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Citation for final published version:

Jones, Hannah J., Hammerton, Gemma, McCloud, Tayla, Hines, Lindsey A., Wright, Caroline, Gage, Suzanne H., Holmans, Peter, Jones, Peter B., Smith, George Davey, Linden, David E. J., O'Donovan, Michael C., Owen, Michael J., Walters, James T., Munafò, Marcus R., Heron, Jon and Zammit, Stanley 2022. Examining pathways between genetic liability for schizophrenia and patterns of tobacco and cannabis use in adolescence. *Psychological Medicine* 52 (1), pp. 132-139. 10.1017/S0033291720001798

Publishers page: <http://dx.doi.org/10.1017/S0033291720001798>

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1 Examining pathways between genetic liability for schizophrenia and 2 patterns of tobacco and cannabis use in adolescence

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23 **Abstract word count: 246**

24 **Main body word count: 4348 (including in-text references)**

25

26

27 Abstract

28 **Background**

29 It is not clear to what extent associations between schizophrenia, cannabis use and cigarette use are due
30 to a shared genetic etiology. We therefore examined whether schizophrenia genetic risk associates with
31 longitudinal patterns of cigarette and cannabis use in adolescence, and mediating pathways for any
32 association to inform potential reduction strategies.

33 **Methods**

34 Associations between schizophrenia polygenic scores and longitudinal latent classes of cigarette and
35 cannabis use from ages 14 years to 19 years were investigated in up to 3925 individuals in the Avon
36 Longitudinal Study of Parents and Children. Mediation models were estimated to assess the potential
37 mediating effects of a range of cognitive, emotional, and behavioral phenotypes.

38 **Results**

39 The schizophrenia polygenic score, based on single nucleotide polymorphisms meeting a training-set
40 threshold of 0.05, was associated with late-onset cannabis use (OR=1.23; 95% CI=1.08,1.41), but not with
41 cigarette or early-onset cannabis classes. This association was not mediated through lower IQ,
42 victimization, emotional difficulties, antisocial behavior, impulsivity, or poorer social relationships during
43 childhood. Sensitivity analyses adjusting for genetic liability to cannabis or cigarette use, using polygenic
44 scores excluding the *CHRNA5-A3-B4* gene cluster, or basing scores on a 0.5 training-set p-threshold,
45 provided results consistent with our main analyses.

46 **Conclusions**

47 Our study provides evidence that genetic risk for schizophrenia is associated with patterns of cannabis
48 use during adolescence. Investigation of pathways other than the cognitive, emotional, and behavioural
49 phenotypes examined here is required to identify modifiable targets to reduce the public health burden
50 of cannabis use in the population.

51

52

53 **Keywords:** ALSPAC, polygenic score, cigarette-use, cannabis-use, schizophrenia, mediation

54

55

56 Introduction

57 Schizophrenia is a highly heritable, severe psychiatric disease with typical symptoms including positive
58 symptoms such as hallucinations, delusions and thought disorder, negative symptoms such as apathy
59 and avolition, and cognitive dysfunction. Genome-wide association studies (GWAS) provide strong
60 evidence of multiple independent loci contributing to the etiology of schizophrenia (Pardiñas et al.,
61 2018; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). However, whilst
62 individual loci have small effects on risk, multi-locus approaches show that cumulatively, even
63 moderately associated alleles explain at least a third of schizophrenia genetic risk (Purcell et al., 2009;
64 Ripke et al., 2013). Based on these alleles, an individual's genetic liability can be quantified using a
65 polygenic score, a valuable tool when investigating shared genetics between disorders and how genetic
66 risk is manifest throughout the life course (Hubbard et al., 2016; Jones et al., 2016).

67 Cannabis use is more common in individuals with schizophrenia than in the general population (Green,
68 Young, & Kavanagh, 2005), and a large body of evidence from observational (Moore et al., 2007) and
69 experimental (D'Souza et al., 2004) studies support a causal effect of cannabis use on psychosis.

70 However, some recent studies (Carey et al., 2016; Power et al., 2014; Reginsson et al., 2017; Verweij et
71 al., 2017), though not all (Guloksuz et al., 2019), have found that genetic liability to schizophrenia (as
72 captured by polygenic scores) is associated with cannabis use, suggesting that the association between
73 cannabis and schizophrenia might be partly genetically confounded, or represent a pathway from
74 schizophrenia risk to cannabis use. The latter may result from early manifestations of schizophrenia
75 liability that may increase an individual's likelihood to start using cannabis, for example, experiencing
76 difficulties with peers (Cannon et al., 2001; Malmberg, Lewis, David, & Allebeck, 1998). If a bi-directional
77 relationship does exist, then identifying the mechanisms by which schizophrenia genetic risk increases
78 risk of cannabis use could provide important insights about targets to prevent cannabis use in the
79 population, and particularly in those at genetically high risk for schizophrenia where cannabis reduction
80 is likely to lead to the greatest benefit in reducing population levels of schizophrenia.

81 Schizophrenia is also associated with a higher prevalence of tobacco smoking behaviors compared with
82 the general population (de Leon & Diaz, 2005; Dickerson et al., 2013). As such, the possibility that
83 cigarette smoking might increase risk for schizophrenia has gained attention (Gurillo, Jauhar, Murray, &
84 MacCabe, 2015), although recent work shows that evidence consistent with causal effects on psychotic
85 experiences are much stronger for cannabis use than they are for tobacco use (Jones et al., 2018).
86 Schizophrenia polygenic risk, and a schizophrenia GWAS hit in the *CHRNA5-A3-B4* gene cluster, are
87 associated with cigarette smoking phenotypes, including initiation, dependence and heaviness
88 (Reginsson et al., 2017; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014)
89 which, similar to the findings for cannabis use, raises questions regarding a shared genetic etiology and
90 direction of effect between cigarette use and schizophrenia, and the potential to gain insights into
91 mechanisms leading to cigarette use in the population.

92 We identified, *a priori* to any analyses, a number of potentially modifiable pathways through which
93 genetic risk for schizophrenia could theoretically lead to adolescent substance use. Based on evidence of
94 association with both genetic/familial risk for schizophrenia, and with substance use, these included
95 peer-victimization, poorer social relationships, deficits in cognitive ability and impulsivity, and emotional
96 or behavioral problems during childhood (Courtney, Mejia, & Jacobus, 2017; Varese et al., 2012;
97 Welham, Isohanni, Jones, & McGrath, 2009).

98 Whilst understanding whether genetic risk for schizophrenia is associated with specific patterns of
99 substance use, and the pathways involved in these relationships, could provide important insights into
100 the etiology of both schizophrenia and substance use disorders, disentangling such associations may be
101 hindered by measurement error in the outcomes, the high correlation between cigarette and cannabis
102 use that makes it difficult to study independent effects of these substances, and by experimental and
103 fluctuating use over time which are difficult to capture with single time-point assessments. To overcome
104 some of these difficulties, we previously used longitudinal latent class analysis (LLCA) of repeated
105 measurements of adolescent cigarette and cannabis use to identify subgroups of individuals based on
106 their use or co-use of cigarettes and cannabis and capture information on persistent use as opposed to

107 brief experimentation with these substances (Jones et al., 2018). The current study therefore aims to
108 use these latent classes to: i) examine whether schizophrenia genetic risk is associated with patterns of
109 cigarette and cannabis use in adolescence, and ii) examine whether genetic effects on substance use are
110 mediated via cognitive, social, emotional or behavioral pathways during childhood.

111

112 **Methods**

113 **Participants**

114 The sample consisted of participants from the Avon Longitudinal Study of Parents and Children (ALSPAC)
115 (see Supplementary Methods) (Boyd et al., 2013; Fraser et al., 2013). Details of available data are
116 accessible through a searchable data dictionary and variable search tool
117 (<http://www.bristol.ac.uk/alspac/researchers/data-access/data-dictionary>). Ethical approval for the
118 study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics
119 Committees (<http://www.bristol.ac.uk/alspac/researchers/research-ethics/>). Consent for biological
120 samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the
121 use of data collected via questionnaires and clinics was obtained from participants following the
122 recommendations of the ALSPAC Ethics and Law Committee at the time.

123 **Genetic data**

124 Genetic data were acquired using the Illumina HumanHap550 quad genome-wide single nucleotide
125 polymorphism (SNP) genotyping platform from 9912 participants. Following quality control assessment
126 and imputation (see Supplementary Methods), genetic data was available for 7977 ALSPAC individuals.

127 **Measures**

128 **Polygenic scores**

129 Polygenic scores for schizophrenia were constructed for each ALSPAC individual using data from the
130 most recent schizophrenia GWAS based on 40 675 cases and 64 643 controls (Pardiñas et al., 2018) as a

131 training set. Following quality control (see Supplementary Methods), polygenic scores were calculated
132 using the PLINK (v1.9) (Chang et al., 2015; Purcell et al., 2007) 'score' command following the
133 methodology described previously (Purcell et al., 2009).

134 For the primary analysis, scores were constructed using a list of SNPs with a GWAS training set p-value
135 threshold ≤ 0.05 , which optimally captures phenotypic variance in schizophrenia (Schizophrenia Working
136 Group of the Psychiatric Genomics Consortium, 2014). Scores were weighted by the logarithm of the
137 odds ratio (OR) for schizophrenia reported by the training set.

138 For sensitivity analyses, additional polygenic scores were created based on different GWAS training set
139 p-value thresholds ($P \leq 0.5$, $1e^{-5}$ and $5e^{-8}$ [genome-wide significant]) and after excluding the *CHRNA5-A3-*
140 *B4* nicotinic receptor gene cluster (chromosome 15: 78- 79.5Mb), a loci which is strongly associated with
141 smoking cigarette quantity and nicotine dependence (Saccone et al., 2009; Tobacco Genetics
142 Consortium, 2010) and also genome-wide significantly associated with schizophrenia (Pardiñas et al.,
143 2018; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Weighted polygenic
144 scores were also constructed for cigarette smoking initiation and cannabis use initiation using results
145 from the GWAS and Sequencing Consortium of Alcohol and Nicotine use (GSCAN) tobacco and alcohol
146 use GWAS ($n = 1\,232\,091$) (Liu et al., 2019) and from a cannabis use GWAS meta-analysis using data
147 from the International Cannabis Consortium (ICC) and UK Biobank ($n = 184\,765$) (Pasman et al., 2018),
148 respectively, using SNPs meeting a p-value threshold ≤ 0.5 in the training set GWAS. As ALSPAC was a
149 part of the GSCAN and ICC GWAS samples (Liu et al., 2019; Stringer et al., 2016), the SNPs and log ORs
150 used to generate and weight the polygenic scores were from results after removal of ALSPAC and, due
151 to data permissions, 23andMe from the GWAS meta-analyses.

152 Repeated measures of cigarette and/or cannabis use

153 Repeated measures of cigarette and/or cannabis use in ALSPAC were collected from clinic visits and
154 questionnaires between approximate ages 14 and 19 years. At each time point, individuals were asked
155 questions relating to their current use and frequency of use (see Supplementary Methods for more
156 detail). At each time point, data on cigarette and cannabis use were combined into a 3-category nominal

157 variable: “Non-users”, “Cigarette-only users” and “Cannabis users (either with or without cigarettes)” as
158 previously described (Jones et al., 2018).

159 Potential mediators

160 Potential mediators were selected on the basis that they are all premorbid antecedents of
161 schizophrenia, associated with familial risk of schizophrenia, and/or associated with substance use.
162 These included: IQ (assessed via the Wechsler Intelligence Scale for Children (Wechsler, Golombok, &
163 Rust, 1992) at age 8 years), victimization (from the Bullying and Friendship Interview Schedule (Wolke,
164 Woods, Bloomfield, & Karstadt, 2000) at age 8 years), emotional problems (Strengths and Difficulties
165 Questionnaire [SDQ] (Goodman, 1999) sub-scale score at age 9 years), antisocial behavior (assessed via
166 a short structured interview at age 10 years), impulsivity (number of incorrect items on the stop signal
167 task (150ms delay) (Handley, Capon, Beveridge, Dennis, & Evans, 2004) at age 10 years), friendship
168 quality (based on 5 items from the Cambridge Friendship Questionnaire (Baron-Cohen & Wheelwright,
169 2003) at age 12 years), and psychotic experiences (Psychosis-Like Symptom Interview [PLIKSi] (Horwood
170 et al., 2008) at age 12 years). For more information, see Supplementary Methods.

171 Statistical analysis

172 Longitudinal latent class analysis

173 Using the repeated, 3-category nominal variable of cigarette and cannabis use described above, LLCA
174 was used to derive distinct behavior patterns of cigarette and/or cannabis use as previously described
175 (Howe et al., 2017; Jones et al., 2018; Taylor et al., 2017) (see Supplementary Methods). Briefly,
176 Individuals were included in the analysis if they had cigarette and cannabis use data present for 3 or
177 more time points. Starting with one class, additional classes were added, and each time the model fit
178 assessed using proportion of individuals in each class, sample size adjusted Bayesian Information
179 Criterion (SSABIC) and Lo-Mendell-Rubin likelihood ratio test (LMR-LRT). The optimal number of classes
180 that explained the variation within the data was achieved. LLCA was performed using MPlus version 8
181 (Muthén & Muthén, 1998-2017).

182 Our previous study using the same data found that a 5-class solution adequately describes the combined
183 cigarette and cannabis use data between ages 14 to 19 years (Jones et al., 2018). The classes were
184 defined as: non-users, early-onset cigarette-only users, early-onset cannabis users (with or without
185 cigarette use), late-onset cigarette-only users and late-onset cannabis users (with or without cigarette
186 use)(Jones et al., 2018). Based on the patterns of class membership across time (see Jones *et al.*, 2018),
187 early-onset and late-onset substance use are approximately defined as higher probability of use
188 between approximate ages 14-16 years and higher probability of use between approximate ages 16-19
189 years, respectively (see Supplementary Figure 1).

190 Association analyses

191 Multinomial logistic regression was used to assess whether polygenic scores predicted latent class
192 membership. Associations were assessed using a manual implementation of the bias-adjusted three-
193 step method in MPlus (see Supplementary Methods and Heron *et al.* (2015) for more detail). Association
194 analyses were conducted using individuals who had cigarette and cannabis use data present for 3 or
195 more time points and genetic data.

196 To investigate whether associations between schizophrenia polygenic scores and latent class
197 membership were influenced by genetic overlap between variants associated with both schizophrenia
198 and cannabis or cigarette use, analyses were also adjusted for the cigarette smoking initiation and
199 cannabis use initiation polygenic scores.

200 As it was not possible to incorporate information on frequency of substance use in our 5-class latent
201 class approach as this resulted in an unstable model (Jones et al., 2018), we examined whether
202 schizophrenia polygenic scores were associated with frequency of cannabis or cigarette use using data
203 from single time-points. To aid future meta-analyses, the association between schizophrenia polygenic
204 scores and cannabis and cigarette ever versus never use were also investigated. The association
205 between schizophrenia polygenic scores and ever/never use and frequency of use were assessed using
206 logistic regression and ordered logistic regression, respectively, in Stata statistical software (version 15;
207 StataCorp LLC).

208 Mediation analysis

209 Mediation models were used to assess the direct effects of polygenic risk for schizophrenia on latent
210 class membership and indirectly through each potential mediator. Mediation models were run in MPlus
211 using a maximum likelihood estimator, and standard errors for indirect effects were calculated using a
212 non-parametric bootstrapping approach with 100 replications. As two of the mediators were
213 dichotomous measures, a counterfactual approach was implemented to allow for incorporation of the
214 dichotomous mediators with effect estimates that are easily interpretable (Valeri & VanderWeele,
215 2013). However, it is noted that for the models incorporating continuous mediators, this approach
216 simplifies to product of coefficient strategy as we did not allow for an interaction between exposure and
217 mediator.

218 Class reparameterization

219 As the main analyses were performed using multinomial logistic regression, the effect estimates are
220 interpreted as the strength of association between the exposure and each outcome class in relation to a
221 reference class, rather than the effect of the exposure on class membership in the whole population. To
222 address whether this influenced our results, we repeated all analyses after reparametrizing the
223 longitudinal latent classes (maintaining uncertainty in class membership) to examine, primarily, the
224 effects for late-onset cannabis use as compared to all other classes combined in a logistic regression.
225 Effects from these analyses therefore represent odds for membership in late-onset cannabis use class
226 compared to membership in any other latent class.

227

228 Results

229 There was strong evidence that genetic risk for schizophrenia differed across the combined cigarette use
230 and cannabis use latent classes (omnibus $p = 0.004$; Table 1). The schizophrenia polygenic score based
231 on SNPs meeting a training sample p -threshold of 0.05 was associated with late-onset cannabis use as
232 compared to non-use (OR = 1.23; 95% CI = 1.08, 1.41). There was also weak evidence of association with

233 decreased odds of late-onset cigarette-only use (OR = 0.87; 95% CI = 0.76, 1.00) as compared to non-
234 use, but little evidence of association with increased odds of early-onset cigarette-only use (OR = 1.13;
235 95% CI = 0.94, 1.36) or early-onset cannabis use (OR = 1.08; 95% CI = 0.87, 1.33). These associations
236 persisted after adjusting for cigarette smoking initiation and cannabis use initiation polygenic scores
237 (Table 1) which both showed evidence of association with the cigarette use and cannabis use latent
238 classes (omnibus $p < 0.001$; Supplementary Table 1).

239 Results were similar when excluding the *CHRNA5-A3-B4* gene cluster, and when using a more relaxed p-
240 value threshold for inclusion of SNPs into the schizophrenia polygenic score (p-value threshold ≤ 0.5).
241 However, evidence was weaker when using polygenic scores based on more stringent p-value
242 thresholds ($p \leq 1e^{-5}$ or $p \leq 5e^{-8}$) for SNP inclusion, that capture very little variance in liability to
243 schizophrenia (Supplementary Tables 2 and 3).

244 Results were also similar following reparameterization of classes with evidence of an increased genetic
245 liability for schizophrenia (p-value threshold ≤ 0.05) being associated with a 1.2-fold increase in odds
246 (95% CI = 1.05, 1.37) of late-onset cannabis use as compared to all other classes combined
247 (Supplementary Table 4).

248 Evidence of association between the schizophrenia polygenic score and ever/never substance use as
249 well as frequency of substance use was generally stronger for cannabis use than for cigarette use, and
250 also stronger for measures of frequency of use in late adolescence and early adulthood than for
251 measures of use in early adolescence (Supplementary Tables 5 and 6).

252 There was weak evidence that genetic risk for schizophrenia was associated with lower quality of
253 friendships (higher score indicates a lower friendship quality) (Beta = 0.06; 95% CI = -0.01, 0.13), and
254 lower IQ score in childhood (Beta = -0.05; 95% CI = -0.07, -0.02), but less so with emotional symptoms,
255 victimization, antisocial behavior or impulsivity (Supplementary Figures 2 and 3). There was evidence
256 that higher IQ and engagement in antisocial behavior were associated with an increased odds of late-
257 onset cannabis use (IQ: OR = 1.39; 95% CI = 1.18, 1.64; antisocial behavior: OR = 1.62; 95% CI = 1.02,

258 2.56). There was weaker evidence that a higher emotional symptoms score was associated with a
259 reduction in late-onset cannabis use (OR = 0.91; 95% CI = 0.82, 1.01) (Supplementary Figure 2).

260 In the mediation analysis, there was weak evidence that the effect of schizophrenia polygenic score on
261 IQ score at age 8 years acts to reduce the effect of schizophrenia genetic risk on late-onset cannabis use
262 (indirect effect through IQ at age 8 years: OR = 0.99; 95% CI = 0.97, 1.00), but little evidence that any
263 other mediators affected this pathway (Table 2). Results were also similar following reparameterization
264 of classes (Supplementary Table 7).

265

266 Discussion

267 We examined whether genetic risk for schizophrenia was associated with cigarette and cannabis use
268 during adolescence within a general population cohort and, where appropriate, tested for mediating
269 effects of a range of factors measured prior to our outcome measures. Our primary outcome measures
270 were latent classes summarizing the use of cigarettes and cannabis between ages 14 and 19 years. As
271 previously reported (Jones et al., 2018), our data was best summarized by 5 classes comprising
272 individuals with early-onset cigarette-only use, late-onset cigarette-only use, early-onset cannabis use,
273 late-onset cannabis use, and no use of either substance. In our primary analysis, using a training sample
274 p-threshold of 0.05 that optimally captures variance in schizophrenia liability, we found that
275 schizophrenia polygenic risk was most strongly associated with late-onset cannabis use. Early-onset
276 cigarette and cannabis use class estimates were compatible with the late-onset cannabis use estimate.
277 However, these estimates were less precise as the classes were substantially smaller and therefore
278 analyses had lower power. Interestingly, we found that schizophrenia polygenic risk was also associated
279 with a decreased odds of late-onset cigarette only use, however, this weak association did not survive
280 after class reparameterization.

281 Our findings are consistent with other studies showing that schizophrenia polygenic risk is associated
282 with cannabis use (Carey et al., 2016; Power et al., 2014; Reginsson et al., 2017; Verweij et al., 2017).

283 Furthermore, results from both our primary results and sensitivity analyses provide evidence that
284 genetic risk of schizophrenia is more strongly associated with cannabis use than with cigarette use.

285 One interpretation of our findings is that genetic risk for schizophrenia confers a risk of substance use
286 that is more specific for some drug classes than others, perhaps due to pleiotropic effects on more
287 substance-specific biological pathways than ones that are common across addictive behaviors. However,
288 as almost all individuals within the cannabis use class also use tobacco this class could just index a more
289 severe phenotype. Therefore, genetic risk for schizophrenia could confer a risk of multiple substance
290 use, for example through dopaminergic or opioid function that are biological pathways strongly
291 implicated across all addictive behaviours (Koob & Volkow, 2016).

292 It is also possible that the association with late-onset cannabis use is not due to pleiotropic effects of
293 addiction-related biological pathways, but due to behavioral manifestations of schizophrenia genetic risk
294 leading to adolescent use of cannabis. To explore this possibility we examined if the strongest
295 association we observed in our primary analysis, between schizophrenia genetic risk and late-onset
296 cannabis use, was mediated by lower childhood IQ, emotional problems, victimization, engagement in
297 antisocial behavior, impulsivity or poorer social relationships, all of which are characteristics associated
298 with increased risk of schizophrenia incidence or cannabis use (Courtney et al., 2017; Varese et al., 2012;
299 Welham et al., 2009). Our results suggested that little to none of this association was mediated through
300 these pathways, and indeed that 'direct' effects of schizophrenia genetic risk on late-onset cannabis use
301 may be stronger than first observed. However, this does not exclude the possibility that other variables
302 that we did not test mediate this relationship.

303 Whilst the cognitive, emotional, and behavioral characteristics we examined did not mediate the
304 relationship between schizophrenia genetic risk and cannabis use, identifying mediating phenotypes
305 expressed in childhood or adolescence is important not just for understanding the mechanisms
306 underlying addictive behavior, but also to inform potential targets for early intervention to prevent
307 substance use and harmful consequences of this. The mediators we examined were measured in
308 childhood, to ensure they occurred prior to substance use, hence minimizing bias in our models.

309 However, a potential limitation of this is that our results might not adequately reflect the relationship of
310 schizophrenia genetic risk with those same characteristics in adolescence, when they might have a more
311 immediate effect on substance use behavior.

312 The association we observe here between schizophrenia genetic risk and cannabis use suggests either
313 that the association between cannabis use and psychosis observed consistently in epidemiological
314 studies (Gage, Hickman, & Zammit, 2016; Moore et al., 2007) is, at least in part, due to pleiotropy, or
315 that cannabis has a causal effect on schizophrenia (and therefore risk variants for cannabis use will also
316 be identified as risk variants for schizophrenia (Gage, Davey Smith, Ware, Flint, & Munafò, 2016) in
317 adequately-powered GWASs where there would be many more cannabis users among cases than
318 controls). In fact, despite the finding from this and other studies that schizophrenia genetic risk is
319 associated with cannabis use, there is little evidence that shared genetic effects confound associations
320 between cannabis use and risk of psychotic outcomes in epidemiological studies. For example, in a
321 recent study we found strong evidence that classes of cannabis use were associated with subsequent
322 risk of psychotic experiences, and that this was not attenuated after adjusting for family history of
323 schizophrenia (Jones et al., 2018) or schizophrenia genetic risk score (Supplementary Table 8).

324 One approach that has been used to examine causal effects of cannabis use on schizophrenia and assess
325 the presence of genetic confounding (horizontal pleiotropy) is Mendelian randomization (MR). Evidence
326 consistent with a causal effect of schizophrenia risk on likelihood of cannabis initiation, as well as weak
327 evidence of a causal effect from cannabis initiation to schizophrenia has been reported (Gage, Jones, et
328 al., 2016; Pasman et al., 2018). Similarly analyses have reported a bidirectional relationship between a
329 measure of lifetime cigarette smoking (capturing smoking duration, heaviness and cessation) (Wootton
330 et al., 2018) and schizophrenia risk. However, when there is little understanding of the biological effects
331 of the genetic instruments used in MR analyses, bidirectional relationships such as these can be difficult
332 to interpret (Davey Smith & Hemani, 2014), and therefore neither MR studies to date, nor our results
333 here, lead to substantially stronger conclusions about the causal effects of cannabis and cigarettes on
334 psychosis than those from more traditional epidemiology designs.

335 Whilst our findings cannot address whether cannabis use has a causal effect on schizophrenia, our
336 results show that schizophrenia genetic liability does not lead to increased cannabis use through the
337 mechanisms examined here, and that the investigation of other pathways is required to identify
338 potentially modifiable targets to reduce the public health burden of cannabis use in the population.

339 **Strengths and limitations**

340 One of the strengths of our study is that we use a large, population-based cohort, with multiple
341 measures of cigarette and cannabis use data over the whole adolescent period, and thus our results are
342 much less prone to measurement error than if we had used single time-point measures of substance
343 use, although it likely still exists to some extent. Furthermore, using a latent class approach with
344 longitudinal data allows us to maximize use of data for individuals even where participation and
345 question response has been sporadic, and hence minimize potential selection bias, despite the
346 considerable levels of attrition over time. We also used the largest, most recent published GWASs of
347 schizophrenia, cigarette use and cannabis use as training sets for derivation of our polygenic scores.
348 Nevertheless, there are a number of limitations with our study.

349 Whilst our use of latent classes derived from information on the combined use of cigarettes and
350 cannabis use is useful for teasing out independent effects of schizophrenia genetic risk on these
351 outcomes, it was not possible to define a class of individuals who use cannabis without tobacco, as most
352 cannabis users smoke cannabis in combination with tobacco (Amos, Wiltshire, Bostock, Haw, & McNeill,
353 2004), even when they self-report as being cigarette non-smokers (Gage et al., 2014). Furthermore, we
354 have previously found that a substantial proportion of the people who smoke cigarettes most heavily
355 also use cannabis (Gage et al., 2014), and thus the cigarette-only class might not include those who have
356 been most heavily exposed to tobacco. Therefore, we cannot rule out whether the associations
357 observed between schizophrenia genetic risk and the late-onset cannabis use class is driven by heavier
358 cigarette use in these individuals than in those within the early-onset cigarette only or late-onset
359 cigarette only classes (although this would not be consistent with our sensitivity analyses).

360 Another limitation is that it was not possible to incorporate information on frequency of substance use
361 per time point within the combined cannabis and cigarette use model due to model instability. We
362 therefore also examined frequency of cigarette use and cannabis use using single time-point measures
363 and found no consistent evidence of association with genetic liability of schizophrenia, with the
364 exception of increase odds of cannabis use frequency at ages 17 to 19 years.

365 Furthermore, although we attempted to minimize genetic confounding by adjusting for cigarette and
366 cannabis initiation polygenic scores, heterogeneity between training set GWAS samples (i.e. differing
367 ages of participants) and substance use measures (i.e. measures combined experimental and regular
368 users into a single group) may have reduced their power to detect genetic associations. Furthermore,
369 polygenic scores for cigarette and cannabis use initiation explain only a small proportion of the variance
370 for these phenotypes in independent samples. Hence, adjusting for cigarette and cannabis initiation
371 polygenic scores may have not adequately removed confounding effects resulting from pleiotropy. It is
372 also possible that our mediation effects are underestimated due to residual confounding.

373 Finally, as our cohort only included data up to 19 years of age, it was not possible to examine effects of
374 schizophrenia genetic risk on longer-term patterns, or long-term cumulative use of cannabis or
375 cigarettes. Addressing these model limitations may become more tractable in the future.

376 **Conclusion**

377 In conclusion, our study provides evidence that genetic risk for schizophrenia is associated with patterns
378 of cannabis use during adolescence, and that this is not mediated through other measured phenotypic
379 manifestations of genetic risk for schizophrenia during childhood, including lower IQ, victimization,
380 increased emotional difficulties, antisocial behavior, impulsivity, or poorer social relationships. Evidence
381 of association between genetic risk for schizophrenia and cigarette use was weaker. Further studies
382 need to examine longer-term patterns of use of these substances over time to minimize measurement
383 error in allocation of substance use classes, and to establish the mechanisms by which these
384 associations arise to inform substance use reduction strategies.

385 Acknowledgments

386 We are extremely grateful to all the families who took part in this study, the midwives for their help in
387 recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory
388 technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

389 Financial support

390 The UK Medical Research Council (MRC) and Wellcome (grant number 102215/2/13/2) and the
391 University of Bristol provide core support for ALSPAC. A [comprehensive list of grants funding](#) is available
392 on the ALSPAC website. Measures used were specifically funded by the MRC (grant number
393 G0800612/86812) and the Wellcome Trust (grant numbers 076467/Z/05/Z, 086684, 092731). GWAS
394 data were generated by Sample Logistics and Genotyping Facilities at the Wellcome Trust Sanger
395 Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe. This study was
396 supported by MRC (grant number MR/M006727/1) and the National Institute for Health Research
397 (NIHR) Biomedical Research Centre at the University Hospitals Bristol National Health Service
398 Foundation Trust and the University of Bristol. The views expressed in this publication are those of the
399 author(s) and not necessarily those of the National Health Service, the National Institute for Health
400 Research or the Department of Health. HJJ, GH and MRM are members of the Medical Research Council
401 Integrative Epidemiology Unit at the University of Bristol funded by the MRC (grant numbers
402 MC_UU_00011/1, MC_UU_00011/7). GH is funded by a Sir Henry Wellcome Postdoctoral Fellowship
403 (grant number 209138/Z/17/Z). CW is funded by a Cancer Research UK Population Research
404 Postdoctoral Fellowship (grant number C60153/A23895).

405 Conflict of Interest

406 Professor O'Donovan received a consultancy fee from Roche in July 2015. All other authors have
407 declared no conflicts of interest.

408 Ethical standards

409 The authors assert that all procedures contributing to this work comply with the ethical standards of the
410 relevant national and institutional committees on human experimentation and with the Helsinki
411 Declaration of 1975, as revised in 2008.

412 References

- 413 Amos, A., Wiltshire, S., Bostock, Y., Haw, S., & McNeill, A. (2004). 'You can't go without a fag ... you need
414 it for your hash' - a qualitative exploration of smoking, cannabis and young people. *Addiction*,
415 99(1), 77-81. doi:DOI 10.1111/j.1360-0443.2004.00531.x
- 416 Baron-Cohen, S., & Wheelwright, S. (2003). The friendship questionnaire: an investigation of adults with
417 Asperger syndrome or high-functioning autism, and normal sex differences. *Journal of Autism*
418 *and Developmental Disorders*, 33(5), 509-517. Retrieved from
419 <https://www.ncbi.nlm.nih.gov/pubmed/14594330>
- 420 Boyd, A., Golding, J., Macleod, J., Lawlor, D. A., Fraser, A., Henderson, J., . . . Davey Smith, G. (2013).
421 Cohort Profile: the 'children of the 90s' - the index offspring of the Avon Longitudinal Study of
422 Parents and Children. *International Journal of Epidemiology*, 42, 111-127.
423 doi:10.1093/ije/dys064
- 424 Cannon, M., Walsh, E., Hollis, C., Kargin, M., Taylor, E., Murray, R. M., & Jones, P. B. (2001). Predictors of
425 later schizophrenia and affective psychosis among attendees at a child psychiatry department.
426 *British Journal of Psychiatry*, 178, 420-426. doi:DOI 10.1192/bjp.178.5.420
- 427 Carey, C. E., Agrawal, A., Bucholz, K. K., Hartz, S. M., Lynskey, M. T., Nelson, E. C., . . . Bogdan, R. (2016).
428 Associations between polygenic risk for psychiatric disorders and substance involvement.
429 *Frontiers in Genetics*, 7. doi:10.3389/fgene.2016.00149
- 430 Chang, C. C., Chow, C. C., Tellier, L. C. A. M., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-
431 generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*, 4.
432 doi:10.1186/s13742-015-0047-8
- 433 Courtney, K. E., Mejia, M. H., & Jacobus, J. (2017). Longitudinal studies on the etiology of cannabis use
434 disorder: a review. *Current addiction reports*, 4(2), 43-52. doi:10.1007/s40429-017-0133-3
- 435 D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., . . . Krystal, J. H. (2004).
436 The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals:
437 implications for psychosis. *Neuropsychopharmacology*, 29(8), 1558-1572.
438 doi:10.1038/sj.npp.1300496
- 439 Davey Smith, G., & Hemani, G. (2014). Mendelian randomization: genetic anchors for causal inference in
440 epidemiological studies. *Human Molecular Genetics*, 23(R1), R89-98. doi:10.1093/hmg/ddu328
- 441 de Leon, J., & Diaz, F. J. (2005). A meta-analysis of worldwide studies demonstrates an association
442 between schizophrenia and tobacco smoking behaviors. *Schizophrenia Research*, 76(2-3), 135-
443 157. doi:10.1016/j.schres.2005.02.010
- 444 Dickerson, F., Stallings, C. R., Origoni, A. E., Vaughan, C., Khushalani, S., Schroeder, J., & Yolken, R. H.
445 (2013). Cigarette smoking among persons with schizophrenia or bipolar disorder in routine
446 clinical settings, 1999-2011. *Psychiatric Services*, 64(1), 44-50. doi:10.1176/appi.ps.001432012
- 447 Fraser, A., Macdonald-Wallis, C., Tilling, K., Boyd, A., Golding, J., Davey Smith, G., . . . Lawlor, D. A.
448 (2013). Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers
449 cohort. *International Journal of Epidemiology*, 42, 97-110. doi:10.1093/ije/dys066
- 450 Gage, S. H., Davey Smith, G., Ware, J. J., Flint, J., & Munafò, M. R. (2016). G = E: what GWAS can tell us
451 about the environment. *Plos Genetics*, 12(2). doi:10.1371/journal.pgen.1005765
- 452 Gage, S. H., Hickman, M., Heron, J., Munafò, M. R., Lewis, G., Macleod, J., & Zammit, S. (2014).
453 Associations of cannabis and cigarette use with psychotic experiences at age 18: findings from

454 the Avon Longitudinal Study of Parents and Children. *Psychological Medicine*, 44(16), 3435-
455 3444. doi:10.1017/S0033291714000531

456 Gage, S. H., Hickman, M., & Zammit, S. (2016). Association between cannabis and psychosis:
457 epidemiologic evidence. *Biological Psychiatry*, 79(7), 549-556.
458 doi:10.1016/j.biopsych.2015.08.001

459 Gage, S. H., Jones, H. J., Burgess, S., Bowden, J., Davey Smith, G., Zammit, S., & Munafò, M. R. (2016).
460 Assessing causality in associations between cannabis use and schizophrenia risk: a two-sample
461 Mendelian randomization study. *Psychological Medicine*, 1-10.
462 doi:10.1017/S0033291716003172

463 Goodman, R. (1999). The extended version of the strengths and difficulties questionnaire as a guide to
464 child psychiatric caseness and consequent burden. *Journal of Child Psychology and Psychiatry,*
465 *and Allied Disciplines*, 40(5), 791-799. doi:Doi 10.1017/S0021963099004096

466 Green, B., Young, R., & Kavanagh, D. (2005). Cannabis use and misuse prevalence among people with
467 psychosis. *British Journal of Psychiatry*, 187, 306-313. doi:DOI 10.1192/bjp.187.4.306

468 Guloksuz, S., Pries, L. K., Delespaul, P., Kenis, G., Luyckx, J. J., Lin, B. C. D., . . . van Os, J. (2019). Examining
469 the independent and joint effects of molecular genetic liability and environmental exposures in
470 schizophrenia: results from the EUGEI study. *World Psychiatry*, 18(2), 173-182.
471 doi:10.1002/wps.20629

472 Gurillo, P., Jauhar, S., Murray, R. M., & MacCabe, J. H. (2015). Does tobacco use cause psychosis?
473 Systematic review and meta-analysis. *Lancet Psychiatry*, 2(8), 718-725. doi:10.1016/S2215-
474 0366(15)00152-2

475 Handley, S. J., Capon, A., Beveridge, M., Dennis, I., & Evans, J. S. T. (2004). Working memory, inhibitory
476 control and the development of children's reasoning. *Thinking & Reasoning*, 10(2), 175-195.
477 doi:10.1080/13546780442000051

478 Heron, J. E., Croudace, T. J., Barker, E. D., & Tilling, K. (2015). A comparison of approaches for assessing
479 covariate effects in latent class analysis. 2015, 6(4), 15. doi:10.14301/llcs.v6i4.322

480 Horwood, J., Salvi, G., Thomas, K., Duffy, L., Gunnell, D., Hollis, C., . . . Harrison, G. (2008). IQ and non-
481 clinical psychotic symptoms in 12-year-olds: results from the ALSPAC birth cohort. *British Journal*
482 *of Psychiatry*, 193, 185-191. doi:10.1192/bjp.bp.108.051904

483 Howe, L. J., Trela-Larsen, L., Taylor, M., Heron, J., Munafò, M. R., & Taylor, A. E. (2017). Body mass index,
484 body dissatisfaction and adolescent smoking initiation. *Drug and Alcohol Dependence*, 178, 143-
485 149. doi:10.1016/j.drugalcdep.2017.04.008

486 Hubbard, L., Tansey, K. E., Rai, D., Jones, P., Ripke, S., Chambert, K. D., . . . Zammit, S. (2016). Evidence of
487 common genetic overlap between schizophrenia and cognition. *Schizophrenia Bulletin*, 42(3),
488 832-842. doi:10.1093/schbul/sbv168

489 Jones, H. J., Gage, S. H., Heron, J., Hickman, M., Lewis, G., Munafò, M. R., & Zammit, S. (2018).
490 Association of combined patterns of tobacco and cannabis use in adolescence with psychotic
491 experiences. *JAMA Psychiatry*, 75(3), 240-246. doi:10.1001/jamapsychiatry.2017.4271

492 Jones, H. J., Stergiakouli, E., Tansey, K. E., Hubbard, L., Heron, J., Cannon, M., . . . Zammit, S. (2016).
493 Phenotypic manifestation of genetic risk for schizophrenia during adolescence in the general
494 population. *JAMA Psychiatry*, 73(3), 221-228. doi:10.1001/jamapsychiatry.2015.3058

495 Koob, G. F., & Volkow, N. D. (2016). Neurobiology of addiction: a neurocircuitry analysis. *Lancet*
496 *Psychiatry*, 3(8), 760-773. Retrieved from <Go to ISI>://WOS:000382276500025

497 Liu, M. Z., Jiang, Y., Wedow, R., Li, Y., Brazel, D. M., Chen, F., . . . Vrieze, S. (2019). Association studies of
498 up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol
499 use. *Nature Genetics*, 51(2), 237-244. doi:10.1038/s41588-018-0307-5

500 Malmberg, A., Lewis, G., David, A., & Allebeck, P. (1998). Premorbid adjustment and personality in
501 people with schizophrenia. *British Journal of Psychiatry*, 172, 308-313; discussion 314-305.
502 Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/9715332>

503 Moore, T. H. M., Zammit, S., Lingford-Hughes, A., Barnes, T. R. E., Jones, P. B., Burke, M., & Lewis, G.
504 (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic
505 review. *Lancet*, 370(9584), 319-328. doi:Doi 10.1016/S0140-6736(07)61162-3

506 Muthén, L. K., & Muthén, B. O. (1998-2017). *MPlus user's guide. Eighth Edition*. Los Angeles, CA: Muthén
507 & Muthén.

- 508 Pardiñas, A. F., Holmans, P., Pocklington, A. J., Escott-Price, V., Ripke, S., Carrera, N., . . . Walters, J. T. R.
509 (2018). Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions
510 under strong background selection. *Nature Genetics*, *50*(3), 381-389. doi:10.1038/s41588-018-
511 0059-2
- 512 Pasman, J. A., Verweij, K. J. H., Gerring, Z., Stringer, S., Sanchez-Roige, S., Treur, J. L., . . . Vink, J. M.
513 (2018). GWAS of lifetime cannabis use reveals new risk loci, genetic overlap with psychiatric
514 traits, and a causal influence of schizophrenia. *Nature Neuroscience*, *21*(9), 1161 - 1170.
515 doi:10.1038/s41593-018-0206-1
- 516 Power, R. A., Verweij, K. J. H., Zuhair, M., Montgomery, G. W., Henders, A. K., Heath, A. C., . . . Martin, N.
517 G. (2014). Genetic predisposition to schizophrenia associated with increased use of cannabis.
518 *Molecular Psychiatry*, *19*(11), 1201-1204. doi:10.1038/mp.2014.51
- 519 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., . . . Sham, P. C. (2007).
520 PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J*
521 *Hum Genet*, *81*, 559-575. doi:10.1086/519795
- 522 Purcell, S., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., Sullivan, P. F., . . . International
523 Schizophrenia Consortium. (2009). Common polygenic variation contributes to risk of
524 schizophrenia and bipolar disorder. *Nature*, *460*, 748-752. doi:10.1038/nature08185
- 525 Reginsson, G. W., Ingason, A., Euesden, J., Bjornsdottir, G., Olafsson, S., Sigurdsson, E., . . . Stefansson, K.
526 (2017). Polygenic risk scores for schizophrenia and bipolar disorder associate with addiction.
527 *Addiction Biology*. doi:10.1111/adb.12496
- 528 Ripke, S., O'Dushlaine, C., Chambert, K., Moran, J. L., Kähler, A. K., Akterin, S., . . . Sullivan, P. F. (2013).
529 Genome-wide association analysis identifies 14 new risk loci for schizophrenia. *Nature Genetics*,
530 *45*(10), 10.1038/ng.2742. doi:10.1038/ng.2742
- 531 Saccone, N. L., Wang, J. C., Breslau, N., Johnson, E. O., Hatsukami, D., Saccone, S. F., . . . Bierut, L. J.
532 (2009). The CHRNA5-CHRNA3-CHRNA4 nicotinic receptor subunit gene cluster affects risk for
533 nicotine dependence in African-Americans and in European-Americans. *Cancer Research*, *69*(17),
534 6848-6856. doi:10.1158/0008-5472.CAN-09-0786
- 535 Schizophrenia Working Group of the Psychiatric Genomics Consortium. (2014). Biological insights from
536 108 schizophrenia-associated genetic loci. *Nature*, *511*, 421-427. doi:10.1038/nature13595
- 537 Stringer, S., Minica, C. C., Verweij, K. J. H., Mbarek, H., Bernard, M., Derringer, J., . . . Vink, J. M. (2016).
538 Genome-wide association study of lifetime cannabis use based on a large meta-analytic sample
539 of 32330 subjects from the International Cannabis Consortium. *Translational Psychiatry*, *6*.
540 doi:10.1038/tp.2016.36
- 541 Taylor, M., Collin, S. M., Munafò, M. R., MacLeod, J., Hickman, M., & Heron, J. (2017). Patterns of
542 cannabis use during adolescence and their association with harmful substance use behaviour:
543 findings from a UK birth cohort. *Journal of Epidemiology and Community Health*.
544 doi:10.1136/jech-2016-208503
- 545 Tobacco Genetics Consortium. (2010). Genome-wide meta-analyses identify multiple loci associated
546 with smoking behavior. *Nature Genetics*, *42*(5), 441-447. doi:10.1038/ng.571
- 547 Valeri, L., & VanderWeele, T. J. (2013). Mediation analysis allowing for exposure-mediator interactions
548 and causal interpretation: theoretical assumptions and implementation with SAS and SPSS
549 macros. *Psychological Methods*, *18*(2), 137-150. doi:10.1037/a0031034
- 550 Varese, F., Smeets, F., Drukker, M., Lieveise, R., Lataster, T., Viechtbauer, W., . . . Bentall, R. P. (2012).
551 Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control,
552 prospective- and cross-sectional cohort Studies. *Schizophrenia Bulletin*, *38*(4), 661-671.
553 doi:10.1093/schbul/sbs050
- 554 Verweij, K. J. H., Abdellaoui, A., Nivard, M. G., Cort, A. S., Ligthart, L., Draisma, H. H. M., . . . Vink, J. M.
555 (2017). Short communication: Genetic association between schizophrenia and cannabis use.
556 *Drug and Alcohol Dependence*, *171*, 117-121. doi:10.1016/j.drugalcdep.2016.09.022
- 557 Wechsler, D., Golombok, S., & Rust, J. (1992). *Wechsler Intelligence Scale for Children – Third Edition UK*
558 *Manual*. Sidcup, UK: The Psychological Corporation.
- 559 Welham, J., Isohanni, M., Jones, P., & McGrath, J. (2009). The antecedents of schizophrenia: a review of
560 birth cohort studies. *Schizophrenia Bulletin*, *35*(3), 603-623. doi:10.1093/schbul/sbn084

561 Wolke, D., Woods, S., Bloomfield, L., & Karstadt, L. (2000). The association between direct and relational
562 bullying and behaviour problems among primary school children. *Journal of Child Psychology*
563 *and Psychiatry*, 41(8), 989-1002. doi:Doi 10.1017/S0021963099006381
564 Wootton, R. E., Richmond, R. C., Stuijzand, B. G., Lawn, R. B., Sallis, H. M., Taylor, G. M. J., . . . Munafò,
565 M. R. (2018). Causal effects of lifetime smoking on risk for depression and schizophrenia:
566 evidence from a Mendelian randomisation study. *bioRxiv*.

567

569 Table 1. Associations between polygenic score for schizophrenia and subsequent cigarette and/or
 570 cannabis use as compared to non-use ($N = 3925$)

P-value threshold for inclusion of SNPs into polygenic score (P_T)	Early cigarette only users	Early cannabis with/without cigarette users	Late cigarette only users	Late cannabis with/without cigarette users	P
	(4.3%) ¹	(3.4%) ¹	(15.2%) ¹	(11.8%) ¹	
	OR (95% CI) ²	OR (95% CI) ²	OR (95% CI) ²	OR (95% CI) ²	
Unadjusted					
$P_T = 0.05$	1.13 (0.94, 1.36)	1.08 (0.87, 1.33)	0.87 (0.76, 1.00)	1.23 (1.08, 1.41)	0.004
Adjusted³					
$P_T = 0.05$	1.11 (0.91, 1.34)	1.07 (0.86, 1.33)	0.85 (0.74, 0.99)	1.22 (1.07, 1.40)	0.006

571 **Note:** SNPs, single nucleotide polymorphisms; OR, odds ratio; 95% CI, 95% confidence interval; P, omnibus P-value
 572 for association between polygenic score and cigarette/cannabis use classes

573 ¹ Class proportions for latent class membership based on the estimated model

574 ² Compared to non-use class (class proportion for latent class membership based on the estimated model: 65.3%).

575 ³ Adjusted for polygenic scores for cigarette smoking initiation and cannabis use initiation ($P_T = 0.5$).

576

577 *Table 2. Total effect, direct effect and indirect effect of schizophrenia polygenic score ($P_T = 0.05$) on late-*
 578 *onset cannabis with/without cigarette use as compared to non-use through a range of potential*
 579 *mediators*

Mediator	N	Total Effect	Direct Effect	Indirect Effect via mediator
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Standardized measure of IQ at age 8 years	3468	1.23 (1.06,1.44)	1.25 (1.07,1.46)	0.99 (0.97,1.00)
Victimization at age 8 years	3371	1.22 (1.07,1.38)	1.22 (1.07,1.38)	1.00 (1.00,1.01)
Emotional symptoms at age 9 years	3522	1.20 (1.04,1.39)	1.20 (1.04,1.39)	1.00 (0.99,1.00)
Antisocial behavior at age 10 years	3533	1.26 (1.09,1.46)	1.26 (1.09,1.46)	1.00 (1.00,1.01)
Impulsivity at age 10 years	3344	1.22 (1.06,1.41)	1.22 (1.06,1.41)	1.00 (1.00,1.00)
Friendship quality at age 12 years	3542	1.27 (1.09,1.48)	1.27 (1.09,1.48)	1.00 (0.99,1.00)
Psychotic experiences at age 12 years	3572	1.26 (1.12,1.42)	1.26 (1.12,1.42)	1.00 (1.00,1.00)

580 **Note:** OR, odds ratio; 95% CI, 95% confidence interval; P_T , p-value threshold for inclusion of SNPs into polygenic
 581 score. Within the mediation models, higher emotional, impulsivity and friendship quality scores indicate more
 582 emotional problems, a higher level of impulsivity and worse friendship quality, respectively.

583

584

Supplementary material -- Examining pathways between genetic liability for schizophrenia and patterns of tobacco and cannabis use in adolescence

Supplementary Methods

Participants

The sample consisted of participants from the Avon Longitudinal Study of Parents and Children (ALSPAC) longitudinal birth cohort which recruited 14,541 pregnant women residing in the former Avon Health Authority area with an expected delivery date between April 1991 and December 1992. Of the initial 14,541 pregnancies, 14,062 were live births and 13,988 were alive at 1 year (Boyd *et al.*, 2013, Fraser *et al.*, 2013). When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally resulting in an additional 913 children being enrolled. The total sample size for analyses using any data collected after the age of 7 years is therefore 15,454 pregnancies, resulting in 15,589 fetuses. Of these 14,901 were alive at 1 year of age. Collection of a range of measures from ALSPAC mothers and their children is still ongoing and details of available data are accessible through a fully searchable data dictionary and variable search tool (<http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary>). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees (<http://www.bristol.ac.uk/alspac/researchers/research-ethics/>). Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Genetic data

Avon Longitudinal Study of Parents and Children (ALSPAC) participants genetic data were acquired using the Illumina HumanHap550 quad genome-wide single nucleotide polymorphism (SNP) genotyping platform from 9912 participants. Individuals were excluded from further analysis on the basis of gender mismatches, minimal or excessive heterozygosity, disproportionate levels of individual missingness (>3%), evidence of cryptic relatedness (>10% of alleles identical by descent), and being of non-European ancestry (assessed by multidimensional scaling analysis including HapMap 2 individuals). SNPs with a minor allele frequency (MAF) of < 1%, Impute2 information quality metric of < 0.8, a call rate of < 95% or evidence for violations of Hardy-Weinberg equilibrium (p value < 5×10^{-7}) were removed. Imputation of the target data was performed using Impute V2.2.2 against the 1000 genomes reference panel (Phase 1, Version 3; all polymorphic SNPs excluding singletons), using 2,186 reference haplotypes (including non-Europeans). Following quality control assessment and imputation and restricting to 1 young person per family, genetic data was available for 7,977 ALSPAC individuals.

Polygenic scores

Polygenic scores for schizophrenia were constructed for each ALSPAC individual using data from the most recent schizophrenia GWAS based on 40,675 cases and 64,643 controls (Pardiñas *et al.*, 2018) as a training set. Polygenic scores were calculated using the PLINK (v1.9)(Chang *et al.*, 2015, Purcell *et al.*, 2007) ‘score’ command following the methodology described by the International Schizophrenia Consortium (ISC) (Purcell *et al.*, 2009). Prior to construction of scores, SNPs were removed from the analysis if they had a minor allele frequency less than 0.01, an imputation quality less than 0.8 or if there was allelic mismatch between samples. Due to the high linkage disequilibrium (LD) within the extended major histocompatibility complex (MHC; chromosome 6: 25-34Mb) only a single SNP was included to represent this region. SNPs were pruned for LD using the PLINK ‘clump’ command to remove SNPs in LD ($r^2 > 0.25$) with a more significant SNP in the training set. Windows of 500kb were used to assess inter-SNP LD for pruning.

Repeated measures of cigarette and/or cannabis use

Measures taken at approximate age 14 years, 16 years and 18 years were collected as part of ALSPAC assessment clinics using a computerized interview. Measures taken at approximate age 15 years, 17 years and 19 years were collected via ALSPAC postal questionnaires. For each time point, individuals were deemed as cigarette users if they were current smokers who smoked at least 1-3 in the previous 6 months (age 14 years), who smoked less than once a week, weekly or daily (age 15 and 16 years), had smoked less than once a week, weekly or daily in the last 30 days (age 16, 18 and 19 years). Individuals were deemed as non-cigarette users if they had never smoked a cigarette, if they had only tried cigarettes once or twice (age 15 and 17 years) or if they had not smoked in the last 6 months (age 14 years) or last 30 days (age 16, 18 and 19 years). For each time point, individuals were deemed as cannabis users if they had used or taken cannabis at least 1-3 times in the past 6 months (age 14 years), currently take cannabis less than weekly, weekly or daily (age 15, 16 and 17 years) or at least monthly or less in the last 12 months (age 18 and 19 years). Individuals were deemed as non-cannabis users if they had never tried cannabis, if they had only ever tried cannabis once or twice or if they used to sometimes use or take cannabis but had since stopped.

For generation of longitudinal latent classes, cigarette and cannabis use data were then combined into a 3-category nominal variable for each time point: “Non-users”, “Cigarette-only users” and “Cannabis users (either with or without cigarettes)” as previously described (Jones *et al.*, 2018).

To assess the associations between polygenic scores for schizophrenia and frequency of cigarette and cannabis use, responses to one or more questions at each time point were used to derive two 3-level ordinal variables for cigarette use and cannabis use: “Non-user”, “Occasional user” (typically less than once per week) and “Frequent user” (typically once a week or more) as previously described (Howe *et al.*, 2017, Taylor *et al.*, 2017).

Association analyses

Multinomial logistic regression was used to assess whether polygenic scores predicted latent class membership. Associations were assessed using a manual implementation of the bias-adjusted three-step method in MPlus (see Heron *et al.* (2015) for more detail and example of code). The latent classes were first derived without the presence of the predictor. The resulting logit parameters defining the relationship between modal and latent classes were used as constraints allowing odds ratios (ORs) and confidence intervals (CIs) for the associations to be calculated without influencing latent class membership. Association analyses were conducted using individuals who had cigarette and cannabis use data present for 3 or more time points and genetic data.

Potential mediators

A number of potential mediators were examined: IQ at age 8 years (assessed via the Wechsler Intelligence Scale for Children (Wechsler *et al.*, 1992); this measure was standardized [mean =0, standard deviation = 1] before use), victimization at age 8 years (a dichotomous measure relating to whether individual experienced relational or overt victimization, assessed via a modified version of the Bullying and Friendship Interview Schedule (Wolke *et al.*, 2000) at age 8 years), Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1999) score at age 9 years relating to emotional symptoms (total scores assessed via parental-completed questionnaire when child was age 9 years with higher score indicating emotional difficulties), antisocial behavior (a dichotomous measure relating to whether individual engaged in any antisocial activities, assessed via a short structured interview at age 10 years), impulsivity (assessed using the number of incorrect stop signal trials at a 150ms delay during the stop signal task (Handley *et al.*, 2004) administered at age 10 years with a higher score indicating a higher level of impulsivity), friendship quality score (total score based on 5 items from the Cambridge Friendship Questionnaire (Baron-Cohen and Wheelwright, 2003) at age 12 years with a higher score indicating worse friendship quality), and psychotic experiences (a dichotomous measure relating to whether individual experienced hallucinations (visual and auditory), delusions (spied on, persecution, thoughts read, reference, control, grandiosity, other) and experiences of thought interference (broadcasting, insertion and withdrawal), assessed via the semi-structured Psychosis-Like Symptom Interview (PLIKSi) (Horwood *et al.*, 2008) at age 12 years).

Supplementary References

- Baron-Cohen, S. & Wheelwright, S.** (2003). The friendship questionnaire: an investigation of adults with Asperger syndrome or high-functioning autism, and normal sex differences. *Journal of Autism and Developmental Disorders* **33**, 509-17.
- Boyd, A., Golding, J., Macleod, J., Lawlor, D. A., Fraser, A., Henderson, J., Molloy, L., Ness, A., Ring, S. & Davey Smith, G.** (2013). Cohort Profile: the 'children of the 90s' - the index offspring of the Avon Longitudinal Study of Parents and Children. *International Journal of Epidemiology* **42**, 111-27.
- Chang, C. C., Chow, C. C., Tellier, L. C. A. M., Vattikuti, S., Purcell, S. M. & Lee, J. J.** (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**.
- Fraser, A., Macdonald-Wallis, C., Tilling, K., Boyd, A., Golding, J., Davey Smith, G., Henderson, J., Macleod, J., Molloy, L., Ness, A., Ring, S., Nelson, S. M. & Lawlor, D. A.** (2013). Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *International Journal of Epidemiology* **42**, 97-110.

- Goodman, R.** (1999). The extended version of the strengths and difficulties questionnaire as a guide to child psychiatric caseness and consequent burden. *Journal of Child Psychology and Psychiatry, and Allied Disciplines* **40**, 791-799.
- Handley, S. J., Capon, A., Beveridge, M., Dennis, I. & Evans, J. S. T.** (2004). Working memory, inhibitory control and the development of children's reasoning. *Thinking & Reasoning* **10**, 175-195.
- Heron, J. E., Croudace, T. J., Barker, E. D. & Tilling, K.** (2015). A comparison of approaches for assessing covariate effects in latent class analysis. *2015* **6**, 15.
- Horwood, J., Salvi, G., Thomas, K., Duffy, L., Gunnell, D., Hollis, C., Lewis, G., Menezes, P., Thompson, A., Wolke, D., Zammit, S. & Harrison, G.** (2008). IQ and non-clinical psychotic symptoms in 12-year-olds: results from the ALSPAC birth cohort. *British Journal of Psychiatry* **193**, 185-91.
- Howe, L. J., Trela-Larsen, L., Taylor, M., Heron, J., Munafò, M. R. & Taylor, A. E.** (2017). Body mass index, body dissatisfaction and adolescent smoking initiation. *Drug and Alcohol Dependence* **178**, 143-149.
- Jones, H. J., Gage, S. H., Heron, J., Hickman, M., Lewis, G., Munafò, M. R. & Zammit, S.** (2018). Association of combined patterns of tobacco and cannabis use in adolescence with psychotic experiences. *JAMA Psychiatry* **75**, 240-246.
- Pardiñas, A. F., Holmans, P., Pocklington, A. J., Escott-Price, V., Ripke, S., Carrera, N., Legge, S. E., Bishop, S., Cameron, D., Hamshere, M. L., Han, J., Hubbard, L., Lynham, A., Mantripragada, K., Rees, E., MacCabe, J. H., McCarroll, S. A., Baune, B. T., Breen, G., Byrne, E. M., Dannlowski, U., Eley, T. C., Hayward, C., Martin, N. G., McIntosh, A. M., Plomin, R., Porteous, D. J., Wray, N. R., Caballero, A., Geschwind, D. H., Huckins, L. M., Ruderfer, D. M., Santiago, E., Sklar, P., Stahl, E. A., Won, H., Agerbo, E., Als, T. D., Andreassen, O. A., Bækvad-Hansen, M., Mortensen, P. B., Pedersen, C. B., Børglum, A. D., Bybjerg-Grauholm, J., Djurovic, S., Durmishi, N., Pedersen, M. G., Golimbet, V., Grove, J., Hougaard, D. M., Mattheisen, M., Molden, E., Mors, O., Nordentoft, M., Pejovic-Milovancevic, M., Sigurdsson, E., Silagadze, T., Hansen, C. S., Stefansson, K., Stefansson, H., Steinberg, S., Tosato, S., Werge, T., GERAD1 Consortium, CRESTAR Consortium, Collier, D. A., Rujescu, D., Kirov, G., Owen, M. J., O'Donovan, M. C. & Walters, J. T. R.** (2018). Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. *Nature Genetics* **50**, 381-389.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., Maller, J., Sklar, P., de Bakker, P. I., Daly, M. J. & Sham, P. C.** (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559-75.
- Purcell, S. M., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., Sullivan, P. F., Sklar, P. & International Schizophrenia Consortium** (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**, 748-52.
- Taylor, M., Collin, S. M., Munafò, M. R., MacLeod, J., Hickman, M. & Heron, J.** (2017). Patterns of cannabis use during adolescence and their association with harmful substance use behaviour: findings from a UK birth cohort. *Journal of Epidemiology and Community Health*.
- Wechsler, D., Golombok, S. & Rust, J.** (1992). *Wechsler Intelligence Scale for Children – Third Edition UK Manual*. The Psychological Corporation: Sidcup, UK.
- Wolke, D., Woods, S., Bloomfield, L. & Karstadt, L.** (2000). The association between direct and relational bullying and behaviour problems among primary school children. *Journal of Child Psychology and Psychiatry* **41**, 989-1002.

Supplementary Tables

Supplementary Table 1. Associations between polygenic score for cigarette smoking initiation and cannabis use initiation and subsequent cigarette and/or cannabis use as compared to non-use (N = 3925)

P_T	Early cigarette only users	Early cannabis with/without cigarette users	Late cigarette only users	Late cannabis with/without cigarette users	P
	OR (95% CI) ¹	OR (95% CI) ¹	OR (95% CI) ¹	OR (95% CI) ¹	
Cigarette smoking initiation polygenic score associations					
0.5	1.68 (1.34, 2.10)	1.33 (1.03, 1.71)	1.46 (1.25, 1.70)	1.23 (1.05, 1.45)	<0.001
Cannabis use initiation polygenic score associations					
0.5	1.16 (0.94, 1.44)	1.15 (0.93, 1.42)	1.13 (0.98, 1.32)	1.42 (1.24, 1.62)	<0.001

Note: SNPs, single nucleotide polymorphisms; OR, odds ratio; 95% CI, 95% confidence interval; P, omnibus P-value for association between polygenic score and cigarette/cannabis use classes; P_T , p-value threshold for inclusion of SNPs into the cigarette use and cannabis use polygenic scores.

¹ Compared to all other classes combined.

Supplementary Table 2. Associations between polygenic risk score for schizophrenia minus the CHRNA5-CHRNA3-CHRNA4 gene cluster on chromosome 15 and cigarette and/or cannabis use as compared to non-use

P_T	Early cigarette only users	Early cannabis with/without cigarette users	Late cigarette only users	Late cannabis with/without cigarette users	P
	OR (95% CI) ¹	OR (95% CI) ¹	OR (95% CI) ¹	OR (95% CI) ¹	
0.5	1.13 (0.93, 1.37)	1.08 (0.88, 1.32)	0.87 (0.75, 1.00)	1.25 (1.09, 1.44)	0.004
0.05	1.13 (0.94, 1.36)	1.08 (0.87, 1.33)	0.87 (0.76, 1.00)	1.23 (1.08, 1.41)	0.004
1e ⁻⁵	1.00 (0.82, 1.22)	1.20 (0.98, 1.47)	0.93 (0.81, 1.08)	1.03 (0.90, 1.18)	0.344
5e ⁻⁸	0.96 (0.75, 1.23)	1.14 (0.92, 1.41)	1.10 (0.94, 1.29)	1.00 (0.87, 1.16)	0.632

Note: OR, odds ratio; 95% CI, 95% confidence interval; P, omnibus P-value for association between polygenic risk score and substance use classes; P_T , p-value threshold for inclusion of SNPs into the schizophrenia polygenic score.

¹ Compared to non-use class.

Supplementary Table 3. Associations between polygenic score for schizophrenia and cigarette and/or cannabis use as compared to non-use

P_T	Early cigarette only users	Early cannabis with/without cigarette users	Late cigarette only users	Late cannabis with/without cigarette users	P
	OR (95% CI) ¹	OR (95% CI) ¹	OR (95% CI) ¹	OR (95% CI) ¹	
0.5	1.13 (0.93, 1.37)	1.08 (0.88, 1.32)	0.87 (0.75, 1.00)	1.25 (1.09, 1.44)	0.003
1e ⁻⁵	0.99 (0.81, 1.21)	1.19 (0.97, 1.47)	0.94 (0.81, 1.09)	1.03 (0.91, 1.18)	0.377
5e ⁻⁸	0.95 (0.74, 1.21)	1.14 (0.92, 1.41)	1.12 (0.95, 1.31)	1.01 (0.87, 1.16)	0.562

Note: SNPs, single nucleotide polymorphisms; OR, odds ratio; 95% CI, 95% confidence interval; P , omnibus P -value for association between polygenic score and cigarette/cannabis use classes; P_T , p -value threshold for inclusion of SNPs into the schizophrenia polygenic score.

¹ Compared to non-use class.

Supplementary Table 4. Logistic regression associations between polygenic score for schizophrenia and cigarette and/or cannabis use after reparameterization of classes into a 2-category outcome (N = 3925)

P_T	Early cigarette only users	Early cannabis with/without cigarette users	Late cigarette only users	Late cannabis with/without cigarette users	P
	OR (95% CI) ¹	OR (95% CI) ¹	OR (95% CI) ¹	OR (95% CI) ¹	
Unadjusted					
0.05	1.07 (0.88, 1.31)	1.10 (0.88, 1.36)	0.89 (0.77, 1.02)	1.20 (1.05, 1.37)	0.032
Adjusted²					
0.05	1.06 (0.86, 1.3)	1.09 (0.87, 1.35)	0.87 (0.75, 1.01)	1.19 (1.04, 1.37)	0.040

Note: SNPs, single nucleotide polymorphisms; OR, odds ratio; 95% CI, 95% confidence interval; P , omnibus P -value for association between polygenic score and cigarette/cannabis use classes; P_T , p -value threshold for inclusion of SNPs into the schizophrenia polygenic score.

¹ Compared to all other classes combined.

² Adjusted for polygenic scores for cigarette smoking initiation and cannabis use initiation ($P_T = 0.5$).

Supplementary Table 5. Associations between polygenic score for schizophrenia and cannabis and cigarette use (ever versus never) at single time-points

P_T	OR (95% CI) for cigarette ever versus never use					
	14 years (N = 4567)	15 years (N = 4150)	16 years (N = 1896)	17 years (N = 3579)	18 years (N = 3043)	19 years (N = 2402)
0.5	1.10 (1.02, 1.18)	1.08 (1.01, 1.16)	0.94 (0.86, 1.04)	1.12 (1.05, 1.20)	1.13 (1.05, 1.21)	1.08 (1.00, 1.17)
0.05	1.08 (1.01, 1.17)	1.10 (1.03, 1.18)	0.95 (0.86, 1.05)	1.09 (1.02, 1.16)	1.11 (1.03, 1.19)	1.05 (0.97, 1.14)
1e ⁻⁵	1.07 (0.99, 1.15)	1.07 (0.99, 1.14)	1.03 (0.94, 1.14)	1.06 (1.00, 1.14)	1.06 (0.98, 1.13)	1.04 (0.96, 1.13)
5e ⁻⁸	1.07 (0.99, 1.15)	1.06 (0.98, 1.13)	1.00 (0.91, 1.10)	1.02 (0.95, 1.09)	1.06 (0.99, 1.14)	0.98 (0.91, 1.07)
P_T	OR (95% CI) for cannabis ever versus never use					
	14 years (N = 4551)	15 years (N = 4164)	16 years (N = 3957)	17 years (N = 3580)	18 years (N = 3015)	19 years (N = 2405)
0.5	1.18 (1.03, 1.35)	1.13 (1.02, 1.26)	1.17 (1.09, 1.25)	1.16 (1.08, 1.25)	1.19 (1.10, 1.28)	1.17 (1.08, 1.27)
0.05	1.15 (1.00, 1.31)	1.12 (1.01, 1.25)	1.18 (1.10, 1.27)	1.13 (1.05, 1.22)	1.14 (1.06, 1.23)	1.13 (1.04, 1.23)
1e ⁻⁵	1.19 (1.04, 1.36)	1.09 (0.98, 1.22)	1.09 (1.01, 1.17)	1.08 (1.01, 1.17)	1.02 (0.95, 1.10)	1.10 (1.01, 1.19)
5e ⁻⁸	1.12 (0.98, 1.28)	1.06 (0.96, 1.18)	1.05 (0.97, 1.12)	1.02 (0.95, 1.1)	1.02 (0.95, 1.10)	1.01 (0.93, 1.10)

Note: P_T , p-value threshold for inclusion of SNPs into the schizophrenia polygenic score; OR, odds ratio; 95% CI, 95% confidence interval.

Supplementary Table 6. Associations between polygenic score for schizophrenia and frequency of cannabis and cigarette use (non-use, occasional use, frequent use) at single time-points

P_{τ}	OR (95% CI) for increasing frequency of cigarette use					
	14 years (N = 3578)	15 years (N = 3403)	16 years (N = 3400)	17 years (N = 3105)	18 years (N = 2714)	19 years (N = 2166)
0.5	1.14 (0.91, 1.41)	0.96 (0.83, 1.12)	1.02 (0.93, 1.12)	1.06 (0.97, 1.16)	1.06 (0.98, 1.16)	1.09 (1.00, 1.20)
0.05	1.15 (0.92, 1.43)	0.95 (0.81, 1.10)	1.05 (0.95, 1.15)	1.07 (0.98, 1.17)	1.04 (0.95, 1.13)	1.08 (0.98, 1.18)
1e ⁻⁵	1.01 (0.81, 1.26)	0.94 (0.81, 1.10)	1.01 (0.92, 1.11)	0.97 (0.89, 1.06)	1.01 (0.93, 1.1)	1.08 (0.98, 1.18)
5e ⁻⁸	1.04 (0.84, 1.30)	0.97 (0.84, 1.13)	1.01 (0.92, 1.10)	1.00 (0.91, 1.09)	1.02 (0.94, 1.11)	1.02 (0.93, 1.12)
P_{τ}	OR (95% CI) for increasing frequency of cannabis use					
	14 years (N = 3557)	15 years (N = 3388)	16 years (N = 3373)	17 years (N = 3098)	18 years (N = 2690)	19 years (N = 2160)
0.5	1.19 (0.99, 1.44)	1.00 (0.80, 1.25)	1.10 (0.98, 1.24)	1.23 (1.10, 1.39)	1.11 (1.01, 1.23)	1.22 (1.09, 1.37)
0.05	1.13 (0.94, 1.37)	1.01 (0.81, 1.27)	1.11 (0.98, 1.25)	1.21 (1.08, 1.36)	1.10 (1.00, 1.22)	1.20 (1.07, 1.34)
1e ⁻⁵	1.19 (0.99, 1.44)	1.04 (0.82, 1.31)	1.08 (0.96, 1.21)	1.02 (0.91, 1.15)	1.06 (0.96, 1.17)	1.04 (0.93, 1.17)
5e ⁻⁸	1.11 (0.92, 1.34)	0.99 (0.79, 1.24)	1.06 (0.94, 1.19)	1.00 (0.89, 1.13)	1.00 (0.91, 1.10)	0.94 (0.84, 1.06)

Note: P_{τ} , p-value threshold for inclusion of SNPs into the schizophrenia polygenic score; OR, odds ratio; 95% CI, 95% confidence interval.

Supplementary Table 7. Total effect, direct effect and indirect effect of schizophrenia polygenic score ($P_T = 0.05$) on late-onset cannabis with/without cigarette use as compared to all other classes combined (after class reparameterization) through a range of potential mediators

Mediator	N	Total Effect	Direct Effect	Indirect Effect via mediator
		OR (95% CI)	OR (95% CI)	OR (95% CI)
IQ at age 8 years	3468	1.20 (1.03,1.39)	1.22 (1.05,1.41)	0.98 (0.97,1.00)
Victimization at age 8 years	3371	1.18 (1.04,1.33)	1.18 (1.04,1.33)	1.00 (1.00,1.01)
Emotional symptoms at age 9 years	3522	1.18 (1.02,1.36)	1.18 (1.02,1.36)	1.00 (0.99,1.00)
Antisocial behavior at age 10 years	3533	1.22 (1.06,1.40)	1.21 (1.05,1.40)	1.00 (1.00,1.01)
Impulsivity at age 10 years	3344	1.17 (1.02,1.35)	1.17 (1.02,1.35)	1.00 (1.00,1.00)
Friendship quality at age 12 years	3542	1.22 (1.06,1.41)	1.22 (1.06,1.41)	1.00 (0.99,1.00)
Psychotic experiences at age 12 years	3572	1.22 (1.08,1.37)	1.22 (1.08,1.38)	1.00 (1.00,1.00)

Note: OR, odds ratio; 95% CI, 95% confidence interval; P_T , p-value threshold for inclusion of SNPs into polygenic score. Within the mediation models, higher emotional, impulsivity and friendship quality scores indicate more emotional problems, a higher level of impulsivity and worse friendship quality, respectively.

Supplementary Table 8. Unadjusted and adjusted associations between cigarette and/or cannabis use and psychotic experiences at age 18 years (N = 2923)

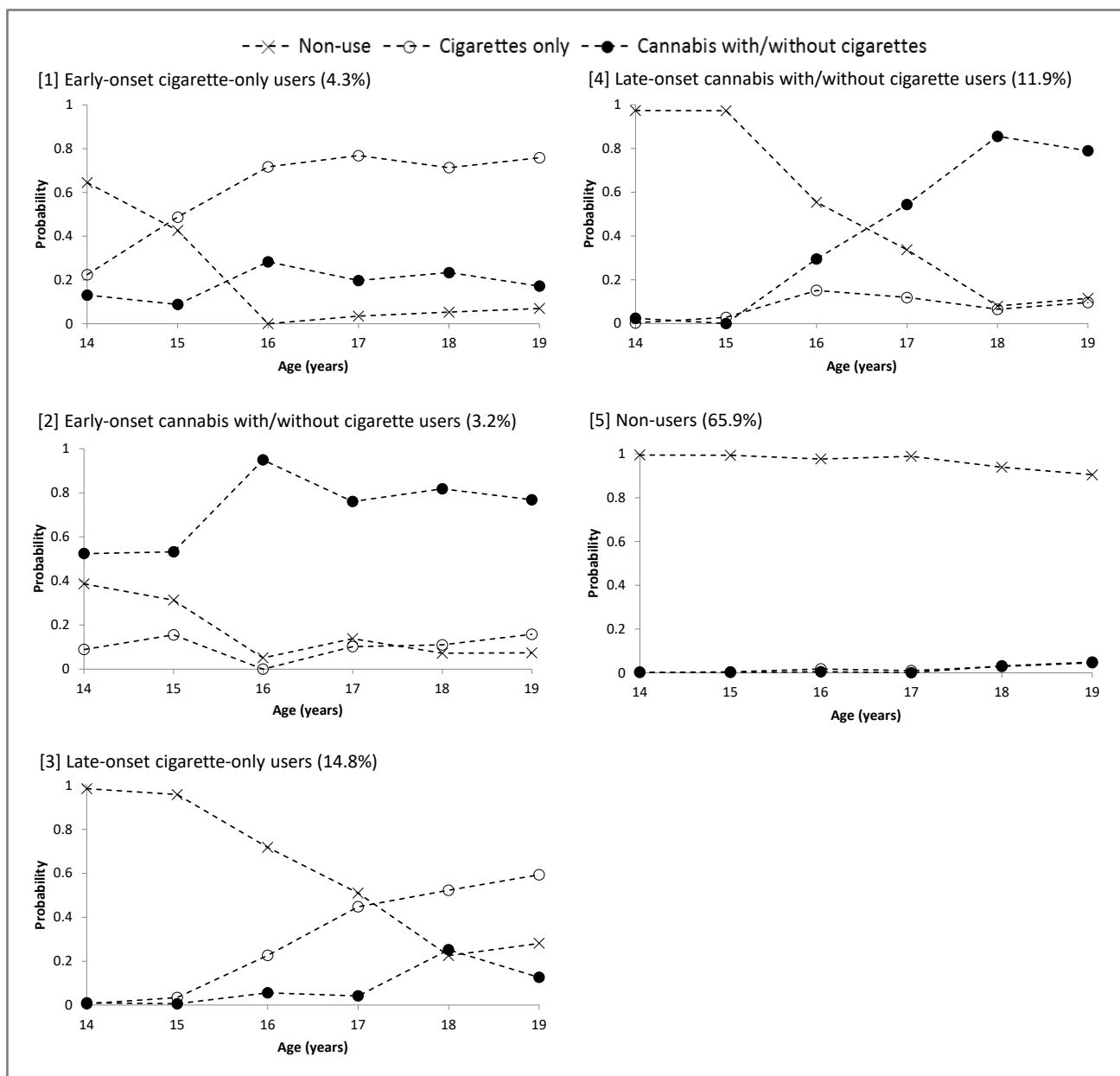
	Definite PE (4.48% definite PEs versus 95.52% suspected PEs or none)			
	Unadjusted		Adjusted ¹	
	OR (95% CI) ²	<i>P</i>	OR (95% CI) ²	<i>P</i>
Early-onset cigarette-only	2.98 (1.14, 7.78)	<0.001	2.96 (1.14, 7.69)	<0.001
Early-onset cannabis	3.28 (1.35, 7.97)		3.28 (1.35, 7.95)	
Late-onset cigarette-only	0.58 (0.16, 2.05)		0.59 (0.17, 2.06)	
Late-onset cannabis	2.76 (1.49, 5.11)		2.75 (1.48, 5.10)	

Note: PE, psychotic experiences; OR, odds ratio; 95% CI, 95% confidence interval; *P*, omnibus *P* value for association between cigarette/cannabis use classes and psychotic experiences at age 18 years.

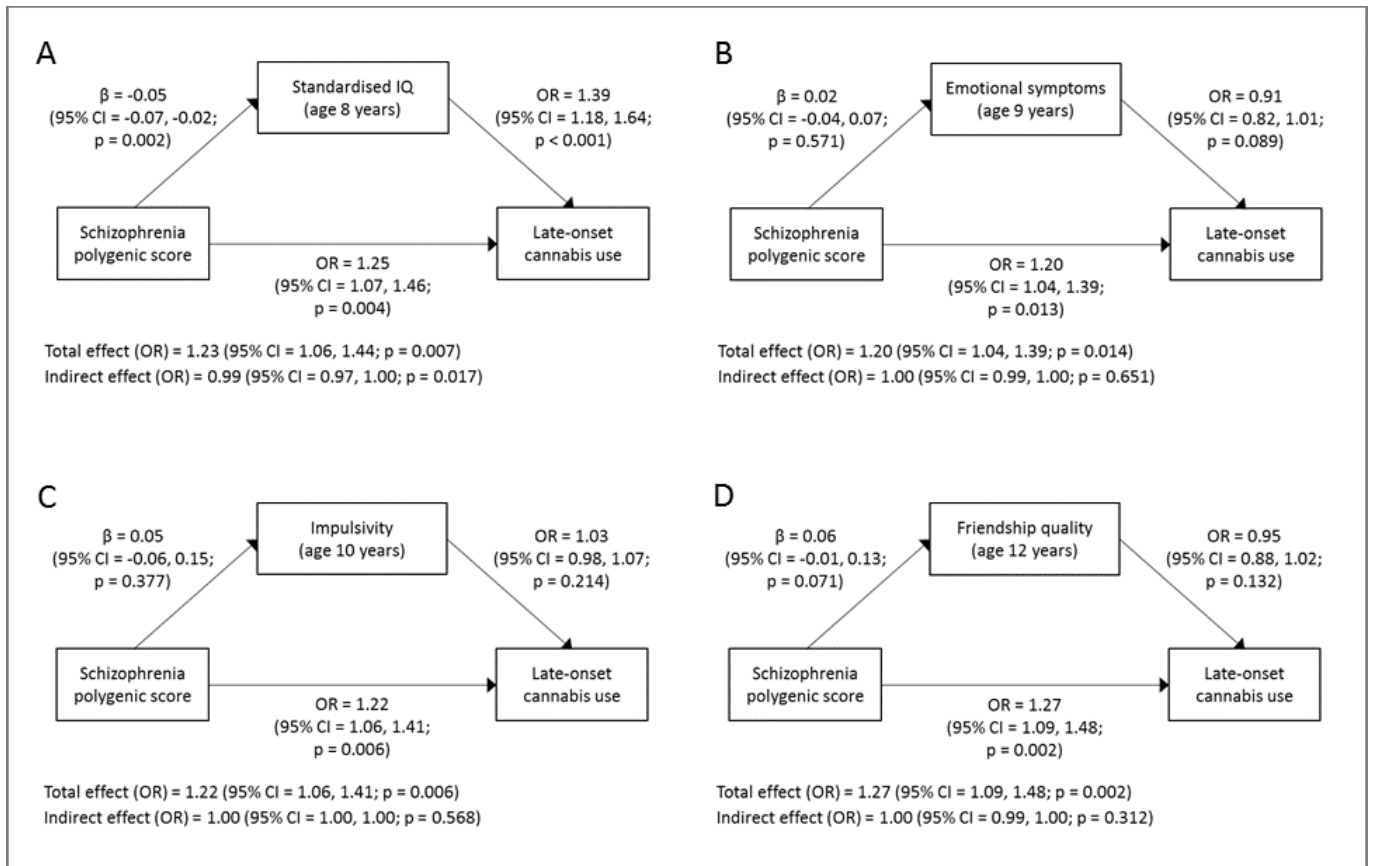
¹ Adjusted for schizophrenia polygenic risk score ($P_T = 0.05$).

² Compared to non-use class.

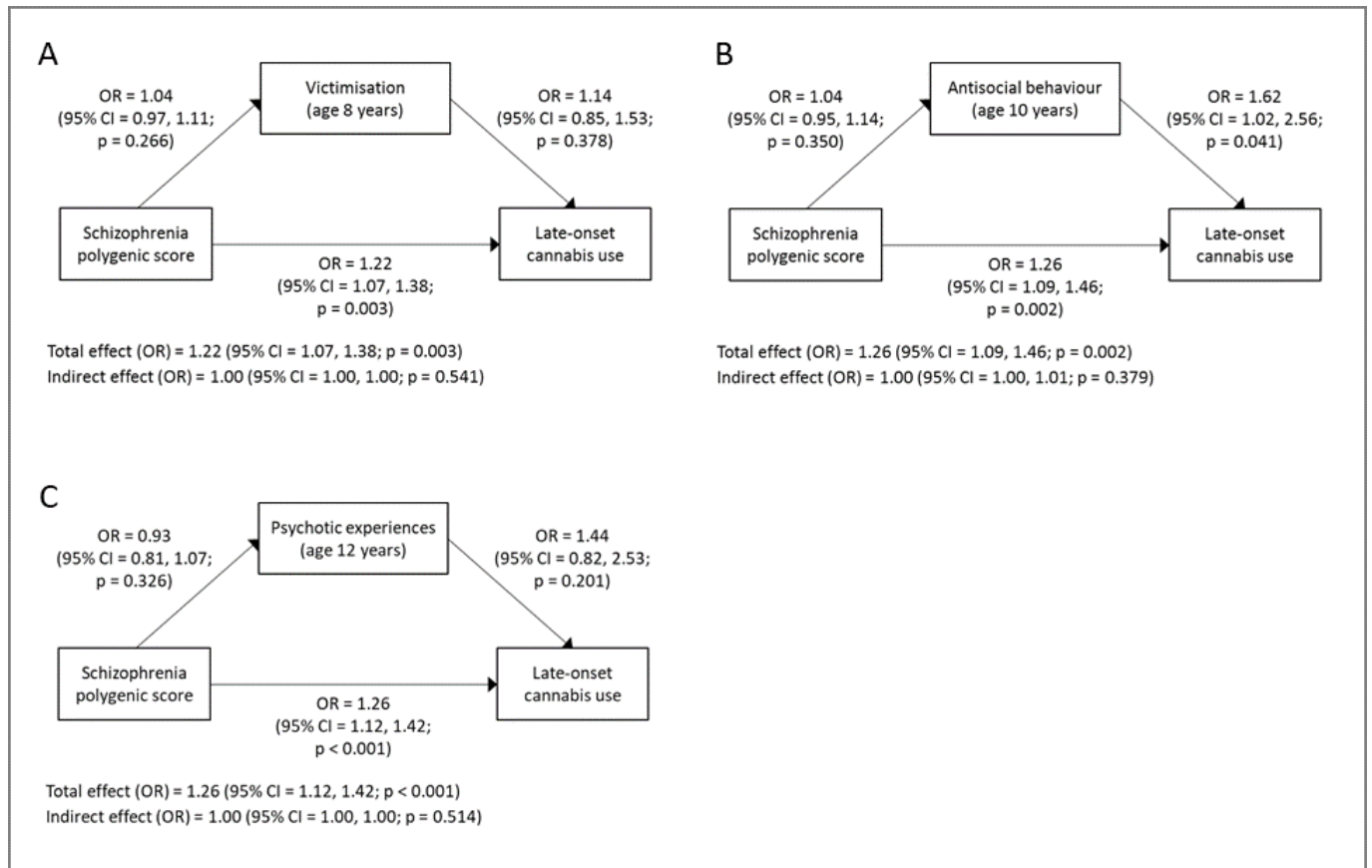
Supplementary Figures



Supplementary Figure 1. Five-class model of cigarette/cannabis use patterns from a sample of 5,300 individuals (Jones *et al.*, 2018). The probability axis represents the probability of a class member being a non-user, a cigarette-only user or a cannabis with/without cigarette user at each time point. Class proportions are show as percentages (%) after each class description.



Supplementary Figure 2. Total effect, direct effect and indirect effects of schizophrenia polygenic score ($P_T = 0.05$) on late-onset cannabis with/without cigarette use as compared to non-use through the following continuous mediators: a) IQ, b) emotional symptoms, c) impulsivity, and d) friendship quality. Note that higher emotional, impulsivity and friendship quality scores indicate more emotional problems, a higher level of impulsivity and worse friendship quality, respectively.



Supplementary Figure 3. Total effect, direct effect and indirect effects of schizophrenia polygenic score ($P_T = 0.05$) on late-onset cannabis with/without cigarette use as compared to non-use through dichotomous measures of a) experiencing victimization, b) participating in antisocial behavior, and c) experiencing psychotic experiences.