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Investigating possible causal effects of externalising behaviours on tobacco initiation: A Mendelian randomisation analysis

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Abstract

Observational studies suggest childhood externalising disorders are associated with increased smoking and early age of initiation. However, causality cannot be inferred from observational data alone. The current study uses two-sample MR to examine the causal relationship between externalising behaviours and tobacco use.

Single nucleotide polymorphisms (SNPs) associated with aggression were obtained from The Early Life Epidemiology Consortium (mean age 8 years), ADHD from The Integrative Psychiatric Research and Psychiatrics Genomics Consortium (age range 6 to 18 years), and tobacco initiation and age of onset from The Tobacco and Genetics Consortium. SNPs were combined using inverse variance weighted approach, weighted median approach, and MR-Egger regression.

There was no clear evidence of an effect of aggression on tobacco initiation or age of onset for either childhood aggression (initiation: β -0.002, 95% CI -0.005, 0.001, P=0.286; age: β -0.001 95% CI -0.002, 0.000, P=0.310) or adolescent aggression (initiation: β -0.001, 95% CI -0.006, 0.003, P=0.610; age: β 0.000, 95% CI 0.000, 0.001, P=0.183)]. However, there was some evidence of an association of ADHD on tobacco initiation (OR 1.23, 95% CI 1.10, 1.35, P = 0.016), although no clear evidence of an effect of ADHD on age of onset (OR= 1.022, 95% CI 0.992, 1.052, P=0.215).

Our results provide some evidence that genetic risk of childhood ADHD is causally related to increased risk of tobacco initiation; however, the causal estimate is relatively small. We found no clear evidence that genetic risk of childhood aggression is causally related to risk of tobacco initiation or age of onset.

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Introduction

Externalising disorders such as conduct disorder (CD) and attention-deficit hyperactivity disorder (ADHD) are characterised by behaviours including aggressiveness, impulsivity, sensation seeking, and often criminal behaviour at older ages (Brazil et al., 2016, Goldstein et al., 2017, Holmes et al., 2001). Externalising disorders are strongly associated with substance use, including tobacco use (Brook et al., 2014, Kim et al., 2009, Brook et al., 2010), and externalising behaviours or symptoms below a clinical threshold are similarly associated with increased risk of smoking (Kollins et al., 2005). Individuals with externalising disorders have similar rates of smoking as other psychiatric disorders (~>40%), experience higher rates of smoking, earlier onset of smoking, and greater nicotine dependency (Pomerleau et al., 1995, John et al., 2004, Lasser et al., 2000, Bagot et al., 2007).

Externalising disorders are associated with deficits in reward function – reduced sensitivity to favourable stimuli and increased reward sensitivity. (Verdejo-Garcia et al., 2008). For example, ADHD is characterised by abnormal functioning of the striatal dopaminergic system and disruptions in dopaminergic transmission within corticostriatal circuits, resulting in executive functioning deficits (McClernon and Kollins, 2008, Solanto, 1998). These individuals display decreased dopamine (DA) tone from below-normal presynaptic activation of DA autoreceptors resulting in exaggerated DA release to salient stimuli (McClernon and Kollins, 2008). In both clinical and preclinical studies nicotine is shown to stimulate striatal DA release, suggesting nicotine-stimulated DA release may be more rewarding in individuals with ADHD (McClernon and Kollins, 2008, Corrigall et al., 1994, Brody et al., 2004). Therefore, individuals with ADHD may find higher levels of reward reinforcement from initial tobacco use, which may in turn facilitate the transition to continued use (McClernon and Kollins, 2008).

Additionally, externalising disorders are characterised by impulsive behaviour (behaviour that lacks foresight, evaluation, and inhibitory control). These individuals typically prefer immediate rewards (over possible larger rewards in the future) (Verdejo-Garcia et al., 2008). Therefore, impulsivity may lead individuals to choose rewarding stimuli despite the possibility of negative outcomes. A hypersensitive reward system may predispose individuals to early-onset substance use or mediate the transition from experimental to more frequent/ daily use (Aklin et al., 2009). Animal studies have supported this hypothesis, as rodents divided into 'high-impulsivity' and 'low impulsivity' groups based on attentional tasks show differential response to drug self-administration. High-impulsive displayed more rapid acquisition and higher rates of drug self-administration in response to decreased levels of dopamine D2-receptor binding (Dalley et al., 2007). These findings are mirrored in human PET studies where decreased striatum D2-receptor density is observed in addicted individuals, resulting in increased positive psychological and physiological response to initial drug exposure (Volkow et al., 1993, Volkow et al., 1996, Wang et al., 1996).

Tobacco may also be used for self-medication in individuals with ADHD to alleviate ADHD symptoms through negative reinforcement (Laucht et al., 2007, Rodriguez et al., 2008). Studies using transdermal nicotine patches alongside stimulant medicine have displayed increased attention capabilities in adult ADHD patients (Gehricke et al., 2006). Furthermore, studies using nicotine patches in non-smoking ADHD patients have displayed increases in attention and cognition similar to methylphenidate (Potter and Newhouse, 2004), improved behavioural inhabitation (Potter and Newhouse, 2004) and increased positive affect (Levin et al., 1996, Rodriguez et al., 2008, McClernon and Kollins, 2008).

However, the causal nature of this relationship, and the direction of causality, remain unclear. Evidence from longitudinal studies suggests that externalising behaviours in children are associated with subsequent early onset tobacco use (Korhonen et al., 2010, Brook et al., 2010, Crone and Reijneveld, 2007, Audrain-McGovern et al., 2004), and while reverse causality is unlikely in this context due to the onset of externalising disorders in

young children (prior to average smoking initiation ~age 15), residual confounding may still be operating (e.g. via intrauterine tobacco exposure). Experimental studies are typically impossible in substance use research for long-term outcomes for obvious ethical and practical reasons. While we can examine the association between externalising behaviours and tobacco use in observational studies, these have provided conflicting evidence on the temporal direction of association (Korhonen et al., 2010, Cadoret et al., 1983, Brook et al., 2010, Crone and Reijneveld, 2007, Hicks et al., 2009). Ultimately, however, conventional epidemiological methods using observational data cannot support strong causal inference as problems such as selection bias, reverse causation, and residual confounding are inherent in observational data (Davey Smith and Ebrahim, 2001). It is therefore important to consider alternative methods to support stronger causal inference, such as the use of negative controls, cross-contextual designs, and instrumental variable analysis (Gage et al., 2016b). One type of instrumental variable analysis is Mendelian randomisation (MR), in which genetic variants robustly predicting an exposure of interest are used as an unconfounded proxy for that exposure. Therefore, any associations found between these genetic variants and the outcome provide evidence of a casual effect of the exposure captured by the genetic variants on the outcome, without the risk of reverse causation or confounding. Two-sample MR uses variant-exposure associations identified via genome-wide association studies (GWAS) , and variant-outcome associations from another independent GWAS, using publicly-available summary data (Burgess et al., 2015). The current study uses two-sample MR to examine the causal relationship between externalising behaviours and tobacco initiation (Bowden et al., 2015).To compare across the spectrum of externalising disorders we used data available from both an aggression and an ADHD GWAS.

Methods

Exposure measures

For our externalising behaviours exposure we used summary data from the Early Life Epidemiology Consortium (EAGLE) consortium GWAS of aggressive behaviour on a sample of 18,988 individuals. Aggression was measured using continuous study-specific scales (Pappa et al., 2016). Six SNPs were identified as the top SNPs approaching genomewide significance ($P < 5 \times 10^{-5}$) for aggression. The GWAS further identified top SNPs stratified by age group, with five SNPs approaching genomewide significance for early childhood (mean age 5.36 years, SD 1.5), and six SNPs for middle childhood/early adolescence (mean age 11.39 years, SD 1.86) (see Supplementary Table S1).

For our ADHD exposure we used summary data from the Initiative for Integrative Psychiatric Research (iPSYCH) and Psychiatrics Genomics Consortium (PGC) GWAS of ADHD on 55,354 individuals (ages 6 to 19) (Demontis et al., 2017). ADHD was measured using binary cohort-specific diagnosis of ADHD. Fourteen independent SNPs (were identified as genomewide significant ($P < 5 \times 10^{-8}$) (see Supplementary Table S2).

Outcome measures

For our tobacco initiation outcome we used summary data from the Tobacco and Genetics Consortium (TAG) GWAS of smoking behaviour (Tobacco and Genetics, 2010). Tobacco initiation (n = 74,053) was a binary ever/never measure and age of initiation (n = 24,114) was the reported age at which participants started smoking.

Statistical analyses

SNPs associated with aggression and ADHD were identified in their respective GWAS and subsequently extracted from the tobacco GWAS (see Supplementary Tables S1-S2). Where original SNPs were unavailable, proxy SNPs were identified using SNiPA (http://snipa.helmholtz-muenchen.de/snipa3/) with an r^2 threshold of ≥ 0.9. SNP-exposure and SNP-outcome associations were combined using an inverse-variance weighted approach (IVW), weighted median approach, and MR-Egger regression. Here, we use

multiple methods, each with differing underlying assumptions regarding instrument validity, to triangulate our results (Lawlor et al., 2016). IVW weights regression of SNP-exposure and SNP-outcome coefficients restricting the intercept to zero, and assumes all instruments are valid with no pleiotropy (Burgess et al., 2013). Weighed median provides a causal estimate if at least 50% of the instruments are valid (Mostafavi, 2016). MR-Egger uses an intercept coefficient in the weighted regression to relax the assumption that the outcome works strictly via the exposure (i.e., up to 100% of the instruments may be invalid). The intercept term displays the overall pleiotropic effect, while the slope (β) coefficient displays a causal estimate under the assumption the pleiotropic effects of the SNP on the outcome are unrelated to the associations between the SNP and exposure (Corbin et al., 2016). Finally, I^2 statistics were calculated to determine heterogeneity between estimates from multiple genetic instruments, with higher numbers indicating greater heterogeneity which could indicate potential violations of the MR assumptions. Main findings are presented as IVW within the text**.** To avoid inference based simply on P-value thresholds, the direction and strength of effect for each association, together with the corresponding P-value, is presented (Sterne and Davey Smith, 2001). All analyses were conducted in R (version 3.3.2). Additionally, there was no direct overlap between samples. However, it is possible some parents in TAG may have offspring included in EAGLE.

Results

Aggression and tobacco initiation.

There was no clear evidence of an effect from genetic risk of aggression to tobacco use initiation (β_{IVW}<0.001, 95% CI -0.003 to 0.002, P = 0.810). While the MR-Egger analysis suggested there is some evidence of an effect on initiation, the effect estimates were inconsistent with those from the IVW and weighted median analyses (see Table 1 for full results). Findings were similar when looking at the effect from genetic risk of aggression to

age at onset, with no clear evidence of an effect observed (β_{IVW}<0.001, 95% CI: 0.000 to 0.001, P = 0.125). These findings were consistent when restricting to early childhood and late adolescence (see Table 1). There was moderate to high heterogeneity (see Table 1 for full results).

Inset Table 1 about here.

ADHD and tobacco initiation.

There was weak evidence of an association from genetic risk of ADHD to tobacco initiation (OR_{IVW} 1.23, 95% CI 1.10 to 1.35, P = 0.016), and low heterogeneity (I^2 = 29%). Although MR-Egger analysis indicated that there was some evidence of pleiotropy ($OR_{Intercept}$ 1.06, 95% CI 1.03 to 1.09, P = 0.020), the bias-adjusted effect estimate remained consistent with that estimated using the IVW approach and suggested some weak evidence of a causal effect (OR_{Slope} 1.40, 95% CI 1.10 to 1.78, P = 0.087). There was no clear evidence of an effect from genetic risk of ADHD to age of onset (see Table 2 for full results).

Insert Table 2 about here.

Power calculation.

Post-hoc power calculations were conducted for our IVW analyses using an online Mendelian randomisation power calculation tool (https://sb452.shinyapps.io/power/) (Burgess, 2014). For the analysis investigating the effect of aggression with tobacco use initiation, using a genetic instrument that explains 1% of the variation in aggression and a sample containing 28% smoking cases, our study has 77% power to detect a causal effect of

β = 0.1. Power was similar when looking at the effect of aggression on age at onset.- For the analysis investigating the effect of ADHD with tobacco use initiation, using a genetic instrument which explains 2% of the variation in ADHD and a sample containing 28% smoking cases, our study has 73% power to detect a causal effect of OR = 1.2. Power was similar when looking at the effect of ADHD on age at onset.

Discussion

Our results provide some evidence that genetic risk of childhood ADHD is causally related to increased risk of tobacco initiation; however, the causal estimate is relatively small. We found no clear evidence that genetic risk of childhood aggression is causally related to risk of tobacco initiation or age of onset. Therefore, the observational evidence relating to these associations (Kretschmer et al., 2014, Disney et al., 1999, Korhonen et al., 2010, Rohde et al., 2004) may not be causal and may be due to environmental or confounding factors.

Our findings with respect to genetic risk of ADHD and tobacco use initiation correspond with observational evidence (Breyer et al., 2014, Milberger et al., 1997, Kollins et al., 2005, Lambert and Hartsough, 1998), and the converging evidence from different MR methodologies provides further confidence in these findings. ~~that these findings are robust.~~ Neurobiological evidence also supports these causal associations (McClernon et al., 2008). However, as the MR-Egger analyses suggest some evidence of horizontal pleiotropy, the use of alternative causal inference methods relying on different assumptions (e.g. cross-contextual comparisons across populations where confounding structures differ) will allow triangulation to determine whether these results do reflect true causal relationships (Gage et al., 2015, Munafo and Davey Smith, 2018).

Within individuals with ADHD a hypoactive striatal dopaminergic system produces increased dopamine transporter within corticostrital circuits. This subsequently decreases

dopamine tone and activation of presynaptic autoreceptors, resulting in exaggerated dopamine response to salient stimuli. As nicotine stimulates response in the striatum of smokers, nicotine-stimulated phasic dopamine releases may be more rewarding for individuals with ADHD (Dresel et al., 2000, Krause et al., 2002, Krause et al., 2003, Grace, 2001). Therefore, this heighted reward response to dopamine in individuals with ADHD, may be responsible for reward and maintenance effects (Krause et al., 2000, Grace, 2001).

These findings may help identify individuals particularly vulnerable to tobacco use behaviours, and therefore provide interventions from an early age. For example, previous evidence indicates that strong familial bonds and open communication within families and schools may serve as a protective factor, or help to delay adolescent substance initiation (McArdle et al., 2002, Kliewer and Murrelle, 2007, Farrell and White, 1998, Spoth et al., 2004).

There are a number of important strengths to this study. The use of Mendelian randomisation analysis strengthens causal inference and removes the possibility of reverse causation, confounding, and bias (Gage et al., 2015, Gage et al., 2016a). Furthermore, we integrated multiple methods with varying assumptions and strengths to formulate more robust causal inferences (Gage et al., 2015). The use of two-sample MR utilizes large sample sizes to provide the sufficient power required to detect small effects in complex phenotypes. However, there are also some limitations to consider. Unlike childhood aggression, which was a measurable trait of externalising disorders, ADHD was only measured as binary diagnosis. Previous evidence has suggested that particular traits of ADHD (i.e., inattention or impulsivity/hyperactivity) are more associated with the increased likelihood of tobacco initiation (McClernon et al., 2008). However, the current GWAS only examined ADHD diagnosis and not by individual symptomology. A GWAS of these individual traits has been conducted in adulthood (Mick et al., 2014), but sample sizes were small and summary statistics were unavailable. Finally, although we've conducted

several analyses rather than correcting with a Bonferroni, we instead rely on consistent direction of effect rather than p values.

Overall our results provided some evidence that there may be a causal association of genetic risk of ADHD on tobacco initiation, these results are in line with previous longitudinal and neurobiological evidence. However, there was no association of early aggressive behaviour on tobacco initiation, suggesting these observed associations may be a result of additional variables. Our results suggest that prevention efforts should target these risk groups, and explore whether interventions to reduce these behaviours influence subsequent tobacco use initiation. By identifying at-risk individuals, prevention behaviours such as strengthening familial bonds, open communication within families and schools, and early intervention education may help delay or prevent adolescent substance use initiation. (Steinberg et al., 1994, McArdle et al., 2002, Dishion et al., 2002, Kliewer and Murrelle, 2007, Farrell and White, 1998, Spoth et al., 2004).

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