ORIGINAL INVESTIGATION

# A large-scale meta-analysis of the association between the *ANKK1/ DRD2* Taq1A polymorphism and alcohol dependence

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**Abstract** Alcohol dependence (AD) is a common neuropsychiatric disorder with high heritability. A number of studies have analyzed the association between the Taq1A polymorphism (located in the gene cluster *ANKK1/DRD2*) and AD. In the present study, we conducted a large-scale meta-analysis to confirm the association between the Taq1A polymorphism and the risk for AD in over 18,000 subjects included in 61 case–control studies that were published up to August 2012. Our meta-analysis demonstrated both allelic and genotypic association between the Taq1A polymorphism and AD susceptibility [allelic: $P(Z) = 1.1 \times 10^{-5}$, OR $= 1.19$; genotypic: $P(Z) = 3.2 \times 10^{-5}$, OR $= 1.24$]. The association remained significant after adjustment for publication bias using the trim and fill method. Sensitivity analysis showed that the effect size of the Taq1A polymorphism on AD risk was moderate and not influenced by any individual study. The pooled odds ratio from published studies decreased with the year of publication, but stabilized after the year 2001. Subgroup analysis indicated that publication bias could be influenced by racial ancestry. In summary, this large-scale meta-analysis confirmed the association between the Taq1A polymorphism and AD. Future studies are required to investigate the functional significance of the *ANKK1/DRD2* Taq1A polymorphism in AD.

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## Introduction

Dopamine plays a key role in alcohol dependence (AD) pathophysiology due to its involvement in reward behavior (Wise and Rompre 1989). Dysfunction of dopaminergic neurotransmission in the brain likely contributes to the pathophysiology of other neuropsychiatric disorders as well (Hummel and Unterwald 2002; Kienast and Heinz 2006). Family, twin, and adoption studies suggest that more than 50 % of the population variance in AD is due to genetic factors (Prescott and Kendler 1999). Variation in genes encoding proteins involved in dopaminergic neurotransmission has been found to contribute to the risk for developing alcohol dependence, most likely through altered reward processing and sensitivity to alcohol exposure (Bontempi et al. 2007; Fiorentini et al. 2002). The dopamine receptor D2, encoded by the gene *DRD2,* is a presynaptic as well as postsynaptic G protein-coupled receptor located on dopaminergic neurons. Both in vivo and in vitro experiments have demonstrated that *DRD2* is a susceptibility gene for AD (Kienast and Heinz 2006; Wise and Rompre 1989).

The Taq1A polymorphism [also known as rs1800497 (C/T)] is located in the gene cluster *ANKK1/DRD2* on chromosome 11q23.2. The minor A1 allele of the Taq1A

polymorphism (or the T allele of rs1800497) was found to be associated with a reduced number of dopamine binding sites in the brain (Pohjalainen et al. 1998). Altered D2 receptor expression due to the Taq1A polymorphism may confer vulnerability to substance (alcohol or drug) dependence and certain neuropsychiatric disorders. A number of studies have analyzed the association between this polymorphism and AD. Blum et al. (1990) investigated the association between the Taq1A polymorphism and AD in a sample of 35 alcoholics and 35 non-alcoholics and found an over eightfold increased risk of AD in subjects carrying the A1 allele (or the T allele) of the Taq1A polymorphism. This finding was supported by several follow-up studies (Amadeo et al. 1993; Berggren et al. 2006; Comings et al. 1991; Hietala et al. 1997; Ovchinnikov et al. 1999; Parsian et al. 1991). Nevertheless, conflicting results have also been reported (Anghelescu et al. 2001; Bolos et al. 1990; Cook et al. 1992; Gelernter et al. 1991; Goldman et al. 1992; Sander et al. 1999). The above studies were mainly conducted in European (and European American) populations. Additionally, the association of the Taq1A polymorphism and AD was examined in Asian and other non-European populations; however, the results were negative (Arinami et al. 1993; Chen et al. 1996; Lee et al. 1997; Lu et al. 1996; Matsushita et al. 2001; Shaikh et al. 2001). As of 2006, over 40 studies had examined the potential role of the Taq1A polymorphism in AD, yielding inconsistent results.

Subsequently, three relatively large meta-analyses examined the association between the Taq1A polymorphism and AD by combing data from studies published between 1990 and 2006. Munafo et al. (2007) analyzed the data from 40 published studies including 4,962 alcoholic and 5,253 comparison controls, and found that the A1 allele of the Taq1A polymorphism conferred a moderate risk for AD in both European (OR = 1.19) and East Asian (OR = 1.17) populations. Smith et al. (2008) included over 9,000 participants from 44 published studies, and found that subjects with the presence of the A1 allele of the Taq1A polymorphism (i.e., carrying genotype A1A1 or A1A2) had a significantly higher risk of AD than those with absence of the A1 allele (i.e., carrying genotype A2A2). Le Foll et al. (2009) re-analyzed the data from 5,395 patients and 4,304 controls recruited for 40 published studies and observed similar results. The three meta-analyses provided further evidence of a moderate effect of the Taq1A polymorphism on the risk for AD. They also demonstrated a significant between-study heterogeneity and publication bias, which could possibly be explained by different ethnic backgrounds or lacking of ethnic-matched controls.

Since 2006, 16 new studies evaluating the association of the Taq1A polymorphism with AD have been published. These studies included 7,756 new subjects (3,807 cases and 3,949 controls). However, no further meta-analyses have been conducted to investigate whether the association between the Taq1A polymorphism and AD remains significant. In the present study, we performed a large-scale meta-analysis to validate the association between the Taq1A polymorphism and AD by including data from studies that were published from 1990 until now (August 2012).

## Methods

### Literature search and inclusion of eligible studies

Studies that investigated the association of the TaqA1 polymorphism with AD were selected from the electronic database PubMed/MEDLINE (the US National Library of Medicine) and included in the present meta-analysis. They were published from 1990 to August, 2012. The search strategy was based on the following terms: "dopamine receptor D2", "DRD2", "alcohol", "alcoholics", "alcohol dependence", "association", and "associated". Abstracts of studies retrieved from the primary search were then browsed to see whether these studies analyzed the association between the Taq1A polymorphism and AD.

Once the published studies were selected, all reference papers (including reviewed papers) that were cited in these studies were further examined to identify additional articles that were not indexed by the PubMed/MEDLINE database. Duplications were discarded. Studies were considered to be eligible for the present meta-analysis if the following information was available: (1) a valid diagnostic tool (e.g., DSM-III-R, DSM-IV, and ICD-10) for AD; (2) genotyping methods [e.g., the restriction fragment length polymorphism (RFLP) technique or the TaqMan Method] were described; (3) genotyping data of the Taq1A polymorphism were provided to calculate odds ratios (ORs) and 95 % confidential intervals (95 % CIs); and (4) consent forms from study subjects and ethics approvals from local committees were obtained. If two studies contained overlapping samples, the study with a larger sample size was kept for this meta-analysis.

### Data extraction

For each eligible study, the following data were extracted using standard forms: (1) authors and publication year; (2) ancestry or race information; (3) diagnostic criteria for alcoholics and comparison controls; (4) number of patients and comparison controls; (5) genotyping data of the Taq1A polymorphism in case and control groups; (6) mean age and sex ratio in case and control groups; and (7) statement of hardy–Weinberg equilibrium (HWE) test. In our meta-analysis, ancestry or race was coded as European, Asian

or Others (American Indians, mixed population, or not stated).

## Statistical analysis

Allelic and genotypic data from eligible studies were summarized in two by two tables for meta-analyses. Odds ratios (ORs) and 95 % confidential intervals (95 % CIs) were calculated for individual studies using Pearson's Chi-squared test. Since previous studies found a significant effect of the A1 allele of the Taq1A polymorphism on risk for AD, we carried out both allelic [the A1 allele (or the T allele) vs. the A2 allele (or the C allele)] and genotypic (dominant model: A1A1 + A1A2 vs.A2A2) meta-analysis using data from all eligible studies. We also performed subgroup analyses stratified by ancestry (i.e., European or Asian) or diagnostic criteria (i.e., DSM-III-R, DSM-IV, or ICD-10). Heterogeneity between studies was estimated using two methods (Kienast and Heinz 2006): the Cochran's $Q$ test and the $I$-square ($I^2$) test. Cochran's $Q$ test was calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies, weighting the contribution of each study by its inverse variance; the $Q$ statistics followed a Chi-square distribution with $n - 1$ degrees of freedom, where $n$ was the number of studies. The $I^2$ statistic [$I^2 = 100\% \times (Q - df)/Q$] described the percentage of variation across studies due to heterogeneity rather than chance. It indicated an evidence of heterogeneity between studies if $I^2 \geq 40\%$.

When between-study heterogeneity existed, the random effect model (or the DerSimonian and Laird method) was applied (DerSimonian and Laird 1986); otherwise, the fixed model (the Mantel–Haenszel method) was applied (DerSimonian and Laird 1986). The significance of the pooled ORs was examined by the $Z$ test. To check whether the result was excessively influenced by any individual study, sensitivity analyses were conducted by repeating the meta-analysis with one study being omitted at a time. To assess publication bias, the graphic method (or the funnel plot) (Egger et al. 1997; Galbraith 1988) was used. If there was evidence of publication bias, the trim and fill method (Duval and Tweedie 2000) was applied to adjust the meta-analysis results by imputing data from presumed missing studies.

## Results

### Description of eligible studies

A total of 504 articles were retrieved. Sixty-seven studies met the inclusion criteria for this meta-analysis. Three studies (Cruz et al. 1995; Karaoguz et al. 2004; Yang et al. 2007) were excluded because genotyping data were not available. Three other studies (Comings 1998; Konishi et al. 2004a; Noble et al. 1991) were also excluded because of sample overlapping with previous studies. After filtration, 61 studies (refer to Supplementary Table S1) were considered eligible for the present meta-analysis. Among these 61 studies, 36 analyzed the association between the TaqA1 polymorphism and AD in the European population (Amadeo et al. 1993, 2000; Anghelescu et al. 2001; Bau et al. 2000; Berggren et al. 2006; Bolos et al. 1990; Comings et al. 1991, 1994; Cook et al. 1992; Finckh et al. 1996; Foley et al. 2004; Freire et al. 2006; Geijer et al. 1994; Gelernter and Kranzler 1999; Gelernter et al. 1991; Goldman et al. 1992; Gorwood et al. 2000a, b; Heinz et al. 1996; Hietala et al. 1997; Kasiakogia-Worlley et al. 2011; Konishi et al. 2004b; Kovanen et al. 2010; Kraschewski et al. 2009; Landgren et al. 2011; Lawford et al. 1997; Limosin et al. 2002; Ovchinnikov et al. 1999; Parsian et al. 1991; Pastorelli et al. 2001; Ponce et al. 2008; Samochowiec et al. 2000, 2008; Sander et al. 1995, 1999; Schellekens et al. 2012); 18 analyzed the association between the TaqA1 polymorphism and AD in the Asian population (Arinami et al. 1993; Bhaskar et al. 2010; Chen et al. 1996, 1997; Huang et al. 2007; Ishiguro et al. 1998; Joe et al. 2008; Kono et al. 1997; Lee et al. 1997; Lu et al. 1996, 2001, 2012; Matsushita et al. 2001; Namkoong et al. 2008; Prasad et al. 2010; Shaikh et al. 2001; Wang et al. 2007; Wu et al. 2008); and seven analyzed the association between the TaqA1 polymorphism and AD in American Indians (Goldman et al. 1993, 1997), a mix population (Blum et al. 1990, 1991; Sakai et al. 2007), or other populations that were not stated (Neiswanger et al. 1995; Noble et al. 1994).

A total of 9,590 alcoholic cases and 9,140 comparison controls recruited from the above 61 case–control studies were included in the present meta-analysis. The frequency of the minor A1 allele of the Taq1A polymorphism varied substantially in different populations. In European control subjects, the frequency of the A1 allele was about 19 % (6–44 %), which was significantly lower than that in the Asian control subjects (about 38 %, 22–47 %; $P < 0.001$, data not show). Moreover, both European and Asian alcoholic subjects showed a higher frequency of the A1 allele (European 22 %, 12–45 %; Asian 42 %, 27–51 %) when compared with their respective ethnic control subjects. Additionally, in two American Indian studies, the A1 allele was the major allele of the Taq1A polymorphism (cases 58–66 %; controls 61–67 %).

### Meta-analysis results

The allelic and genotypic meta-analysis results are summarized in Table 1. Among the 61 studies included in this

**Table 1** Results of meta-analyses including all and subgroup studies stratified by ancestry and diagnosis criteria

| | $n^a$ | $Z^b$ | $P(Z)^c$ | OR (95 %CI)$^d$ | $P(Q)^e$ | $I^2$ (95 %CI)$^f$ | $P(B)^g$ |
|---|---|---|---|---|---|---|---|
| **Allelic association analysis (A1 vs. A2)** | | | | | | | |
| All studies$^h$ | 56 | 4.41 | $1.1 \times 10^{-5}$ | 1.19 (1.10–1.29) | <0.0001 | 46.6 (26.8–61.0) | 0.010 |
| Allelic studies with diagnosis 1$^i$ | 51 | 4.34 | $1.4 \times 10^{-5}$ | 1.21 (1.11–1.31) | <0.0001 | 47.2 (26.7–62.0) | 0.054 |
| Allelic studies with diagnosis 2$^j$ | 47 | 5.45 | $<6.0 \times 10^{-7}$ | 1.18 (1.11–1.25) | <0.0001 | 49.2 (28.8–63.8) | 0.069 |
| European studies | 34 | 2.93 | 0.003 | 1.16 (1.06–1.29) | 0.002 | 45.7 (18.7–63.8) | 0.183 |
| Allelic studies with diagnosis 1$^i$ | 30 | 2.71 | 0.007 | 1.17 (1.05–1.32) | 0.003 | 46.4 (17.7–65.1) | 0.681 |
| Allelic studies with diagnosis 2$^j$ | 27 | 2.19 | 0.029 | 1.15 (1.02–1.31) | 0.003 | 47.8 (18.2–66.7) | 0.856 |
| Asian studies | 15 | 3.32 | $9.0 \times 10^{-4}$ | 1.17 (1.07–1.28) | 0.813 | 0.0 (0.0–30.0) | 0.794 |
| Allelic studies with diagnosis 1$^i$ | 14 | 3.46 | $5.0 \times 10^{-4}$ | 1.18 (1.07–1.30) | 0.824 | 0.0 (0.0–29.5) | 0.633 |
| Allelic studies with diagnosis 2$^j$ | 13 | 3.52 | $4.0 \times 10^{-4}$ | 1.19 (1.08–1.31) | 0.797 | 0.0 (0.0–33.6) | 0.427 |
| **Genotypic association analysis (A1A1 + A1A2 vs. A2A2):** | | | | | | | |
| All studies$^h$ | 56 | 4.16 | $3.2 \times 10^{-5}$ | 1.24 (1.13–1.38) | 0.0001 | 45.9 (25.9–60.6) | 0.014 |
| Genotypic studies with diagnosis 1$^i$ | 52 | 4.02 | $5.8 \times 10^{-5}$ | 1.25 (1.13–1.46) | <0.0001 | 49.1 (29.7–63.1) | 0.016 |
| Genotypic studies with diagnosis 2$^j$ | 48 | 3.66 | $3.0 \times 10^{-4}$ | 1.24 (1.10–1.39) | <0.0001 | 50.6 (31.0–64.6) | 0.034 |
| European studies | 34 | 3.42 | $6.0 \times 10^{-4}$ | 1.23 (1.09–1.38) | 0.0187 | 36.6 (3.80–58.2) | 0.316 |
| Genotypic studies with diagnosis 1$^i$ | 31 | 3.13 | 0.002 | 1.23 (1.08–1.40) | 0.009 | 41.4 (10.0–61.9) | 0.375 |
| Genotypic studies with diagnosis 2$^j$ | 28 | 2.69 | 0.007 | 1.21 (1.05–1.39) | 0.011 | 42.3 (9.5–63.2) | 0.677 |
| Asian studies | 16 | 1.73 | 0.083 | 1.13 (0.98–1.29) | 0.186 | 23.6 (0.0–58.0) | 0.191 |
| Genotypic studies with diagnosis 1$^i$ | 15 | 1.83 | 0.067 | 1.14 (0.99–1.31) | 0.156 | 27.2 (0.0–60.8) | 0.175 |
| Genotypic studies with diagnosis 2$^j$ | 14 | 1.93 | 0.538 | 1.15 (0.99–1.32) | 0.132 | 30.5 (0.0–63.3) | 0.129 |

[a] Number of studies included

[b] Statistics of $Z$ test

[c] Significance of pooled effect size

[d] Odds ratio with 95 % confidential interval

[e] Significance of heterogeneity test between studies using Cochran's Q test

[f] $I^2$ statistic with 95 % confidential interval (test of heterogeneity between studies)

[g] Significance of publication bias test

[h] Studies including all eligible European, Asian and other studies

[i] Subjects were screened using stated diagnostic criteria

[j] Subjects were screened using the standard DSM-III-R, DSM-IV, or ICD10 diagnostic tool

meta-analysis, five studies only had allelic data (A1 vs. A2) and five studies only had genotypic data (A11 + A12 vs. A12). Other 51 studies had both allelic and genotypic data. Thus, 56 studies were included in allelic meta-analysis. The OR of individual studies and the pooled OR are shown in a forest plot (Fig. 1). There was strong evidence of heterogeneity between studies [$P(Q) < 0.0001$, $I^2 = 46.6$ %]. The random effects model was, therefore, applied in the data analysis. The A1 allele showed a significant association with AD [$Z = 4.41$, $P(Z) = 1.1 \times 10^{-5}$, OR = 1.19] (Table 1). Similarly, 56 studies were included in genotypic meta-analysis. The OR of individual studies and the pooled OR are shown in a forest plot (Fig. 2). There was also strong evidence of heterogeneity between studies [$P(Q) < 0.0001$, $I^2 = 45.9$ %]. Under the random effects model, subjects carrying the A1 allele (or with genotype A1A1 or A1A2) showed a higher risk for AD compared to subjects without

the allele A1 (i.e., with genotype A2A2) [$Z = 4.16$, $P(Z) = 3.2 \times 10^{-5}$, OR = 1.24] (Table 1).

Subgroup analyses stratified by race and diagnostic criteria

When the meta-analysis was limited to subgroups according to ethnic (ancestral) background (i.e., European or Asian), only the Asian population studies did not show between-study heterogeneity [Allelic: $P(Q) = 0.813$, $I^2 = 0.0$ %; Genotypic: $P(Q) = 0.186$, $I^2 = 23.6$ %]. Allelic meta-analysis indicated that the association between the Taq1A polymorphism and AD was positive in both European and Asian subgroups [European: $Z = 2.93$, $P(Z) = 0.003$, OR = 1.16; Asian: $Z = 3.32$, $P(Z) = 9.0 \times 10^{-4}$, OR = 1.17). Similar results were obtained from genotypic meta-analysis in the European subgroup but not in the Asian
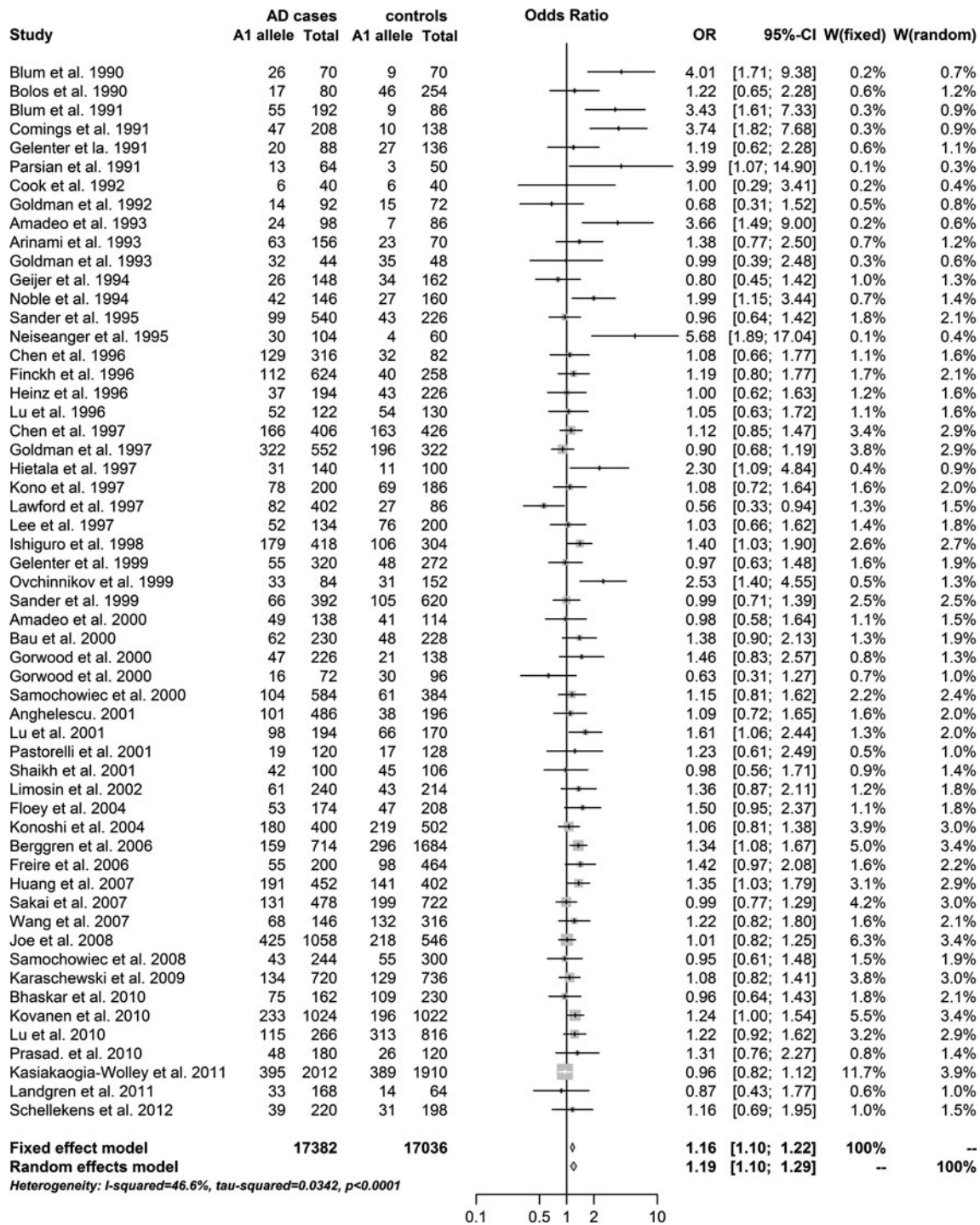
| Study | AD cases A1 allele | AD cases Total | controls A1 allele | controls Total | Odds Ratio | OR | 95%-CI | W(fixed) | W(random) |
|---|---|---|---|---|---|---|---|---|---|
| Blum et al. 1990 | 26 | 70 | 9 | 70 | | 4.01 | [1.71; 9.38] | 0.2% | 0.7% |
| Bolos et al. 1990 | 17 | 80 | 46 | 254 | | 1.22 | [0.65; 2.28] | 0.6% | 1.2% |
| Blum et al. 1991 | 55 | 192 | 9 | 86 | | 3.43 | [1.61; 7.33] | 0.3% | 0.9% |
| Comings et al. 1991 | 47 | 208 | 10 | 138 | | 3.74 | [1.82; 7.68] | 0.3% | 0.9% |
| Gelenter et la. 1991 | 20 | 88 | 27 | 136 | | 1.19 | [0.62; 2.28] | 0.6% | 1.1% |
| Parsian et al. 1991 | 13 | 64 | 3 | 50 | | 3.99 | [1.07; 14.90] | 0.1% | 0.3% |
| Cook et al. 1992 | 6 | 40 | 6 | 40 | | 1.00 | [0.29; 3.41] | 0.2% | 0.4% |
| Goldman et al. 1992 | 14 | 92 | 15 | 72 | | 0.68 | [0.31; 1.52] | 0.5% | 0.8% |
| Amadeo et al. 1993 | 24 | 98 | 7 | 86 | | 3.66 | [1.49; 9.00] | 0.2% | 0.6% |
| Arinami et al. 1993 | 63 | 156 | 23 | 70 | | 1.38 | [0.77; 2.50] | 0.7% | 1.2% |
| Goldman et al. 1993 | 32 | 44 | 35 | 48 | | 0.99 | [0.39; 2.48] | 0.3% | 0.6% |
| Geijer et al. 1994 | 26 | 148 | 34 | 162 | | 0.80 | [0.45; 1.42] | 1.0% | 1.3% |
| Noble et al. 1994 | 42 | 146 | 27 | 160 | | 1.99 | [1.15; 3.44] | 0.7% | 1.4% |
| Sander et al. 1995 | 99 | 540 | 43 | 226 | | 0.96 | [0.64; 1.42] | 1.8% | 2.1% |
| Neiseanger et al. 1995 | 30 | 104 | 4 | 60 | | 5.68 | [1.89; 17.04] | 0.1% | 0.4% |
| Chen et al. 1996 | 129 | 316 | 32 | 82 | | 1.08 | [0.66; 1.77] | 1.1% | 1.6% |
| Finckh et al. 1996 | 112 | 624 | 40 | 258 | | 1.19 | [0.80; 1.77] | 1.7% | 2.1% |
| Heinz et al. 1996 | 37 | 194 | 43 | 226 | | 1.00 | [0.62; 1.63] | 1.2% | 1.6% |
| Lu et al. 1996 | 52 | 122 | 54 | 130 | | 1.05 | [0.63; 1.72] | 1.1% | 1.6% |
| Chen et al. 1997 | 166 | 406 | 163 | 426 | | 1.12 | [0.85; 1.47] | 3.4% | 2.9% |
| Goldman et al. 1997 | 322 | 552 | 196 | 322 | | 0.90 | [0.68; 1.19] | 3.8% | 2.9% |
| Hietala et al. 1997 | 31 | 140 | 11 | 100 | | 2.30 | [1.09; 4.84] | 0.4% | 0.9% |
| Kono et al. 1997 | 78 | 200 | 69 | 186 | | 1.08 | [0.72; 1.64] | 1.6% | 2.0% |
| Lawford et al. 1997 | 82 | 402 | 27 | 86 | | 0.56 | [0.33; 0.94] | 1.3% | 1.5% |
| Lee et al. 1997 | 52 | 134 | 76 | 200 | | 1.03 | [0.66; 1.62] | 1.4% | 1.8% |
| Ishiguro et al. 1998 | 179 | 418 | 106 | 304 | | 1.40 | [1.03; 1.90] | 2.6% | 2.7% |
| Gelenter et al. 1999 | 55 | 320 | 48 | 272 | | 0.97 | [0.63; 1.48] | 1.6% | 1.9% |
| Ovchinnikov et al. 1999 | 33 | 84 | 31 | 152 | | 2.53 | [1.40; 4.55] | 0.5% | 1.3% |
| Sander et al. 1999 | 66 | 392 | 105 | 620 | | 0.99 | [0.71; 1.39] | 2.5% | 2.5% |
| Amadeo et al. 2000 | 49 | 138 | 41 | 114 | | 0.98 | [0.58; 1.64] | 1.1% | 1.5% |
| Bau et al. 2000 | 62 | 230 | 48 | 228 | | 1.38 | [0.90; 2.13] | 1.3% | 1.9% |
| Gorwood et al. 2000 | 47 | 226 | 21 | 138 | | 1.46 | [0.83; 2.57] | 0.8% | 1.3% |
| Gorwood et al. 2000 | 16 | 72 | 30 | 96 | | 0.63 | [0.31; 1.27] | 0.7% | 1.0% |
| Samochowiec et al. 2000 | 104 | 584 | 61 | 384 | | 1.15 | [0.81; 1.62] | 2.2% | 2.4% |
| Anghelescu. 2001 | 101 | 486 | 38 | 196 | | 1.09 | [0.72; 1.65] | 1.6% | 2.0% |
| Lu et al. 2001 | 98 | 194 | 66 | 170 | | 1.61 | [1.06; 2.44] | 1.3% | 2.0% |
| Pastorelli et al. 2001 | 19 | 120 | 17 | 128 | | 1.23 | [0.61; 2.49] | 0.5% | 1.0% |
| Shaikh et al. 2001 | 42 | 100 | 45 | 106 | | 0.98 | [0.56; 1.71] | 0.9% | 1.4% |
| Limosin et al. 2002 | 61 | 240 | 43 | 214 | | 1.36 | [0.87; 2.11] | 1.2% | 1.8% |
| Floey et al. 2004 | 53 | 174 | 47 | 208 | | 1.50 | [0.95; 2.37] | 1.1% | 1.8% |
| Konoshi et al. 2004 | 180 | 400 | 219 | 502 | | 1.06 | [0.81; 1.38] | 3.9% | 3.0% |
| Berggren et al. 2006 | 159 | 714 | 296 | 1684 | | 1.34 | [1.08; 1.67] | 5.0% | 3.4% |
| Freire et al. 2006 | 55 | 200 | 98 | 464 | | 1.42 | [0.97; 2.08] | 1.6% | 2.2% |
| Huang et al. 2007 | 191 | 452 | 141 | 402 | | 1.35 | [1.03; 1.79] | 3.1% | 2.9% |
| Sakai et al. 2007 | 131 | 478 | 199 | 722 | | 0.99 | [0.77; 1.29] | 4.2% | 3.0% |
| Wang et al. 2007 | 68 | 146 | 132 | 316 | | 1.22 | [0.82; 1.80] | 1.6% | 2.1% |
| Joe et al. 2008 | 425 | 1058 | 218 | 546 | | 1.01 | [0.82; 1.25] | 6.3% | 3.4% |
| Samochowiec et al. 2008 | 43 | 244 | 55 | 300 | | 0.95 | [0.61; 1.48] | 1.5% | 1.9% |
| Karaschewski et al. 2009 | 134 | 720 | 129 | 736 | | 1.08 | [0.82; 1.41] | 3.8% | 3.0% |
| Bhaskar et al. 2010 | 75 | 162 | 109 | 230 | | 0.96 | [0.64; 1.43] | 1.8% | 2.1% |
| Kovanen et al. 2010 | 233 | 1024 | 196 | 1022 | | 1.24 | [1.00; 1.54] | 5.5% | 3.4% |
| Lu et al. 2010 | 115 | 266 | 313 | 816 | | 1.22 | [0.92; 1.62] | 3.2% | 2.9% |
| Prasad. et al. 2010 | 48 | 180 | 26 | 120 | | 1.31 | [0.76; 2.27] | 0.8% | 1.4% |
| Kasiakaogia-Wolley et al. 2011 | 395 | 2012 | 389 | 1910 | | 0.96 | [0.82; 1.12] | 11.7% | 3.9% |
| Landgren et al. 2011 | 33 | 168 | 14 | 64 | | 0.87 | [0.43; 1.77] | 0.6% | 1.0% |
| Schellekens et al. 2012 | 39 | 220 | 31 | 198 | | 1.16 | [0.69; 1.95] | 1.0% | 1.5% |
| **Fixed effect model** | | 17382 | | 17036 | | 1.16 | [1.10; 1.22] | 100% | -- |
| **Random effects model** | | | | | | 1.19 | [1.10; 1.29] | -- | 100% |

Heterogeneity: I-squared=46.6%, tau-squared=0.0342, p<0.0001

0.1   0.5 1 2   10

**Fig. 1** Forest plot of allelic meta-analysis results across all studies The counts of the A1 allele and total alleles of the Taq1A polymorphism in alcohol dependence (AD) and control groups from eligible studies were presented by *rows*. The *bars with squares* in the middle represent 95 % confidence intervals (CIs) and odds ratios (ORs). The *central vertical solid line* indicates the ORs (equal to 1) for the null hypothesis. The estimated pooled effect size (represented by the diamond symbol) underneath the plot was calculated under the fixed effects model and the random effects model

subgroup [European: $Z = 3.42$, $P(Z) = 6 \times 10^{-4}$; Asian: $Z = 1.73$, $P(Z) = 0.083$] (Table 1). When the meta-analysis was categorized in terms of clearly stated diagnostic criteria (diagnosis 1) or the standard DSM-III-R,

DSM-IV, or ICD-10 criteria (diagnosis 2), between-study heterogeneity still existed in European studies [Allelic (diagnosis 1): $P(Q) = 0.003$ and $I^2 = 46.4$ %; Allelic (diagnosis 2): $P(Q) = 0.003$ and $I^2 = 47.8$ %; Genotypic
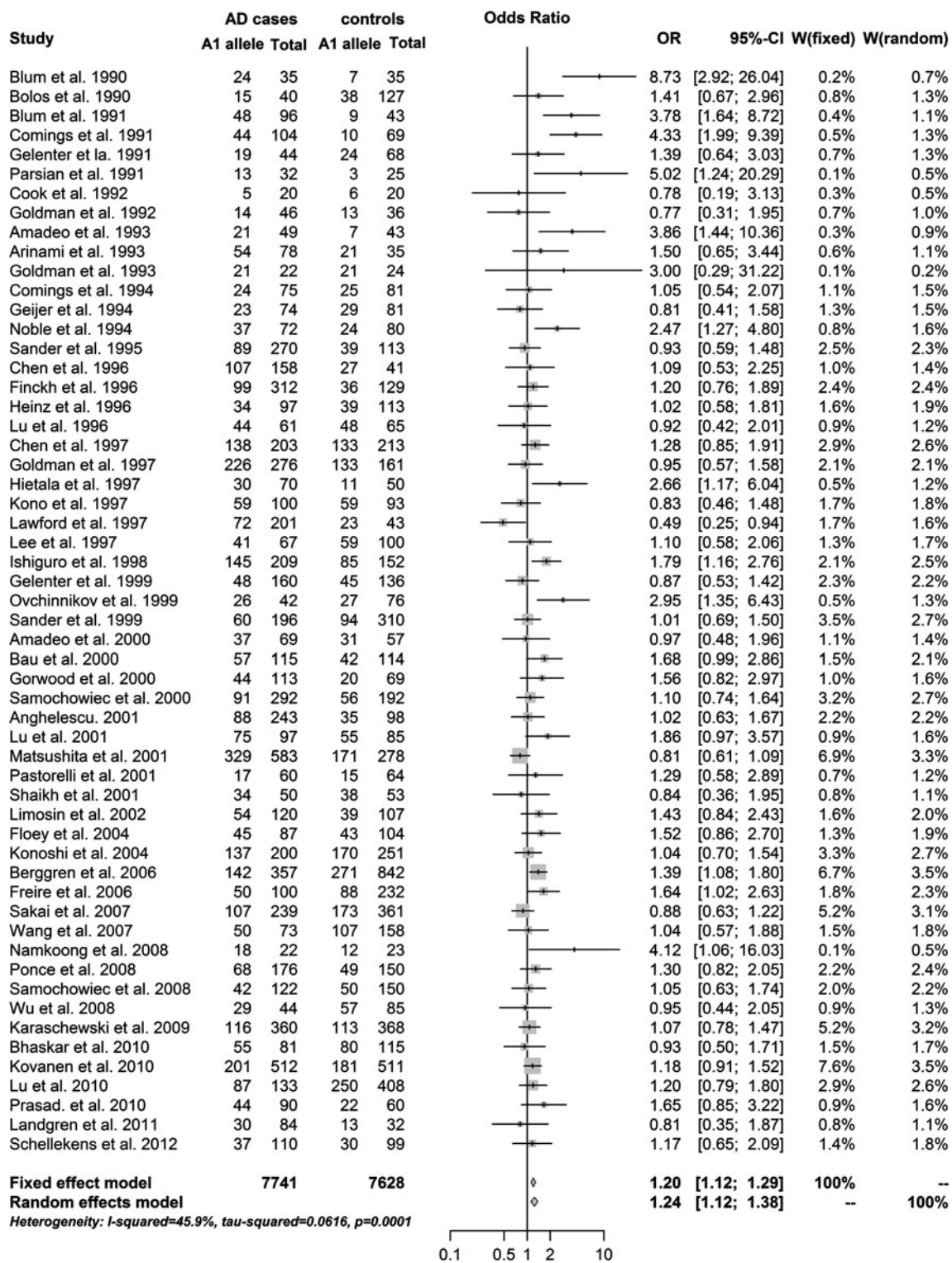
| Study | AD cases | | controls | | Odds Ratio | OR | 95%-CI | W(fixed) | W(random) |
|---|---|---|---|---|---|---|---|---|---|
| | A1 allele | Total | A1 allele | Total | | | | | |
| Blum et al. 1990 | 24 | 35 | 7 | 35 | | 8.73 | [2.92; 26.04] | 0.2% | 0.7% |
| Bolos et al. 1990 | 15 | 40 | 38 | 127 | | 1.41 | [0.67; 2.96] | 0.8% | 1.3% |
| Blum et al. 1991 | 48 | 96 | 9 | 43 | | 3.78 | [1.64; 8.72] | 0.4% | 1.1% |
| Comings et al. 1991 | 44 | 104 | 10 | 69 | | 4.33 | [1.99; 9.39] | 0.5% | 1.3% |
| Gelenter et la. 1991 | 19 | 44 | 24 | 68 | | 1.39 | [0.64; 3.03] | 0.7% | 1.3% |
| Parsian et al. 1991 | 13 | 32 | 3 | 25 | | 5.02 | [1.24; 20.29] | 0.1% | 0.5% |
| Cook et al. 1992 | 5 | 20 | 6 | 20 | | 0.78 | [0.19; 3.13] | 0.3% | 0.5% |
| Goldman et al. 1992 | 14 | 46 | 13 | 36 | | 0.77 | [0.31; 1.95] | 0.7% | 1.0% |
| Amadeo et al. 1993 | 21 | 49 | 7 | 43 | | 3.86 | [1.44; 10.36] | 0.3% | 0.9% |
| Arinami et al. 1993 | 54 | 78 | 21 | 35 | | 1.50 | [0.65; 3.44] | 0.6% | 1.1% |
| Goldman et al. 1993 | 21 | 22 | 21 | 24 | | 3.00 | [0.29; 31.22] | 0.1% | 0.2% |
| Comings et al. 1994 | 24 | 75 | 25 | 81 | | 1.05 | [0.54; 2.07] | 1.1% | 1.5% |
| Geijer et al. 1994 | 23 | 74 | 29 | 81 | | 0.81 | [0.41; 1.58] | 1.3% | 1.5% |
| Noble et al. 1994 | 37 | 72 | 24 | 80 | | 2.47 | [1.27; 4.80] | 0.8% | 1.6% |
| Sander et al. 1995 | 89 | 270 | 39 | 113 | | 0.93 | [0.59; 1.48] | 2.5% | 2.3% |
| Chen et al. 1996 | 107 | 158 | 27 | 41 | | 1.09 | [0.53; 2.25] | 1.0% | 1.4% |
| Finckh et al. 1996 | 99 | 312 | 36 | 129 | | 1.20 | [0.76; 1.89] | 2.4% | 2.4% |
| Heinz et al. 1996 | 34 | 97 | 39 | 113 | | 1.02 | [0.58; 1.81] | 1.6% | 1.9% |
| Lu et al. 1996 | 44 | 61 | 48 | 65 | | 0.92 | [0.42; 2.01] | 0.9% | 1.2% |
| Chen et al. 1997 | 138 | 203 | 133 | 213 | | 1.28 | [0.85; 1.91] | 2.9% | 2.6% |
| Goldman et al. 1997 | 226 | 276 | 133 | 161 | | 0.95 | [0.57; 1.58] | 2.1% | 2.1% |
| Hietala et al. 1997 | 30 | 70 | 11 | 50 | | 2.66 | [1.17; 6.04] | 0.5% | 1.2% |
| Kono et al. 1997 | 59 | 100 | 59 | 93 | | 0.83 | [0.46; 1.48] | 1.7% | 1.8% |
| Lawford et al. 1997 | 72 | 201 | 23 | 43 | | 0.49 | [0.25; 0.94] | 1.7% | 1.6% |
| Lee et al. 1997 | 41 | 67 | 59 | 100 | | 1.10 | [0.58; 2.06] | 1.3% | 1.7% |
| Ishiguro et al. 1998 | 145 | 209 | 85 | 152 | | 1.79 | [1.16; 2.76] | 2.1% | 2.5% |
| Gelenter et al. 1999 | 48 | 160 | 45 | 136 | | 0.87 | [0.53; 1.42] | 2.3% | 2.2% |
| Ovchinnikov et al. 1999 | 26 | 42 | 27 | 76 | | 2.95 | [1.35; 6.43] | 0.5% | 1.3% |
| Sander et al. 1999 | 60 | 196 | 94 | 310 | | 1.01 | [0.69; 1.50] | 3.5% | 2.7% |
| Amadeo et al. 2000 | 37 | 69 | 31 | 57 | | 0.97 | [0.48; 1.96] | 1.1% | 1.4% |
| Bau et al. 2000 | 57 | 115 | 42 | 114 | | 1.68 | [0.99; 2.86] | 1.5% | 2.1% |
| Gorwood et al. 2000 | 44 | 113 | 20 | 69 | | 1.56 | [0.82; 2.97] | 1.0% | 1.6% |
| Samochowiec et al. 2000 | 91 | 292 | 56 | 192 | | 1.10 | [0.74; 1.64] | 3.2% | 2.7% |
| Anghelescu. 2001 | 88 | 243 | 35 | 98 | | 1.02 | [0.63; 1.67] | 2.2% | 2.2% |
| Lu et al. 2001 | 75 | 97 | 55 | 85 | | 1.86 | [0.97; 3.57] | 0.9% | 1.6% |
| Matsushita et al. 2001 | 329 | 583 | 171 | 278 | | 0.81 | [0.61; 1.09] | 6.9% | 3.3% |
| Pastorelli et al. 2001 | 17 | 60 | 15 | 64 | | 1.29 | [0.58; 2.89] | 0.7% | 1.2% |
| Shaikh et al. 2001 | 34 | 50 | 38 | 53 | | 0.84 | [0.36; 1.95] | 0.8% | 1.1% |
| Limosin et al. 2002 | 54 | 120 | 39 | 107 | | 1.43 | [0.84; 2.43] | 1.6% | 2.0% |
| Floey et al. 2004 | 45 | 87 | 43 | 104 | | 1.52 | [0.86; 2.70] | 1.3% | 1.9% |
| Konoshi et al. 2004 | 137 | 200 | 170 | 251 | | 1.04 | [0.70; 1.54] | 3.3% | 2.7% |
| Berggren et al. 2006 | 142 | 357 | 271 | 842 | | 1.39 | [1.08; 1.80] | 6.7% | 3.5% |
| Freire et al. 2006 | 50 | 100 | 88 | 232 | | 1.64 | [1.02; 2.63] | 1.8% | 2.3% |
| Sakai et al. 2007 | 107 | 239 | 173 | 361 | | 0.88 | [0.63; 1.22] | 5.2% | 3.1% |
| Wang et al. 2007 | 50 | 73 | 107 | 158 | | 1.04 | [0.57; 1.88] | 1.5% | 1.8% |
| Namkoong et al. 2008 | 18 | 22 | 12 | 23 | | 4.12 | [1.06; 16.03] | 0.1% | 0.5% |
| Ponce et al. 2008 | 68 | 176 | 49 | 150 | | 1.30 | [0.82; 2.05] | 2.2% | 2.4% |
| Samochowiec et al. 2008 | 42 | 122 | 50 | 150 | | 1.05 | [0.63; 1.74] | 2.0% | 2.2% |
| Wu et al. 2008 | 29 | 44 | 57 | 85 | | 0.95 | [0.44; 2.05] | 0.9% | 1.3% |
| Karaschewski et al. 2009 | 116 | 360 | 113 | 368 | | 1.07 | [0.78; 1.47] | 5.2% | 3.2% |
| Bhaskar et al. 2010 | 55 | 81 | 80 | 115 | | 0.93 | [0.50; 1.71] | 1.5% | 1.7% |
| Kovanen et al. 2010 | 201 | 512 | 181 | 511 | | 1.18 | [0.91; 1.52] | 7.6% | 3.5% |
| Lu et al. 2010 | 87 | 133 | 250 | 408 | | 1.20 | [0.79; 1.80] | 2.9% | 2.6% |
| Prasad. et al. 2010 | 44 | 90 | 22 | 60 | | 1.65 | [0.85; 3.22] | 0.9% | 1.6% |
| Landgren et al. 2011 | 30 | 84 | 13 | 32 | | 0.81 | [0.35; 1.87] | 0.8% | 1.1% |
| Schellekens et al. 2012 | 37 | 110 | 30 | 99 | | 1.17 | [0.65; 2.09] | 1.4% | 1.8% |
| **Fixed effect model** | | 7741 | | 7628 | | 1.20 | [1.12; 1.29] | 100% | -- |
| **Random effects model** | | | | | | 1.24 | [1.12; 1.38] | -- | 100% |
| Heterogeneity: I-squared=45.9%, tau-squared=0.0616, p=0.0001 | | | | | | | | | |

0.1   0.5 1 2    10

**Fig. 2** Forest plot of genotypic meta-analysis results across all studies The counts of genotypes A1A1 + A1A2 and all genotypes of the Taq1A polymorphism in alcohol dependence (AD) and control groups from eligible studies were presented by *rows*. The *bars with squares* in the middle represent 95 % confidence intervals (CIs) and odds ratios (ORs). The *central vertical solid line* represents ORs (equal to 1) for the null hypothesis. The estimated pooled effect size (represented by the *diamond symbol*) underneath the plot was calculated under the fixed effects model and the random effects model

**Fig. 3** Plots of pooled effect size with the publication year The pooled effect size [Ln(OR)] of theTaq1A polymorphism for risk of alcohol dependence was plotted against the publication year in allelic (Fig. 3a) and genotypic (Fig. 3b) meta-analyses. The *X* axis represents the year when all studies were included to calculate the pooled effect size. *Each vertical line with a diamond symbol* represents the 95 % confidential interval and the pooled effect size
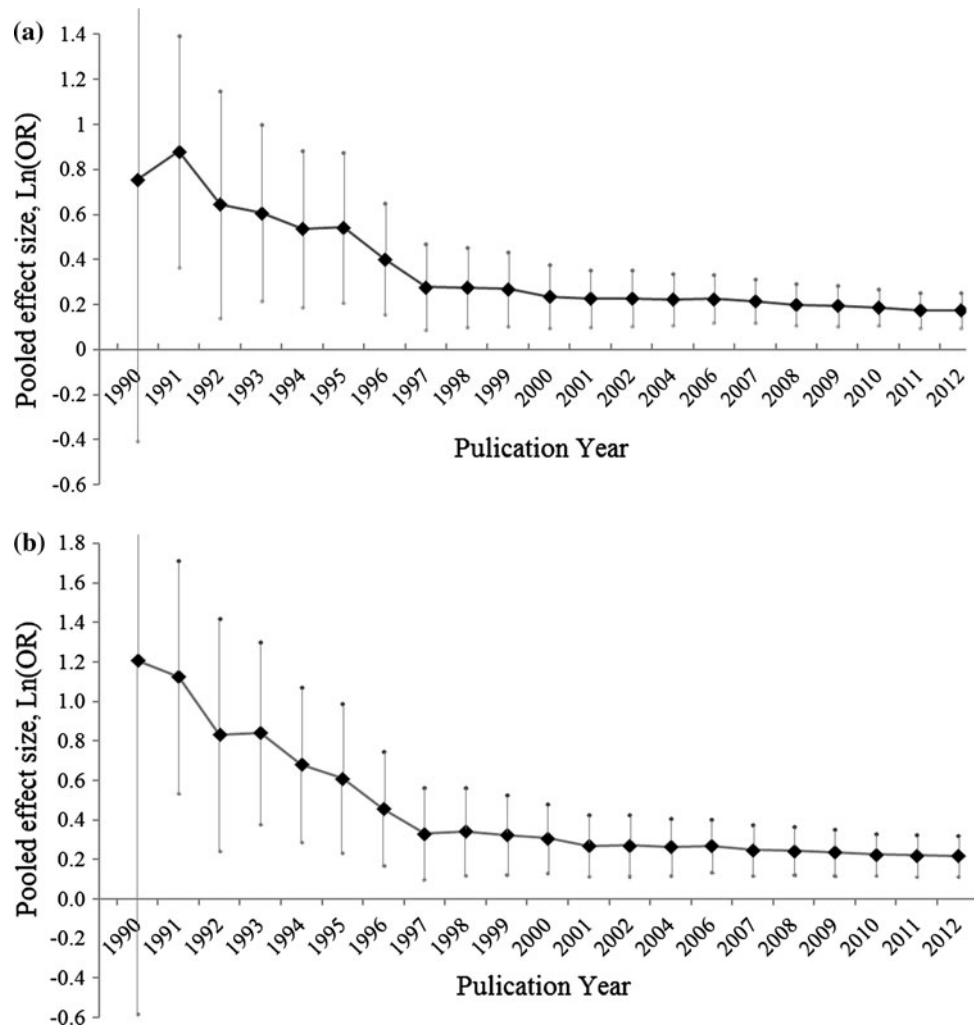


(diagnosis 1): $P(Q) = 0.009$ and $I^2 = 41.4$ %; Genotypic (diagnosis 2): $P(Q) = 0.011$ and $I^2 = 42.3$ %] but not in Asian studies [$P(Q) > 0.05$ for both allelic and genotypic studies with either diagnosis 1 or diagnosis 2] (Table 1). Nevertheless, both allelic and genotypic meta-analysis results remained significant when studies were stratified by either diagnosis 1 or diagnosis 2 [$P(Z) < 0.01$], except genotypic analysis in Asian studies [diagnosis 1: $Z = 1.83$, $P(Z) = 0.067$; diagnosis 2: $Z = 1.93$, $P(Z) = 0.538$] (Table 1).

Sensitivity and accumulative analysis

To examine whether the association between the TaqA1 polymorphism and AD was influenced by individual studies, both allelic and genotypic meta-analyses were repeated under the random effects model after omitting one individual study at a time. Sensitivity analysis showed that our meta-analysis was stable and the results remained unchanged (Supplementary Figure S1).

To test whether the pooled effect of the Taq1A polymorphism on the risk for AD varied by the publication year, pooled ORs and 95 % CIs from all studies were calculated along with the year of publication. As shown in Fig. 3, the pooled OR was high in early studies published in 1990 and was then decreased substantially in later years. The OR became stable after the year 1997. A similar cumulative curve was observed in genotypic meta-analyses.

Identification of publication bias

Both graphic and statistical methods were applied to analyze the publication bias that potentially existed in the global as well as the subgroup analyses (Supplementary Figure S2). In the funnel plot, the effect size (OR) of each study was plotted against standard errors of the effect size (OR). If publication bias exists, we would expect the funnel plot to be asymmetrical. The Egger test (Egger et al. 1997) was also used to quantitatively estimate the symmetry of the funnel plot by linear regression analysis. As shown in Table 1, publication

bias existed in both allelic and genotypic meta-analyses when all studies were included [allelic: $P(B) = 0.010$; genotypic: $P(B) = 0.014$]. However, when the analysis was conducted in studies grouped by racial ancestry, the publication bias disappeared in both European [allelic: $P(B) = 0.183$; genotypic: $P(B) = 0.316$] and Asian [allelic: $P(B) = 0.794$; genotypic: $P(B) = 0.191$] studies. Furthermore, diagnostic criteria were not found to cause significant publication bias in either European or Asian studies [$P(B) > 0.05$, for both allelic and genotypic analyses].

Since publication bias was shown in studies selected for this meta-analysis, the Trim and Fill method was applied to adjust for the meta-analysis results by adding missing negative results. In allelic meta-analysis, the association between the Taq1A polymorphism and AD was still positive in all studies and studies with the two diagnostic criteria [All studies: $Z = 2.78$, $P(Z) = 0.005$, OR = 1.13; diagnostic 1: $Z = 4.06$, $P(Z) = 5.0 \times 10^{-5}$, OR = 1.20; diagnostic 2: $Z = 3.69$, $P(Z) = 2.0 \times 10^{-4}$, OR = 1.19]. In genotypic meta-analysis, the association between the Taq1A polymorphism and AD also remained significant [All studies: $Z = 2.83$, $P(Z) = 0.005$, OR = 1.18; diagnostic 1: $Z = 2.66$, $P(Z) = 0.008$, OR = 1.18; diagnostic 2: $Z = 2.67$, $P(Z) = 0.007$, OR = 1.19].

## Discussion

Since Blum et al. (1990) initially reported a large effect of the A1 allele of the Taq1A polymorphism on risk for AD, a number of follow-up studies successfully validated this finding, although negative results were also obtained in some studies. Moreover, three meta-analyses of studies confirmed the association and demonstrated a moderate effect of the Taq1A polymorphism on AD (Le Foll et al. 2009; Munafo et al. 2007; Smith et al. 2008). However, the conclusion was far from certain because of inconsistent findings and the limited sample size of previous studies. We performed the largest meta-analysis so far concerning the association between the Taq1A polymorphism and AD. Sixty-one eligible studies published up to 2012 were considered, gathering a total of 18,730 subjects including 9,590 cases and 9,140 comparison controls. Our results provide strong evidence of the association between the Taq1A polymorphism and AD, especially in the European population. The association between the Taq1A polymorphism and AD remains significant after adjustment for publication bias using the trim and fill method. Sensitivity analysis shows that the results from both allelic and genotypic meta-analyses are stable and not influenced by any individual study. As the year of publication progressed, and more eligible studies were included, pooled ORs decreased remarkably compared to the first positive report in

1990 and become constant after the year 2001 (Fig. 3). The trend curve showed that the Taq1A polymorphism has a moderate impact on AD in the population, across all studies.

Both our allelic and genotypic meta-analyses confirmed previous findings concerning the significant association between the Taq1A polymorphism and AD, except the genotypic meta-analysis in Asian studies (refer to Table 1). The reason for the negative finding obtained in the genotypic subgroup meta-analysis in subjects with Asian ancestry is unknown, but here we present some possible explanations. First, allelic and genotypic analyses are two different but related methods used in genetic association studies. Allelic association studies are based on comparing allele frequencies, while genotypic association studies are based on comparing genotype distributions between cases and controls. Moreover, genotypic analysis can examine the effect of polymorphisms on diseases under different genetic models (e.g., dominant, recessive, or co-dominant). Second, the frequency of the minor A1 allele of the Taq1A polymorphism varies substantially in different populations and a higher frequency (over 40 %) of the A1 allele was shown in the Asian population, suggesting that the association between the Taq1A polymorphism and AD could be race-specific. Third, the observed association between the Taq1A polymorphism and AD may result from functional variants that are in tight linkage disequilibrium (LD) with the TaqA1 polymorphism, which was originally considered to be the cause for the *DRD2* restriction fragment length polymorphism (RFLP) (Blum et al. 1990; Bolos et al. 1990).

The exact location of the TaqA1 polymorphism was not specified until the ankyrin repeat and kinase domain containing-1 gene (*ANKK1*) was identified (Neville et al. 2004). Now it is known that the Taq1A polymorphism causes a missense mutation (Glu713Lys) in the conserved 11th ankyrin repeat site. According to the *ANKK1* and *DRD2* gene cluster LD information from the HapMap database, the Taq1A polymorphism is actually located in a LD block that extends to the *DRD2* gene region, especially in the European population. However, it was not found to be in close LD with other AD-associated variants in *ANKK1* (Dick et al. 2007). Since the Taq1A polymorphism does not affect the DRD2 protein sequence, Le Foll et al. (2009) proposed that the Taq1A polymorphism might simply be a marker linked to a functional polymorphism for AD. Nevertheless, positron emission tomography (PET) and high performance liquid chromatography (HPLC) studies showed that the Taq1A polymorphism could affect DRD2 availability (Hirvonen et al. 2004) and the dopamine metabolite homovanillic acid level (Ponce et al. 2004), suggesting a functional role of Taq1A polymorphism in regulating *DRD2* expression either directly or indirectly. Moreover, functional variants in *ANKK1* were found to influence expression levels of nuclear factor-kappaB (NF-

κB) (Huang et al. 2009), which can regulate the transcription of *DRD2* (Bontempi et al. 2007; Fiorentini et al. 2002). Additionally, the Taq1A polymorphism was predicted to influence the aggregation of the ANKK1 protein (Ghosh et al. 2012). Thus, the positive association between the Taq1A polymorphism and AD is likely due to three possible reasons: (1) the Taq1A polymorphism is in tight LD with causative variants for AD, as suggested by Le Foll et al. (2009); (2) the Taq1A polymorphism, which is located in the downstream region of *DRD2* (Yang et al. 2007), is potentially being harbored in the regulatory region of *DRD2*; and (3) the Taq1A polymorphism, which is a nonsynonymous variant in exon 8 of *ANKK1* (Neville et al. 2004), may influence the activity of *ANKK1* which subsequently affects *DRD2* expression. Additionally, besides *ANKK1* and *DRD2*, other genes grouped in the same chromosomal region harboring the Taq1A polymorphism (such as *TTC12* and *NCAM1*) might be involved in AD (Dick et al. 2007; Gelernter et al. 2006).

Publication bias in meta-analyses may be partially influenced by population admixture. In the present meta-analysis, 7 of 61 studies analyzed populations that were classified as "Others" (American Indians, mixed population, or not stated). When the meta-analysis was performed using all studies including different ethnic groups, publication bias was shown to exist; however, publication bias disappeared when studies were limited to European or Asian subgroup studies. Additionally, we did not observe a significant effect of diagnostic criteria on publication bias.

To estimate the type II error, a retrospective statistical power analysis was calculated. For allelic analysis, given the effect size of 1.19 and the type I error of 0.05, the minimal sample size would be 3,201 subjects for the European population and 2,162 subjects for the Asian population to achieve an expected statistical power of 80 %. For genotypic analysis, given the effect size of 1.24 and the type I error of 0.05, the minimal sample size would be about 1,500 subjects for both European and Asian populations to achieve an expected statistical power of 80 %. The above results suggest that most studies included in this meta-analysis did not have sufficient statistical power to detect the association between the TaqA1 polymorphism and AD because of a moderate effect size of the TaqA1 polymorphism. This likely explains the inconsistent findings in previous studies.

Two limitations should be addressed in this meta-analysis. First, a large heterogeneity was observed in European studies. This could be due to other potential differences between studies, e.g., sex, age, family history, severity of AD, or unscreened controls. Only a few studies included in this meta-analysis provided information regarding these variables. It is, therefore, difficult to investigate the influence of the above covariates in causing heterogeneity between studies. Moreover, the potential interaction of *ANKK1/DRD2* with other genes may also lead to heterogeneity between studies. Recent studies demonstrated that dopamine receptor D2 (coded by *DRD2*) and dopamine receptor D4 (coded by *DRD4*) were able to form functional heteromers and the heteromerization process was modulated by polymorphisms in *DRD2* and *DRD4* (Gonzalez et al. 2012; Mota et al. 2012). Interestingly, the *DRD2/DRD4* heteromers were found to influence genetic susceptibility to AD (Mota et al. 2012). Second, publication bias is not the only explanation for the asymmetric funnel plot. The afore-mentioned covariates could contribute to publication bias as well, resulting in false positive findings. Additionally, selection bias could also have occurred because only studies published in English were taken into consideration. Thus, to have a better understanding of the association between the Taq1A polymorphism and AD, an improved meta-analysis could be conducted using more sophisticated analysis methods for controlling of between-study heterogeneity and publication bias.

## Conclusions

In summary, our meta-analysis provides further evidence supporting a moderate effect size of the Taq1A polymorphism on AD across populations. Some of the heterogeneity between studies and publication bias might be due to differences in the racial ancestry of the different populations studied. Retrospective statistical power analysis suggested that the sample size for future studies of the association between the Taq1A polymorphism and AD should be at least 1,500 subjects to have sufficient statistical power. Moreover, findings from previous studies and this large meta-analysis suggest that, in future studies, we need to (1) explore the molecular mechanism by which the TaqA1 polymorphism influences risk for AD, (2) examine the biological function of those variants which are located in the *ANKK1/DRD2* gene cluster region and in tight LD with the TaqA1 polymorphism, and (3) deep-sequence the *ANKK1/DRD2* gene region to identify novel functional variants which are potentially linked to the TaqA1 polymorphism.

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