

# 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 vs. ≤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.

**Keywords** Addiction · Substance dependence · *OPRM1* · Opioid receptor · Single nucleotide polymorphism (SNP) · Genetic association

## 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](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

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et al. 2009; Ray et al. 2012; Wang et al. 2014, 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.

## Methods

### Samples and Study Design

Twenty-five datasets contributed a starting sample of 28,689 study participants of European ancestry. Invitations

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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.

## 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 (non-specific) 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.

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**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 <sup>a</sup>	3999	0.14	0.13	0.17	0.83	18	25	28	30	58
CADD <sup>b</sup>	409	0.13	0.12	0.18	0.56	12	21	28	47	72
CATS <sup>c</sup>	1748	0.12	0.12	0.13	0.57	18	29	36	43	65
CEDAR-SADS <sup>d</sup>	747	0.12	0.12	0.12	0.35	14	18	37	42	65
Cinciripini <sup>e</sup>	627	0.11	0.09	0.15	0.48	18	33	42	50	74
COGA <sup>f</sup>	1024	0.12	0.11	0.13	0.46	18	36	43	51	77
COGEN <sup>g</sup>	2024	0.13	0.13	0.15	0.61	25	32	37	41	65
Finnish Health 2000 <sup>h</sup>	1025	0.21	0.20	0.22	0.8	30	37	46	54	87
FSCD <sup>j</sup>	558	0.13	0.13	0.10	0.5	18	25	34	40	54
FT12 <sup>j</sup>	617	0.22			0.48	20	22	22	23	27
GADD <sup>k</sup>	281	0.12	0.13	0.15	0.57	12	15	16	19	61
GESGA <sup>l</sup>	3501	0.12	0.11	0.11	0.65	18	39	47	56	84
Kreek <sup>m</sup>	750	0.11	0.10	0.13	0.59	17	31	43	52	82
MCTFR-Parents <sup>n</sup>	3842	0.11	0.10	0.11	0.46	30	43	46	50	72
NAG-AUS <sup>o</sup>	1281	0.12	0.12	0.12	0.59	18	36	43	50	82
NAG-FIN <sup>p</sup>	879	0.21			0.7	42	52	55	58	77
NYS <sup>q</sup>	552	0.1	0.07	0.10	0.49	35	37	39	41	44
OYSUP <sup>r</sup>	357	0.13			0.5	20	21	21	21	23
PAGES <sup>s</sup>	409	0.11			0.68	17	27	37	45	68
Pip <sup>t</sup>	809	0.11	0.11	0.15	0.48	18	35	43	53	79
ROMA <sup>u</sup>	732	0.21	0.16	0.38	0.83	18	25	28	32	53
SMOFAM <sup>v</sup>	166	0.12	0.18	0.12	0.63	26	27	29	30	62
Utah <sup>w</sup>	463	0.13	0.12	0.14	0.59	25	54	61	67	86

Table 1 continued

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
VA-Twin <sup>x</sup>	672	0.12	0.15	0.10	0.32	21	30	38	46	58
Yale-Penn <sup>y</sup>	1217	0.12			0.41	16	30	39	46	72
<b>Total</b>	28,689	(0.10–0.22)			(0.32–0.83)	(12–42)	(15–54)	(16–61)	(19–67)	(23–87)

<sup>a</sup> (Nikolov et al. 2011)

<sup>b</sup> (Stallings et al. 2005, 2003)

<sup>c</sup> (Nelson et al. 2014, 2013)

<sup>d</sup> (Maher et al. 2011)

<sup>e</sup> (Carter et al. 2008; Cincipini et al. 2006, 2005; Lam et al. 2012; Minnix et al. 2011; Robinson et al. 2007, 2011)

<sup>f</sup> (Edenberg 2002; Edenberg et al. 2010)

<sup>g</sup> (Saccone et al. 2009a, 2009b)

<sup>h</sup> (Aromaa and Koskinen 2004; Rouvinen-Lagerstrom et al. 2013)

<sup>i</sup> (Bierut et al. 2010, 2008)

<sup>j</sup> (Broms et al. 2012; Kaprio et al. 2002)

<sup>k</sup> (Kamens et al. 2013)

<sup>l</sup> (Frank et al. 2012; Treutlein et al. 2009)

<sup>m</sup> (Levrain et al. 2008)

<sup>n</sup> (Iacono et al. 1999; Keyes et al. 2009; McGue et al. 2007; Miller et al. 2012)

<sup>o</sup> (Loukola et al. 2008; Saccone et al. 2007)

<sup>p</sup> (Loukola et al. 2008)

<sup>q</sup> (Elliott et al. 1985, 1989; Hofst et al. 2009)

<sup>r</sup> (Andrews et al. 2003)

<sup>s</sup> (Van den Oord et al. 2006)

<sup>t</sup> (David et al. 2011)

<sup>u</sup> (Nikolov et al. 2011)

<sup>v</sup> (Hops et al. 2000)

<sup>w</sup> (Weiss et al. 2008)

<sup>x</sup> (Chen et al. 2009; Kendler et al. 2007; Zhang et al. 2006b)

<sup>y</sup> (Gelernter et al. 2014, 2013a, b)

**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

Datasets	Alcohol			Cigarettes per day (CPD)			Cannabis			Cocaine			Opioid			General substance dependence	
	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	
																	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	
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	14,535	6396	3674	1797	2497	11,331	7077	2223	12,527	3119	2860	11,920	1746	1,1648	7834	

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.

### 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.

### 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<sup>®</sup> 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 2009; 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<sup>2</sup> 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<sup>2</sup> values were small with no values greater than 26 % (Supplementary Table S1).

### 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

**Table 3** Summary of the effect of rs1799971 on general substance dependence

Model	Cases	Controls	Cochrane’s Q	Q-p value	Odds Ratio	L95 %-U95 %	OR-p value
Gen-Dep = age sex rs1799971	9064	7844	20.13	0.387	0.90	0.83–0.97	0.952
Alcohol = age sex rs1799971	5086	7623	12.08	0.672	0.92	0.83–1.01	0.696
Nicotine = age sex rs1799971	3358	2670	16.84	0.265	0.93	0.83–1.05	0.244
Cannabis = age sex rs1799971	2077	5115	7.63	0.746	0.83	0.71–0.98	0.279
Cocaine = age sex rs1799971	1307	5313	7.68	0.809	0.87	0.73–1.04	0.132
Opioid = age sex rs1799971	2139	5168	7.87	0.641	0.84	0.70–1.00	0.557

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 shown 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-p value	Odds Ratio	L95 %-U95 %	OR-p value
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

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).

### 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.

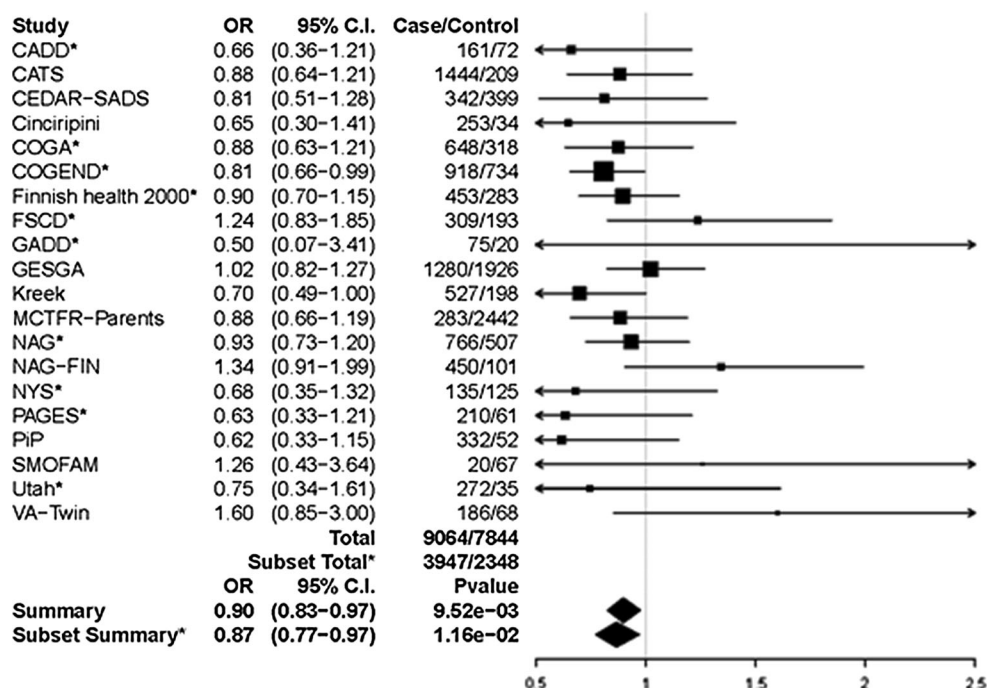
### Multiple Test Correction

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.

## Results

### 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 (Fig. 1). Based on 9064 cases and 7844 age-filtered controls from 20 datasets, the G



**Fig. 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. *Asterisk* indicates the subset of 10 studies that had all five specific substance dependence diagnoses, examined in

allele showed a modest protective effect (OR = 0.90, 95 % C.I. [0.83–0.97],  $p$  value =  $9.52 \times 10^{-3}$ ,  $N = 16,908$ ); 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 =  $1.06 \times 10^{-2}$ ), 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 five cases and five 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.

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

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 0.83–0.93) 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  $\geq 4$ , control  $\leq 1$ ; 13 studies,  $N = 8481$ ) 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.

#### 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 nine 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 nine 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).

## 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, 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  $9.76 \times 10^{-3}$ . 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 ( $\alpha = 5 \times 10^{-8}$ ) 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  $\beta$ -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, 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 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 comorbid 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.

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#### Compliance with Ethical Standards

**Conflict of interest** Dr. Bierut is listed as an inventor on Issued U.S. Patent 8 080 371, “Markers for Addiction” covering the use 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.

#### References

- American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders* (5th Edition), Washington, DC
- Andrews JA, Tildesley E, Hops H, Duncan SC, Severson HH (2003) Elementary school age children’s future intentions and use of substances. *J Clin Child Adolesc Psychol* 32(4):556–567
- Arias A, Feinn R, Kranzler HR (2006) Association of an Asn40Asp (A118G) polymorphism in the mu-opioid receptor gene with substance dependence: a meta-analysis. *Drug Alcohol Depend* 83(3):262–268
- Aromaa A, Koskinen S (2004) Health and functional capacity in Finland: Baseline Results of the Health 2000 Health Examination Survey. National Public Health Institute, Helsinki
- Bart G, Kreek MJ, Ott J, LaForge KS, Proudnikov D, Pollak L, Heilig M (2005) Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. *Neuropsychopharmacology* 30(2):417–422
- Beftor K, Filliol D, Decaillet FM, Gaveriaux-Ruff C, Hoehe MR, Kieffer BL (2001) A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs receptor signaling. *J Biol Chem* 276(5):3130–3137
- Bergen AW, Kokoszka J, Peterson R, Long JC, Virkkunen M, Linnoila M, Goldman D (1997) Mu opioid receptor gene variants: lack of association with alcohol dependence. *Mol Psychiatry* 2(6):490–494
- Beyer A, Koch T, Schroder H, Schulz S, Hollt V (2004) Effect of the A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human mu-opioid receptor. *J Neurochem* 89(3):553–560
- Bierut LJ, Dinwiddie SH, Begleiter H, Crowe RR, Hesselbrock V, Nurnberger JI Jr, Porjesz B, Schuckit MA, Reich T (1998) Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the

- Collaborative Study on the Genetics of Alcoholism. *Arch Gen Psychiatry* 55(11):982–988
- Bierut LJ, Strickland JR, Thompson JR, Afful SE, Cottler LB (2008) Drug use and dependence in cocaine dependent subjects, community-based individuals, and their siblings. *Drug Alcohol Depend* 95(1–2):14–22
- Bierut LJ, Agrawal A, Bucholz KK, Doheny KF, Laurie C, Pugh E, Fisher S, Fox L, Howells W, Bertelsen S, Hinrichs AL, Almasy L, Breslau N, Culverhouse RC, Dick DM, Edenberg HJ, Foroud T, Gruzca RA, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Krueger RF, Kuperman S, Lynskey M, Mann K, Neuman RJ, Nothen MM, Nurnberger JI Jr, Porjesz B, Ridinger M, Saccone NL, Saccone SF, Schuckit MA, Tischfield JA, Wang JC, Rietschel M, Goate AM, Rice JP (2010) A genome-wide association study of alcohol dependence. *Proc Natl Acad Sci USA* 107(11):5082–5087
- Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L (1998) Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci USA* 95(16):9608–9613
- Broms U, Wedenoja J, Largeau MR, Korhonen T, Pitkanieni J, Kesitalo-Vuokko K, Happola A, Heikkila KH, Heikkila K, Ripatti S, Sarin AP, Salminen O, Paunio T, Pergadia ML, Madden PA, Kaprio J, Loukola A (2012) Analysis of detailed phenotype profiles reveals CHRNA5-CHRNA3-CHRNA4 gene cluster association with several nicotine dependence traits. *Nicotine Tob Res* 14(6):720–733
- Carter BL, Lam CY, Robinson JD, Paris MM, Waters AJ, Wetter DW, Cinciripini PM (2008) Real-time craving and mood assessments before and after smoking. *Nicotine Tob Res* 10(7):1165–1169
- Chen X, Chen J, Williamson VS, An SS, Hetttema JM, Aggen SH, Neale MC, Kendler KS (2009) Variants in nicotinic acetylcholine receptors alpha5 and alpha3 increase risks to nicotine dependence. *Am J Med Genet B Neuropsychiatr Genet*
- Chen D, Liu L, Xiao Y, Peng Y, Yang C, Wang Z (2012a) Ethnic-specific meta-analyses of association between the OPRM1 A118G polymorphism and alcohol dependence among Asians and Caucasians. *Drug Alcohol Depend* 123(1–3):1–6
- Chen LS, Saccone NL, Culverhouse RC, Bracci PM, Chen CH, Dueker N, Han Y, Huang H, Jin G, Kohno T, Ma JZ, Przybeck TR, Sanders AR, Smith JA, Sung YJ, Wenzlaff AS, Wu C, Yoon D, Chen YT, Cheng YC, Cho YS, David SP, Duan J, Eaton CB, Furberg H, Goate AM, Gu D, Hansen HM, Hartz S, Hu Z, Kim YJ, Kittner SJ, Levinson DF, Mosley TH, Payne TJ, Rao DC, Rice JP, Rice TK, Schwantes-An TH, Shete SS, Shi J, Spitz MR, Sun YV, Tsai FJ, Wang JC, Wrensch MR, Xian H, Gejman PV, He J, Hunt SC, Kardia SL, Li MD, Lin D, Mitchell BD, Park T, Schwartz AG, Shen H, Wiencke JK, Wu JY, Yokota J, Amos CI, Bierut LJ (2012b) Smoking and genetic risk variation across populations of European, Asian, and African American ancestry—a meta-analysis of chromosome 15q25. *Genet Epidemiol* 36(4):340–351
- Cheverud JM (2001) A simple correction for multiple comparisons in interval mapping genome scans. *Heredity* 87(Pt 1):52–58
- Cinciripini PM, Tsoh JY, Wetter DW, Lam C, de Moor C, Cinciripini L, Baile W, Anderson C, Minna JD (2005) Combined effects of venlafaxine, nicotine replacement, and brief counseling on smoking cessation. *Exp Clin Psychopharmacol* 13(4):282–292
- Cinciripini PM, Robinson JD, Carter BL, Lam C, Wu X, de Moor CA, Baile WF, Wetter DW (2006) The effects of smoking deprivation and nicotine administration on emotional reactivity. *Nicotine Tob Res* 8(3):379–392
- Clarke TK, Crist RC, Kampman KM, Dackis CA, Pettinati HM, O'Brien CP, Oslin DW, Ferraro TN, Lohoff FW, Berrettini WH (2013) Low frequency genetic variants in the mu-opioid receptor (OPRM1) affect risk for addiction to heroin and cocaine. *Neurosci Lett* 54271–75
- Coller JK, Beardsley J, Bignold J, Li Y, Merg F, Sullivan T, Cox TC, Somogyi AA (2009) Lack of association between the A118G polymorphism of the mu opioid receptor gene (OPRM1) and opioid dependence: A meta-analysis. *Pharmacogenomics Pers Med* 29–19
- Crist RC, Berrettini WH (2013) Pharmacogenetics of OPRM1. *Pharmacol Biochem Behav*
- Cross-Disorder Group of the Psychiatric Genomics Consortium (2013) Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* 381(9875):1371–1379
- Crowley JJ, Oslin DW, Patkar AA, Gottheil E, DeMaria PA Jr, O'Brien CP, Berrettini WH, Grice DE (2003) A genetic association study of the mu opioid receptor and severe opioid dependence. *Psychiatr Genet* 13(3):169–173
- David SP, Johnstone EC, Churchman M, Aveyard P, Murphy MF, Munafò MR (2011) Pharmacogenetics of smoking cessation in general practice: results from the Patch II and Patch in Practice trials. *Nicotine Tob Res* 13(3):157–167
- Deb I, Chakraborty J, Gangopadhyay PK, Choudhury SR, Das S (2010) Single-nucleotide polymorphism (A118G) in exon 1 of OPRM1 gene causes alteration in downstream signaling by mu-opioid receptor and may contribute to the genetic risk for addiction. *J Neurochem* 112(2):486–496
- Development Core Team R (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Drakenberg K, Nikoshkov A, Horvath MC, Fagergren P, Gharibyan A, Saarelainen K, Rahman S, Nylander I, Bakalkin G, Rajs J, Keller E, Hurd YL (2006) Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proc Natl Acad Sci USA* 103(20):7883–7888
- Edenberg HJ (2002) The collaborative study on the genetics of alcoholism: an update. *Alcohol Res Health* 26(3):214–218
- Edenberg HJ, Koller DL, Xuei X, Wetherill L, McClintick JN, Almasy L, Bierut LJ, Bucholz KK, Goate A, Aliev F, Dick D, Hesselbrock V, Hinrichs A, Kramer J, Kuperman S, Nurnberger JI Jr, Rice JP, Schuckit MA, Taylor R, Todd Webb B, Tischfield JA, Porjesz B, Foroud T (2010) Genome-wide association study of alcohol dependence implicates a region on chromosome 11. *Alcohol Clin Exp Res* 34(5):840–852
- Elliott DS, Huizinga D, Ageton SS (1985) Explaining delinquency and drug use. Sage Publications, Beverly Hills
- Elliott DS, Huizinga D, Menard S (1989) Multiple Problem Youth: Delinquency, Drugs and Mental Health Problems, Springer
- Frank J, Cichon S, Treutlein J, Ridinger M, Mattheisen M, Hoffmann P, Herms S, Wodarz N, Soyka M, Zill P, Maier W, Mossner R, Gaebel W, Dahmen N, Scherbaum N, Schmal C, Steffens M, Lucae S, Ising M, Muller-Myhsok B, Nothen MM, Mann K, Kiefer F, Rietschel M (2012) Genome-wide significant association between alcohol dependence and a variant in the ADH gene cluster. *Addict Biol* 17(1):171–180
- Franke P, Wang T, Nothen MM, Knapp M, Neidt H, Albrecht S, Jahnes E, Propping P, Maier W (2001) Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *Am J Med Genet* 105(1):114–119
- Gelernter J, Kranzler H, Cubells J (1999) Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population

- studies, and allele frequencies in alcohol- and drug-dependent subjects. *Mol Psychiatry* 4(5):476–483
- Gelernter J, Kranzler HR, Sherva R, Koesterer R, Almasy L, Zhao H, Farrer LA (2013a) Genome-Wide association study of opioid dependence: multiple associations mapped to calcium and potassium pathways. *Biol Psychiatry*
- Gelernter J, Sherva R, Koesterer R, Almasy L, Zhao H, Kranzler HR, Farrer L (2013b) Genome-wide association study of cocaine dependence and related traits: FAM53B identified as a risk gene. *Mol Psychiatry*
- Gelernter J, Kranzler HR, Sherva R, Almasy L, Koesterer R, Smith AH, Anton R, Preuss UW, Ridinger M, Rujescu D, Wodarz N, Zill P, Zhao H, Farrer LA (2014) Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci. *Mol Psychiatry* 19(1):41–49
- Glatt SJ, Bousman C, Wang RS, Murthy KK, Rana BK, Lasky-Su JA, Zhu SC, Zhang R, Li J, Zhang B, Lyons MJ, Faraone SV, Tsuang MT (2007) Evaluation of OPRM1 variants in heroin dependence by family-based association testing and meta-analysis. *Drug Alcohol Depend* 90(2–3):159–165
- Gruza RA, Wang JC, Stitzel JA, Hinrichs AL, Saccone SF, Saccone NL, Bucholz KK, Cloninger CR, Neuman RJ, Budde JP, Fox L, Bertelsen S, Kramer J, Hesselbrock V, Tischfield J, Nurnberger JJ Jr, Almasy L, Porjesz B, Kuperman S, Schuckit MA, Edenberg HJ, Rice JP, Goate AM, Bierut LJ (2008) A risk allele for nicotine dependence in CHRNA5 is a protective allele for cocaine dependence. *Biol Psychiatry* 64(11):922–929
- Haerian BS, Haerian MS (2013) OPRM1 rs1799971 polymorphism and opioid dependence: evidence from a meta-analysis. *Pharmacogenomics* 14(7):813–824
- Hall FS, Drgonova J, Jain S, Uhl GR (2013) Implications of genome wide association studies for addiction: are our a priori assumptions all wrong? *Pharmacol Ther* 140(3):267–279
- Hancock DB, Levy JL, Gaddis NC, Glasheen C, Saccone NL, Page GP, Hulse GK, Wildenauer D, Keltz EA, Schwab SG, Degenhardt L, Martin NG, Montgomery GW, Attia J, Holliday EG, McEvoy M, Scott RJ, Bierut LJ, Nelson EC, Kral AH, Johnson EO (2015) Cis-Expression Quantitative Trait Loci Mapping Reveals Replicable Associations with Heroin Addiction in OPRM1. *Biol Psychiatry*
- Hardin J, He Y, Javitz HS, Wessel J, Krasnow RE, Tildesley E, Hops H, Swan GE, Bergen AW (2009) Nicotine withdrawal sensitivity, linkage to chr6q26, and association of OPRM1 SNPs in the SMOKING in FAMILIES (SMOFAM) sample. *Cancer Epidemiol Biomarkers Prev* 18(12):3399–3406
- Hart AB, de Wit H, Palmer AA (2013) Candidate gene studies of a promising intermediate phenotype: failure to replicate. *Neuropsychopharmacology* 38(5):802–816
- Hartz SM, Short SE, Saccone NL, Culverhouse R, Chen L, Schwantes-An TH, Coon H, Han Y, Stephens SH, Sun J, Chen X, Ducci F, Dueker N, Franceschini N, Frank J, Geller F, Gubjartsson D, Hansel NN, Jiang C, Keskitalo-Vuokko K, Liu Z, Lyytikainen LP, Michel M, Rawal R, Rosenberger A, Scheet P, Shaffer JR, Teumer A, Thompson JR, Vink JM, Vogelzangs N, Wenzlaff AS, Wheeler W, Xiao X, Yang BZ, Aggen SH, Balmforth AJ, Baumeister SE, Beaty T, Bennett S, Bergen AW, Boyd HA, Broms U, Campbell H, Chatterjee N, Chen J, Cheng YC, Cichon S, Couper D, Cucca F, Dick DM, Foroud T, Furberg H, Giegling I, Gu F, Hall AS, Hallfors J, Han S, Hartmann AM, Hayward C, Heikkila K, Hewitt JK, Hottenga JJ, Jensen MK, Jousilahti P, Kaakinen M, Kittner SJ, Konte B, Korhonen T, Landi MT, Laatikainen T, Leppert M, Levy SM, Mathias RA, McNeil DW, Medland SE, Montgomery GW, Muley T, Murray T, Nauck M, North K, Pergadia M, Polasek O, Ramos EM, Ripatti S, Risch A, Ruczinski I, Rudan I, Salomaa V, Schlessinger D, Styrkarsdottir U, Terracciano A, Uda M, Willemsen G, Wu X, Abecasis G, Barnes K, Bickeboller H, Boerwinkle E, Boomsma DI, Caporaso N, Duan J, Edenberg HJ, Francks C, Gejman PV, Gelernter J, Grabe HJ, Hops H, Jarvelin MR, Viikari J, Kahonen M, Kendler KS, Lehtimäki T, Levinson DF, Marazita ML, Marchini J, Melbye M, Mitchell BD, Murray JC, Nothen MM, Penninx BW, Raitakari O, Rietschel M, Rujescu D, Samani NJ, Sanders AR, Schwartz AG, Shete S, Shi J, Spitz M, Stefansson K, Swan GE, Thorgeirsson T, Volzke H, Wei Q, Wichmann HE, Amos CI, Breslau N, Cannon DS, Ehringer M, Gruza R, Hatsukami D, Heath A, Johnson EO, Kaprio J, Madden P, Martin NG, Stevens VL, Stitzel JA, Weiss RB, Kraft P, Bierut LJ (2012) Increased Genetic Vulnerability to Smoking at CHRNA5 in Early-Onset Smokers. *Arch Gen Psychiatry* 69(8):854–860
- Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO (1991) The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict* 86(9):1119–1127
- Hoft NR, Corley RP, McQueen MB, Schlaepfer IR, Huizinga D, Ehringer MA (2009) Genetic association of the CHRNA6 and CHRN3 genes with tobacco dependence in a nationally representative sample. *Neuropsychopharmacology* 34(3):698–706
- Hops H, Andrews JA, Duncan SC, Duncan TE, Tildesley E (2000) Adolescent drug use development: a social interactional and contextual perspective. In: Sameroff AJ, Lewis M (eds) *Handbook of Developmental Psychopathology*. Cluwer Academic/Plenum, New York, pp 589–605
- Huang P, Chen C, Mague SD, Blendy JA, Liu-Chen LY (2012) A common single nucleotide polymorphism A118G of the mu opioid receptor alters its N-glycosylation and protein stability. *Biochem J* 441(1):379–386
- Iacono WG, Carlson SR, Taylor J, Elkins IJ, McGue M (1999) Behavioral disinhibition and the development of substance-use disorders: findings from the Minnesota Twin Family Study. *Dev Psychopathol* 11(4):869–900
- Kamens HM, Corley RP, McQueen MB, Stallings MC, Hopfer CJ, Crowley TJ, Brown SA, Hewitt JK, Ehringer MA (2013) Nominal association with CHRNA4 variants and nicotine dependence. *Genes Brain Behav* 12(3):297–304
- Kaprio J, Pulkkinen L, Rose RJ (2002) Genetic and environmental factors in health-related behaviors: studies on Finnish twins and twin families. *Twin Res* 5(5):366–371
- Kendler KS, Myers J, Prescott CA (2007) Specificity of genetic and environmental risk factors for symptoms of cannabis, cocaine, alcohol, caffeine, and nicotine dependence. *Arch Gen Psychiatry* 64(11):1313–1320
- Keyes MA, Malone SM, Elkins IJ, Leland LN, McGue M, Iacono WG (2009) The enrichment study of the Minnesota twin family study: increasing the yield of twin families at high risk for externalizing psychopathology. *Twin Res Hum Genet* 12(5):489–501
- Kim SG, Kim CM, Kang DH, Kim YJ, Byun WT, Kim SY, Park JM, Kim MJ, Oslin DW (2004) Association of functional opioid receptor genotypes with alcohol dependence in Koreans. *Alcohol Clin Exp Res* 28(7):986–990
- Kreek MJ, Koob GF (1998) Drug dependence: stress and dysregulation of brain reward pathways. *Drug Alcohol Depend* 51(1–2):23–47
- Lam CY, Robinson JD, Versace F, Minnix JA, Cui Y, Carter BL, Wetter DW, Cinciripini PM (2012) Affective reactivity during smoking cessation of never-quitters as compared with that of abstainers, relapsers, and continuing smokers. *Exp Clin Psychopharmacol* 20(2):139–150
- Lessov CN, Martin NG, Statham DJ, Todorov AA, Slutske WS, Bucholz KK, Heath AC, Madden PA (2004) Defining nicotine



- dependence for genetic research: evidence from Australian twins. *Psychol Med* 34(5):865–879
- Levrano O, Londono D, O'Hara K, Nielsen DA, Peles E, Rotrosen J, Casadonte P, Linzy S, Randesi M, Ott J, Adelson M, Kreek MJ (2008) Genetic susceptibility to heroin addiction: a candidate gene association study. *Genes Brain Behav* 7(7):720–729
- Levrano O, Yufarov V, Kreek MJ (2012) The genetics of the opioid system and specific drug addictions. *Hum Genet* 131(6):823–842
- Li J, Ji L (2005) Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* 95(3):221–227
- Liu JZ, Tozzi F, Waterworth DM, Pillai SG, Muglia P, Middleton L, Berrettini W, Knouff CW, Yuan X, Waeber G, Vollenweider P, Preisig M, Wareham NJ, Zhao JH, Loos RJ, Barroso I, Khaw KT, Grundy S, Barter P, Mahley R, Kesaniemi A, McPherson R, Vincent JB, Strauss J, Kennedy JL, Farmer A, McGuffin P, Day R, Matthews K, Bakke P, Gulsvik A, Lucae S, Ising M, Brueckl T, Horstmann S, Wichmann HE, Rawal R, Dahmen N, Lamina C, Polasek O, Zgaga L, Huffman J, Campbell S, Kooner J, Chambers JC, Burnett MS, Devaney JM, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Epstein S, Wilson JF, Wild SH, Campbell H, Vitart V, Reilly MP, Li M, Qu L, Wilensky R, Matthaï W, Hakonarson HH, Rader DJ, Franke A, Wittig M, Schafer A, Uda M, Terracciano A, Xiao X, Busonero F, Scheet P, Schlessinger D, St Clair D, Rujescu D, Abecasis GR, Grabe HJ, Teumer A, Volzke H, Petersmann A, John U, Rudan I, Hayward C, Wright AF, Kolcic I, Wright BJ, Thompson JR, Balmforth AJ, Hall AS, Samani NJ, Anderson CA, Ahmad T, Mathew CG, Parkes M, Satsangi J, Caulfield M, Munroe PB, Farrall M, Dominiczak A, Worthington J, Thomson W, Eyre S, Barton A, Mooser V, Francks C, Marchini J (2010) Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nat Genet* 42(5):436–440
- Loukola A, Broms U, Maunu H, Widen E, Heikkilä K, Siivola M, Salo A, Pergadia ML, Nyman E, Sammalisto S, Perola M, Agrawal A, Heath AC, Martin NG, Madden PA, Peltonen L, Kaprio J (2008) Linkage of nicotine dependence and smoking behavior on 10q, 7q and 11p in twins with homogeneous genetic background. *Pharmacogenomics* 8(3):209–219
- Lumley T (2009) *rmeta: Meta-analysis, R package version 2.15*. <http://CRAN.R-project.org/package=rmeta>
- Mague SD, Blendy JA (2010) OPRM1 SNP (A118G): involvement in disease development, treatment response, and animal models. *Drug Alcohol Depend* 108(3):172–182
- Mague SD, Isiegas C, Huang P, Liu-Chen LY, Lerman C, Blendy JA (2009) Mouse model of OPRM1 (A118G) polymorphism has sex-specific effects on drug-mediated behavior. *Proc Natl Acad Sci USA* 106(26):10847–10852
- Maher BS, Vladimirov VI, Latendresse SJ, Thiselton DL, McNamee R, Kang M, Bigdeli TB, Chen X, Riley BP, Hettema JM, Chilcoat H, Heibredere C, Muglia P, Murrelle EL, Dick DM, Aliev F, Agrawal A, Edenberg HJ, Kramer J, Nurnberger J, Tischfield JA, Devlin B, Ferrell RE, Kirillova GP, Tarter RE, Kendler KS, Vanyukov MM (2011) The AVPR1A gene and substance use disorders: association, replication, and functional evidence. *Biol Psychiatry* 70(6):519–527
- McGue M, Keyes M, Sharma A, Elkins I, Legrand L, Johnson W, Iacono WG (2007) The environments of adopted and non-adopted youth: evidence on range restriction from the Sibling Interaction and Behavior Study (SIBS). *Behav Genet* 37(3):449–462
- Medland SE, Neale MC (2010) An integrated phenomic approach to multivariate allelic association. *Eur J Hum Genet* 18(2):233–239
- Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, Zhang H, O'Malley SS, Rounsaville BJ (1998) Familial transmission of substance use disorders. *Arch Gen Psychiatry* 55(11):973–979
- Miller MB, Basu S, Cunningham J, Eskin E, Malone SM, Oetting WS, Schork N, Sul JH, Iacono WG, McGue M (2012) The Minnesota Center for Twin and Family Research genome-wide association study. *Twin Res Hum Genet* 15(6):767–774
- Minnix JA, Robinson JD, Lam CY, Carter BL, Foreman JE, Vandenberg DJ, Tomlinson GE, Wetter DW, Cinciripini PM (2011) The serotonin transporter gene and startle response during nicotine deprivation. *Biol Psychol* 86(1):1–8
- Miranda R Jr, Reynolds E, Ray L, Justus A, Knopik VS, McGeary J, Meyerson LA (2013) Preliminary evidence for a gene-environment interaction in predicting alcohol use disorders in adolescents. *Alcohol Clin Exp Res* 37(2):325–331
- Munafò MR, Johnstone EC, Aveyard P, Marteau T (2013) Lack of association of OPRM1 genotype and smoking cessation. *Nicotine Tob Res* 15(3):739–744
- Neale MC, Harvey E, Maes HH, Sullivan PF, Kendler KS (2006) Extensions to the modeling of initiation and progression: applications to substance use and abuse. *Behav Genet* 36(4):507–524
- Nelson EC, Lynskey MT, Heath AC, Wray N, Agrawal A, Shand FL, Henders AK, Wallace L, Todorov AA, Schrage AJ, Saccone NL, Madden PA, Degenhardt L, Martin NG, Montgomery GW (2013) ANKK1, TTC12, and NCAM1 polymorphisms and heroin dependence: importance of considering drug exposure. *JAMA Psychiatry* 70(3):325–333
- Nelson EC, Lynskey MT, Heath AC, Wray N, Agrawal A, Shand FL, Henders AK, Wallace L, Todorov AA, Schrage AJ, Madden PA, Degenhardt L, Martin NG, Montgomery GW (2014) Association of OPRD1 polymorphisms with heroin dependence in a large case-control series. *Addict Biol* 19(1):111–121
- Nikolov MA, Beltcheva O, Galabova A, Ljubenova A, Jankova E, Gergov G, Russev AA, Lynskey MT, Nelson EC, Nesheva E, Krasteva D, Lazarov P, Mitev VI, Kremensky IM, Kaneva RP, Todorov AA (2011) No evidence of association between 118A > G OPRM1 polymorphism and heroin dependence in a large Bulgarian case-control sample. *Drug Alcohol Depend* 117(1):62–65
- Nyholt DR (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 74(4):765–769
- Palmer AA, de Wit H (2012) Translational genetic approaches to substance use disorders: bridging the gap between mice and humans. *Hum Genet* 131931–939
- Ramchandani VA, Umhau J, Pavon FJ, Ruiz-Velasco V, Margas W, Sun H, Damadzic R, Eskay R, Schoor M, Thorsell A, Schwandt ML, Sommer WH, George DT, Parsons LH, Herscovitch P, Hommer D, Heilig M (2011) A genetic determinant of the striatal dopamine response to alcohol in men. *Mol Psychiatry* 16(8):809–817
- Ray LA, Hutchison KE (2004) A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. *Alcohol Clin Exp Res* 28(12):1789–1795
- Ray LA, Hutchison KE (2007) Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response: a double-blind placebo-controlled study. *Arch Gen Psychiatry* 64(9):1069–1077
- Ray R, Jepsen C, Patterson F, Strasser A, Rukstalis M, Perkins K, Lynch KG, O'Malley S, Berrettini WH, Lerman C (2006) Association of OPRM1 A118G variant with the relative reinforcing value of nicotine. *Psychopharmacology* 188(3):355–363
- Ray LA, Miranda R Jr, Tidey JW, McGeary JE, MacKillop J, Gwaltney CJ, Rohsenow DJ, Swift RM, Monti PM (2010) Polymorphisms of the mu-opioid receptor and dopamine D4 receptor genes and subjective responses to alcohol in the natural environment. *J Abnorm Psychol* 119(1):115–125

- Ray R, Ruparel K, Newberg A, Wileyto EP, Loughhead JW, Divgi C, Blendy JA, Logan J, Zubieta JK, Lerman C (2011) Human Mu Opioid Receptor (OPRM1 A118G) polymorphism is associated with brain mu-opioid receptor binding potential in smokers. *Proc Natl Acad Sci USA* 108(22):9268–9273
- Ray LA, Barr CS, Blendy JA, Oslin D, Goldman D, Anton RF (2012) The role of the Asn40Asp polymorphism of the mu opioid receptor gene (OPRM1) on alcoholism etiology and treatment: a critical review. *Alcohol Clin Exp Res* 36(3):385–394
- Robinson JD, Cinciripini PM, Carter BL, Lam CY, Wetter DW (2007) Facial EMG as an index of affective response to nicotine. *Exp Clin Psychopharmacol* 15(4):390–399
- Robinson JD, Lam CY, Carter BL, Minnix JA, Cui Y, Versace F, Wetter DW, Cinciripini PM (2011) A multimodal approach to assessing the impact of nicotine dependence, nicotine abstinence, and craving on negative affect in smokers. *Exp Clin Psychopharmacol* 19(1):40–52
- Rouvinen-Lagerstrom N, Lahti J, Alho H, Kovanen L, Aalto M, Partonen T, Silander K, Sinclair D, Raikkonen K, Eriksson JG, Palotie A, Koskinen S, Saarikoski ST (2013) mu-Opioid receptor gene (OPRM1) polymorphism A118G: lack of association in Finnish populations with alcohol dependence or alcohol consumption. *Alcohol Alcohol* 48(5):519–525
- Saccone SF, Pergadia ML, Loukola A, Broms U, Montgomery GW, Wang JC, Agrawal A, Dick DM, Heath AC, Todorov AA, Maunu H, Heikkila K, Morley KI, Rice JP, Todd RD, Kaprio J, Peltonen L, Martin NG, Goate AM, Madden PA (2007) Genetic linkage to chromosome 22q12 for a heavy-smoking quantitative trait in two independent samples. *Am J Hum Genet* 80(5):856–866
- Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML, Agrawal A, Breslau N, Gruzca RA, Hatsukami D, Johnson EO, Madden PA, Swan GE, Wang JC, Goate AM, Rice JP, Bierut LJ (2009a) Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. *Am J Med Genet B Neuropsychiatr Genet* 150B(4):453–466
- Saccone NL, Wang JC, Breslau N, Johnson EO, Hatsukami D, Saccone SF, Gruzca RA, Sun L, Duan W, Budde J, Culverhouse RC, Fox L, Hinrichs AL, Steinbach JH, Wu M, Rice JP, Goate AM, Bierut LJ (2009b) The CHRNA5-CHRNA3-CHRNA4 nicotinic receptor subunit gene cluster affects risk for nicotine dependence in African-Americans and in European-Americans. *Cancer Res* 69(17):6848–6856
- Saccone NL, Culverhouse RC, Schwantes-An TH, Cannon DS, Chen X, Cichon S, Giegling I, Han S, Han Y, Keskitalo-Vuokko K, Kong X, Landi MT, Ma JZ, Short SE, Stephens SH, Stevens VL, Sun L, Wang Y, Wenzlaff AS, Aggen SH, Breslau N, Broderick P, Chatterjee N, Chen J, Heath AC, Heliövaara M, Hoft NR, Hunter DJ, Jensen MK, Martin NG, Montgomery GW, Niu T, Payne TJ, Peltonen L, Pergadia ML, Rice JP, Sherva R, Spitz MR, Sun J, Wang JC, Weiss RB, Wheeler W, Witt SH, Yang BZ, Caporaso NE, Ehringer MA, Eisen T, Gapstur SM, Gelernter J, Houlston R, Kaprio J, Kendler KS, Kraft P, Leppert MF, Li MD, Madden PA, Nothen MM, Pillai S, Rietschel M, Rujescu D, Schwartz A, Amos CI, Bierut LJ (2010) Multiple Independent Loci at Chromosome 15q25.1 Affect Smoking Quantity: a Meta-Analysis and Comparison with Lung Cancer and COPD. *PLoS Genet* 6(8):e1001053
- Sander T, Gscheidel N, Wendel B, Samochowiec J, Smolka M, Rommelspacher H, Schmidt LG, Hoehe MR (1998) Human mu-opioid receptor variation and alcohol dependence. *Alcohol Clin Exp Res* 22(9):2108–2110
- Schinka JA, Town T, Abdullah L, Crawford FC, Ordorica PI, Francis E, Hughes P, Graves AB, Mortimer JA, Mullan M (2002) A functional polymorphism within the mu-opioid receptor gene and risk for abuse of alcohol and other substances. *Mol Psychiatry* 7(2):224–228
- Stallings MC, Corley RP, Hewitt JK, Krauter KS, Lessem JM, Mikulich SK, Rhee SH, Smolen A, Young SE, Crowley TJ (2003) A genome-wide search for quantitative trait loci influencing substance dependence vulnerability in adolescence. *Drug Alcohol Depend* 70(3):295–307
- Stallings MC, Corley RP, Dennehey B, Hewitt JK, Krauter KS, Lessem JM, Mikulich-Gilbertson SK, Rhee SH, Smolen A, Young SE, Crowley TJ (2005) A genome-wide search for quantitative trait loci that influence antisocial drug dependence in adolescence. *Arch Gen Psychiatry* 62(9):1042–1051
- Swan GE, Carmelli D, Cardon LR (1997) Heavy consumption of cigarettes, alcohol and coffee in male twins. *J Stud Alcohol* 58(2):182–190
- Tan EC, Tan CH, Karupathivan U, Yap EP (2003) Mu opioid receptor gene polymorphisms and heroin dependence in Asian populations. *NeuroReport* 14(4):569–572
- The Tobacco and Genetics Consortium (2010) Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nat Genet* 42(5):441–447
- Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, Sulem P, Rafnar T, Esko T, Walter S, Gieger C, Rawal R, Mangino M, Prokopenko I, Magi R, Keskitalo K, Gudjonsdottir IH, Gretarsdottir S, Stefansson H, Thompson JR, Aulchenko YS, Nelis M, Aben KK, den Heijer M, Dirksen A, Ashraf H, Soranzo N, Valdes AM, Steves C, Uitterlinden AG, Hofman A, Tonjes A, Kovacs P, Hottenga JJ, Willemsen G, Vogelzang N, Doring A, Dahmen N, Nitz B, Pergadia ML, Saez B, De Diego V, Lezcano V, Garcia-Prats MD, Ripatti S, Perola M, Kettunen J, Hartikainen AL, Pouta A, Laitinen J, Isohanni M, Huei-Yi S, Allen M, Krestyaninova M, Hall AS, Jones GT, van Rij AM, Mueller T, Dieplinger B, Haltmayer M, Jonsson S, Matthiasson SE, Oskarsson H, Tyrfingsson T, Kiemeny LA, Mayordomo JI, Lindholt JS, Pedersen JH, Franklin WA, Wolf H, Montgomery GW, Heath AC, Martin NG, Madden PA, Giegling I, Rujescu D, Jarvelin MR, Salomaa V, Stumvoll M, Spector TD, Wichmann HE, Metspalu A, Samani NJ, Penninx BW, Oostra BA, Boomsma DI, Tiemeier H, van Duijn CM, Kaprio J, Gulcher JR, McCarthy MI, Peltonen L, Thorsteinsdottir U, Stefansson K (2010) Sequence variants at CHRNA3-CHRNA4 and CYP2A6 affect smoking behavior. *Nat Genet* 42(5):448–453
- Town T, Abdullah L, Crawford F, Schinka J, Ordorica PI, Francis E, Hughes P, Duara R, Mullan M (1999) Association of a functional mu-opioid receptor allele (+118A) with alcohol dependency. *Am J Med Genet* 88(5):458–461
- Treutlein J, Cichon S, Ridinger M, Wodarz N, Soyka M, Zill P, Maier W, Moessner R, Gaebel W, Dahmen N, Fehr C, Scherbaum N, Steffens M, Ludwig KU, Frank J, Wichmann HE, Schreiber S, Dragano N, Sommer WH, Leonardi-Essmann F, Lourdasamy A, Gebicke-Haerter P, Wienker TF, Sullivan PF, Nothen MM, Kiefer F, Spanagel R, Mann K, Rietschel M (2009) Genome-wide association study of alcohol dependence. *Arch Gen Psychiatry* 66(7):773–784
- Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J, True W, Lin N, Toomey R, Eaves L (1998) Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch Gen Psychiatry* 55(11):967–972
- Van den Oord EJ, Rujescu D, Robles JR, Giegling I, Birrell C, Bukszar J, Murrelle L, Moller HJ, Middleton L, Muglia P (2006) Factor structure and external validity of the PANSS revisited. *Schizophr Res* 82(2–3):213–223
- Vanyukov MM, Tarter RE, Kirisci L, Kirillova GP, Maher BS, Clark DB (2003) Liability to substance use disorders: I. Common mechanisms and manifestations. *Neurosci Biobehav Rev* 27(6):507–515

- Vanyukov MM, Tarter RE, Kirillova GP, Kirisci L, Reynolds MD, Kreek MJ, Conway KP, Maher BS, Iacono WG, Bierut L, Neale MC, Clark DB, Ridenour TA (2012) Common liability to addiction and “gateway hypothesis”: theoretical, empirical and evolutionary perspective. *Drug Alcohol Depend* 123 Suppl 1S3–17
- Wang YJ, Huang P, Ung A, Blendy JA, Liu-Chen LY (2012) Reduced expression of the mu opioid receptor in some, but not all, brain regions in mice with OPRM1 A112G. *Neuroscience* 205:178–184
- Wang YJ, Huang P, Blendy JA, Liu-Chen LY (2014) Brain region- and sex-specific alterations in DAMGO-stimulated [(35)S]GTPgammaS binding in mice with Oprm1 A112G. *Addict Biol* 19(3):354–361
- Weiss RB, Baker TB, Cannon DS, von Niederhausern A, Dunn DM, Matsunami N, Singh NA, Baird L, Coon H, McMahon WM, Piper ME, Fiore MC, Scholand MB, Connett JE, Kanner RE, Gahring LC, Rogers SW, Hoidal JR, Leppert MF (2008) A candidate gene approach identifies the *CHRNA5-A3-B4* region as a risk factor for age-dependent nicotine addiction. *PLoS Genet* 4(7):e1000125
- Wetherill L, Agrawal A, Kapoor M, Bertelsen S, Bierut LJ, Brooks A, Dick D, Hesselbrock M, Hesselbrock V, Koller DL, Le N, Nurnberger JI Jr, Salvatore JE, Schuckit M, Tischfield JA, Wang JC, Xuei X, Edenberg HJ, Porjesz B, Bucholz K, Goate AM, Foroud T (2015) Association of substance dependence phenotypes in the COGA sample. *Addict Biol* 20(3):617–627
- Xuei X, Flury-Wetherill L, Bierut L, Dick D, Nurnberger J Jr, Foroud T, Edenberg HJ (2007) The opioid system in alcohol and drug dependence: family-based association study. *Am J Med Genet B Neuropsychiatr Genet* 144B(7):877–884
- Zhang Y, Wang D, Johnson AD, Papp AC, Sadee W (2005) Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. *J Biol Chem* 280(38):32618–32624
- Zhang H, Luo X, Kranzler HR, Lappalainen J, Yang BZ, Krupitsky E, Zvartau E, Gelernter J (2006a) Association between two mu-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Hum Mol Genet* 15(6):807–819
- Zhang L, Kendler KS, Chen X (2006b) The mu-opioid receptor gene and smoking initiation and nicotine dependence. *Behav Brain Funct* 228