Furthermore, even though the diagnoses in the national Danish Patient Registry are of high quality in general, the validity has not been evaluated for psychiatric supplementary diagnoses (two-thirds of our diagnoses), which might have a less certain diagnostic validity. Finally, for both diseases but especially for schizophrenia, there can be a pronounced diagnostic delay and the diagnoses might change over time, which also contributes to the misclassification of the diagnoses. However, in general smoking onset preceded onset of all endpoints by many years in our study, arguing against reverse causation (that is disease leading to smoking, observationally or in the genetic analyses). We cannot, however, totally exclude that smoking onset occurred secondary to onset of the endpoints in a few individuals.

Another possible limitation of this study is the assumption that the rs1051730 genotype should influence the outcomes only through smoking. Studies have suggested that the rs1051730 genotype is associated with body mass index in both smokers and never-smokers, suggesting a pleiotropic effect of the genotype. In our own studies, however, we have found that the genotype was associated with body mass index in current smokers only, but not in former or never-smokers. We therefore cannot exclude that the association between antipsychotic medication use, schizophrenia and the genotype could be mediated through endocrine pathways (e.g. by lower body mass index) rather than through smoking, or that the genotype is in linkage disequilibrium with other variants which may influence
risk of antipsychotic medication use, schizophrenia, anti-depressant medication use, depression or chronic obstructive pulmonary disease through mechanisms other than through higher tobacco use. However, when we adjusted for body mass index in our genetic analyses, results were similar.

Furthermore, although this study is large, statistical power may nevertheless be limited given that common genetic variants account for only a small proportion of phenotypic variance, and that some of the outcomes investigated in this study (e.g. schizophrenia) are rare. It is notable that even for chronic obstructive pulmonary disease, the P-value for the interaction term is large, which may be due to limited power. This is probably because smoking is such a strong risk factor for chronic obstructive pulmonary disease, so that relatively few never-smokers will get chronic obstructive pulmonary disease. Thus, to detect an interaction of smoking on the association between genotype and chronic obstructive pulmonary disorder, we will need more never-smokers who develop chronic obstructive pulmonary disease. Also, we did not have information on drug abuse which could have confounded the observational associations. However, unless drug abuse is also associated with the rs1051730 genotype, this might not affect the Mendelian randomization analysis, but information on cannabis use in smokers vs non-smokers could help explain whether the association between the genotypes and psychosis was mediated by cannabis use. Finally, as we only included White participants, our results may not necessarily apply to other races; however, we are not aware of data to suggest that the present results should not be applicable to individuals of most races.

In conclusion, our data suggest that tobacco smoking could influence the development of psychotic conditions causally, whereas an influence on depression seems unlikely from the present data.

**Supplementary Data**

Supplementary data are available at IJE online.

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