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Association of the *OPRM1* variant rs1799971 (A118G) with non-specific liability to substance dependence in a collaborative *de novo* meta-analysis of European-ancestry cohorts

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Abstract

The mu1 opioid receptor gene, *OPRM1*, has long been a high-priority candidate for human genetic studies of addiction. Because of its potential functional significance, the non-synonymous variant rs1799971 (A118G, Asn40Asp) in *OPRM1* has been extensively studied, yet its role in addiction has remained unclear, with conflicting association findings. To resolve the question of what effect, if any, rs1799971 has on substance dependence risk, we conducted collaborative meta-analyses of 25 datasets with over 28,000 European-ancestry subjects. We investigated non-specific risk for "general" substance dependence, comparing cases dependent on any substance to controls who were non-dependent on all assessed substances. We also examined five specific substance dependence diagnoses: DSM-IV alcohol, opioid, cannabis, and cocaine dependence, and nicotine dependence defined by the proxy of heavy/light smoking (cigarettes-per-day > 20 versus ≤ 10). The G allele showed a modest protective effect on general substance dependence (OR = 0.90, 95% C.I. [0.83–0.97], p-value = 0.0095, N = 16,908). We observed similar effects for each individual substance, although these were not statistically significant, likely because of reduced sample sizes. We conclude that rs1799971 contributes to mechanisms of addiction liability that are shared across different addictive substances. This project highlights the benefits of examining addictive behaviors collectively and the power of collaborative data sharing and meta-analyses.

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Conflict of Interest Disclosures: Dr. Bierut is listed as an inventor on Issued U.S. Patent 8,080,371, "Markers for Addiction" covering the used of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction, and served as a consultant for Pfizer in 2008. Dr. NL Saccone is the spouse of Dr. SF Saccone, who is also listed as an inventor on the above patent. Dr. Cinciripini served on the scientific advisory board of Pfizer, conducted educational talks sponsored by Pfizer on smoking cessation (2006–2008), and has received grant support from Pfizer. Dr. Degenhardt has no relevant disclosures for this specific project; however, for general pharmaceutical company disclosures, Dr. Degenhardt has received untied educational grants from Reckitt Benckiser to conduct post-marketing surveillance of the diversion and injection of opioid substitution therapy medications in Australia. Although these activities are unrelated to the current study, Dr. Kranzler has been a consultant or advisory board member for Alkermes, Lilly, Lundbeck, Otsuka and Pfizer; he is also a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which is supported by Ethypharm, Lilly, Lundbeck, AbbVie, and Pfizer. Dr. Ridinger is member of the advisory board of Lundbeck referring to Nalmefene. Prof. Dr. N. Scherbaum received honoraria for several activities (advisory boards, lectures, manuscripts and educational material) by the factories Sanofi-Aventis, Reckitt-Benckiser, Lundbeck, and Janssen-Cilag. During the last three years he participated in clinical trials financed by the pharmaceutical industry. The remaining authors declare no conflict of interest.

Informed Consent. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008. All participants provided informed consent.

Keywords

Addiction; substance dependence; *OPRM1*; opioid receptor; single nucleotide polymorphism (SNP); genetic association

1. INTRODUCTION

The mu opioid receptors are part of a family of G protein-coupled receptors that are expressed in the brain and bind endogenous and exogenous opioids. The mu1 opioid receptor gene (*OPRM1*) has been one of the most studied genes in psychoactive substance research. It is a receptor for opioid analgesic agents and is involved in reward and analgesic pathways (Kreek and Koob 1998). The non-synonymous single nucleotide polymorphism (SNP) rs1799971 (A118G) in exon 1 of *OPRM1* causes an asparagine to aspartic acid substitution at the fortieth amino acid residue (Asn40Asp). The G (Asp) allele is the minor allele across multiple human populations, with frequencies ranging from 4% in African-American samples to ~16% in European-ancestry samples to over 40% in some Asian samples (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1799971). Multiple studies have examined the functional effects of this amino acid change on expression levels and receptor properties such as binding affinity and signaling (Befort et al. 2001; Beyer et al. 2004; Bond et al. 1998; Deb et al. 2010; Mague and Blendy 2010; Mague et al. 2009; Ray et al. 2012; Wang et al. 2014; Wang et al. 2012; Zhang et al. 2005).

Because of its potential functional significance, many human genetic studies of substance dependence have targeted rs1799971. However, the role, if any, of rs1799971 in substance dependence remains unclear (Crist and Berrettini 2013; Levran et al. 2012; Mague and Blendy 2010). In studies of opioid dependence, results have been mixed, with the minor (G) allele reported to have no effect in some studies (Crowley et al. 2003; Levran et al. 2008; Nelson et al. 2014; Nikolov et al. 2011) and to decrease risk in others (Bond et al. 1998; Tan et al. 2003). Similarly, analyses of alcohol dependence have reported increased risk (Bart et al. 2005; Kim et al. 2004), no effect (Bergen et al. 1997; Rouvinen-Lagerstrom et al. 2013; Sander et al. 1998; Xuei et al. 2007), and decreased risk (Schinka et al. 2002; Town et al. 1999) for this allele. Analyses of rs1799971 with other addictive substances also show no consensus (Clarke et al. 2013; Crist and Berrettini 2013; Franke et al. 2001; Gelernter et al. 1999; Hardin et al. 2009; Munafo et al. 2013).

Literature-based meta-analyses have evaluated the association of rs1799971 with substance dependence (Arias et al. 2006), opioid dependence (Coller et al. 2009; Glatt et al. 2007; Haerian and Haerian 2013), and alcohol dependence (Chen et al. 2012a). Three of these meta-analyses reported no association (Arias et al. 2006; Coller et al. 2009; Glatt et al. 2007), while among Asian samples the G allele was reported to increase risk for alcohol (Chen et al. 2012a) and opioid dependence (Haerian and Haerian 2013). Although these meta-analyses attained large samples by combining published information, they were subject to heterogeneity from multiple sources, including differing phenotypes, ascertainment schemes, and statistical analysis models across the meta-analyzed publications.

To clarify the effect of rs1799971 on substance dependence risk, we conducted collaborative meta-analyses based on new analyses of multiple datasets. Our data-driven approach moves beyond the limitations of literature-based meta-analyses by (1) defining consistent phenotypes across studies, (2) performing new, uniform analyses across datasets as in our previous meta-analyses (Chen et al. 2012b; Hartz et al. 2012; Saccone et al. 2010), and (3) inviting investigators to contribute analyses from established studies with relevant phenotype and genotype data, irrespective of prior publication on rs1799971.

2. METHODS

2.1. Samples and Study design

Twenty-five datasets contributed a starting sample of 28,689 study participants of European ancestry. Invitations to participate were sent to all studies in the NIDA Genetics Consortium, which NIDA formed to facilitate collaboration among investigators in addiction genetics, as documented by the NIDA Center for Genetic Studies (https://nidagenetics.org/studies). We extended invitations to additional studies suggested by consortium members as likely to have relevant data, and to collaborators on a previous meta-analysis of smoking quantity and lung disease (Saccone et al. 2010). NIDA further advertised the opportunity to participate in this meta-analysis project with a web announcement at http://www.drugabuse.gov/researchers/research-resources/genetics-research-resources/collaborative-opportunities-genetics-research. Dataset inclusion criteria were: (1) rs1799971 must have been genotyped, and (2) at least one of these five phenotypes must have been assessed: DSM-IV defined alcohol, cannabis, cocaine, or opioid dependence, or categorized cigarettes per day (CPD) (0–10, 11–20, 21–30, and 31+ CPD).

Study participants with a history of abstinence from alcohol (never drank) were excluded prior to all analyses, so that included participants satisfied a minimum exposure to alcohol. For the main analyses, we filtered out study participants if they had no known substance dependence and were also under the age of 25. Thus, we included non-dependent (control) participants only if they were old enough to have passed through the period of highest risk, so as to reduce phenotypic misclassification. For each dataset, Table 1 gives demographic characteristics, the allele frequency of rs1799971, and key publications. Supplementary text S1 provides additional details for each dataset, including study recruitment, genotyping methods, and data quality control.

2.2. Phenotypes

We analyzed six primary dichotomous phenotypes: a “general” substance dependence diagnosis (lifetime dependence on any of five substances: alcohol, nicotine, cannabis, cocaine, and opioids), plus the corresponding five individual substance-specific lifetime dependence diagnoses. General substance dependence controls were required to be non-dependent on all substances assessed in that dataset; not all studies assessed all five substances. For each substance, individuals who did not meet dependence criteria were classified as non-dependent; abuse criteria were not considered. These phenotypes allowed us to examine the general (nonspecific) liability to substance dependence and compare non-specific and substance-specific associations.

DSM-IV criteria were used to define dependent cases for alcohol, cannabis, cocaine, and opioids. For nicotine dependence, we defined the proxy of heavy smoking cases (CPD > 20) and light smoking controls (CPD ≤ 10) for current and former smokers, based on CPD when they were smoking; if multiple measurements were available the maximum value was used. Heavy versus light CPD is more commonly measured than nicotine dependence and has been an informative proxy for nicotine dependence in large meta-analyses (Chen et al. 2012b; Hartz et al. 2012; Saccone et al. 2010); smokers meeting this threshold strongly overlap with nicotine dependent smokers. Because CPD does not account for dependence items such as withdrawal (Lessov et al. 2004), secondary analyses examined the effect of redefining general dependence using standard definitions of nicotine dependence (Fagerström Test for Nicotine Dependence (Heatherton et al. 1991) and DSM-IV), in the subset of studies for which these were available.

In addition to filtering out subjects who did not meet minimum exposure to alcohol, we also defined analysis variables for exposure to each of the other four substances. For cannabis, cocaine, and opioids, the exposure threshold was “at least one lifetime use.” For nicotine, we used “at least 100 cigarettes smoked lifetime,” a commonly used threshold to define smoking exposure in epidemiological studies.

Table 2 shows dataset-specific counts for cases, controls, and exposed controls. Individuals dependent on multiple substances are counted and analyzed in the corresponding multiple categories.

2.3. SNP for analysis

We required rs1799971 to be genotyped in each dataset. For analyses, we coded rs1799971 as the number of copies of the G (minor) allele.

2.4. Statistical analyses and meta-analysis

We conducted six correlated discovery tests corresponding to the six primary phenotypes: the general substance dependence diagnosis and the five specific substance dependence diagnoses. To limit the number of tests, we focused on testing for a main effect of rs1799971 on these outcomes. All discovery analyses filtered out study participants under the age of 25 with no known substance dependence to ensure that controls had passed the typical age of dependence onset; cases are dependent and thus have had sufficient exposure regardless of age. Additional interpretive tests examined the robustness and consistency of discovery test results, and included analyses without age filtering for comparison.

To ensure uniform analyses across datasets, the coordinating site at Washington University developed analysis scripts in SAS® and R. Scripts were distributed to collaborating sites, which then analyzed their datasets locally. Results were returned to the coordinating site for meta-analyses. We used standard inverse-variance-weighted meta-analysis as implemented in the *rmeta* package in R (Lumley; R Development Core Team 2012). Additionally, to be included in the meta-analysis of a given model, each dataset was required to have at least five cases and five controls available. This requirement was intended to reduce noise when some subgroups became very small after phenotypic filtering. All samples included for

general dependence in fact met a higher threshold of at least 20 cases and 20 controls. We report fixed effect estimates together with Cochran's Q and I^2 to evaluate heterogeneity for each meta-analyzed model. No significant heterogeneity was observed among the studies analyzed (p-value for $Q > 0.05$, Table S1 and Tables 3 and 4). Correspondingly, Q values were close to the respective degrees of freedom (number of studies) and I^2 values were small with no values greater than 26% (Supplementary Table S1).

2.5. General substance dependence analyses

Logistic regression was used to estimate the effect of rs1799971 on general substance dependence with covariates for sex and age. Of the 25 available datasets, 20 had at least five cases (dependent at least one of the five substances) and five controls (no known substance dependence diagnoses and exposed to alcohol) for analysis of general substance dependence.

Our interpretive tests examined the robustness of the general substance dependence results and compared them to substance-specific effects. Specifically, to assess the influence of each individual dataset, each of the 20 contributing datasets was, in turn, left out of the meta-analysis. In this leave-one-out test, observing consistency of summary odds ratios would suggest that it is unlikely that the overall meta-analysis result is primarily due to a single study. Also, we meta-analyzed only studies that had assessed all five substances to examine consistency of results; the general dependence controls in these studies were assessed for all five substances and thus more homogenous. Finally, to compare the effect of rs1799971 on general substance dependence liability with its effect on the constituent substance-specific diagnoses, we tested for association using individuals dependent on each specific substance as cases compared to the same controls used in the general dependence analysis (non-dependent on all assessed substances).

2.6. Specific substance dependence analyses

To test the association of rs1799971 with each specific dependence diagnoses while accounting for the remaining diagnoses, our primary analysis used ordinal logistic regression with additively coded rs1799971 as the dependent variable and the five dependence diagnoses, four exposures, sex, and age as explanatory variables. This model simultaneously estimates association of rs1799971 with each substance while accounting for co-morbidity (Grucza et al. 2008). This analysis used only the datasets that had all five substance dependence diagnoses and all four exposure variables because the model required that there be no missing variables.

To interpret and examine the robustness of these results, we evaluated traditional logistic regression models on the same datasets, also accounting for co-morbidity: each specific substance dependence was tested as the outcome, with log-additively coded rs1799971, age, sex, and the remaining specific substance dependence diagnoses as explanatory variables. Here, cases were dependent on a given substance, and controls were exposed but not dependent on that substance regardless of diagnoses for the remaining four substances. Additionally, to test equivalence of regression coefficients from ordinal regression analyses of individual substances, we conducted a two sample t-test assuming unequal variance.

To examine whether substance-specific results remained consistent with a larger number of datasets, we used all datasets that had assessed each substance for additional interpretive tests, with the dependence diagnosis as outcome and additively coded rs1799971, sex, and age as explanatory variables.

2.7. Multiple test correction

To estimate the effective number of independent tests corresponding to the six correlated discovery tests, we used matSpD [http://gump.qimr.edu.au/general/daleN/matSpD/], which accounts for correlations among phenotypes (Cheverud 2001; Li and Ji 2005; Nyholt 2004). Using Pearson correlations among the five dependence diagnoses from the studies with all five phenotypes assessed (see Table S3), plus one additional test for general substance dependence, we obtained a conservative estimate of 5.1218 independent tests, corresponding to a Bonferroni-corrected p-value threshold of $\alpha' = 9.76 \times 10^{-3}$ for statistical significance.

3. RESULTS

3.1. The G (Asp) allele of rs1799971 shows a modest protective effect on general substance dependence

We observed a significant association between rs1799971 and general substance dependence (Figure 1). Based on 9064 cases and 7844 age-filtered controls from 20 datasets, the G allele showed a modest protective effect (OR = 0.90, 95% C.I. [0.83–0.97], p-value = 9.52×10^{-3}, N = 16,908); 15 of the 20 studies showed a protective direction of the G allele. Heterogeneity variance was not statistically significant (Q=20.13, p-value = 0.39). A secondary analysis that did not require controls to be over 25 years old yielded a similar odds ratio (OR = 0.90, 95% C.I. (0.84–0.98), N = 17,918), but was not statistically significant after multiple correction in this larger sample (p-value = 1.06×10^{-2}), consistent with our hypothesis that it is important for controls to be past the typical age of risk.

Leave-one-out test of robustness yielded odds ratio estimates ranging from 0.88 to 0.92, with none of the 20 iterations showing significant heterogeneity. This tight range of ORs centered on the overall odds ratio indicates that our finding was not driven by a single dataset. Only a few of the individual iterations showed significant association (e.g. 4 of 20 when using $\alpha' = 9.76 \times 10^{-3}$ as the significance threshold), likely due to the reduced sample size.

To reduce potential heterogeneity among the general dependence controls, we meta-analyzed the 10 datasets that had all five substance-specific dependence diagnoses and at least 5 cases and 5 controls. For these 10 datasets (3947 cases and 2348 controls), the summary odds ratio was 0.87 (p-value = 0.01), very similar to the discovery result based on 20 studies.

Additionally, to aid interpretation, we compared the cases for each specific substance to the general dependence controls. We found that the G allele of rs1799971 was consistently protective (odds ratio of 0.83 to 0.93) across all five substances (Table 3), consistent with the interpretation that this allele is a non-substance-specific protective factor.

To further confirm robustness, we examined the effect of redefining general dependence using alternative definitions for nicotine dependence, namely the Fagerström Test for Nicotine Dependence (FTND) (case ≥ 4, control ≤ 1; 13 studies, N = 8,481) or DSM-IV nicotine dependence (14 studies, N = 11,711), in place of our CPD-based heavy/light phenotype (20 studies, N = 16,908). Analyses of these smaller samples gave similar protective odds ratios for general dependence, though results were not statistically significant: OR = 0.91, 95% C.I. (0.81–1.02) for FTND and OR = 0.94, 95% C.I. (0.85–1.03) for DSM-IV nicotine dependence.

3.2. For each substance-specific dependence, the G allele of rs1799971 is similarly protective but non-significant

In our primary test of rs1799971 genotype as the dependent variable on the 9 datasets that had assessed all five substance dependence diagnoses and exposures, we obtained odds ratios that ranged from 0.89 (nicotine dependence) to 0.92 (cocaine dependence). The odds ratio for each specific substance showed the same protective direction as that for general substance dependence, though none was statistically significant in these smaller samples (Table 4). Also, odds ratios for specific substances did not differ significantly from each other (Table S2), suggesting consistency across substances.

We also examined traditional logistic regression in these 9 datasets. Each substance dependence diagnosis was examined as the outcome (cases dependent on that substance and controls required to be non-dependent but exposed to that substance), with rs1799971 as the predictor and the remaining diagnoses as covariates. Results were similar to those from our ordinal logistic model (Table 4, bottom half). Finally, analyzing all available datasets for these same case/control outcomes (cases dependent on each specific substance, controls non-dependent and exposed to that substance) also showed protective, but non-significant, odds ratios consistent with those seen in the datasets that assessed all dependence diagnoses and exposures (Supplementary Figures S1–S5).

4. DISCUSSION

This project, the first collaborative genetic meta-analysis to investigate specific and general liability for these substance dependence diagnoses, has demonstrated that the G allele of rs1799971 has a modest protective effect on general substance dependence liability (OR = 0.90, 95% C.I. (0.83–0.97), p-value $=9.52 \times 10^{-3}$) in samples of European ancestry. This is the first meta-analysis to show that this non-synonymous variant, which has been heavily studied for functional effects, is significantly associated in European ancestry samples with liability to substance dependence. The small but significant effect size of rs1799971 suggests that variability in previous association reports may be due in part to sampling variation. This collaborative meta-analysis benefited from the opportunity to define uniform phenotypes across studies, perform coordinated, *de novo* analyses to test our hypotheses, and include existing datasets that have not yet focused on the question of rs1799971 and addiction.

The protective effect of this allele on substance dependence liability appears to be non-specific: it is not driven primarily by dependence on any particular substance. For each

substance-specific subset of cases compared to the general dependence controls, we observed a protective effect of similar size to that observed for general dependence. Additional substance-specific analyses similarly showed consistent protective effects of the G allele. These substance-specific odds ratios were not statistically significant, but this may have been largely due to reduced sample size and power.

These findings indicate that rs1799971 in *OPRM1* may contribute to mechanisms of addiction liability that are shared across different addictive substances, consistent with the high genetic correlation between the traits, high co-morbidity, and with prior studies showing that both substance-specific and non-specific genetic effects on addiction liability can be expected (Bierut et al. 1998; Kendler et al. 2007; Merikangas et al. 1998; Swan et al. 1997; Tsuang et al. 1998; Vanyukov et al. 2012; Vanyukov et al. 2003). Rs1799971 is now one of the few examples of a genetic factor that demonstrates a similar, general effect across multiple substances, albeit of modest magnitude. In this sense, our study is similar to a genome-wide association study of multiple psychiatric disorders that identified variants having a common, cross-disorder genetic effect on five major psychiatric diseases (Cross-Disorder Group of the Psychiatric Genomics Consortium 2013). Both studies underscore the value of investigating the genetics of general liability underlying related diseases. Genetic studies of addiction would therefore benefit from including measures pertaining to multiple substances that can then be analyzed collectively. Indeed, a very recent genome-wide study of general substance dependence liability using four of the five substances studied here (alcohol, cannabis, cocaine, opioids) reported novel associations (Wetherill et al. 2015), further supporting the potential benefits.

Our results are compatible with negative results from prior genome-wide meta-analyses of cigarettes-per-day (Liu et al. 2010; The Tobacco and Genetics Consortium 2010; Thorgeirsson et al. 2010). Our hypothesis-driven analyses of a single SNP translate to a study-wide required significance threshold of 9.76×10^{-3}. This led to statistically significant evidence for a modest effect (OR=0.90) of rs1799971 on general substance dependence liability, in N=16,908 subjects (Table 3). The three genome-wide smoking consortia tested *OPRM1* only in each consortium separately (N=38,000, N=31,000, and N=16,000 smokers with cigarettes-per-day); estimated power to have detected the nicotine-specific odds ratio of 0.93 (Table 3) in at least one of the three consortia with genome-wide significance (alpha=5×10^{-8}) is only 4%. Power details are in Supplementary Text S2. Hence it is not surprising that these smoking consortia did not report an *OPRM1* effect.

This study contributes valuable information to connect functional findings to the clinically important outcome of addiction in humans. Several neurobiological, functional, and physiological changes have been demonstrated for the rs1799971 (A118G) amino acid change and a corresponding mutation in a similar region of the receptor in mice (A112G) (Drakenberg et al. 2006; Huang et al. 2012; Mague and Blendy 2010; Palmer and de Wit 2012; Ray et al. 2012; Wang et al. 2014). *In vitro* studies of the G allele have reported increased binding to β- endorphin (Bond et al. 1998), altered downstream signaling (Deb et al. 2010), and decreased mu opioid receptor expression (Zhang et al. 2005). In human brain imaging, the G allele is associated with striatal dopamine response to alcohol (Ramchandani et al. 2011) and increased mu opioid receptor binding potential (Ray et al. 2011). In mouse

knock-in models (A112G), the G/G knock-in has shown reduced receptor protein levels overall and reduced reinforcing value of morphine in female mice (Mague et al. 2009), reduced G-protein signaling (Wang et al. 2014), and increased peak dopamine response to alcohol challenge (Ramchandani et al. 2011); changes are often brain-region specific.

It is important to note that some functional and neurobiological findings have been interpreted as indicating that the G allele of rs1799971 should increase risk for addiction, for example due to its association with greater alcohol-induced reinforcement and reward (Ramchandani et al. 2011; Ray and Hutchison 2004; Ray and Hutchison 2007; Ray et al. 2010). Our data-driven evidence of a modest *protective* effect of this allele on substance dependence liability is thus surprising and all the more important to integrate with functional findings to understand downstream contributions to human substance dependence. A protective effect of the G allele on addiction may be consistent with either increased or decreased reward/reinforcement, for example due to varying roles of positive versus negative reinforcement at different stages in the transition from use to dependence. Modeling these connections remains an open area to be worked out by neurobiological theories of addiction (Ray et al. 2012).

This project demonstrates the value of collaborative data sharing and meta-analysis, as the modest odds ratio of rs1799971 would be challenging to detect and consistently replicate in modestly sized candidate gene studies (Hall et al. 2013; Hart et al. 2013). Also important was our approach of defining consistent phenotypes across all datasets. In particular, careful definition of controls can help to detect associations (Nelson et al. 2013; Schinka et al. 2002). In our case, requiring controls to be at least 25 years of age led to stronger association results even with the reduced the number of controls.

This study has limitations. First, as in any meta-analysis, sample heterogeneity could not be completely avoided. Studies had diverse ascertainment schemes, with some designed to recruit dependent cases for one particular substance. Some studies recruited from the general population while others recruited potentially more extreme cases from treatment centers. Hence, over- and under-representation of phenotypes were present in contributing datasets, and the severity of dependence, degree of co-morbid dependence, and prevalence of substance exposure varied. Reduced proportions of exposed controls would reduce effective sample size and power for a study. But overall, uniform phenotype definitions were an important design feature to ameliorate effects of heterogeneity. Although some bias may have occurred, it seems unlikely to have been systematic in either direction. Similarly, it seems unlikely that systematic bias would have occurred due to differences between studies that contributed to this meta-analysis and those that declined to participate.

Second, this project interrogated only the non-synonymous variant rs1799971. As with any statistical association, our finding may reflect a proxy association for which the true functional variant(s) remain to be recognized. Other *OPRM1* variants have been associated with addiction and merit consideration for future study (Clarke et al. 2013; Hancock et al. 2015; Zhang et al. 2006a). Analyses of multiple SNPs and haplotypes will also be of future interest: recent evidence indicates an important role in heroin addiction for the haplotype structure of *OPRM1*, with the A allele of rs1799971 showing association only in the

presence of the C allele of rs3778150 (Hancock et al. 2015). Importantly, (Hancock et al. 2015) also found that the G allele of rs1799971 is protective (A allele confers risk) on that background, agreeing with the direction of effect observed in our meta-analysis of general substance dependence.

Third, further phenotypic refinement is possible. We did not consider substance abuse criteria, nor did we use the newer diagnostic system, DSM-5 (American Psychiatric Association 2013). Our threshold for exposure was a single use for all substances except nicotine; therefore, the genetic effect of rs1799971 detected by our analyses may involve a combination of effects on development of regular/repeated use and effects on dependence. We focused on dichotomous diagnoses for each substance. For nicotine, we examined heavy/light smoking as the most widely available nicotine trait in our datasets. Consistency of results was confirmed using DSM-IV and Fagerström Test of Nicotine Dependence criteria when available. Because we focused on dichotomous diagnoses that could then be combined into the general substance dependence diagnosis, we did not examine quantitative or categorical cigarettes-per-day.

Fourth, we focused on main effects of rs1799971 to limit multiple testing. Thus, we did not examine gene-environment interactions (e.g., sex-specific effects) or gene-gene interactions. We did adjust statistically for sex, which showed no evidence for a main effect on general substance dependence ($p = 0.57$). Interactions likely have roles in a complex trait such as addiction, and could attenuate the genetic main effect when not accounted for (e.g. when the effect occurs only in a specific stratum). Thus, it is possible that the modest main effect that we detected could translate to a stronger effect if particular genetic or environmental backgrounds are considered. Future work could examine interactions nominated in the literature (Mague et al. 2009; Miranda et al. 2013; Ray et al. 2006).

Finally, a model that explicitly partitions the association between a general factor for any substance dependence and substance-specific components was not fitted to these data. Although such a model would allow a more refined distinction between general and specific associations (Medland and Neale 2010; Neale et al. 2006), we chose not to apply this because of the complexities of running and integrating such analyses across sites.

In closing, this data-driven, collaborative meta-analysis has demonstrated a modest protective effect of the G allele of rs1799971 on general liability to substance dependence. This work highlights the benefits of jointly studying related disorders: larger samples and insight into factors involved in underlying shared liability. An important strength of our approach is that the analyses of our datasets were designed and conducted in collaboration with the originating investigators. Thus, we benefited from collaborators' deep knowledge of their own data and our combined expertise on addiction. This effort underscores the value of collaboratively sharing data and expertise to accelerate discoveries.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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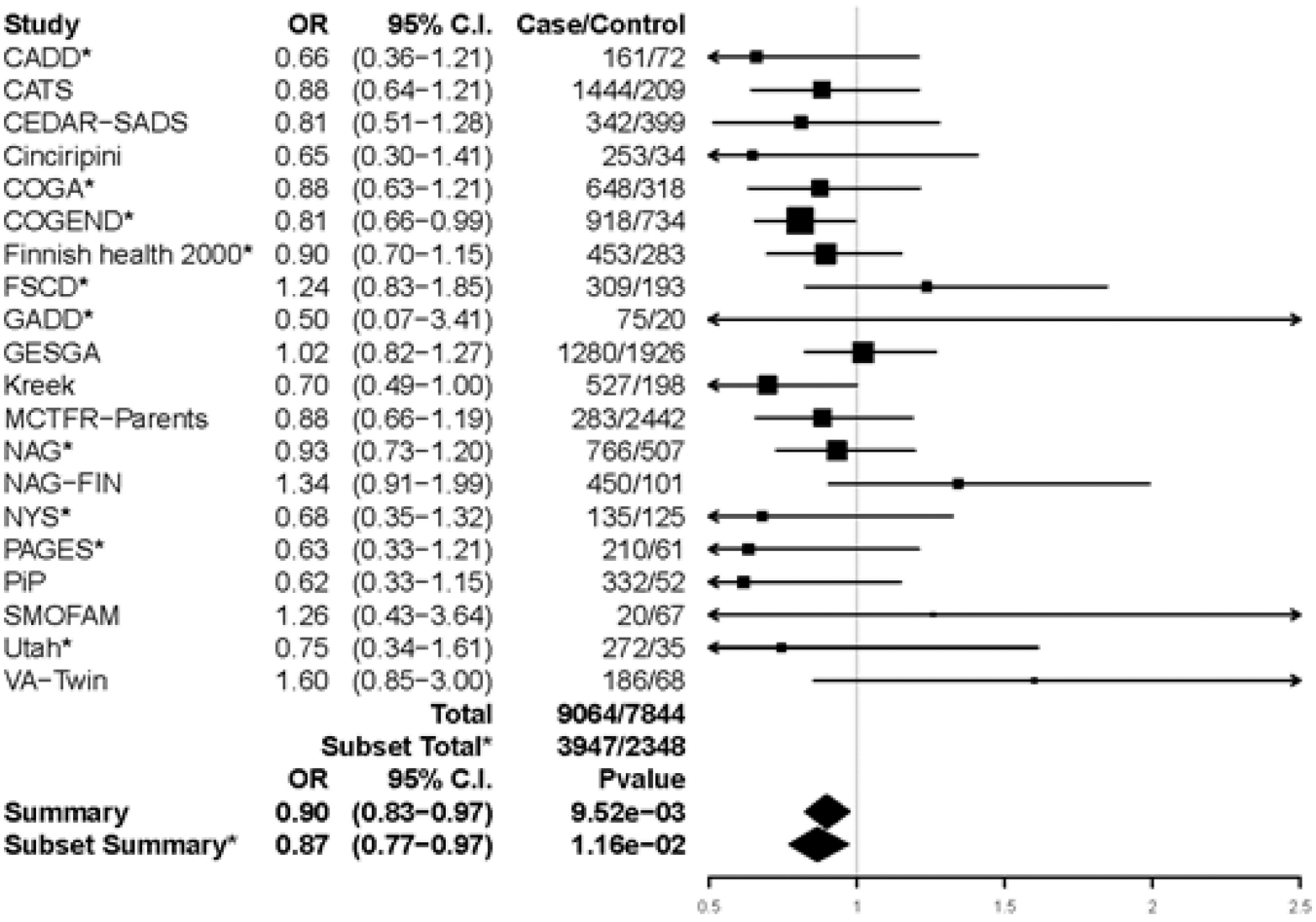

Figure 1.

Forest plot of general substance dependence and rs1799971 across studies that had at least 5 cases and 5 controls. Summary odds ratio, 95% Confidence Interval, and p-values are based on fixed effect meta-analysis. *indicates the subset of 10 studies that had all five specific substance dependence diagnoses, examined in secondary analyses to confirm consistency of results. Estimated heterogeneity variance was Q = 20.13 with a p-value of 0.387 among all 20 studies and Q = 6.49 with a p-value of 0.69 among the subset of 10 studies.

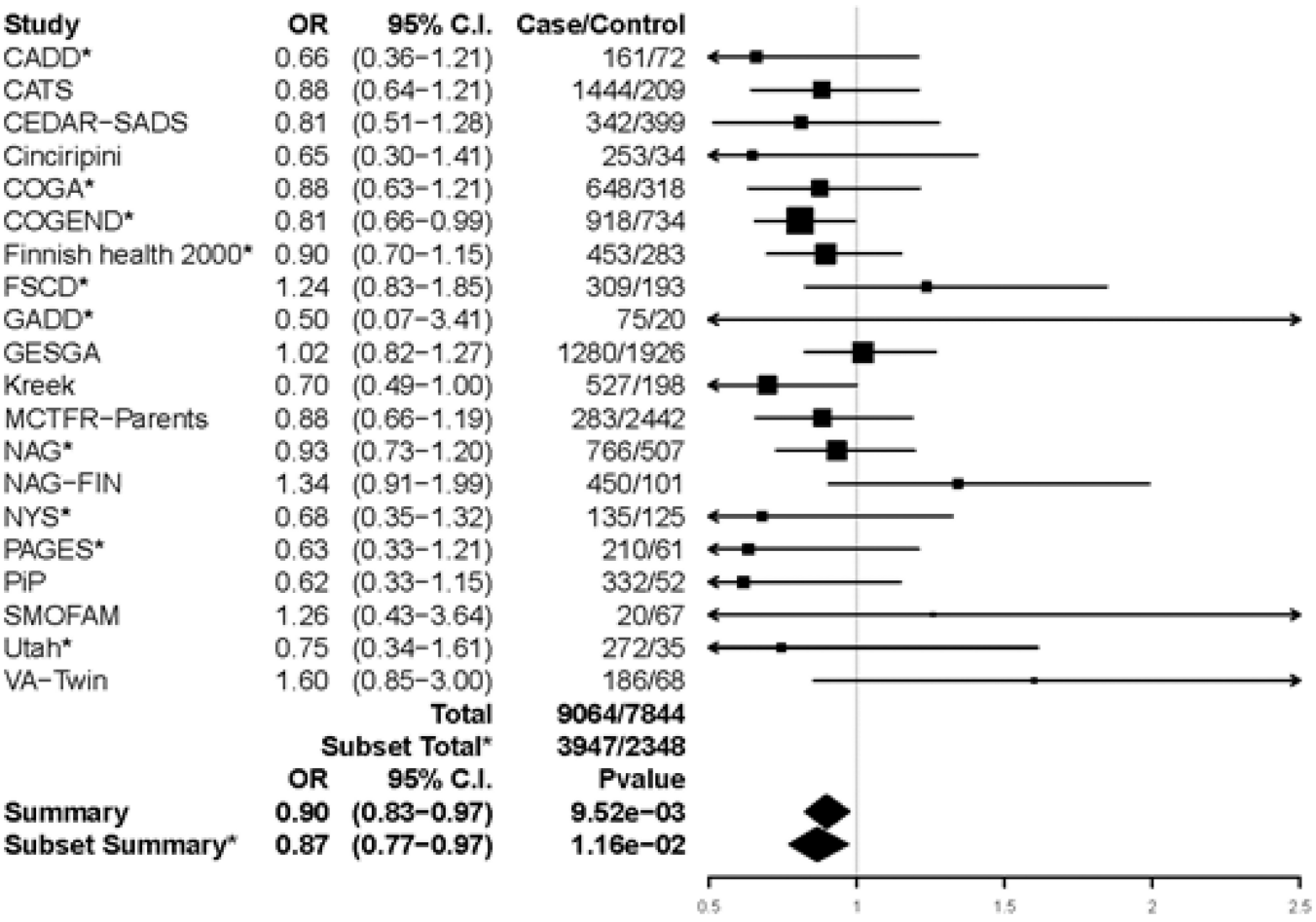

Figure 2.

Forest plot of general substance dependence and rs1799971 across studies that had at least 5 cases and 5 controls. Summary odds ratio, 95% Confidence Interval, and p-values are based on fixed effect meta-analysis. *indicates the subset of 10 studies that had all five specific substance dependence diagnoses, examined in secondary analyses to confirm consistency of results. Estimated heterogeneity variance was Q = 20.13 with a p-value of 0.387 among all 20 studies and Q = 6.49 with a p-value of 0.69 among the subset of 10 studies.

Table 1

Study descriptions: sample sizes, G (minor) allele frequencies (overall, in general dependence cases, in general dependence controls), proportions of male participants, and age distribution (minimum, first quartile, median, third quartile, and maximum) of each study.

Study	Total N	G-allele-freq	Case G-allele-freq	Control G-allele-freq	proport-male	age min	age_q1	age_med	age_q3	age_max
BG [a]	3999	0.14	0.13	0.17	0.83	18	25	28	30	58
CADD [b]	409	0.13	0.12	0.18	0.56	12	21	28	47	72
CATS [c]	1748	0.12	0.12	0.13	0.57	18	29	36	43	65
CEDAR-SADS [d]	747	0.12	0.12	0.12	0.35	14	18	37	42	65
Cinciripini [e]	627	0.11	0.09	0.15	0.48	18	33	42	50	74
COGA [f]	1024	0.12	0.11	0.13	0.46	18	36	43	51	77
COGEND [g]	2024	0.13	0.13	0.15	0.61	25	32	37	41	65
Finnish Health 2000 [h]	1025	0.21	0.20	0.22	0.8	30	37	46	54	87
FSCD [i]	558	0.13	0.13	0.10	0.5	18	25	34	40	54
FT12 [j]	617	0.22			0.48	20	22	22	23	27
GADD [k]	281	0.12	0.13	0.15	0.57	12	15	16	19	61
GESGA [l]	3501	0.12	0.11	0.11	0.65	18	39	47	56	84
Kreek [m]	750	0.11	0.10	0.13	0.59	17	31	43	52	82
MCTFR-Parents [n]	3842	0.11	0.10	0.11	0.46	30	43	46	50	72
NAG-AUS [o]	1281	0.12	0.12	0.12	0.59	18	36	43	50	82
NAG-FIN [p]	879	0.21			0.7	42	52	55	58	77
NYS [q]	552	0.1	0.07	0.10	0.49	35	37	39	41	44
OYSUP [r]	357	0.13			0.5	20	21	21	21	23
PAGES [s]	409	0.11			0.68	17	27	37	45	68
PiP [t]	809	0.11	0.11	0.15	0.48	18	35	43	53	79
ROMA [u]	732	0.21	0.16	0.38	0.83	18	25	28	32	53

Study	Total N	G-allele-freq	Case G-allele-freq	Control G-allele-freq	proport-male	age min	age_q1	age_med	age_q3	age_max
SMOFAM [v]	166	0.12	0.18	0.12	0.63	26	27	29	30	62
Utah [w]	463	0.13	0.12	0.14	0.59	25	54	61	67	86
VA-Twin [x]	672	0.12	0.15	0.10	0.32	21	30	38	46	58
Yale-Penn [y]	1217	0.12			0.41	16	30	39	46	72
Total	**28689**	**(0.10–0.22)**			**(0.32–0.83)**	**(12–42)**	**(15–54)**	**(16–61)**	**(19–67)**	**(23–87)**

References:

[a](Nikolov et al. 2011);

[b](Stallings et al. 2005; Stallings et al. 2003);

[c](Nelson et al. 2014; Nelson et al. 2013);

[d](Maher et al. 2011);

[e](Carter et al. 2008; Cinciripini et al. 2006; Cinciripini et al. 2005; Lam et al. 2012; Minnix et al. 2011; Robinson et al. 2007; Robinson et al. 2011);

[f](Edenberg 2002; Edenberg et al. 2010);

[g](Saccone et al. 2009a; Saccone et al. 2009b);

[h](Aromaa and Koskinen 2004; Rouvinen-Lagerstrom et al. 2013);

[i](Bierut et al. 2010; Bierut et al. 2008);

[j](Broms et al. 2012; Kaprio et al. 2002);

[k](Kamens et al. 2013);

[l](Frank et al. 2012; Treutlein et al. 2009);

[m](Levran et al. 2008);

[n](Iacono et al. 1999; Keyes et al. 2009; McGue et al. 2007; Miller et al. 2012);

[o](Loukola et al. 2008; Saccone et al. 2007);

[p](Loukola et al. 2008);

[q](Elliott et al. 1985; Elliott et al. 1989; Hoft et al. 2009);

[r] (Andrews et al. 2003);

[s] (Van den Oord et al. 2006);

[t] (David et al. 2011);

[u] (Nikolov et al. 2011);

[v] (Hops et al. 2000);

[w] (Weiss et al. 2008);

[x] (Chen et al. 2009; Kendler et al. 2007; Zhang et al. 2006b);

[y] (Gelernter et al. 2014; Gelernter et al. 2013a; Gelernter et al. 2013b)

Table 2

Numbers of cases, total controls, and controls with exposure to each substance. These numbers were based on a filtered sample that removed participants not exposed to alcohol, and participants who are less than 25 years of age and have with no dependence to any assessed substances. NA indicates that the substance was not assessed in the study.

	Alcohol		Cigarettes Per Day (CPD)			Cannabis			Cocaine			Opioid			General Substance Dependence	
Datasets	**Cases**	**Total Controls**	**Heavy Smokers**	**Total Controls**	**Exposed Controls**	**Cases**	**Total Controls**	**Exposed Controls**	**Cases**	**Total Controls**	**Exposed Controls**	**Cases**	**Total Controls**	**Exposed Controls**	**Cases**	**Controls**
BG	277	1278	1449	4	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	948	3
CADD	93	264	54	109	57	72	285	218	35	306	141	6	351	106	161	72
CATS	651	1032	NA	NA	NA	857	831	765	416	1272	875	1259	429	102	1444	209
CEDAR-SADS	179	562	NA	NA	NA	255	486	338	86	655	263	114	627	418	342	399
Cinciripini	NA	NA	253	34	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	253	34
COGA	612	410	242	483	140	196	823	475	224	795	204	86	933	193	648	318
COGEND	463	1529	584	935	923	192	1808	1543	132	1871	560	34	1970	191	918	734
Finnish Health 2000	417	512	89	463	180	3	1011	0	0	1014	0	2	1012	0	453	283
FSCD	280	230	124	276	58	170	340	213	237	273	55	81	429	100	309	193
FT12	93	470	198	15	15	NA	NA	NA	NA	NA	NA	NA	NA	NA	251	0
GADD	43	110	11	57	NA	45	108	78	9	144	29	NA	153	35	75	20
GESGA	1333	1933	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1280	1926
Kreek	42	684	NA	NA	NA	NA	NA	NA	82	644	41	528	198	NA	527	198
MCTFR-Parents	226	2504	NA	NA	NA	93	2637	1637	37	2695	452	12	2722	125	283	2442
NAG-AUS	359	972	737	594	102	79	1249	694	5	1322	74	17	1310	137	766	507
NAG-FIN	221	513	354	115	115	NA	NA	NA	NA	NA	NA	NA	NA	NA	450	101
NYS	65	466	71	135	63	25	508	368	26	507	157	1	532	86	135	125
OYSUP	All participants were under 25 years of age															
PAGES	64	335	132	83	40	77	320	160	7	391	67	8	390	30	210	61
PiP	NA	NA	347	54	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	332	52
ROMA	18	240	209	7	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	177	4
SMOFAM	NA	NA	20	67	34	NA	NA	NA	NA	NA	NA	NA	NA	NA	20	67

	Alcohol		Cigarettes Per Day (CPD)			Cannabis			Cocaine			Opioid			General Substance Dependence	
Datasets	**Cases**	**Total Controls**	**Heavy Smokers**	**Total Controls**	**Exposed Controls**	**Cases**	**Total Controls**	**Exposed Controls**	**Cases**	**Total Controls**	**Exposed Controls**	**Cases**	**Total Controls**	**Exposed Controls**	**Cases**	**Controls**
Utah	112	283	235	60	60	36	359	88	16	379	34	9	386	9	272	35
VA-Twin	NA	NA	193	70	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	186	68
Yale-Penn	799	208	1094	113	NA	397	566	500	911	259	167	703	478	214	1208	3
Total	6347	14535	6396	3674	1797	2497	11331	7077	2223	12527	3119	2860	11920	1746	11648	7834

Table 3

Summary of the effect of rs1799971 on general substance dependence.

Model	Cases	Controls	Cochrane's Q	Q-Pvalue	Odds Ratio	L95%-U95%
Gen-Dep = age sex rs1799971	9064	7844	20.13	0.387	0.90	0.83–0.97
Alcohol = age sex rs1799971	5086	7623	12.08	0.672	0.92	0.83–1.01
Nicotine = age sex rs1799971	3358	2670	16.84	0.265	0.93	0.83–1.05
Cannabis = age sex rs1799971	2077	5115	7.63	0.746	0.83	0.71–0.98
Cocaine = age sex rs1799971	1307	5313	7.68	0.809	0.87	0.73–1.04
Opioid = age sex rs1799971	2139	5168	7.87	0.641	0.84	0.70–1.00

Model column shows what outcome phenotype was tested for each model. Gen-Dep denotes general substance dependence. Each substance denotes the subsets of general substance dependence that were tested in interpretative phase of the analysis. All effects shows are fixed effect estimates. Controls were filtered for age and exposure to alcohol.

Table 4

Summary of the effect of rs1799971 on specific substance dependence diagnoses in 9 studies that assessed all five substance dependence diagnoses and exposures.

Ordinal Logistic Regression Results							
Substance	**Cases**	**Controls**	**Cochrane's Q**	**Q-Pvalue**	**Odds Ratio**	**L95%-U95%**	**OR-Pvalue**
Alcohol	2031	3361	8.90	0.351	0.90	0.76–1.06	0.218
Nicotine	2718	2674	7.78	0.455	0.89	0.74–1.07	0.216
Cannabis	839	4553	10.76	0.216	0.91	0.73–1.14	0.420
Cocaine	992	4085	0.86	0.990	0.92	0.69–1.24	0.593
Opioid	607	4274	3.12	0.682	0.91	0.65–1.27	0.577
Traditional Logistic Regression Results (Dependence as outcome variable)							
Alcohol	2051	3430	10.66	0.222	0.88	0.76–1.02	0.974
Nicotine	2066	1412	8.69	0.276	0.91	0.76–1.08	0.267
Cannabis	861	3036	9.08	0.336	0.90	0.74–1.09	0.283
Cocaine	1011	899	0.85	0.997	0.91	0.70–1.19	0.492
Opioid	600	577	2.31	0.679	0.91	0.67–1.24	0.547

Substance column shows the tested outcome phenotype. All effects shows are fixed effect estimates. In the traditional logistic regression results, controls were required to be exposed each tested substance, in addition to meeting the previously applied filters for age and exposure to alcohol.