

The role of conduct disorder in the relationship between alcohol, nicotine and cannabis use disorders

J. D. Grant^{1*}, M. T. Lynskey², P. A. F. Madden¹, E. C. Nelson¹, L. R. Few¹, K. K. Bucholz¹,
D. J. Statham³, N. G. Martin⁴, A. C. Heath¹ and A. Agrawal¹

¹Department of Psychiatry, Washington University School of Medicine, St Louis, MO, USA

²Institute of Psychiatry, Psychology & Neuroscience, Addictions Department, King's College London, London, UK

³University of the Sunshine Coast, Queensland, Australia

⁴QIMR Berghofer Medical Research Institute, Queensland, Australia

Background. Genetic influences contribute significantly to co-morbidity between conduct disorder and substance use disorders. Estimating the extent of overlap can assist in the development of phenotypes for genomic analyses.

Method. Multivariate quantitative genetic analyses were conducted using data from 9577 individuals, including 3982 complete twin pairs and 1613 individuals whose co-twin was not interviewed (aged 24–37 years) from two Australian twin samples. Analyses examined the genetic correlation between alcohol dependence, nicotine dependence and cannabis abuse/dependence and the extent to which the correlations were attributable to genetic influences shared with conduct disorder.

Results. Additive genetic ($a^2 = 0.48$ – 0.65) and non-shared environmental factors explained variance in substance use disorders. Familial effects on conduct disorder were due to additive genetic ($a^2 = 0.39$) and shared environmental ($c^2 = 0.15$) factors. All substance use disorders were influenced by shared genetic factors ($r_g = 0.38$ – 0.56), with all genetic overlap between substances attributable to genetic influences shared with conduct disorder. Genes influencing individual substance use disorders were also significant, explaining 40–73% of the genetic variance per substance.

Conclusions. Among substance users in this sample, the well-documented clinical co-morbidity between conduct disorder and substance use disorders is primarily attributable to shared genetic liability. Interventions targeted at generally reducing deviant behaviors may address the risk posed by this shared genetic liability. However, there is also evidence for genetic and environmental influences specific to each substance. The identification of these substance-specific risk factors (as well as potential protective factors) is critical to the future development of targeted treatment protocols.

Received 4 October 2014; Revised 16 July 2015; Accepted 17 July 2015

Key words: Alcohol, cannabis, conduct disorder, genetic overlap, nicotine, substance use disorders, twins.

Introduction

There is substantial evidence that similar genetic factors influence liability to multiple substance use disorders (e.g. Kendler *et al.* 2003a, 2007; Rhee *et al.* 2006; Young *et al.* 2006; Xian *et al.* 2008; Sartor *et al.* 2010; Palmer *et al.* 2013). Most notably, Kendler *et al.* (2007) identified two genetic factors, corresponding to the covariance across licit (alcohol, nicotine, caffeine) and illicit (cannabis, cocaine) drugs; however, the genetic factors were highly correlated ($r = 0.82$). Conduct disorder has been repeatedly implicated as a co-morbid feature of substance use disorders and there is substantial evidence that genetic influences contribute to this

co-morbidity. For instance, another study by Kendler and colleagues found that alcohol and drug dependence shared a sizeable proportion of their genetic liability with conduct and antisocial personality disorder, and less so with internalizing disorders, such as depression (Kendler *et al.* 2003b). Button and colleagues (2006) also found significant genetic overlap between conduct disorder and both alcohol and illicit drug dependence in an adolescent sample, and that residual genetic overlap between alcohol and illicit drug dependence, after adjusting for conduct disorder, was non-significant. Similar results indicating the absence of a residual genetic correlation between alcohol and cannabis dependence when accounting for genetic overlap with antisocial personality disorder have also been noted in adult Vietnam era males (Fu *et al.* 2002). Other studies suggest that this shared liability extends to additional aspects of externalizing behavior, including novelty seeking and non-substance-related

* Address for correspondence: Dr J. D. Grant, Department of Psychiatry, Washington University School of Medicine, 660 S. Euclid, CB 8134, St Louis, MO 63110, USA.
(Email: grantj@psychiatry.wustl.edu)

behavioral disinhibition (Krueger *et al.* 2002; Hicks *et al.* 2011).

Based on this extant literature, it remains unanswered the extent to which the genetic covariation across the three most common forms of substance use disorder (i.e. alcohol, nicotine and cannabis) is attributable to genes shared with conduct disorder. Two outcomes might be expected. One possibility is that all the genetic influences on the three substances are overlapping with each other and with conduct disorder, with limited evidence for substance-specific or conduct disorder-specific genetic factors. This is unlikely given prior evidence for significant substance-specific genetic influences on substance dependence symptoms and on conduct disorder (Kendler *et al.* 2003b; Button *et al.* 2006, 2007). Alternatively, a moderate to high genetic correlation across the three substances might be expected, with evidence for substance-specific genes. Whether the entire proportion of this genetic correlation is due to genes shared with conduct disorder or whether some of this genetic correlation is independent of it (i.e. shared across the substances but not with conduct disorder) has not been examined. This study of the extent to which the genetics of conduct disorder contribute to genetic liability to substance use disorders is critical to gene-finding efforts where increasing sample sizes, often capitalizing on differing definitions of phenotypes with overlapping genetic underpinnings, is a necessity (Hicks *et al.* 2011). Equally important is the estimation of substance-specific genetic influences. For instance, Kendler *et al.* (2007) noted that substance-specific genetic variance ranged from 3% for cocaine to 91% for caffeine abuse/dependence symptoms.

To examine this question, we utilized data from 9577 adult male and female twins aged 24–37 years drawn from two samples derived from the Australian Twin Registry. Specifically we fit a quadrivariate model that examined the extent to which genetic and environmental influences were shared across conduct disorder and DSM-IV diagnoses of nicotine dependence (ND) and alcohol dependence (AD), and a modified cannabis abuse/dependence (CAD) diagnosis based on a subset of DSM-IV criteria.

Method and materials

Sample

Twin pairs from two cohorts of the volunteer Australian Twin Registry were included in the present analyses (Maciejewski *et al.* 2014). As described in detail elsewhere, telephone diagnostic interviews were completed for one cohort in 1996–2000 (see Knopik *et al.* 2004 for details) and for the other in 2005–2009

(Lynskey *et al.* 2012). The samples had comparable ages at the time of interview (mean = 29.94 and mean = 31.85, respectively). After removing individuals of unknown zygosity and those with no data for any of the four outcomes, the sample for the present analyses included 9577 individuals (6255 of 6257 interviewed individuals in the first cohort; 3322 of 3348 interviewed individuals in the second cohort). The 3982 complete twin pairs included: 1096 monozygotic (MZ, or identical) female twin pairs, 665 MZ male pairs, 817 dizygotic (DZ, or fraternal) female pairs, 513 DZ male pairs, and 891 male–female twin pairs; data from 1613 individuals without co-twin data, which contribute to estimates of prevalence (but not variance decomposition), were also included. Zygosity was determined using standard items about physical similarity which have been shown in a subset of the present respondents to have 95% agreement with DNA analysis (Medland *et al.* 2009). Verbal informed consent was obtained from all participants, and the procedures were approved by the Human Research Protection Office at Washington University and the Human Research Ethics Committee at the Queensland Institute of Medical Research.

Measures

Both cohorts completed a diagnostic telephone interview based on the Semi-structured Assessment of the Genetics of Alcoholism [Australian version, SSAGA-OZ (Bucholz *et al.* 1994; Heath *et al.* 1997)], which included DSM-IV (APA, 1994) assessments of conduct disorder, and ND, AD, and CAD.

Conduct disorder

Respondents were asked about 15 behaviors associated with DSM-IV conduct disorder (e.g. bullying, threatening, stealing, rule-breaking). Individuals who indicated that ≥ 3 behaviors (without requiring impairment) occurred within a 12-month period prior to age 18 years were coded as having conduct disorder. None of the items used in the conduct disorder section was related to substance use or misuse.

Nicotine

Respondents who had smoked at least 100 cigarettes lifetime or who had smoked 21–99 cigarettes and had smoked for at least ≥ 2 days a week for ≥ 3 weeks, completed a detailed DSM-IV nicotine dependence (ND) assessment. Individuals who endorsed ≥ 3 dependence symptoms (out of seven) within a 12-month period were coded as having a history of ND. Because individuals who do not initiate using a substance (i.e. never user) should be considered

unmeasured for their liability to developing substance use disorders (Neale *et al.* 2006), individuals who had never tried cigarettes were coded as missing for ND ($n = 1001$). Individuals with <3 dependence symptoms, those whose symptoms did not cluster within a 12-month period, and individuals who had tried cigarettes but had only experimented with nicotine (i.e. had smoked <100 cigarettes lifetime and had not smoked for at least ≥ 2 days per week, $n = 3900$) were coded as unaffected for ND.

Alcohol

Respondents were asked about their lifetime alcohol consumption. Individuals who had consumed at least five drinks in a 24-h period at least once completed a detailed DSM-IV alcohol dependence (AD) assessment and were included in analyses of AD. Those individuals who endorsed ≥ 3 AD symptoms (out of seven) within a 12-month period were coded as having a history of AD. Non-drinkers ($n = 105$) and those who had not consumed at least five drinks in a 24-h period ($n = 908$) were coded as missing for AD analyses.

Cannabis

Individuals who had used cannabis ≥ 11 times or who had used cannabis at least monthly during the period of heaviest use were asked questions tapping DSM-IV cannabis abuse and dependence (CAD). The first cohort was only asked about two abuse symptoms (hazardous use and role interference) and four dependence symptoms (tolerance, using more than intended, continued use despite emotional/psychological problems caused/exacerbated by use, and repeated desire or inability to cut down on use). Although the second cohort was asked additional abuse and dependence questions, the present analyses used only the symptoms assessed in both cohorts. Respondents were identified as having CAD if they endorsed any abuse symptom or they endorsed ≥ 2 dependence symptoms (symptoms were not required to have clustered within a 12-month period). Because individuals who do not initiate using a substance (i.e. never user) should be considered unmeasured for their liability to developing substance use disorders (Neale *et al.* 2006), individuals who had never tried cannabis were coded as missing for CAD ($n = 3499$). Individuals who completed the CAD section but did not meet the criteria for CAD listed above as well as those who had minimal cannabis exposure (i.e. had tried cannabis but used it <11 times and had not used it at least monthly during their period of heaviest use; $n = 3114$) were coded as unaffected for CAD.

Statistical analyses

Quantitative genetic analyses were used to estimate the relative contributions of genetic and environmental factors to outcomes, and the overlap between diagnoses. As detailed elsewhere (Neale & Cardon, 1992), twin models allow for the estimation of additive genetic factors (A), non-additive genetic factors (D), shared environmental factors (C, environmental influences that make twins similar to each other), and non-shared environmental factors (E, environmental influences not shared by twins, as well as error variance). Genetic influences are indicated when the identical twin (MZ) correlation is greater than the fraternal twin (DZ) correlation. If all twin pair similarity were attributable to A, the MZ correlation would be about twice the DZ correlation, because MZ twins share all of their genes and DZ twins share half of their segregating genes (on average). D is indicated when the DZ correlation is less than half the MZ correlation (because MZ twins again share all non-additive influences but DZ twins only share one quarter of such influences, on average), and C is indicated when the DZ correlation is more than half the MZ correlation. Models containing only twins reared together cannot estimate both C and D simultaneously, and a decision as to which should be estimated is made based on correlations between identical and fraternal twins.

A quadrivariate Cholesky (lower triangular) decomposition was used to assess the degree of genetic and environmental influence on each measure, as well as the overlap across conduct disorder, ND, AD, and CAD. A Cholesky model allows for influences on the first variable to also load on all subsequent variables, and for novel influences on each subsequent variable to load on all remaining variables. The E parameters in Fig. 1 show a full Cholesky parameterization, and the comparable Cholesky parameterizations for the familial components are shown in Supplementary Fig. S1 [see also work by Neale and colleagues (Neale & Cardon, 1992; Neale, 2004)]. Conduct disorder was entered into the Cholesky model first. Given that individuals become at risk for developing dependence when they initiate substance use and that not all users develop dependence, ordering of the substance diagnosis variables in the Cholesky model (ND, AD, CAD) was determined by the most typical pattern of substance initiation. Of those who had tried all three substances, 60.0% tried cigarettes before both alcohol and cannabis, and an additional 18.3% initiated multiple substances in the same year, with cigarettes being one of those substances; 20.0% initiated alcohol first; 0.8% initiated cannabis first. Of individuals who had tried two of the three substances, 94.6% had used cigarettes and alcohol; 60.7% of those

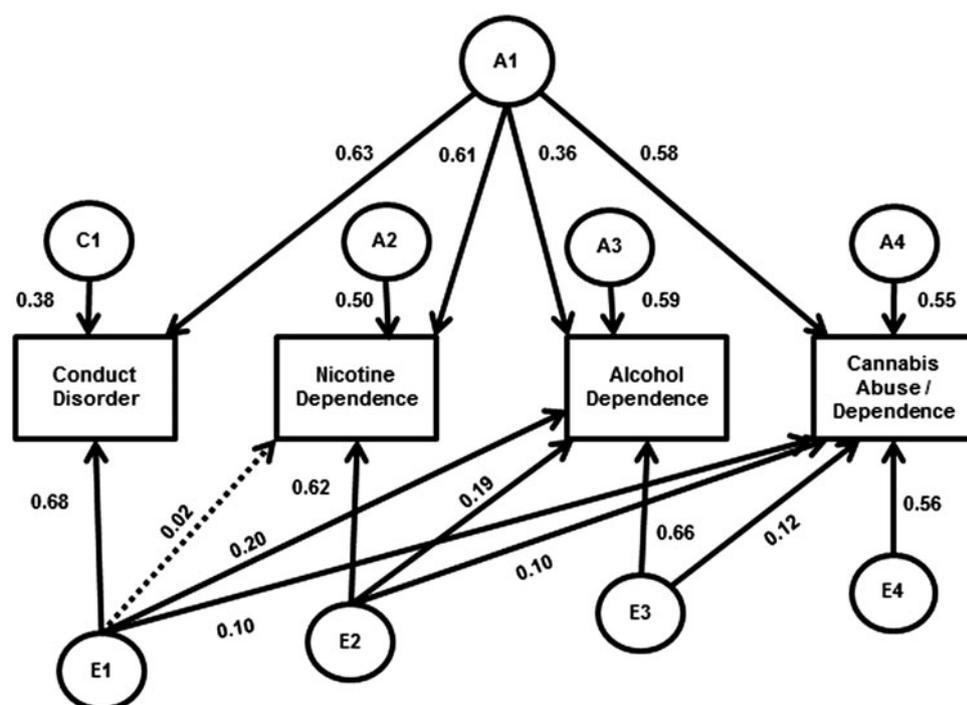


Fig. 1. Final quadrivariate genetic model with standardized parameter estimates. Paths are only shown for one twin; paths for the second twin are equated to the first twin, and connected to each other by fixed values in keeping with quantitative genetic theory (1.0 for genetic paths between MZ twins; 0.5 for genetic paths between DZ twins; 1.0 for shared environmental paths between MZ and DZ twins). Latent variables are indicated by circles; observed variables by rectangles. A, Additive genetic influences; C, shared environmental influences; E, non-shared environmental influences. The subscript 1 indicates factors influencing conduct disorder; 2 is additional factors for nicotine dependence; 3 is additional factors for alcohol dependence; 4 is additional factors for cannabis abuse/dependence; A₁ is a genetic factor common to all measures. Diagonal lines indicate paths from one measure that load on other measures. Solid lines indicate significant paths. Parameter estimates can be squared to determine the proportion of variance in the observed construct attributable to that path.

who had used two substances initiated cigarettes before alcohol, 21.1% initiated alcohol before cigarettes, and 12.9% initiated cigarettes and alcohol within the same year. This ordering of variables in the Cholesky model (conduct disorder, ND, AD, CAD) was also supported by the mean ages of initiation (see Table 1).

The fit of the full Cholesky model was compared to a saturated model, which allowed us to test whether the assumptions of twin modeling were met. Sub-models dropping specific paths were used to test the significance of familial influences, and the overlap in influences across measures. To minimize the large number of tests that would need to be conducted to examine combinations of paths that could be dropped from the model, omnibus tests that constrained a family of paths were first fit to the data in a manner that fit with the correlation patterns and with conceptually meaningful tests (single-parameter tests, presented in Supplementary Table S2, confirmed that no individual path in an omnibus test was statistically significant). The significance of paths was tested by comparing the fit of each sub-model to the preceding model and calculating the difference in -2 times the log-likelihood of the prior model and the sub-model,

which is interpreted as a χ^2 test for the given degrees of freedom (df). Models were fitted using the statistical package Mx (Neale, 2004) using full information maximum-likelihood estimation with raw data. Analyzing raw data in Mx (Neale, 2004) allowed us to utilize data from conduct disorder for everyone, and substance dependence data from each individual for the substances he/she had used more times than the cut-off established. All quadrivariate models included gender and cohort as covariates.

Ethical standards

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

Results

Demographic and substance use characteristics are shown in Table 1. Alcohol initiation was endorsed by

Table 1. Demographic and substance use characteristics of 9577 members of Australian twin pairs aged 24–37 years

	Years, mean (S.D.)
Mean age at interview	30.60 (2.6)
Mean age at conduct disorder onset	13.84 (2.4)
Mean age at cigarette initiation	14.06 (3.4)
Mean age at nicotine dependence onset	21.96 (4.1)
Mean age at alcohol initiation	15.83 (2.6)
Mean age at alcohol dependence onset	21.87 (3.9)
Mean age at cannabis initiation	18.50 (3.4)
Mean age at cannabis dependence onset ^a	21.36 (3.9)
	No. (%)
Male	3972 (41.5)
Monozygotic	4102 (42.8)
DSM-IV conduct disorder	1100 (11.6)
Nicotine use and dependence	
Ever smoked a cigarette	8470 (88.5)
DSM-IV nicotine dependence	
Population prevalence	2770 (28.9)
Of ever smokers	2770 (32.7)
Alcohol use and dependence	
Ever had a full drink of alcohol	9456 (98.9)
Ever had ≥ 5 drinks in a 24-h period	8544 (89.2)
DSM-IV alcohol dependence	
Population prevalence	2219 (23.2)
Of those having consumed at least 5 drinks in a 24 h period	2219 (26.0)
Cannabis use and abuse/dependence	
Ever used cannabis	6017 (63.2)
Cannabis abuse/dependence (modified DSM-IV)	
Population prevalence	1683 (17.7)
Of ever users	1683 (28.0)

Data from $n=9577$ individuals; $n=9501$ individuals for conduct disorder, $n=8470$ for nicotine dependence, 8544 for alcohol dependence, and $n=6017$ for cannabis abuse/dependence.

^a Age at clustering for $n=311$ individuals in the second cohort who met the modified DSM-IV cannabis abuse/dependence diagnosis (CAD). The $n=1683$ with CAD included $n=1122$ from the first cohort (age at diagnosis not assessed); of the $n=561$ cohort 2 individuals with the modified CAD diagnosis, age was not assessed for the $n=177$ with abuse only or the $n=73$ with the modified ≥ 2 symptom dependence diagnosis but not the full DSM-IV dependence diagnosis with ≥ 3 symptoms and clustering.

almost 99% of the respondents, with 89% of the sample reporting having consumed at least five drinks in a 24-h period and 26% of those having consumed ≥ 5 drinks in a 24-h period reporting a lifetime history of AD. Over 88% of respondents had tried cigarettes, with 33% of initiators having a history of ND. Cannabis initiation was endorsed by 63% of respondents, with 28% of initiators having a history of CAD. Mean age of onset of conduct disorder was

13.84 years, which predated the average age of initiation for cigarettes (mean = 14.06), alcohol (mean = 15.83), and cannabis (mean = 18.50).

Because all univariate genetic analyses indicated that the parameter estimates could be equated for men and women [$\Delta\chi^2(3)$ range: 2.30–6.38, all $p > 0.05$], quadri-variate analyses were collapsed across gender and gender was included as a covariate (see Supplementary Table S1 for the correlations for all five zygosity groups). MZ and DZ twin-pair correlations, tested using SAS software version 9.2 (SAS Institute Inc., 2002–2008), indicated significant familiarity for all measures (see Table 2). Univariate genetic models confirmed that additive genetic influences and non-shared environmental influences were significant for all measures, with shared environmental influences not reaching significance for any measure, but reaching $p=0.06$ for conduct disorder (see Table 2).

A saturated model was used to estimate the tetrachoric correlations in Mx (Neale, 2004), and served as a baseline from which to test the assumptions of the genetic models (-2 times the log-likelihood = 31 827.135 with 72 estimated parameters). The initial genetic model, which included additive genetic, shared environmental, and non-shared environmental paths in a Cholesky parameterization, indicated that the Cholesky parameterization was reasonable for the current data ($\Delta\chi^2=19.023$, $\Delta df=30$, $p=0.94$ compared to the saturated model). Given that the correlation pattern suggested genetic influence on all measures, with smaller and less consistent evidence for shared environmental influences, we first tested the significance of the shared environmental paths on and from ND and AD. Eliminating all shared environmental influences on and from ND (c21, c22, c32, and c42 in Supplementary Fig. S1) and AD (c31, c33, and c43 in Supplementary Fig. S1) resulted in a more parsimonious model without a significant decrement in fit (total $\Delta\chi^2=5.948$, $\Delta df=7$, $p=0.55$; also see Supplementary Table S2 for the single-parameter tests of change in model-fit for this and all additional tests). A test of the overlap in shared environmental influences on conduct disorder and CAD indicated that the overlap was not significant (path c41 in Supplementary Fig. S1; $\Delta\chi^2=-2.656$, $\Delta df=1$, $p>0.99$). A sub-model specifying that all genetic overlap between the substances could be explained by genetic influences shared with conduct disorder fit the data well (i.e. deleting paths a43, a42, and a32 from Supplementary Fig. S1; $\Delta\chi^2=0.664$, $\Delta df=3$, $p=0.88$; also see Supplementary Table S2). From this model, confidence intervals and further model testing confirmed that it was possible to delete the remaining CAD-specific shared environmental path (path c44 in Supplementary Fig. S1; $\Delta\chi^2=2.481$, $\Delta df=1$, $p=0.12$),

Table 2. Twin-pair correlations and final univariate genetic models (and 95% confidence intervals) for conduct disorder, nicotine dependence, alcohol dependence, and cannabis abuse/dependence

	r_{MZ}	r_{DZ}	A	C	E
Conduct disorder	0.55* (0.46–0.64)	0.39* (0.30–0.48)	0.32* (0.05–0.59)	0.20 ^a (0.00–0.41)	0.48* (0.38–0.58)
Nicotine dependence	0.63* (0.56–0.69)	0.31* (0.24–0.38)	0.62* (0.57–0.68)	–	0.38* (0.32–0.44)
Alcohol dependence	0.51* (0.43–0.59)	0.22* (0.14–0.30)	0.47* (0.40–0.54)	–	0.53* (0.46–0.60)
Cannabis abuse/dependence	0.63* (0.55–0.71)	0.40* (0.30–0.49)	0.63* (0.56–0.70)	–	0.37* (0.30–0.44)

A, Additive genetic influences; C, shared environmental influences; E, non-shared environmental influences.

Data from $n = 9501$ individuals for conduct disorder, $n = 8470$ for nicotine dependence, $n = 8544$ for alcohol dependence, and $n = 6017$ for cannabis abuse/dependence.

*Significant at $p < 0.05$;

^a $\Delta\chi^2 = 3.59$ when removed from the model, $p = 0.06$.

Table 3. Standardized proportions of variance (95% confidence intervals) attributable to additive genetic, shared environmental, and non-shared environmental influences in the best-fitting quadrivariate model examining the genetic and environmental contributions to the co-morbidity between conduct disorder, nicotine dependence, alcohol dependence, and cannabis abuse/dependence in 9577 Australian twins

	Additive genetic	Shared environment	Non-shared environment
Conduct disorder	0.39 (0.31–0.48)	0.15 (0.07–0.22)	0.46 (0.39–0.53)
Nicotine dependence	0.62 (0.56–0.67)	–	0.38 (0.34–0.44)
Alcohol dependence	0.48 (0.41–0.55)	–	0.52 (0.45–0.59)
Cannabis abuse/dependence	0.65 (0.58–0.71)	–	0.35 (0.29–0.43)

All variance components significant at $p < 0.05$.

but that all remaining genetic influences were statistically significant, as indicated by a significant decrement in fit when any path was deleted ($\Delta\chi^2$ range: 20.093–262.394 with 1 df, all $p < 0.001$). Although modest in magnitude, non-shared environmental influences overlapped across measures, with 5 of 6 paths being statistically significant ($\Delta\chi^2$ range: 5.846–28.275 with 1 df, all $p < 0.005$; the non-shared environmental path from conduct disorder to ND, which could be dropped without a significant decrement in fit ($\Delta\chi^2 = 0.169$ with 1 df, all $p = 0.68$), was retained in the final model but is indicated by the dashed line in Fig. 1). The significance of the three substance-to-substance non-shared environmental parameters (e32, e42, and e43 from Supplementary Fig. S1; also see Supplementary Table S2) indicates that individual-specific effects were shared across substances independent of the effects each substance shared with conduct disorder.

The standardized parameter estimates from the best-fitting model are shown in Fig. 1, with the proportions of variance presented in Table 3 and the genetic and environmental correlations in Table 4. As shown in Table 3, familial influences accounted for substantial variance in all measures, with genetic factors explaining 39% of the variance in conduct disorder, and 62%, 48%, and 65% of the variance in ND, AD, and

CAD, respectively. Shared environmental influences accounted for an additional 15% of the variance in conduct disorder. Genetic correlations were significant and moderate to substantial (ranging from 0.38–0.77; above the diagonal in Table 4), especially between conduct disorder and ND and CAD, where over 50% of the genetic influences were overlapping. Importantly, the genetic correlation across substances was entirely attributable to genes shared with conduct disorder. Non-shared environmental influences were predominantly measure specific, as shown by the modest correlations between individual-specific environmental factors (range: 0.03–0.28; below the diagonal in Table 4).

Discussion

In a large cohort of adult Australian twins, we show that the previously well-documented shared genetic etiology across ND, AD, and CAD was entirely attributable to genes contributed by a shared liability to conduct disorder. Our study extends previous research in that we: (a) examined conduct disorder, ND, AD, and CAD simultaneously in an adult sample [researchers have examined these substances together (Kendler et al. 2007, 2008), and have examined conduct disorder

Table 4. Genetic (above diagonal) and non-shared environmental correlations^a between conduct disorder, nicotine dependence, alcohol dependence, and cannabis abuse/dependence from the best-fitting model examining the genetic and environmental contributions to the co-morbidity between conduct disorder, alcohol dependence, nicotine dependence and cannabis abuse/dependence in 9577 Australian twins

	Conduct disorder	Nicotine dependence	Alcohol dependence	Cannabis abuse/dependence
Conduct disorder	–	0.77* (0.69–0.87)	0.52* (0.44–0.61)	0.72* (0.64–0.81)
Nicotine dependence	0.03 (–0.10 to 0.14)	–	0.40* (0.33–0.48)	0.56* (0.48–0.64)
Alcohol dependence	0.28* (0.19 to 0.38)	0.27* (0.17–0.35)	–	0.38* (0.31–0.45)
Cannabis abuse/dependence	0.17* (0.05 to 0.31)	0.18* (0.06–0.31)	0.28* (0.17–0.39)	–

*Indicates $p < 0.05$.

^a Genetic correlations are above the diagonal; non-shared environmental correlations are below the diagonal.

with AD and general substance use disorders (Krueger *et al.* 2002; Kendler *et al.* 2003b), but not all in the same analysis]; (b) extended Hicks and colleagues analysis of adolescents (aged 16–19 years; Hicks *et al.* 2011), which could yield different patterns of overlap; (c) set to missing the data from individuals who had not initiated a substance and hence had unknown genetic liability. Our results are consistent with previous reports (Fu *et al.* 2002; Kendler *et al.* 2003b; Button *et al.* 2006; Hicks *et al.* 2011) showing that alcohol and drug dependence tend to share an appreciable proportion of their genetic liability with conduct and antisocial personality disorder, and further demonstrating that similar effects extend to ND and to substance use disorders contingent on initiation. The overall structure of genetic overlap (see Fig. 1) is also broadly consistent with numerous twin studies of adolescent and adult populations indexing a general liability factor underlying a host of externalizing disorders, including conduct disorder, substance use disorders and also impulsivity and non-substance-related disinhibition (Krueger *et al.* 2002; Hicks *et al.* 2011).

Such results have key implications for gene-finding efforts such as genome-wide association studies (GWAS). In particular, they imply that, as least when examining initiators, phenotypes that aggregate across various substance use disorders (e.g. Wetherill *et al.* 2015) or examine genetic variants that influence multiple substances (McGue *et al.* 2013; Vrieze *et al.* 2014) may effectively be capturing genetic variation shared with liability to conduct disorder. Thus, approaches of aggregating across substances or of using conduct disorder and other aspects of behavioral disinhibition as proxies may be particularly useful in increasing power to detect genetic variants shared across substances, particularly in adolescent cohorts where subjects may not have fully passed through the period of greatest risk for onset of substance use disorders (Iacono *et al.* 1999; Rose *et al.* 2004; Button *et al.* 2006; Hicks *et al.* 2011). However, at least one study reports

that polygenic scores created for individual substance-related measures and behavioral disinhibition are only modestly ($r = 0.03$ – 0.07) correlated with each other (Vrieze *et al.* 2013).

Developmental changes in the degree to which genetic factors contribute to substance involvement itself also should be considered, although there is disagreement about the pattern of change. For instance, research by Vrieze *et al.* (2012) suggests that the genetic contribution to the overlap across substances may decline from adolescence into adulthood, while research by Kendler and colleagues suggests that the genetic contribution to overlap across substance use remains steady, even increases modestly, across that time span (Kendler *et al.* 2008). There is also evidence that the correlation between conduct disorder and substance involvement is more attributable to shared environmental factors during adolescence (Rose *et al.* 2004). Thus, our finding of strong genetic overlap may be specific to the age of our cohort (young adults) and may not generalize to other developmental settings.

Despite genetic overlap, the statistically significant role of substance-specific genetic influences cannot be ignored. In fact, a significant proportion of the genetic variance in ND (40%), AD (73%), and CAD (47%) was substance-specific. The finding of substance-specific genetic influences corroborates previous research, although the relatively higher proportion of specific genetic influences on AD *v.* ND is in contrast to one prior study that found ND to be most significantly influenced by specific genetic factors (Kendler *et al.* 2007). The magnitude of these specific genetic factors underscores that when studying the genomic underpinnings of individual substance use disorders, variants are not exclusively expected to reflect a general liability to conduct disorder or externalizing problems. Most notably, for AD and ND, the most robustly validated single nucleotide polymorphisms identified via GWAS have been substance specific. For alcohol,

rs1229984 in the alcohol dehydrogenase (*ADH1B*) gene is associated with accelerated conversion of ethanol to acetaldehyde, thus protecting against alcoholism via a specific metabolic pathway (Thomasson *et al.* 1993). This functional polymorphism has been identified at genome-wide significant levels in two independent studies (Bierut *et al.* 2012; Gelernter *et al.* 2014). For tobacco smoking, a similar metabolic variant in the cytochrome P450 A6 (*CYP2A6*) gene has been identified in one large meta-analysis (Thorgeirsson *et al.* 2010). However, the most widely replicated findings are for tobacco smoking and rs16969968 in the cholinergic nicotinic receptor α_5 subunit (*CHRNA5*) (Liu *et al.* 2010; Thorgeirsson *et al.* 2010; Tobacco and Genetics Consortium, 2010) and other variants in the family of genes encoding nicotinic receptor subunits, which have been repeatedly identified at genome-wide significant levels. It is worth noting that the large GWAS meta-analyses of smoking that identified rs16969968 relied on nicotine consumption measures such as cigarettes per day (not dependence) and primarily included samples in which the co-morbid effects of conduct disorder are likely to be minimal. These substance-specific findings have also arisen in a sample with a high degree of drug-related co-morbidity (e.g. Rice *et al.* 2012) but in that study, when studying one substance, diagnoses of other substance use disorders were accounted for, thus likely refining the ability to home in on substance-specific results. Future studies may also wish to control for conduct disorder to further enhance chances of identifying drug-specific variants.

Some limitations of the current study are worth noting. First, this is a sample of adult Caucasian Australians and findings may not generalize to other ethnic groups. Second, in order to test for the role of substance initiation on the genetic overlap with substance use disorder, we set to missing the substance-specific data for individuals who had never tried a given substance (conduct disorder was analyzed for everyone, as was substance dependence data from each individual for the substances he/she had used more times than the cutoff established). An alternate approach would have been to use two-stage twin models where the relationship between substance use and use disorders is expressly modeled (e.g. Kendler *et al.* 1999; Heath *et al.* 2002). However, this would have resulted in a 7-variable multivariate structure which our sample was underpowered to support. Third, a related caveat is that we included nicotine and cannabis experimenters as unaffected for dependence; an alternative would have been to restrict the analyses to those with more substantial exposure, as we did with alcohol. It is possible that the genetic overlap of conduct disorder with ND and CAD might change with a different substance use threshold (e.g. if conduct

disorder is associated more with the transition to 'regular use' than with the transition to 'dependence'). Fourth, the ordering of variables in our Cholesky model reflects the most commonly reported order of onset, but there are individuals who had a different order of onset. Although our variance components are applicable regardless of the order of onset, some of the parameter estimates in the Cholesky model would differ if a different variable order was used. Fifth, there is some evidence that the genetic structure of alcohol problems is not attributable to a single factor; in twin analyses examining the seven individual symptoms of DSM-IV AD, Kendler *et al.* (2012) found that the individual symptoms were best explained using three distinct genetic factors. However, analyzing the individual symptoms for AD, ND and CAD was beyond the scope of our analysis. Sixth, our CAD diagnoses included only a subset of DSM-IV symptoms (1+ of two abuse symptoms or 2+ of four dependence symptoms), which may have affected our prevalence and/or heritability. This was done because only this subset of items was included in the 1996–2000 cohort assessment. However, Lynskey and colleagues (2002) have reported that a diagnosis of CAD based on these six items reasonably approximates the population prevalence and does not attenuate estimates of heritability. Finally, all of our diagnoses are drawn from adult retrospective reports which may have been subject to recall bias.

In this era of big data, several efforts are underway to mega- and meta-analyze genome-wide data for substance use disorders. Our study indicates that looking across substances may provide a unique set of genetic variants that relate to a general vulnerability to conduct problems (at least among substance initiators), whereas co-varying for co-morbid conduct disorder may isolate substance specific findings. Importantly, our study documents the enormous value of twin studies in providing a paradigm for development of substance-related phenotypes for gene-identification efforts.

Supplementary material

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0033291715001518>.

Acknowledgements

This study was supported by grant funding from the National Institute on Drug Abuse (NIDA) grants DA23668 (A.A.), DA32573 (A.A.), DA18267 (M.T.L.) and T32DA007313 (L.F.) as well as from the National Institute of Alcohol Abuse and Alcoholism (NIAAA) including AA11998, AA07728 and AA13221 to A.C.

H. Data collection for sample 2 was also facilitated through access to the Australian Twin Registry, a national resource supported by an Enabling Grant (ID 628911) from the National Health & Medical Research Council. Funding agencies did not participate in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. The authors thank the Australian Twin Registry and the twins themselves for participating in this research.

Declaration of Interest

None.

References

- APA (1994). *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Association: Washington, DC.
- Bierut LJ, Goate AM, Breslau N, Johnson EO, Bertelsen S, Fox L, Agrawal A, Bucholz KK, Gruzza R, Hesselbrock V, Kramer J, Kuperman S, Nurnberger J, Porjesz B, Saccone NL, Schuckit M, Tischfield J, Wang JC, Foroud T, Rice JP, Edenberg HJ (2012). ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. *Molecular Psychiatry* **17**, 445–450.
- Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger Jr. JL, Reich T, Schmidt I, Schuckit MA (1994). A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *Journal of Studies on Alcohol* **55**, 149–158.
- Button TM, Hewitt JK, Rhee SH, Young SE, Corley RP, Stallings MC (2006). Examination of the causes of covariation between conduct disorder symptoms and vulnerability to drug dependence. *Twin Research and Human Genetics* **9**, 38–45.
- Button TM, Rhee SH, Hewitt JK, Young SE, Corley RP, Stallings MC (2007). The role of conduct disorder in explaining the comorbidity between alcohol and illicit drug dependence in adolescence. *Drug and Alcohol Dependence* **87**, 46–53.
- Fu Q, Heath AC, Bucholz KK, Nelson E, Goldberg J, Lyons MJ, True WR, Jacob T, Tsuang MT, Eisen SA (2002). Shared genetic risk of major depression, alcohol dependence, and marijuana dependence: contribution of antisocial personality disorder in men. *Archives of General Psychiatry* **59**, 1125–1132.
- Gelernter J, Kranzler HR, Sherva R, Almasy L, Koesterer R, Smith AH, Anton R, Preuss UW, Ridinger M, Rujescu D, Wodarz N, Zill P, Zhao H, Farrer LA (2014). Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci. *Molecular Psychiatry* **19**, 41–49.
- Heath AC, Bucholz KK, Madden PAF, Dinwiddie SH, Slutske WS, Bierut LJ, Statham DJ, Dunne MP, Whitfield JB, Martin NG (1997). Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. *Psychological Medicine* **27**, 1381–1396.
- Heath AC, Martin NG, Lynskey MT, Todorov AA, Madden PAF (2002). Estimating two-stage models for genetic influences on alcohol, tobacco or drug use initiation and dependence vulnerability in twin and family data. *Twin Research* **5**, 113–124.
- Hicks BM, Schalet BD, Malone SM, Iacono WG, McGue M (2011). Psychometric and genetic architecture of substance use disorder and behavioral disinhibition measures for gene association studies. *Behavior Genetics* **41**, 459–475.
- Iacono WG, Carlson SR, Taylor J, Elkins IJ, McGue M (1999). Behavioral disinhibition and the development of substance-use disorders: findings from the Minnesota Twin Family Study. *Development and Psychopathology* **11**, 869–900.
- Kendler KS, Aggen SH, Prescott CA, Crabbe J, Neale MC (2012). Evidence for multiple genetic factors underlying the DSM-IV criteria for alcohol dependence. *Molecular Psychiatry* **17**, 1306–1315.
- Kendler KS, Jacobson KC, Prescott CA, Neale MC (2003a). Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. *American Journal of Psychiatry* **160**, 687–695.
- Kendler KS, Myers J, Prescott CA (2007). Specificity of genetic and environmental risk factors for symptoms of cannabis, cocaine, alcohol, caffeine, and nicotine dependence. *Archives of General Psychiatry* **64**, 1313–1320.
- Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA (1999). A population-based twin study in women of smoking initiation and nicotine dependence. *Psychological Medicine* **29**, 299–308.
- Kendler KS, Prescott CA, Myers J, Neale MC (2003b). The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Archives of General Psychiatry* **60**, 929–937.
- Kendler KS, Schmitt E, Aggen SH, Prescott CA (2008). Genetic and environmental influences on alcohol, caffeine, cannabis, and nicotine use from early adolescence to middle adulthood. *Archives of General Psychiatry* **65**, 674–682.
- Knopik VS, Heath AC, Madden PAF, Bucholz KK, Slutske WS, Nelson EC, Statham D, Whitfield JB, Martin NG (2004). Genetic effects on alcohol dependence risk: re-evaluating the importance of psychiatric and other heritable risk factors. *Psychological Medicine* **34**, 1519–1530.
- Krueger RF, Hicks BM, Patrick CJ, Carlson SR, Iacono WG, McGue M (2002). Etiologic connections among substance dependence, antisocial behavior, and personality: modeling the externalizing spectrum. *Journal of Abnormal Psychology* **111**, 411–424.
- Liu JZ, Tozzi F, Waterworth DM, Pillai SG, Muglia P, Middleton L, Berrettini W, Knouff CW, Yuan X, Waeber G, Vollenweider P, Preisig M, Wareham NJ, Zhao JH, Loos RJE, Barroso I, Khaw KT, Grundy S, Barter P, Mahley R, Kesaniemi A, McPherson R, Vincent JB, Strauss J, Kennedy JL, Farmer A, McGuffin P, Day R, Matthews K, Bakke P, Gulsvik A, Lucae S, Ising M,

- Brueckl T, Horstmann S, Wichmann HE, Rawal R, Dahmen N, Lamina C, Polasek O, Zgaga L, Huffman J, Campbell S, Kooner J, Chambers JC, Burnett MS, Devaney JM, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Epstein S, Wilson JF, Wild SH, Campbell H, Vitart V, Reilly MP, Li M, Qu L, Wilensky R, Matthaï W, Hakonarson HH, Rader DJ, Franke A, Wittig M, Schafer A, Uda M, Terracciano A, Xiao X, Busonero F, Scheet P, Schlessinger D, St Clair D, Rujescu D, Abecasis GR, Grabe HJ, Teumer A, Volzke H, Petersmann A, John U, Rudan I, Hayward C, Wright AF, Kolcic I, Wright BJ, Thompson JR, Balmforth AJ, Hall AS, Samani NJ, Anderson CA, Ahmad T, Mathew CG, Parkes M, Satsangi J, Caulfield M, Munroe PB, Farrall M, Dominiczak A, Worthington J, Thomson W, Eyre S, Barton A, The Wellcome Trust Case Control Consortium, Mooser V, Francks C, Marchini J (2010). Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nature Genetics* 42, 436–440.
- Lynskey MT, Agrawal A, Henders AK, Nelson EC, Madden PAF, Martin NG (2012). An Australian twin study of cannabis and other illicit drug use and misuse, and other psychopathology. *Twin Research and Human Genetics* 15, 631–641.
- Lynskey MT, Heath AC, Nelson EC, Bucholz KK, Madden PAF, Slutske WS, Statham DJ, Martin NG (2002). Genetic and environmental contributions to cannabis dependence in a national young adult twin sample. *Psychological Medicine* 32, 195–207.
- Maciejewski DF, Creemers HE, Lynskey MT, Madden PAF, Heath AC, Statham DJ, Martin NG, Verweij KJH (2014). Overlapping genetic and environmental influences on nonsuicidal self-injury and suicidal ideation: different outcomes, same etiology? *JAMA Psychiatry* 71, 699–705.
- McGue M, Zhang Y, Miller MB, Basu S, Vrieze S, Hicks B, Malone S, Oetting WS, Iacono WG (2013). A genome-wide association study of behavioral disinhibition. *Behavior Genetics* 43, 363–373.
- Medland SE, Nyholt DR, Painter JN, McEvoy BP, McRae AF, Zhu G, Gordon SD, Ferreira MA, Wright MJ, Henders AK, Campbell MJ, Duffy DL, Hansell NK, Macgregor S, Slutske WS, Heath AC, Montgomery GW, Martin NG (2009). Common variants in the trichohyalin gene are associated with straight hair in Europeans. *American Journal of Human Genetics* 85, 750–755.
- Neale MC (2004). *Statistical Modeling with Mx*. Department of Psychiatry, Box #980710: Richmond, VA 23298.
- Neale MC, Cardon LR (1992). *Methodology for Genetic Studies of Twins and Families*. Kluwer Academic Publishers: The Netherlands.
- Neale MC, Harvey E, Maes HH, Sullivan PF, Kendler KS (2006). Extensions to the modeling of initiation and progression: applications to substance use and abuse. *Behavior Genetics* 36, 507–524.
- Palmer RH, Young SE, Corley RP, Hopfer CJ, Stallings MC, Hewitt JK (2013). Stability and change of genetic and environmental effects on the common liability to alcohol, tobacco, and cannabis DSM-IV dependence symptoms. *Behavior Genetics* 43, 374–385.
- Rhee SH, Hewitt JK, Young SE, Corley RP, Crowley TJ, Neale MC, Stallings MC (2006). Comorbidity between alcohol dependence and illicit drug dependence in adolescents with antisocial behavior and matched controls. *Drug and Alcohol Dependence* 84, 85–92.
- Rice JP, Hartz SM, Agrawal A, Almasy L, Bennett S, Breslau N, Bucholz KK, Doheny KF, Edenberg HJ, Goate AM, Hesselbrock V, Howells WB, Johnson EO, Kramer J, Krueger RF, Kuperman S, Laurie C, Manolio TA, Neuman RJ, Nurnberger JI, Porjesz B, Pugh E, Ramos EM, Saccone N, Saccone S, Schuckit M, Bierut LJ (2012). CHRN3 is more strongly associated with Fagerstrom test for cigarette dependence-based nicotine dependence than cigarettes per day: phenotype definition changes genome-wide association studies results. *Addiction* 107, 2019–2028.
- Rose RJ, Dick DM, Viken RJ, Pulkkinen L, Kaprio J (2004). Genetic and environmental effects on conduct disorder and alcohol dependence symptoms and their covariation at age 14. *Alcoholism, Clinical and Experimental Research* 28, 1541–1548.
- Sartor CE, Grant JD, Bucholz KK, Madden PAF, Heath AC, Agrawal A, Whitfield JB, Statham DJ, Martin NG, Lynskey MT (2010). Common genetic contributions to alcohol and cannabis use and dependence symptomatology. *Alcoholism, Clinical Experimental Research* 34, 545–554.
- SAS Institute, Inc (2002–2008). *SAS User's Guide, Version 9.2*. SAS Institute Inc: Cary, NC.
- Thomasson HR, Crabb DW, Edenberg HJ, Li TK (1993). Alcohol and aldehyde dehydrogenase polymorphisms and alcoholism. *Behavior Genetics* 23, 131–136.
- Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, Sulem P, Rafnar T, Esko T, Walter S, Gieger C, Rawal R, Mangino M, Prokopenko I, Magi R, Keskitalo K, Gudjonsdottir IH, Gretarsdottir S, Stefansson H, Thompson JR, Aulchenko YS, Nelis M, Aben KK, den Heijer M, Dirksen A, Ashraf H, Soranzo N, Valdes AM, Steves C, Uitterlinden AG, Hofman A, Tonjes A, Kovacs P, Hottenga JJ, Willemsen G, Vogelzangs N, Doring A, Dahmen N, Nitz B, Pergadia ML, Saez B, De Diego V, Lezcano V, Garcia-Prats MD, Ripatti S, Perola M, Kettunen J, Hartikainen AL, Pouta A, Laitinen J, Isohanni M, Huei-Yi S, Allen M, Krestyaninova M, Hall AS, Jones GT, van Rij AM, Mueller T, Dieplinger B, Haltmayer M, Jonsson S, Matthiasson SE, Oskarsson H, Tyrifingsson T, Kiemeneý LA, Mayordomo JI, Lindholt JS, Pedersen JH, Franklin WA, Wolf H, Montgomery GW, Heath AC, Martin NG, Madden PAF, Giegling I, Rujescu D, Jarvelin MR, Salomaa V, Stumvoll M, Spector TD, Wichmann HE, Metspalu A, Samani NJ, Penninx BW, Oostra BA, Boomsma DI, Tiemeier H, van Duijn CM, Kaprio J, Gulcher JR, The ENGAGE Consortium, McCarthy ML, Peltonen L, Thorsteinsdottir U, Stefansson K (2010). Sequence variants at CHRN3-CHRNA6 and CYP2A6 affect smoking behavior. *Nature Genetics* 42, 448–453.
- Tobacco and Genetics Consortium (2010). Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nature Genetics* 42, 441–447.

- Vrieze SI, Feng S, Miller MB, Hicks BM, Pankratz N, Abecasis GR, Iacono WG, McGue M** (2014). Rare nonsynonymous exonic variants in addiction and behavioral disinhibition. *Biological Psychiatry* **75**, 783–789.
- Vrieze SI, Hicks BM, Iacono WG, McGue M** (2012). Decline in genetic influence on the co-occurrence of alcohol, marijuana, and nicotine dependence symptoms from age 14 to 29. *American Journal of Psychiatry* **169**, 1073–1081.
- Vrieze SI, McGue M, Miller MB, Hicks BM, Iacono WG** (2013). Three mutually informative ways to understand the genetic relationships among behavioral disinhibition, alcohol use, drug use, nicotine use/dependence, and their co-occurrence: twin biometry, GCTA, and genome-wide scoring. *Behavior Genetics* **43**, 97–107.
- Wetherill L, Agrawal A, Kapoor M, Bertelsen S, Bierut LJ, Brooks A, Dick D, Hesselbrock M, Hesselbrock V, Koller DL, Le N, Nurnberger Jr. JI, Salvatore JE, Schuckit M, Tischfield JA, Wang JC, Xuei X, Edenberg HJ, Porjesz B, Bucholz K, Goate AM, Foroud T** (2015). Association of substance dependence phenotypes in the COGA sample. *Addiction Biology* **20**, 617–627.
- Xian H, Scherrer JF, Grant JD, Eisen SA, True WR, Jacob T, Bucholz KK** (2008). Genetic and environmental contributions to nicotine, alcohol and cannabis dependence in male twins. *Addiction* **103**, 1391–1398.
- Young SE, Rhee SH, Stallings MC, Corley RP, Hewitt JK** (2006). Genetic and environmental vulnerabilities underlying adolescent substance use and problem use: general or specific? *Behavior Genetics* **36**, 603–615.