Molecular Psychiatry (2007) 12, 454–461

www.nature.com/mp

ORIGINAL ARTICLE

Association of the *DRD2* gene Taq1A polymorphism and alcoholism: a meta-analysis of case–control studies and evidence of publication bias

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We investigated the association of the dopamine D2 receptor (*DRD2*) Taq1A polymorphism and alcoholism, using meta-analytic techniques, and specifically undertook an investigation of possible publication bias. Potential publication bias represents a genuine risk to the integrity of published research, but its impact has rarely been documented. We observed a small effect of the *DRD2* Taq1A polymorphism on risk of alcoholism, indicating increased alcoholism in individuals possessing the A1 allele of the Taq1A polymorphism (OR = 1.21, 95% CI 1.13–1.30, $P < 0.001$). This association remained significant when data from samples of European and East Asian ancestry were analyzed separately. We did not find evidence for association in high-severity alcoholism compared to low-severity alcoholism. Removing the first published study significantly reduced the magnitude of the pooled effect size estimate, although the association remained significant. In addition, we observed evidence for possible publication bias and for the strength of individual study effect size to be inversely related to year of publication. These results support the association of the *DRD2* Taq1A polymorphism with alcoholism. This conclusion is qualified by the possibility of publication bias in the literature and the observed between-study heterogeneity, which indicates that the observed association may differ in strength between populations or may not exist at all in some populations.
Molecular Psychiatry (2007) **12,** 454–461. doi:10.1038/sj.mp.4001938; published online 9 January 2007

Keywords: *DRD2*; ANKK1; genetics; alcoholism; meta-analysis; publication bias

Introduction

The human central dopamingeric system is widely considered to play an important role in substance use and the development of subsequent dependence. Evidence for a role for this system extends to a range of psychoactive substances, including opiates, cocaine, nicotine and alcohol.[1–3] In consequence, a great deal of attention has been devoted to determining whether variation in genes with a dopaminergic function could account for the heritable variation in susceptibility to substance abuse. In particular, the dopamine D2 receptor (*DRD2*) gene on chromosome 11 (q22–q23) has been widely studied.[4]

Following a report in 1990[5] that the A1 allele of the Taq1A polymorphism (rs1800497) of the *DRD2* gene, a C > T substitution located in a noncoding region of the *DRD2* locus, was associated with alcoholism, several studies have attempted to replicate the finding. Despite the large number of individual studies, results have been equivocal. A 1993 report found that heterogeneity between populations was considerably greater than differences between alcoholics and controls overall, indicating that the positive findings could be due to sampling error and population stratification.[6] By contrast, a recent meta-analysis,[7] surveying 55 studies involving almost 10 000 participants, found that the A1 allele was significantly more likely to be found in the substance abuse groups than in controls. Nevertheless, despite the large number of studies, the effect was only detected at a 0.05 significance threshold.

It remains unclear whether inconsistency in individual study results arises from ancestral variation, phenotypic variation, sampling variation or some other source of heterogeneity. Identifying these sources is a major challenge for the genetic analysis of any complex trait, not just alcoholism. Fortunately, the large number of studies of the *DRD2* Taq1A1 polymorphism makes it possible to investigate heterogeneity within the context of a meta-analysis. For example, in the meta-analysis reported above, the association was not significant when studied in samples of non-European ancestry, but significant when studies were limited to those assessing the association of the A1 allele and severe substance dependence.[7]

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Received 13 July 2006; revised 6 October 2006; accepted 30 October 2006; published online 9 January 2007

We undertook an investigation of another source of heterogeneity: publication bias. Potential publication bias represents a genuine risk to the integrity of published research. However, although there are grounds for believing publication bias to be present in the psychiatric genetic literature (e.g., because of the relative difficulty in interpreting nonsignificant results compared to statistically significant results within a null hypothesis testing framework), its impact has rarely been documented. This is because methods to detect publication bias require relatively large numbers of published studies.

The methods that may be used to detect possible publication bias are of two types: graphical and statistical. The funnel plot is a commonly used graphical test to assess publication bias in meta-analytic data sets.[8] The rationale behind a funnel plot analysis is that if all studies come from a single population, then the plot should look like a funnel with the diameter of the funnel decreasing (i.e., effect size estimate becoming more accurate) as sample size increases.[9] However, there are often insufficient large studies to form the apex of the predicted funnel. Formal tests of publication bias are statistical analogs of the funnel plot.[8,10] Begg and Mazumdar[10] proposed an adjusted rank correlation method to examine the association between the effect estimates and their variances, whereas Egger *et al*.[8] introduced an approach that tests for asymmetry in the funnel plot and corresponds to a weighted regression of effect sizes on their standard errors, where the weights are inversely proportional to the variance of the effect size. Asymmetry may be a result of the non-publication of nonsignificant studies, and this is a formal test of the null hypothesis that such a bias is not present. The regression method is more sensitive than the rank correlation approach, but the sensitivity of both methods is generally low in meta-analyses based on less than 20 studies.

Although the Egger method is widely used, it has been criticized for being intrinsically biased[11,12] owing to the correlation between the effect size estimate and its standard error when using odds ratios (OR),[13] which may lead to a high type I error rate (i.e., falsely identifying possible publication bias). Alternative methods have been suggested, including the Macaskill method, which corresponds to a weighted regression of effect sizes on their sample sizes,[14] and a modified Macaskill method, which employs the inverse of individual study sample sizes.[13] These methods have been argued to be more conservative than the Egger method, and simulation data suggest that the modified Macaskill method is optimal.[13]

The large number of *DRD2* Taq1A studies means that we are able to apply a formal test of publication bias. We attempted to replicate the findings of a recent meta-analysis[7] and include reports not included in this earlier study (e.g., those published subsequently). We restricted our analysis to case–control studies. Although it has recently been shown that the Taq1A variant alters an amino acid in a protein kinase gene (*ANKK1*) near the *DRD2* locus,[15] we refer to the variant throughout as the *DRD2* Taq1A polymorphism, as this is the nomenclature used in the majority of published studies to date.

Methods

Selection of studies for inclusion

Case–control genetic association studies of the *DRD2* Taq1A polymorphism in healthy controls and clinically diagnosed alcoholic patients were included. Studies reporting data on either single-sex or both male and female participants of any ethnic origin were included. Studies with data for only alcoholic patients or only healthy participants were excluded, as were family-based studies that only reported transmission disequilibrium to affected offspring. The principal outcome measure was the allelic OR for the Taq1A polymorphism and alcoholism case status.

Search strategy

The search was performed on three databases: PubMed, PsycInfo and Medline. These databases were searched from the first date available in each database up to 30 June 2006, using the search terms 'alcohol', 'alcoholism', 'DRD2', 'dopamine D2' and 'Taq1A'. Once articles had been collected, bibliographies were then hand-searched for additional references.

The abstracts of studies identified by these search strategies were then examined with reference to the inclusion and exclusion criteria. Duplications were deleted and the whole text of each reference was then checked to further establish whether the study met the study inclusion criteria. Studies that reported previously published data were excluded.

Data extraction

For each study, the following data were extracted independently by two authors (MM and IM) using standard forms: (1) author(s) and year of publication; (2) methods (country of origin, dominant ancestry of sample, case and control sample size, diagnostic criteria for alcoholism case status, candidate gene, polymorphism, statement of Hardy–Weinberg equilibrium, method of genotyping); (3) data (number of participants in control and case groups, mean age and sex ratio by allele frequency). Genotype frequencies were used to calculate whether or not these deviated significantly from Hardy–Weinberg equilibrium among controls. Ancestry was coded as European, East Asian or Other (which included cases where ancestry was stated as mixed or not stated). Discrepancies were resolved by mutual consent.

Analysis of data

Data were analyzed using the Comprehensive Meta-analysis (v.2) statistical software package. A *P*-value of 0.050 was retained throughout.

Data were initially analyzed within a fixed-effects framework and OR pooled using inverse variance methods to generate a summary OR and 95% confidence interval (CI). A fixed-effects framework assumes that the effect of allele frequency is constant across studies and between-study variation is considered to be due to chance or random variation. The assumption was checked using a χ^2 test of goodness of fit for homogeneity. The significance of the pooled OR was determined using a *Z*-test.

Where there was evidence of a significant association between *DRD2* Taq1A1 allele frequency and alcoholism case status in the presence of significant between-study heterogeneity, a random-effects framework was employed, with ORs pooled using DerSimonian and Laird methods. A random-effects framework assumes that between-study variation is due to both chance or random variation and an individual study effect. Random-effects models are more conservative than fixed-effects models and generate a wider CI. The significance of the pooled OR was determined using a *Z*-test.

Stratified analyses by sample ancestry and alcoholism severity were conducted in order to assess potential moderating effects of these variables. Studies with samples of predominantly European or East Asian ancestry were combined separately and the difference in pooled OR was determined using a *Z*-test. For studies that identified low- and high-severity subgroups and reported data separately for these subgroups (e.g., alcohol abuse vs alcohol dependence), these subgroups were combined separately and compared with each other. Such groupings were made on the basis of the classifications implemented by individual studies.

The OR of the first published study was compared to the pooled OR of the remaining studies using a *Z*-test, as there is evidence for a substantially greater estimate of effect size in the first published study.[16] Funnel plots were created in order to assess potential ascertainment bias by plotting individual study log OR against the standard error of the log OR. Ascertainment bias was also assessed using the Egger test[8] and the modified Macaskill test.[13]

Results

Description of studies

A total of 40 studies published between 1990 and 2006 were identified by the search strategy, met the inclusion criteria and contributed to the meta-analysis.[5,6,17–54] The characteristics of these studies are described in Table 1.

Twenty-five studies reported data on samples of predominantly European ancestry, eight on samples of predominantly East Asian ancestry and seven on samples of Other ancestry. Three studies reported *DRD2* genotype frequencies for controls that deviated significantly from Hardy–Weinberg equilibrium (Bolos *et al*., 1990; Comings *et al*., 1994; Lu *et al*., 1996). Four studies used DSM-IV criteria for assessing schizophrenia case status, whereas 26 used DSM-III-R criteria, five used ICD-10 criteria and one each used Feighner, DIGS and RDC criteria, whereas two did not state the criteria used.

Meta-analysis

When all studies ($k = 40$) were included, there was evidence of a significant association between *DRD2* Taq1A1 allele frequency and alcoholism case status ($Z = 5.50$, $P < 0.001$, OR = 1.21, 95% CI 1.13–1.30). There was evidence of significant between-study heterogeneity ($\chi^2[39] = 92.87$, $P < 0.001$), but when the analysis was re-run within a random-effects framework the evidence for association remained statistically significant ($Z = 4.39$, $P < 0.001$, OR = 1.29, 95% CI 1.15–1.45). These results are presented graphically in Figure 1.

When the first published study[5] was removed from the analysis ($k = 39$), there was evidence of a significant association between *DRD2* Taq1A1 allele frequency and alcoholism case status ($Z = 5.03$, $P < 0.001$, OR = 1.20, 95% CI 1.11–1.28). There was evidence of significant between-study heterogeneity ($\chi^2[38] = 67.68$, $P = 0.002$), but when the analysis was re-run within a random-effects framework the evidence for association remained statistically significant ($Z = 4.23$, $P < 0.001$, OR = 1.23, 95% CI 1.12–1.36).

The comparison of the effect size indicated by the first published study (OR = 8.73) compared to the pooled effect size for subsequent studies (OR = 1.20) indicated a significant difference within both a fixed-effects ($Z = 5.02$, $P < 0.001$) and a random-effects framework ($Z = 4.92$, $P < 0.001$). Meta-regression indicated a significant negative association between year of publication (corrected for month of publication) and individual study effect size ($Z = -2.15$, $P = 0.032$), with this trend reflecting a decrease in individual study effect size over time. These data are presented graphically in Figure 2.

The removal of three studies that reported *DRD2* genotype frequencies for controls that deviated significantly from Hardy–Weinberg equilibrium[23,26,47] did not alter these results substantially.

Ethnicity

When studies that recruited samples of predominantly European ancestry were analyzed separately ($k = 24$), there was evidence for a significant association of *DRD2* Taq1A1 allele frequency and alcoholism case status ($Z = 3.89$, $P < 0.001$, OR = 1.19, 95% CI 1.09–1.29). There was evidence of significant between-study heterogeneity ($\chi^2[24] = 43.44$, $P = 0.009$), but when the analysis was re-run within a random-effects framework the evidence for association remained statistically significant ($Z = 3.16$, $P < 0.001$, OR = 1.22, 95% CI 1.08–1.38).

When studies that recruited samples of predominantly East Asian ancestry were analyzed separately ($k = 8$) there was evidence for a significant association of *DRD2* Taq1A1 allele frequency and alcoholism case status ($Z = 2.11$, $P = 0.034$, OR = 1.17, 95% CI 1.01–

Table 1 Characteristics of included studies

Study	*Year*	*Case* n	*Case* N	*Control* n	*Control* N	*Ancestry*	*Diagnosis*	*HWE*
Blum *et al*.[5]	1990	48	70	14	70	Other	DSM-III-R	Yes
Bolos *et al*.[23]	1990	30	80	76	254	European	DSM-III-R	No
Blum *et al*.[22]	1991	55	192	9	86	Other	DSM-III-R	Yes
Noble *et al*.[48]	1991	24	66	9	66	Other	DSM-III-R	Yes
Parsian *et al*.[51]	1991	13	64	3	50	European	Feighner	Yes
Comings *et al*.[26]	1991	47	208	10	138	European	DSM-III-R	No
Gelernter *et al*.[34]	1991	20	88	27	136	European	DSM-III-R	Yes
Goldman *et al*.[35]	1992	14	92	15	72	European	DSM-III-R	Yes
Cook *et al*.[28]	1992	10	40	12	40	European	DSM-III-R	Yes
Arinami *et al*.[19]	1993	63	156	23	70	East Asian	DSM-III-R	Yes
Goldman *et al*.[6]	1993	23	32	35	48	Other	RDC	Yes
Amadeo *et al*.[17]	1993	23	164	23	174	European	DSM-III-R	Yes
Comings *et al*.[27]	1994	24	98	7	86	European	DSM-III-R	Yes
Geijer *et al*.[32]	1994	56	188	443	1526	European	DSM-III-R	Yes
Higuchi *et al*.[39]	1994	26	148	12	80	East Asian	DSM-III-R	Yes
Noble *et al*.[49]	1994	42	146	27	160	Other	DSM-III-R	Yes
Sander *et al*.[53]	1995	99	540	43	226	European	ICD-10	Yes
Finckh *et al*.[29]	1996	112	624	42	262	European	ICD-10	Yes
Heinz *et al*.[37]	1996	37	194	43	226	European	ICD-10	Yes
Chen *et al*.[24]	1996	129	316	32	82	East Asian	DSM-III-R	Yes
Lu *et al*.[47]	1996	52	122	54	130	East Asian	DSM-III-R	No
Chen *et al*.[25]	1997	166	406	163	426	East Asian	DSM-III-R	Yes
Goldman *et al*.[36]	1997	322	552	196	322	Other	DSM-III-R	Yes
Hietala *et al*.[38]	1997	31	140	11	100	European	DSM-III-R	Yes
Kono *et al*.[43]	1997	78	200	69	186	East Asian	DSM-III-R	Yes
Lawford *et al*.[44]	1997	92	402	17	92	European	DSM-III-R	Yes
Lee *et al*.[45]	1997	52	134	76	200	East Asian	DSM-III-R	Yes
Ishiguro *et al*.[40]	1998	179	418	106	304	East Asian	DSM-III-R	Yes
Gelernter and Kranzler[33]	1999	55	320	48	272	European	DSM-III-R	Yes
Sander *et al*.[54]	1999	110	620	66	392	European	ICD-10	Yes
Ovchinnikov *et al*.[50]	1999	33	84	31	152	European	ICD-10	Yes
Bau *et al*.[20]	2000	62	230	48	228	Other	DSM-III-R	Yes
Samochowiec *et al*.[52]	2000	104	584	61	384	Other	Not stated	Yes
Anghelescu *et al*.[18]	2001	101	486	38	196	European	DSM-IV	Yes
Limosin *et al*.[46]	2002	61	240	43	214	European	DIGS	Yes
Foley *et al*.[30]	2004	52	174	47	218	Other	Not stated	Yes
Karaoguz *et al*.[41]	2004	52	104	88	186	European	DSM-IV	Yes
Konishi *et al*.[42]	2004	148	260	283	502	European	DSM-IV	Yes
Freire *et al*.[31]	2006	88	228	199	466	European	DSM-III-R	Yes
Berggren *et al*.[21]	2006	159	714	296	1684	European	DSM-IV	Yes

n, number of T alleles.
N, number of C and T alleles.

1.35). There was no evidence of significant between-study heterogeneity ($\chi^2[7] = 2.46$, $P = 0.929$). When the analysis was re-run within a random-effects framework, however, there was no evidence for a significant association ($Z = 1.54$, $P = 0.120$, OR = 1.10, 95% CI 0.97–1.25).

The comparison of the pooled effect size for studies of participants of predominantly European ancestry (OR = 1.19) compared to the pooled effect size for studies of participants of predominantly East Asian ancestry (OR = 1.17) did not indicate a significant difference ($Z = 0.17$, $P = 0.865$).

Severity

When studies that explicitly recorded allele frequencies in cases with severe and mild alcoholism separately ($k = 10$) were analyzed with mild alcoholism cases as the comparison group, there was no evidence for a significant difference in *DRD2* Taq1A1 allele frequency and cases of severe and mild alcoholism ($Z = 1.15$, $P = 0.249$, OR = 1.13, 95% CI 0.92–1.40). There was evidence of significant between-study heterogeneity ($\chi^2[9] = 17.36$, $P = 0.043$).

Publication bias

A visual inspection of a funnel plot of 1/S.E. against effect size estimate suggested evidence of ascertainment bias, due to asymmetry in the plot in the predicted direction, with a relative lack of low accuracy (i.e., small) studies indicating no effect or a direction of effect opposite to that reported in the first published study. Both the Egger test ($t[38] = 3.31$,

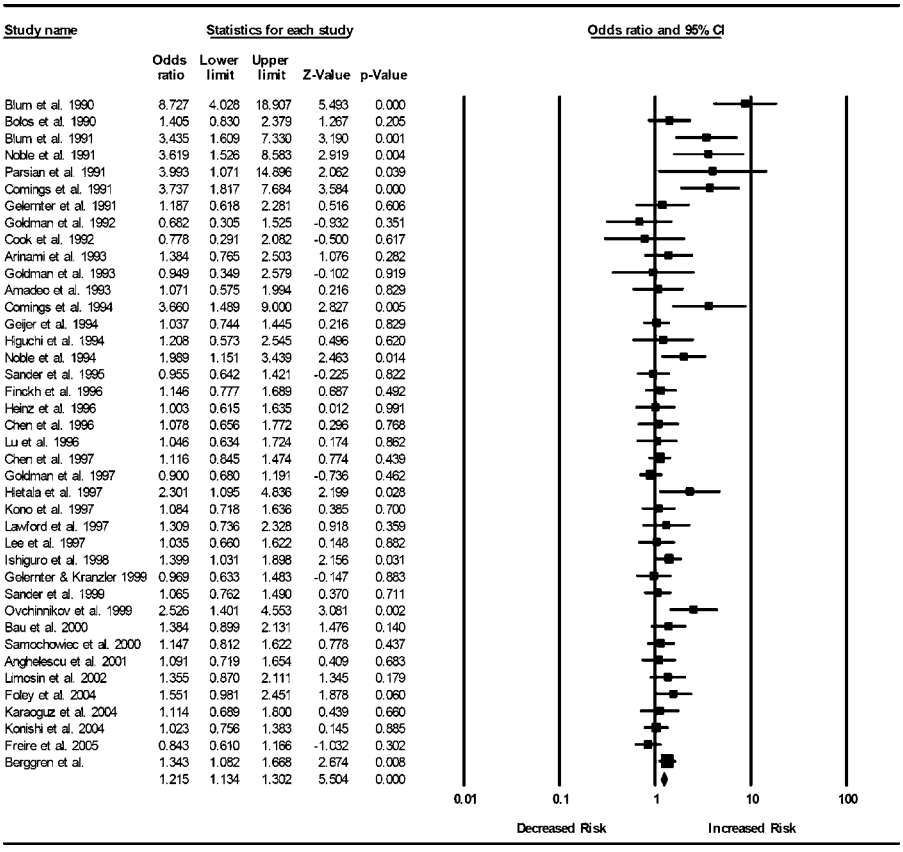

Figure 1 Meta-analysis of case–control studies of *DRD2* Taq1A allele frequency and alcoholism case status. Meta-analysis indicates significant association between *DRD2* Taq1A1 allele frequency and alcoholism case status ($P<0.001$). Bars represent individual study 95% CI, with a central block proportional to study size. The summary diamond bar represents the pooled effect size estimate and 95% CI.

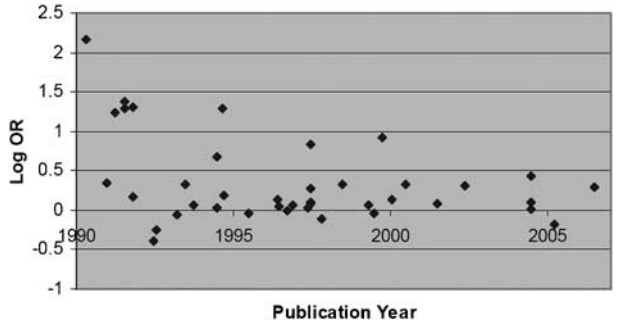

Figure 2 Association between year of publication and effect size estimate (log OR). Year of publication (corrected for month of publication) is negatively associated with individual study effect size ($P=0.032$), with this trend reflecting a decrease in individual study effect size over time.

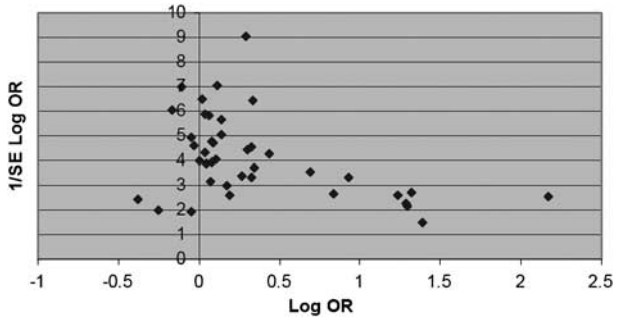

Figure 3 Funnel plot of accuracy (1/S.E.) and effect size estimate (log OR). Asymmetry in the plot in the predicted direction (i.e., a relative lack of low accuracy or small studies which indicate no effect or a direction of effect opposite to that reported in the first published study) suggests possible publication bias.

$P=0.002$) and the modified Macaskill test ($t[38]=2.90$, $P=0.006$) also indicated the presence of such bias. These data are presented graphically in Figure 3.

A pooled OR corrected for possible publication bias was calculated, using Duval and Tweedie's trim-and-fill method,[55] which is an extension of the funnel plot method. This removes studies with outlying effect size values until symmetry is achieved, and then replaces these along with imputed 'mirror' values in order to retain symmetry. This indicated a reduced pooled OR (OR = 1.17, 95% CI 1.09–1.25), although this was still statistically significant, which corresponds to 0.2% of phenotypic variance.

Discussion

The results of our meta-analysis agree with those recently published in finding a significant but small effect of the *DRD2* Taq1A polymorphism on risk of alcoholism. Combining all studies, we found a statistically significant OR of 1.21, indicating increased alcoholism in individuals possessing the A1 allele of the Taq1A polymorphism. This association remained significant when data from samples of European and East Asian ancestry were analyzed separately. We did not find evidence for association in high-severity alcoholism compared to low-severity alcoholism, although the direction of effect was consistent with this possibility. We found that removing the first published study significantly reduced the magnitude of the pooled effect size estimate, although the association remained significant. In all cases except for the analyses of samples of East Asian ancestry, there was evidence of significant between-study heterogeneity, although the observed associations were robust to the application of a random-effects framework.

In addition, we observed evidence for possible publication bias and for the strength of individual study effect size to be inversely related to year of publication. This observation is important as the literature on the association of the *DRD2* Taq1A polymorphism and alcoholism case status is one of the few in the psychiatric genetics literature where publication bias can be examined with reasonable power, given the large number of studies. It is noteworthy that studies of potential publication bias do not themselves appear to demonstrate evidence of publication bias.[56] Once we had corrected the pooled effect size estimate for possible publication bias we found that, if the association between the *DRD2* Taq1A polymorphism and alcoholism case status is real, the single nucleotide polymorphism (SNP) likely accounts for 0.2% of phenotypic variance. In addition, given a minor allele frequency of 0.3 and a prevalence of alcoholism of 5%, for an alpha level of 0.05, in excess of 1500 cases and a similar number of controls would be required in order to achieve 80% power to detect significant association.

It should be noted that publication bias is not the only explanation for an asymmetrical funnel plot, and the results of formal tests of bias based on the funnel plot, such as those used in this study, should therefore be interpreted with caution. Other possibilities include other selection biases (e.g., English language bias or multiple publication of small studies), true heterogeneity (e.g., differences in effect between populations of differing ancestry), data irregularities (e.g., poor methodological quality in small studies), artifacts (e.g., differences due to effect measure employed) or chance[8,57] There are also possible explanations for asymmetry which are specific to genetic studies, such as the violation of Hardy–Weinberg equilibrium.[58] However, we attempted to reduce the impact of these other sources of bias, for example, by not explicitly excluding non-English language journals and by attempting to identify cases of multiple publication.

The between-study heterogeneity observed in the majority of our analyses may be due to any potentially relevant differences between the study designs and methodologies, such as populations from which the study samples are drawn. In the case of genetic association studies, possible causes of between-study heterogeneity include, for example, the possibility that an association exists in one population but not another, that different studies did not use comparable measures of phenotype, or that allelic distributions deviated from Hardy–Weinberg equilibrium in some studies. However, we did not observe a difference in the strength of association between samples of European and East Asian ancestry, nor did we find evidence that samples of low-severity alcoholics differ from those of high-severity alcoholics in prevalence of the A1 allele, and the between-study heterogeneity remained significant in the majority of these analyses, suggesting that the observed heterogeneity is not due to these factors. We attempted to accommodate this heterogeneity in our analyses by implementing a random-effects framework in the presence of significant heterogeneity. It should be noted that fixed-effects and random-effects analyses address fundamentally different research questions. The former asks what the best estimate of the true effect size is in the population studied, whereas the latter asks what the range and distribution of effect sizes is in the distribution of populations studied.[57] Therefore, the calculation of the mean of the distribution of population effect sizes (random-effects model) provides quite different information to the calculation of the mean of the distribution of sample effect sizes (fixed-effects model). In other words, our analyses suggest that there may be populations in which the *DRD2* Taq1A polymorphism is associated with alcoholism and others where it is not, or the strength of this association is different (although we were unable to identify any such populations).

One of the reasons for the interest in the Taq1A variant is that it may alter the function of the nearby *DRD2* gene. The SNP has been reported to affect dopamine receptor DRD2 availability in post-mortem striatal samples[48,59] and there is also evidence from *in vivo* studies for an association between the A1 allele and lower mean relative glucose metabolic rate in dopaminergic regions in the human brain.[60] Positron emission tomography studies have indicated that this allele is also associated with low receptor density.[61] Evidence that the Taq1A variant alters an amino acid in the *ANKK1* protein kinase gene, near the *DRD2* locus,[15] does not rule out an effect on the *DRD2* gene: data from the HapMap project reveal that the variant is in linkage disequilibrium with other variants in the *DRD2* gene, but not with variants in the *ANKK1* gene. Thus, it is possible that additional functional variants in *DRD2* are contributing to the observed association with alcoholism.

Another, more speculative, possibility is that *ANKK1* may exert an effect on dopaminergic neurotransmission itself. The function of many proteins can be influenced or regulated by a process of phosphorylation of key amino-acid residues within the protein. This process can influence factors such as the affinity of the protein for ligands that bind to it, such as dopamine to its transporter in this instance. Phosphorylation can also influence other aspects of activity, and kinases catalyze these phosphorylation processes. ANKK1 might therefore be a kinase that acts on the transporter to influence its activity. If this were to be so, it might explain how a polymorphism in a gene that was not the transporter itself might relate to dopaminergic activity, so that the polymorphism in *ANKK1* may influence the activity or regulation of the kinase, thereby influencing the activity of the transporter (DJK Balfour, personal communication, 23 June 2006). Data do not currently exist to test this possibility directly.

The results of our meta-analysis support the association of the *DRD2* Taq1A polymorphism with alcoholism, suggesting that possession of the A1 allele confers a modest increase in risk. However, this conclusion is qualified by the possibility of publication bias or other bias in the literature and the observed between-study heterogeneity, which indicates that the observed association may differ in strength between populations, or may not exist at all in some populations. Other sources of heterogeneity than ancestry and severity of disease are therefore likely to exist, and further research on adequately large samples is required to explicitly identify these. Publication of nonsignificant results in the psychiatric genetics literature is important to protect against the existence of a biased corpus of data in the public domain.

Acknowledgments

Jonathan Flint is supported by the Wellcome Trust.

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