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Evidence for a two-stage model of dependence using the NESARC and its implications for genetic association studies

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Abstract

Some twin studies suggest that substance initiation and dependence are part of a complex, two-stage process and that some genetic influences are stage-specific, acting on either the transition from abstinence to initiation, or on the transition from use to dependence. However, questions remain about the two-stage model, especially for illicit drugs. Using a familial aggregation design, we tested the hypothesized two-stage model of dependence on illicit substances and alcohol in a large, nationally representative sample. Family history of drug or alcohol problems is significantly associated with initiation that does not progress to dependence (i.e., conditional initiation). Furthermore, family history of drug or alcohol problems is significantly associated with dependence even after conditioning on factors influencing initiation (i.e., conditional dependence). These results suggest that substance initiation and dependence involve at least partially distinct familial factors. The possibility that different genetic factors affect initiation and dependence has important implications for control group selection in case-control genetic association studies, and may explain some inconsistent results for drug dependence. If some genetic factors are stage-specific (i.e., not common across initiation and dependence), inclusion of abstainers in the control group may mix the genetic effects for initiation with those for transition to dependence, providing unclear results. Depending on the specific question about the nature of the genetic effect (whether on initiation, on dependence, or both), investigators designing case-control genetic association studies should carefully consider inclusion and exclusion criteria of the control group.

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Keywords

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1. INTRODUCTION

Twin studies indicate that genetic factors play a significant role in both drug and alcohol (denoted hereafter as substance) initiation and dependence (Lachman, 2006; Merikangas and Avenevoli, 2000). Compared to the large number of twin studies on the general heritability of alcohol and drug disorders, fewer twin studies investigated whether genetic influences are common across the stages of both initiation and dependence, or involved in a specific stage (i.e., dependence only). By conditioning on use of the substance (denoted hereafter as "conditional dependence"), these studies have attempted to partition genetic influences on initiation of use from those unique to dependence (Agrawal et al., 2005; Agrawal and Lynskey, 2006; Kendler et al., 1999; Tsuang et al., 1999; Whitfield et al., 2004). Results in U.S. and Australian samples suggest that initiation and dependence are part of a complex, two-stage process and that some genetic influences are stage-specific, acting on the transition from abstinence to initiation, or on the transition from use to dependence. Genetic influences on initiation may act through disinhibitory psychopathology or temperament traits such as risk-taking and novelty-seeking behavior (Kreek et al., 2005; McGue et al., 2001) whereas influences on dependence may include pharmacokinetic and pharmacodynamic effects (Agrawal et al., 2005; Kendler et al., 1999; Saxon et al., 2005; Tsuang et al., 1999).

Separate genetic effects for initiation and dependence have important implications for control group selection and may explain some inconsistent results in genetic association studies of substance use disorders. Worldwide, case-control genetic association studies for dependence on illicit substances have had somewhat inconsistent results (Saxon et al., 2005). While pitfalls such as small samples and confounding (i.e., population stratification) have been blamed (Lachman, 2006), the lack of replication may also be due, in part, to differences in selection criteria for the control groups across studies, particularly with respect to the inclusion of abstainers (Buckland, 2001; Tsuang et al., 1999), i.e., individuals who never used the substance. Some studies restricted control subjects to abstainers (Gerra et al., 2004; Li et al., 2004; Liu et al., 2005; Uhl et al., 2001) or those with extremely low frequency of use (Morita et al., 2005; Xu et al., 2002). Other studies specifically included users, i.e., those exposed to the substance (Kohnke et al., 2005; Sullivan et al., 2001a) and attempted to match on level of drug use (Franke et al., 2003). Still others did not describe if controls were abstainers or users (Dahl et al., 2005; Luo et al., 2005; Mottagui-Tabar et al., 2005).

While twin studies assessing conditional dependence support the two-stage model of dependence (initiation as distinct from dependence), some aspects of these studies leave the issue unresolved, especially for illicit substances: (a) studies typically had limited statistical power to assess specific drugs types (Kendler et al., 1999), or (b) focused on whether risk factors were common or unique across substances when the two stages (initiation and dependence) were considered separately (e.g., Kendler et al., 2003), or (c) did not focus on illicit substances, but rather, alcohol and tobacco *(*e.g., Heath et al., 1990; Kendler et al., 1999; Madden et al., 1999; Maes et al., 2004; True et al., 1999; Vink et al., 2006). Further, some authors have suggested that the previous twin studies provided only indirect evidence on progression to dependence (Neale et al., 2006), with newer methods only recently introduced to directly address conjoint shared and unique influences on the different stages in twin studies (Agrawal et al., 2005; Neale et al., 2006). More information is needed on support for the two-stage model in the progression from use to dependence for illicit substances. In addition,

concerns are sometimes raised that twin results may not generalize to singletons for complex traits (Hall, 2003; Ronalds et al., 2005).

For all of these reasons, we undertook a familial aggregation study to test the hypothesized two-stage model of dependence in a large, nationally representative sample, focusing largely on dependence on illicit drugs. The familial aggregation design is often used to refine disease phenotypes that appear to have a genetic component in their etiology (Khoury et al., 1993; Zimmerman et al., 2006). The goal was to determine whether or not family history is a risk factor for initiation (denoted hereafter as "initiation"), conditional initiation (i.e., use without dependence), dependence (denoted hereafter as "dependence"), and conditional dependence (i.e., dependence among users). By conditioning on use without progression to dependence, familial effects on initiation are not confounded by factors that affect transition from use to dependence. By conditioning on prior use, familial effects on dependence are not confounded by factors (both genetic and environmental) involved only in initiation. If family factors are found to significantly impact conditional initiation as well as conditional dependence, this would suggest that conditional initiation and conditional dependence involve at least partially distinct familial factors, supporting the two stage model of dependence. Such findings would have important implications for the design of genetic association studies of dependence on illicit substances, for example, whether to include or exclude abstainers in control groups when the goal is to identify genes affecting the risk for dependence.

2. METHODS

2.1 Sample

Subjects were participants in the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC), a nationally representative face-to-face survey of 43,093 respondents aged 18 years and older conducted by the NIAAA in 2001-2002. The target population of the NESARC was the civilian non-institutionalized population residing in the United States and District of Columbia, including Alaska and Hawaii. African-Americans and Hispanics were oversampled, as were young adults. The NESARC also included a group quarters sampling frame. The group quarters sampling frame was derived from the Census 2000 Group Quarters Inventory. This frame captures important subgroups of the population with heavy substance use patterns not often included in general population surveys. These included the military living off base, boarding houses, rooming houses, nontransient hotels and motels, shelters, facilities for housing workers, college quarters, and group homes. Hospitals, jails, and prisons were not among the group quarters sampled. More detailed information of the sample design and sampling frame are described in detail elsewhere (Grant et al., 2003; Grant et al., 2004). The overall survey response rate was 81%. Of the subjects analyzed below, 47.9% were male. White subjects comprised 70.9% of the sample, while 10.8% were African-American, 11.6% Hispanic, 4.5% Asian, and 2.3% Native American. By age, 21.8% were 18 - 29, 30.9% were 30 - 44, 31.1% were 45 - 64 and 16.2% were 65 or older.

2.2 Procedures and quality control

Approximately 1800 professional interviewers from the U.S. Bureau of the Census administered the AUDADIS-IV using laptop computer-assisted software with built-in skip logic and consistency checks (Grant et al., 2004). Interviewers had an average of five years experience on Census and other health-related national surveys. All interviewers completed 10 days of training, standardized across the Census Bureau's 12 regional offices through centralized training sessions directed by NIAAA and Census headquarters staff. For quality control purposes, regional supervisors re-contacted a random 10% of all respondents and re-asked a subset of the interview questions to verify the accuracy of the interviewer's performance. This careful process showed that the interviewers performed at a high level, as

indicated by the high reliability of the instrument (Grant et al., 2003). In the very few cases when the accuracy of the interviews was uncertain, the interview data were discarded and the interview re-done by a supervising interviewer.

2.3 Measures

The data analyzed in this report were generated by the NIAAA Alcohol Use Disorder and Associated Disabilities Interview Schedule - DSM-IV Version (Grant et al., 2001), a fully-structured diagnostic interview designed specifically for non-clinician interviewers and developed to advance measurement of substance use and mental disorders in large, general population surveys.

2.4 Drug and alcohol dependence diagnoses

The AUDADIS-IV includes detailed assessment of DSM-IV abuse and dependence criteria for alcohol as well as for 10 classes of drugs, including sedatives, tranquilizers, opiates (other than heroin or methadone), stimulants, hallucinogens, cannabis, cocaine (including crack cocaine), inhalants/solvents, heroin, and other drugs. Participants were classified as "abstainers" from alcohol if they never consumed a full drink of an alcoholic beverage; and as abstainers from drugs if they responded negatively to all questions about lifetime use for ten categories of psychoactive substances. We report data on NESARC participants' alcohol, cannabis, cocaine, and a composite "any drug" category (which includes all ten illicit drug categories). Lifetime diagnoses of alcohol and drug dependence were derived with computer algorithms that operationalized the DSM-IV (American Psychiatric Association, 1994) criteria; at least 3 of 7 criteria required for DSM-IV alcohol or drug dependence. The reliability and validity of substance diagnoses and symptom items have been examined via test-retest reliability studies, clinical reappraisals conducted by psychiatrists (Canino et al., 1999) and other designs. These had excellent reliability in U.S. and international clinical and general population samples, with alcohol diagnoses having a minimum kappa of 0.74 and drug diagnoses having a minimum reliability of 0.79 (Canino et al., 1999; Chatterji et al., 1997; Grant et al., 1995; Grant et al., 2003; Hasin et al., 1997b). Validity was demonstrated in numerous studies including the World Health Organization/National Institutes of Health and Reliability and Validity Study (Chatterji et al., 1997; Cottler et al., 1997; Hasin et al., 2003; Pull et al., 1997; Vrasti et al., 1998), psychiatrist re-examination (Canino et al., 1999) and others (Grant et al., 1992; Grant, 1996; Grant and Harford, 1988; Grant and Harford, 1990; Hasin and Grant, 1994; Hasin et al., 1997a; Hasin et al., 1997c; Hasin and Paykin, 1999).

2.5 Age at first initiation and age of onset

Age of alcohol and drug initiation (i.e. age of first use) is assessed by the AUDADIS for each substance used by the respondent. The reliability of the age of first initiation variable has been shown to be good to excellent, with ICC ranging from 0.69 for sedatives to 0.94 for opioids (Grant et al., 1995). Age of onset of alcohol or drug dependence was assessed by respondent report of first age at which substance-specific dependence symptoms began to cluster in the same time period. For polydrug users, the age of onset for the first drug dependence was used.

2.6 Family history

Family histories of alcohol and drug problems were ascertained in separate modules of the AUDADIS. Subjects were prompted with a definition that included examples of problems in the alcohol and drug diagnostic criteria, and then asked whether relatives (by category) had experienced the condition as defined. The definitions read to respondents included readily observable manifestations, since these are the mostly likely to be known to family informants and sensitivity is the main issue in family history information (Andreasen et al., 1977; Slutske et al., 1996; Zimmerman et al., 1988). To reduce interview time on questions whose answers

unlikely to be known to respondents, data were not collected on the specific drug types used by relatives, or whether relatives met diagnostic criteria for alcohol or drug dependence. To use variables with the best possible quality information in this report, and to cover relatives sharing the highest proportion of genes in common with the NESARC participant, only first-degree relatives were included in the analyses shown below; more distant relatives were not included as their disorders are less likely to be known to the respondent. With this information, we defined two types of variables for family history of drug and alcohol problems: binary variables (any first-degree relative positive), and ordinal variables (family density) based on the proportion of positive first-degree relatives. The ordinal variable was divided into four ordinal categories: 0% positive, >0% - <25% positive, >25% - <50% positive, and >50% positive. The test-retest reliability of AUDADIS family history variables is very good to excellent (Grant et al., 1995; Grant et al., 2003; Hasin et al., 1997c).

2.7 Statistical analysis

Survival analysis was the main type of analyses used, a commonly used method of addressing the effects of specific risk factors on the occurrence of disorders among right-censored samples where all participants have not yet passed fully through the period of risk. Four types of outcome variables were investigated: initiation, conditional initiation (use but never dependent), dependence, and conditional dependence (dependence only among users). Each was analyzed separately for alcohol, cannabis, cocaine, and the combined drug category.

For initiation, the full NESARC sample was included. Time to the event was defined as age at onset of use and observations were censored among respondents that never used the substance. For censored observations, age at interview represented the censored time.

Conditional initiation was defined the same way as initiation. However, it was analyzed among the subset of the sample that did not progress to dependence (i.e., use without dependence).

For dependence, the full NESARC sample was included. Time to the event was defined as age at onset of dependence and observations were censored if respondents never met criteria for dependence. For censored observations, age at interview represented the censored time.

Conditional dependence was defined the same way as dependence except that the sample was restricted to respondents that had used the substance (i.e., dependence among users).

In all analyses, we tested if family history was an independent predictor for initiation, conditional initiation, dependence and conditional dependence for each substance type. For example, in the analyses for cannabis, we tested if family history of drug problems was an independent predictor for initiation, conditional initiation, dependence and conditional dependence as defined above. Similar analyses were conducted for cocaine, and any drug. For alcohol, we tested if family history of alcohol problems was an independent predictor. All models were tested using Cox proportional hazards models. Model parameters were estimated with SUDAAN software (Research Triangle Institute, 2005) that uses Taylor series linearization to adjust for the design effects of complex sample surveys like the NESARC. In each model, we controlled for respondent's gender, age group (18-29, 30-44, 45-64, 65+), race (white, black, Hispanic, other), and education (college graduate vs. other). For models of drug initiation and dependence, we additionally controlled for alcohol dependence in the proband and first degree relatives, and for models of alcohol initiation and dependence, we additionally controlled for any drug dependence in first degree relatives. We present adjusted hazard ratios and 95% confidence intervals. For the four-level ordinal family history variables (0% positive, >0% - <25% positive, >25% - <50% positive, and >50% positive), the hazards ratio is interpreted as the increase in the risk for the disorder in the NESARC participant for each one-unit change in the four categories representing increasing family density.

3. RESULTS

Distributions of the key variables among the total NESARC sample and the sub-samples of cannabis users, cocaine users, alcohol users and any illicit drug users are shown in Table 1. Among the total NESARC sample, 20.5% reported ever using cannabis, 6.1% reported ever using cocaine, 22.8% reported ever using any illicit drug and 82.7% reported ever using alcohol. Among cannabis users, 6.3% (n=530) met criteria for cannabis dependence. Among cocaine users, 16.0% (n=408) met criteria for cocaine dependence. Among users of any illicit drug, 11.3% (n=881) met criteria for any illicit drug dependence. Among alcohol users, 15.1% (n=4781) met criteria for alcohol dependence. Of the total NESARC sample, 34.0% reported at least one first degree relative with an alcohol problem and 16.3% reported at least one first degree relative with a drug problem. Not shown in Table 1, there were 24.5% lifetime cannabis users among alcohol users, 7.3% were lifetime cocaine users, and 26.9% used any illicit drug in their lifetime. Of the lifetime drug users, 2.6% stated that they were alcohol abstainers.

3.1 Initiation and dependence (unconditional)

Results of the Cox proportional hazards models for time to drug initiation and dependence (unconditional) by family history are shown in Table 2. Family history, as defined as the proportion of first-degree relatives with a drug problem, significantly increased the hazard ratio (HR) of initiation for cannabis (HR = 1.38), cocaine (HR = 1.41), and any illicit drug (HR = 1.38) compared to individuals without a family history. Family history of drug problems also significantly increased the HR of dependence for cannabis (HR = 1.68), cocaine (HR = 1.55), and any illicit drug (HR = 1.68) (Table 2). Similarly, family history of alcohol problems significantly increased the risk for alcohol initiation (HR = 1.18) and dependence (HR = 1.50) compared with individuals without a family history of alcohol problems (Table 3).

The effects of family history on drug (or alcohol) initiation and dependence was significant regardless of whether family history was based on proportion of first-degree relatives with drug (or alcohol) problems (Tables 2 and 3) or defined as a binary variable (data not shown). The magnitude of effect was always higher for dependence than for initiation for each drug and alcohol group. All tests (including those based on binary family history variables) resulted in p-values < 0.0001.

3.2 Conditional initiation and conditional dependence

The results of the Cox proportional hazards models for time to conditional initiation (initiation without progressing onto dependence) are shown in Table 2. Among individuals who did not progress to dependence, family history of drug problems (defined as the proportion of first-degree relatives with a drug problem) significantly increased the HR of initiation for cannabis (HR = 1.36), cocaine (HR = 1.39), and any illicit drug (HR = 1.34) compared with individuals without a family history. Similarly, family history of alcohol problems significantly increased the risk for conditional initiation for alcohol (HR = 1.15) compared with individuals without a family history of alcohol problems (Table 3).

Family history of drug problems significantly increased the HR of conditional dependence for cannabis (HR = 1.44), cocaine (HR = 1.21), and any illicit drug (HR = 1.46) compared with individuals without a family history (Table 2) and family history of alcohol problems significantly increased the HR for conditional dependence for alcohol (HR = 1.46) compared with individuals without a family history of alcohol problems.

The effects of family history on conditional initiation and conditional dependence were significant regardless of whether the family history was based on the proportion of first-degree relatives with drug (or alcohol) problems (Tables 2 and 3) or defined as a binary variable (data

not shown). Except for conditional dependence for cocaine (p-value = 0.014), all comparisons resulted in p-values less than 0.0001. Descriptively, the difference in familial effect between dependence and conditional dependence (HR [dependence] - HR [conditional dependence]) was notably greater for illicit substances (cannabis, 0.24; cocaine, 0.34; any drug, 0.22) than for alcohol (0.04).

4. DISCUSSION

In this study, we found evidence that substance initiation and dependence each involve both common and distinct familial factors. Family history of drug or alcohol problems was significantly associated with dependence without conditioning on use, supporting familial effects common across initiation and dependence. Family history of drug or alcohol problems was significantly associated with initiation that was conditional on not progressing to dependence, supporting other familial factors specific to initiation. Furthermore, family history of drug or alcohol problems remained a significant risk factor for dependence even after conditioning on factors influencing initiation (i.e., conditional dependence), supporting still other family factors specific to dependence.

In this study, the Cox models using conditional initiation (i.e., use without dependence) and conditional dependence (i.e., dependence among users) allowed us to separate familial risk factors specific to initiation (Stage 1 of the two-stage model) from those specific to dependence (Stage 2 of the two-stage model). These Cox models, while different from the structural equation modeling used in twin studies (Agrawal et al., 2005; Agrawal and Lynskey, 2006; Kendler et al., 1999; Tsuang et al., 1999), were also useful in discriminating between the two stages due to the specific nature of the sub-samples analyzed. One of these sub-samples compared individuals that progressed to Stage 1 only with a control group that did not. Another compared individuals that did and did not progress to Stage 2 among those at Stage 1. If the family factor that predicted dependence on illicit substances entirely explained use, the factor would not predict use in non-dependent users when compared to abstainers. Note that this study was not designed to demonstrate that factors affecting initiation and dependence on illicit substances are entirely independent, nor do we claim that this is what we found. Instead, we conclude that somewhat different factors are operating at the two stages, and that therefore the research question in molecular genetics studies involving illicit substances needs to be sufficiently refined to define the control group correctly. More can be learned about Stage 2 in such a study if care is taken in the design to minimize confusion in the results due to unknowing mixing of factors influencing both Stage 1 and Stage 2. Thus, the above results can be used to guide phenotype and sample design in molecular genetic studies.

Examples of processes involved with initiation are sensation-seeking or novelty-seeking; (McGue et al., 2001), while examples of processes involved with transition from initiation to dependence involve pharmacodynamic interaction of the substances with various neurotransmission systems (Li, 2000; Volkow et al., 2004). The distinct nature of these processes, taken in conjunction with the above results, support the design of genetic association studies whose phenotypes clearly differentiate between these different processes.

Interestingly, the absolute reduction in the magnitude of effect between dependence (unconditional) and conditional dependence was greater for the illicit drug categories than for alcohol. The smaller reduction for alcohol may be due, in part, to the normative aspects and legal status of drinking alcohol in the United States. Personality traits with a heritable component such as antisocial traits may have a larger impact on initiation of illicit substances.

Methodological limitations are noted. This study utilized the family history method rather than direct interviews of relatives. However, the NESARC assessed readily observable

manifestations of substance use disorders, an advantage as these are the mostly likely to be known to relatives (Andreasen et al., 1977; Andreasen et al. 1986; Slutske et al., 1996; Zimmerman et al., 1988). Data were not ascertained on the specific drug type used by the relatives, or whether the relatives met diagnostic criteria for alcohol or drug dependence. Thus, this study does not provide information on whether familial factors are drug-specific. Also, since this is a familial aggregation study, the results do not differentiate between genetic and environmental influences. However, the results are consistent with twin studies addressing factors affecting initiation as distinct from dependence (Agrawal et al., 2005; Agrawal and Lynskey, 2006; Kendler et al., 1999; Tsuang et al., 1999). Additionally, while we controlled for race-ethnicity over broad groups, the study was conducted in an entirely U.S. sample. Similar research of this nature is needed in other countries to determine if the results generalize across different cultures. Finally, the definitions of "abstainer" used in the study (one full drink for alcohol, any use over the lifecourse for drugs) may not have identified initial users of very small amounts (e.g., less than a full drink of alcohol or a single puff of cannabis) who had negative reactions that discouraged further use (Eissenberg and Balster, 2000). For example, many Asian studies of *ALDH2*, a gene affecting alcohol metabolism, explicitly included abstainers (e.g., Chen et al., 1999; Shen et al., 1997; Sun et al., 2002), and our understanding of *ALDH2* could not have been gained without abstainers in the samples. Individuals homozygous for *ALDH2*2* have such unpleasant reactions to alcohol that they cannot drink more than a small sip. Such individuals would be classified as lifetime abstainers in most U.S. studies although they were sufficiently exposed to alcohol to determine their response to alcohol and subsequent abstention. Future studies should include measures of alcohol and drug consumption that are sensitive to very limited quantities of use, in order to clarify how this affects the relationship of genetic factors to lifetime minimal use and abstention.

There are also several important strengths of this study. First, this is the first study to address the issue of conditional initiation and conditional dependence on illicit substances using data from a large, nationally representative sample. As such, the increased familial aggregation found could not have been due to selective factors that may bias results when participants have been ascertained from specialized settings such as clinical facilities. Second, strict diagnostic criteria were used to evaluate the respondents. The NESARC operationalized both alcohol and drug dependence as syndromes. Drug and alcohol-specific diagnoses of dependence were independently assessed whether or not the subject had an abuse diagnosis, allowing complete assessment of dependence in all individuals (Hasin and Grant, 2004; Hasin et al., 2005). Third, respondents were interviewed using a highly reliable and valid instrument, the AUDADIS-IV. This state-of-the-art instrument was specifically developed to assess current and lifetime drug and alcohol abuse and dependence. Fourth, perhaps because the AUDADIS measure of family history was designed to tap observable manifestations of alcohol and drug use disorders, the prevalence of family history of alcohol and drug problems in first degree relatives was similar to prevalences of alcohol and drug use disorders in the U.S. general population as a whole (Compton et al., 2007; Hasin et al., 2007). This suggests that sensitivity was good in the AUDADIS family history measure. Fifth, the sample was large enough not only to analyze alcohol and illicit drugs separately, but also to control for alcohol when illicit drug use/dependence was considered, and to control for illicit drug use/dependence when alcohol was considered, producing specificity of the results.

4.1 Implications of this study

Results of this study have important implications in designing case-control genetic association studies of drug dependence. Restricting the control group to abstainers (individuals who never used the substance) may affect results of these studies by mixing the genetic effects for initiation with those specific to dependence. To illustrate, consider the hypothetically expected allele frequencies for initiating drug use and for dependence among abstainers, non-dependent users,

and individuals with dependence (Table 4). Abstainers (i.e., true abstainers that never even tried the substance) would be expected to have a low frequency of alleles increasing the risk for initiation (i.e., initiation-specific genes) because they have not used the substance. However, they would be expected to have the population frequency for alleles that increase the risk for dependence (i.e., dependence-specific genes) since there are no differential pressures to increase or decrease the risk-inducing allele frequencies in these individuals. Since non-dependent users and individuals with dependence have initiated drug use, we would expect individuals in each of these groups to evidence a high frequency of alleles increasing risk of initiation. Lastly, we would expect non-dependent users to have low frequency of alleles increasing risk for dependence, since they did not transition to dependence, whereas individuals with dependence would be expected to have a high frequency of alleles increasing risk for dependence, since only these individuals transitioned to dependence.

As Table 4 illustrates, case-control genetic association studies comparing drug dependent cases with abstainer controls may not provide clear information about polymorphisms increasing the risk of transitioning to dependence because this comparison mixes the genetic effects influencing initiation of drug use with those increasing the risk for dependence. Such a comparison is likely to find associations for polymorphisms involved in initiation (i.e., high frequency in cases vs. low frequency in controls). Observed associations for alleles influencing dependence would be weaker, and would depend on the population frequencies of the alleles influencing dependence (i.e., high frequency in cases vs. population frequency in controls). However, choosing the appropriate control group also depends on how genetic factors affect the risk for initiation and dependence. If, as suggested by results of this study, substance initiation and dependence involve at least partially separate familial factors, then including abstainers in the control group would mix the effects for initiation and dependence. Choosing the appropriate control group may also depend on the type of genetic effect being assessed. Whereas some genetic influences are specific to alcohol dependence or a particular drug type (e.g., nicotine, cannabis, cocaine), other genetic factors are believed to be involved in both drug and alcohol dependence. Computer simulation studies present an important means of clarifying these issues, which may further help guide researchers in choosing the appropriate control group for various study hypotheses. Such studies should be undertaken.

While some genetic factors may have stage-specific effects and others may influence both initiation and dependence, little information presently exists to differentiate between these influences *a priori* when designing a genetic association study. Therefore, the ideal study design would involve three groups: individuals with dependence, non-dependent users, and abstainers. A comparison of drug dependent cases with non-dependent users would address genetic factors influencing only dependence, while a comparison of non-dependent users with abstainers would address initiation-only factors. Furthermore, a design that compares users (both dependent cases and non-dependent users) with abstainers addresses genetic influences that effect both initiation and dependence. A few studies involving nicotine have used this three-subject group design (Chen et al., 2004; Greenbaum et al., 2006; Lerer et al., 2006; Sullivan et al., 2001a; Sullivan et al., 2001b; Zhang et al., 2006) and separately assessed genetic effects for initiation from those for dependence. These studies, conducted in the U.S. and Israel, also supported the two-stage model, providing further evidence of the value of such a design in genetic association studies of illicit substances.

5. CONCLUSION

Results of our study of conditional initiation (i.e., use without dependence) and conditional dependence (i.e., dependence among users) suggest that substance (both drug and alcohol) initiation and dependence involve at least partly distinct familial factors. Thus, the study supports the two-stage model of dependence. The results of this study, twin studies and some

genetic association studies collectively indicate that researchers should consider excluding abstainers from the control group in case-control genetic association studies where dependence is the phenotype of primary interest because, including abstainers, may mix the genetic effects for initiation with those for dependence. Otherwise, researchers should consider a three-arm design of abstainers, non-dependent users, and dependent individuals. Clarification of the control group's inclusion and exclusion criteria should contribute to more consistent and interpretable results in case-control allelic association studies of dependence on alcohol and illicit substances.

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References

Agrawal A, Lynskey MT. The genetic epidemiology of cannabis use, abuse and dependence. Addiction 2006;101:801–812. [PubMed: 16696624]

Agrawal A, Neale MC, Jacobson KC, Prescott CA, Kendler KS. Illicit drug use and abuse/dependence: modeling of two-stage variables using the CCC approach. Addict Behav 2005;30:1043–1048. [PubMed: 15893102]

Andreasen NC, Endicott J, Spitzer RL, Winokur G. The family history method using diagnostic criteria. Reliability and validity Arch Gen Psychiatry 1977;34:1229–1235.

Andreasen NC, Rice J, Endicott J, Reich T, Coryell W. The family history approach to diagnosis. How useful is it? Arch Gen Psychiatry 1986;43:421–429. [PubMed: 3964020]

Buckland PR. Genetic association studies of alcoholism--problems with the candidate gene approach. Alcohol Alcohol 2001;36:99–103. [PubMed: 11259204]

Canino G, Bravo M, Ramirez R, Febo VE, Rubio-Stipec M, Fernandez RL, Hasin D. The Spanish Alcohol Use Disorder and Associated Disabilities Interview Schedule (AUDADIS): reliability and concordance with clinical diagnoses in a Hispanic population. J Stud Alcohol 1999;60:790–799. [PubMed: 10606491]

Chatterji S, Saunders JB, Vrasti R, Grant BF, Hasin D, Mager D. Reliability of the alcohol and drug modules of the Alcohol Use Disorder and Associated Disabilities Interview Schedule--Alcohol/Drug-Revised (AUDADIS-ADR): an international comparison. Drug Alcohol Depend 1997;47:171–185. [PubMed: 9306043]

Chen CC, Lu RB, Chen YC, Wang MF, Chang YC, Li TK, Yin SJ. Interaction between the functional polymorphisms of the alcohol-metabolism genes in protection against alcoholism. Am J Hum Genet 1999;65:795–807. [PubMed: 10441588]

Chen X, Wu B, Kendler KS. Association study of the Epac gene and tobacco smoking and nicotine dependence. Am J Med Genet B Neuropsychiatr Genet 2004;129:116–119. [PubMed: 15274052]

Compton WM, Thomas YF, Stinson FS, Grant BF. Prevalence, correlates, disability, and comorbidity of DSM-IV drug abuse and dependence in the United States: results from the national epidemiologic survey on alcohol and related conditions. Arch Gen Psychiatry 2007;64:566–576. [PubMed: 17485608]

Cottler LB, Grant BF, Blaine J, Mavreas V, Pull C, Hasin D, Compton WM, Rubio-Stipec M, Mager D. Concordance of DSM-IV alcohol and drug use disorder criteria and diagnoses as measured by AUDADIS-ADR, CIDI and SCAN. Drug Alcohol Depend 1997;47:195–205. [PubMed: 9306045]

Dahl JP, Doyle GA, Oslin DW, Buono RJ, Ferraro TN, Lohoff FW, Berrettini WH. Lack of association between single nucleotide polymorphisms in the corticotropin releasing hormone receptor 1 (CRHR1) gene and alcohol dependence. J Psychiatr Res 2005;39:475–479. [PubMed: 15992556]

Eissenberg T, Balster RL. Initial tobacco use episodes in children and adolescents: current knowledge, future directions. Drug Alcohol Depend 2000;59(Suppl 1):S41–S60. [PubMed: 10773437]

Franke P, Wendel B, Knapp M, Schwab SG, Neef D, Maier W, Wildenauer DB, Hoehe MR. Introducing a new recruitment approach to sample collection for genetic association studies in opioid dependence. Eur Psychiatry 2003;18:18–22. [PubMed: 12648891]

Gerra G, Garofano L, Santoro G, Bosari S, Pellegrini C, Zaimovic A, Moi G, Bussandri M, Moi A, Brambilla F, Donnini C. Association between low-activity serotonin transporter genotype and heroin dependence: behavioral and personality correlates. Am J Med Genet B Neuropsychiatr Genet 2004;126:37–42. [PubMed: 15048645]

Grant BF. DSM-IV, DSM-III-R, and ICD-10 alcohol and drug abuse/harmful use and dependence, United States, 1992: a nosological comparison. Alcohol Clin Exp Res 1996;20:1481–1488. [PubMed: 8947328]

Grant, BF.; Dawson, DA.; Hasin, D. The Alcohol Use Disorder and Associated Disabilities Interview Schedule-DSM-IV version (AUDADIS-IV). National Institute on Alcohol Abuse and Alcoholism; Bethesda, MD: 2001.

Grant BF, Dawson DA, Stinson FS, Chou PS, Kay W, Pickering R. The Alcohol Use Disorder and Associated Disabilities Interview Schedule-IV (AUDADIS-IV): reliability of alcohol consumption, tobacco use, family history of depression and psychiatric diagnostic modules in a general population sample. Drug Alcohol Depend 2003;71:7–16. [PubMed: 12821201]

Grant BF, Harford TC. The relationship between ethanol intake and DSM-III alcohol use disorders: a cross-perspective analysis. J Subst Abuse 1988;1:231–252. [PubMed: 2980873]

Grant BF, Harford TC. The relationship between ethanol intake and DSM-III-R alcohol dependence. J Stud Alcohol 1990;51:448–456. [PubMed: 2232799]

Grant BF, Harford TC, Dawson DA, Chou PS, Pickering RP. The Alcohol Use Disorder and Associated Disabilities Interview schedule (AUDADIS): reliability of alcohol and drug modules in a general population sample. Drug Alcohol Depend 1995;39:37–44. [PubMed: 7587973]

Grant BF, Harford TC, Hasin DS, Chou P, Pickering R. DSM-III-R and the proposed DSM-IV alcohol use disorders, United States 1988: a nosological comparison. Alcohol Clin Exp Res 1992;16:215–221. [PubMed: 1590542]

Grant BF, Stinson FS, Dawson DA, Chou SP, Dufour MC, Compton W, Pickering RP, Kaplan K. Prevalence and co-occurrence of substance use disorders and independent mood and anxiety disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Arch Gen Psychiatry 2004;61:807–816. [PubMed: 15289279]

Greenbaum L, Kanyas K, Karni O, Merbl Y, Olender T, Horowitz A, Yakir A, Lancet D, Ben-Asher E, Lerer B. Why do young women smoke? I. Direct and interactive effects of environment, psychological characteristics and nicotinic cholinergic receptor genes. Mol Psychiatry 2006;11:312–22. 223. [PubMed: 16314871]

Hall JG. Twinning. Lancet 2003;362:735–743. [PubMed: 12957099]

Hasin D, Carpenter KM, McCloud S, Smith M, Grant BF. The alcohol use disorder and associated disabilities interview schedule (AUDADIS): reliability of alcohol and drug modules in a clinical sample. Drug Alcohol Depend 1997a;44:133–141. [PubMed: 9088785]

Hasin D, Grant BF, Cottler L, Blaine J, Towle L, Ustun B, Sartorius N. Nosological comparisons of alcohol and drug diagnoses: a multisite, multi-instrument international study. Drug Alcohol Depend 1997b;47:217–226. [PubMed: 9306047]

Hasin D, Paykin A. Alcohol dependence and abuse diagnoses: concurrent validity in a nationally representative sample. Alcohol Clin Exp Res 1999;23:144–150. [PubMed: 10029216]

Hasin D, Stinson FS, Ogburn E, Grant BF. Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Arch Gen Psychiatry 2007;64:830–842. [PubMed: 17606817]

Hasin D, Van Rossem R, McCloud S, Endicott J. Alcohol dependence and abuse diagnoses: validity in community sample heavy drinkers. Alcohol Clin Exp Res 1997c;21:213–219. [PubMed: 9113255]

Hasin DS, Grant BF. Nosological comparisons of DSM-III-R and DSM-IV alcohol abuse and dependence in a clinical facility: comparison with the 1988 National Health Interview Survey results. Alcohol Clin Exp Res 1994;18:272–279. [PubMed: 8048727]

Hasin DS, Grant BF. The co-occurrence of DSM-IV alcohol abuse in DSM-IV alcohol dependence: results of the National Epidemiologic Survey on Alcohol and Related Conditions on heterogeneity that differ by population subgroup. Arch Gen Psychiatry 2004;61:891–896. [PubMed: 15351767]

Hasin DS, Hatzenbuehler M, Smith S, Grant BF. Co-occurring DSM-IV drug abuse in DSM-IV drug dependence: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Drug Alcohol Depend 2005;80:117–123. [PubMed: 16157234]

Hasin DS, Schuckit MA, Martin CS, Grant BF, Bucholz KK, Helzer JE. The validity of DSM-IV alcohol dependence: what do we know and what do we need to know? Alcohol Clin Exp Res 2003;27:244–252. [PubMed: 12605073]

Heath AC. Persist or quit? Testing for a genetic contribution to smoking persistence. Acta Genet Med Gemellol (Roma) 1990;39:447–58. [PubMed: 2102589]

Kendler KS, Jacobson KC, Prescott CA, Neale MC. Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. Am J Psychiatry 2003;160:687–695. [PubMed: 12668357]

Kendler KS, Karkowski LM, Corey LA, Prescott CA, Neale MC. Genetic and environmental risk factors in the aetiology of illicit drug initiation and subsequent misuse in women. Br J Psychiatry 1999;175:351–356. [PubMed: 10789303]

Khoury, MJ.; Cohen, BH.; Beaty, TH. Fundamentals of Genetic Epidemiology. Oxford University Press; Oxford: 1993.

Kohnke MD, Batra A, Kolb W, Kohnke AM, Lutz U, Schick S, Gaertner I. Association of the dopamine transporter gene with alcoholism. Alcohol Alcohol 2005;40:339–342. [PubMed: 15996968]

Kreek MJ, Nielsen DA, Butelman ER, LaForge KS. Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nat Neurosci 2005;8:1450–1457. [PubMed: 16251987]

Lachman HM. An overview of the genetics of substance use disorders. Curr Psychiatry Rep 2006;8:133–143. [PubMed: 16539891]

Lerer E, Kanyas K, Karni O, Ebstein RP, Lerer B. Why do young women smoke? II Role of traumatic life experience, psychological characteristics and serotonergic genes. Mol Psychiatry 2006;11:771–781. [PubMed: 16770336]

Li T, Chen CK, Hu X, Ball D, Lin SK, Chen W, Sham PC, Loh E, Murray RM, Collier DA. Association analysis of the DRD4 and COMT genes in methamphetamine abuse. Am J Med Genet B Neuropsychiatr Genet 2004;129:120–124. [PubMed: 15274053]

Li TK. Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. J Stud Alcohol 2000;61:5–12. [PubMed: 10627090]

Liu QR, Drgon T, Walther D, Johnson C, Poleskaya O, Hess J, Uhl GR. Pooled association genome scanning: validation and use to identify addiction vulnerability loci in two samples. Proc Natl Acad Sci USA 2005;102:11864–11869. [PubMed: 16091475]

Luo X, Kranzler HR, Zuo L, Wang S, Blumberg HP, Gelernter J. CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: results from an extended case-control structured association study. Hum Mol Genet 2005;14:2421–2434. [PubMed: 16000316]

Madden PA, Heath AC, Pedersen NL, Kaprio J, Koskenvuo MJ, Martin NG. The genetics of smoking persistence in men and women: a multicultural study. Behav Genet 1999;29:423–431. [PubMed: 10857247]

Maes HH, Sullivan PF, Bulik CM, Neale MC, Prescott CA, Eaves LJ, Kendler KS. A twin study of genetic and environmental influences on tobacco initiation, regular tobacco use and nicotine dependence. Psychol Med 2004;34:1251–1261. [PubMed: 15697051]

McGue M, Iacono WG, Legrand LN, Elkins I. Origins and consequences of age at first drink. II Familial risk and heritability. Alcohol Clin Exp Res 2001;25:1166–1173. [PubMed: 11515563]

Merikangas KR, Avenevoli S. Implications of genetic epidemiology for the prevention of substance use disorders. Addict Behav 2000;25:807–820. [PubMed: 11125772]

Morita Y, Ujike H, Tanaka Y, Uchida N, Nomura A, Otani K, Kishimoto M, Morio A, Inada T, Harano M, Komiyama T, Yamada M, Sekine Y, Iwata N, Iyo M, Sora I, Ozaki N. The X-box binding protein 1 (XBP1) gene is not associated with methamphetamine dependence. Neurosci Lett 2005;383:194–198. [PubMed: 15936534]

Mottagui-Tabar S, Prince JA, Wahlestedt C, Zhu G, Goldman D, Heilig M. A novel single nucleotide polymorphism of the neuropeptide Y (NPY) gene associated with alcohol dependence. Alcohol Clin Exp Res 2005;29:702–707. [PubMed: 15897713]

Neale MC, Harvey E, Maes HH, Sullivan PF, Kendler KS. Extensions to the modeling of initiation and progression: applications to substance use and abuse. Behav Genet 2006;36:507–524. [PubMed: 16770695]

Pull CB, Saunders JB, Mavreas V, Cottler LB, Grant BF, Hasin DS, Blaine J, Mager D, Ustun BT. Concordance between ICD-10 alcohol and drug use disorder criteria and diagnoses as measured by the AUDADIS-ADR, CIDI and SCAN: results of a cross-national study. Drug Alcohol Depend 1997;47:207–216. [PubMed: 9306046]

Research Triangle Institute. Software for Survey Data Analysis (SUDAAN), Version 9.0. Research Triangle Institute; Research Triangle Park, NC: 2005.

Ronalds GA, De Stavola BL, Leon DA. The cognitive cost of being a twin: evidence from comparisons within families in the Aberdeen children of the 1950s cohort study. BMJ 2005;331:1306. [PubMed: 16299014]

Saxon A, Oreskovich M, Brkanac Z. Genetic determinants of addiction to opioids and cocaine. Harv Rev Psychiatry 2005;13:218–232. [PubMed: 16126608]

Shen YC, Fan JH, Edenberg HJ, Li TK, Cui YH, Wang YF, Tian CH, Zhou CF, Zhou RL, Wang J, Zhao ZL, Xia GY. Polymorphism of ADH and ALDH genes among four ethnic groups in China and effects upon the risk for alcoholism. Alcohol Clin Exp Res 1997;21:1272–1277. [PubMed: 9347089]

Slutske WS, Heath AC, Madden PA, Bucholz KK, Dinwiddie SH, Dunne MP, Statham DJ, Martin NG. Reliability and reporting biases for perceived parental history of alcohol-related problems: agreement between twins and differences between discordant pairs. J Stud Alcohol 1996;57:387–395. [PubMed: 8776680]

Sullivan PF, Jiang Y, Neale MC, Kendler KS, Straub RE. Association of the tryptophan hydroxylase gene with smoking initiation but not progression to nicotine dependence. Am J Med Genet 2001a; 105:479–484. [PubMed: 11449402]

Sullivan PF, Neale MC, Silverman MA, Harris-Kerr C, Myakishev MV, Wormley B, Webb BT, Ma Y, Kendler KS, Straub RE. An association study of DRD5 with smoking initiation and progression to nicotine dependence. Am J Med Genet 2001b;105:259–265. [PubMed: 11353446]

Sun F, Tsuritani I, Yamada Y. Contribution of genetic polymorphisms in ethanol-metabolizing enzymes to problem drinking behavior in middle-aged Japanese men. Behav Genet 2002;32:229–236. [PubMed: 12211622]

True WR, Xian H, Scherrer JF, Madden PA, Bucholz KK, Heath AC, Eisen SA, Lyons MJ, Goldberg J, Tsuang M. Common genetic vulnerability for nicotine and alcohol dependence in men. Arch Gen Psychiatry 1999;56:655–661. [PubMed: 10401514]

Tsuang MT, Lyons MJ, Harley RM, Xian H, Eisen S, Goldberg J, True WR, Faraone SV. Genetic and environmental influences on transitions in drug use. Behav Genet 1999;29:473–479. [PubMed: 10857252]

Uhl GR, Liu QR, Walther D, Hess J, Naiman D. Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. Am J Hum Genet 2001;69:1290–1300. [PubMed: 11704927]

Volkow ND, Fowler JS, Wang GJ. The addicted human brain viewed in the light of imaging studies: brain circuits and treatment strategies. Neuropharmacol 2004;47(Suppl 1):3–13.

Vink JM, Willemsen G, Boomsma DI. Heritability of smoking initiation and nicotine dependence. Behav Genet 2005;35:397–406. [PubMed: 15971021]

Vrasti R, Grant BF, Chatterji S, Ustun BT, Mager D, Olteanu I, Badoi M. Reliability of the Romanian version of the alcohol module of the WHO Alcohol Use Disorder and Associated Disabilities: Interview Schedule --Alcohol/Drug-Revised. Eur Addict Res 1998;4:144–149. [PubMed: 9852366]

Whitfield JB, Zhu G, Madden PA, Neale MC, Heath AC, Martin NG. The genetics of alcohol intake and of alcohol dependence. Alcohol Clin Exp Res 2004;28:1153–1160. [PubMed: 15318113]

Xu K, Liu XH, Nagarajan S, Gu XY, Goldman D. Relationship of the delta-opioid receptor gene to heroin abuse in a large Chinese case/control sample. Am J Med Genet 2002;110:45–50. [PubMed: 12116270]

Zhang L, Kendler KS, Chen X. Association of the phosphatase and tensin homolog gene (PTEN) with smoking initiation and nicotine dependence. Am J Med Genet B Neuropsychiatr Genet 2006;141:10–14. [PubMed: 16331670]

Zimmerman M, Coryell W, Pfohl B, Stangl D. The reliability of the family history method for psychiatric diagnoses. Arch Gen Psychiatry 1988;45:320–322. [PubMed: 3281625]

Zimmerman, R.; Ahsan, H.; Susser, E. Modern family history studies. In: Susser, E.; Schwartz, S.; Morabia, A.; Bromet, EJ., editors. Psychiatric Epidemiology: Searching for Causes of Mental Disorders. Oxford University Press; New York: 2006. p. 361-374.

Table 1

Alcohol and drug use, dependence and family history in the NESARC sample (N=43,093): percent (s.e.)

	Total NESARC sample (n=43,093)	**Subset of Alcohol users (n=34,827)**	**Subset of Cannabis users (n=8,172)**	**Subset of Cocaine users (n=2,528)**	**Subset of Any illicit drug users† (n=9,140)**
Total		82.72 (0.62)	20.55 (0.50)	06.15 (0.23)	22.80 (0.50)
Lifetime Dependence*					
Alcohol	12.48 (0.35)	15.09 (0.39)	35.36 (0.76)	49.31 (1.26)	33.99 (0.72)
Cannabis	01.30 (0.08)	01.55 (0.10)	06.31 (0.35)	12.01 (0.81)	05.69 (0.32)
Cocaine	00.98 (0.06)	01.17 (0.07)	04.56 (0.27)	15.97 (0.9)	04.31 (0.25)
Any Drug	02.59 (0.13)	03.09 (0.15)	11.74 (0.49)	27.28 (1.19)	11.35 (0.45)
Family History					
Family History of Alcohol Problems	33.95 (0.61)	36.14 (0.55)	49.08 (0.74)	58.06 (1.19)	48.89 (0.71)
Family History of Drug Problems	16.35 (0.36)	17.74 (0.37)	29.41 (0.64)	35.92 (1.21)	28.68 (0.61)

† Composite variable containing cocaine, hallucinogens, heroin, cannabis, opioids, sedatives, inhalants, stimulants, tranquilizers, and other drugs

* Percentages reported for the number of respondents in each column

Table 2

Hazard ratios of substance initiation, conditional initiation, dependence and conditional dependence by a one-unit change in the proportion of relatives having a drug problem[1,2]

	Cannabis HR (95% CI)	Cocaine HR (95% CI)	Any illicit drug[3] HR (95% CI)
Initiation	1.38 (1.33 - 1.43)	1.41 (1.31 - 1.51)	1.38 (1.33 - 1.43)
Dependence	1.68 (1.51 - 1.88)	1.55 (1.34 - 1.79)	1.68 (1.55 - 1.81)
Conditional Initiation	1.36 (1.31 - 1.42)	1.39 (1.28 - 1.51)	1.34 (1.28 - 1.40)
Conditional Dependence	1.44 (1.30 - 1.61)	1.21 (1.04 - 1.41)[4]	1.46 (1.35 - 1.58)

[1] All analyses adjusted for proband's gender, age group, race, education, alcohol problems in first-degree relatives and proband's alcohol dependence.

[2] Hazard ratios are interpreted as the increase in the rate for each one unit change in the four-category ordinal family history Family history defined as a four-category ordinal variable based the proportion of first-degree relatives with drug problems: 0%, >0% - <25% positive, >25% - <50% positive, and >50% positive.

[3] Composite variable containing cocaine, hallucinogens, heroin, cannabis, opioids, sedatives, inhalants, stimulants, tranquilizers, other drugs

[4] all other comparisons gave p-values < 0.0001; p-value for conditional dependence on cocaine gave a p-value= 0.014

Table 3

Hazard ratios of substance initiation, conditional initiation, dependence and conditional dependence by a one-unit change in the proportion of relatives having an alcohol problem[1,2]

	HR (95% CI)[3]
Initiation	1.18 (1.15 - 1.20)
Dependence	1.50 (1.44 - 1.56)
Conditional Initiation	1.15 (1.12 - 1.17)
Conditional Dependence	1.46 (1.41 - 1.52)

[1] All analyses adjusted for proband's gender, age group, race, education and drug problems in first-degree relatives and proband's drug dependence.

[2] Hazard ratios are interpreted as the increase in the rate for each one unit change in the four-category ordinal family history variable. Family history defined as a four-category ordinal variable based the proportion of first-degree relatives with alcohol problems: 0%, >0% - <25% positive, >25% - <50% positive, and >50% positive.

[3] all other comparisons gave p-values < 0.0001

Table 4

Hypothetical expected allele frequencies for initiation and dependence genes in abstainers, non-dependent users, and individuals with dependence

Population	Initiation-specific alleles	Dependence-specific alleles
Abstainers	Low frequency	+/− Population frequency
Non-dependent users	High frequency	Low frequency
Dependence	High frequency	High frequency