ORIGINAL ARTICLE

Meta-Analysis on the Association Between the TF Gene rs1049296 and AD

Yun Wang, Shunliang Xu, Zhen Liu, Chao Lai, Zhaohong Xie, Cuiping Zhao, Yan Wei, JianZhong Bi

ABSTRACT: ***Background:*** Polymorphisms of genes participating in iron transportation have been associated with Alzheimer's disease (AD) risk. The association between transferrin (TF) gene rs1049296 (P570S) polymorphism and AD is controversial. ***Methods:*** We performed meta analysis on data from 19 studies with 6310 cases and 13661 controls to reexamine the association between the TF gene rs1049296 polymorphism and AD. We applied a fixed-effects model to combine the odds ratio (OR) and 95% confidence intervals (95% CI). Egger's test was carried out to evaluate the potential publication bias. ***Results:*** The overall ORs with 95% CIs showed statistical association between the TF gene rs1049296 polymorphism and the risk of AD in the allele contrast, the recessive model and the dominant model for allele C2 (fixed-effects pooled OR 1.11; 95% CI 1.05 to 1.17, pooled OR 1.13; 95% CI 1.06 to 1.21, and pooled OR 1.23; 95% CI 1.03 to 1.47, respectively). In the contrast of C2C2+C2C1 vs C1C1, large heterogeneity among the Asian subgroup (p=0.041, I^2= 68.6%) was observed but not among the overall population (p = 0.184, I^2= 22.4%). No publication bias was observed. ***Conclusions:*** The present meta analysis demonstrated that TF gene rs1049296 polymorphism is a genetic determinant of AD.

RÉSUMÉ: Méta-analyse portant sur l'association entre le polymorphisme rs1049296 du gène TF et la MA. ***Contexte :*** Les polymorphismes de gènes qui participent au transport du fer ont été associés au risque de présenter la maladie d'Alzheimer (MA). L'association entre le polymorphisme rs1049296 du gène de la transferrine (TF) et la MA demeure controversée. ***Méthode :*** Nous avons effectué une méta-analyse portant sur les données de 19 études regroupant 6 310 cas de MA et 13 661 sujets témoins pour réexaminer l'association entre ce polymorphisme et la MA. Nous avons utilisé un modèle à effets fixes pour combiner les rapports de cotes (RC) et les intervalles de confiance à 95% (IC à 95%). Nous avons utilisé le test de Egger pour évaluer les biais de publication potentiels. ***Résultats :*** Les RC globaux avec les IC à 95% ont montré une association statistique entre le polymorphisme rs1049296 du gène TF et le risque de MA dans la comparaison des allèles, le modèle récessif et le modèle dominant pour l'allèle C2 (effets fixes RC groupé 1,11 ; IC à 95% 1,05 à 1,17, RC groupé 1,13 ; IC à 95% 1,06 à 1.21 et RC groupé 1,23 ; IC à 95% 1,03 à 1,47 respectivement). Par contre, pour C2C2+C2C1 versus C1C1, une hétérogénéité importante dans le sous-groupe asiatique (p = 0,041, 12 = 68,6%) a été observée, ce qui n'était pas le cas dans la population totale (p = 0,184, 12 = 22,4%). Aucun biais de publication n'a été observé. ***Conclusion :*** Cette méta-analyse démontre que le polymorphisme rs1049296 du gène TF est un déterminant génétique de la MA.

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Alzheimer's disease (AD) is the leading cause of dementia in the elderly, and its etiology is still not fully understood. Mutations in the amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) genes cause familial AD[1].

The more common late onset form (LOAD) of the disease is more complex in nature with proposed combined genetic and environmental risk factors. The well-replicated genetic association for LOAD is the ε4 variant of the APOE gene[2]. However, the APOEε4 allele is neither necessary nor sufficient for the expression of AD, suggesting that there might be other genetic factors. With the advent and ongoing improvement of genome-scanning technologies, the search for the remaining genetic risk factors for LOAD is still ongoing[3].

Transferrin (TF), a component of senile plaques[4], is a candidate susceptibility gene of AD since its C2 variant has been reported significantly higher occurrence in AD patients. The defective binding of iron and aluminum by the TF C2 variant was postulated to contribute to the development of AD[5].

Transferrin is the major circulating glycoprotein involved in iron transportation. Several allelic isoforms of TF have been identified with the most common ones being C1 and C2 (C1 Pro 570; C2 Ser 570). C1 and C2 have different iron binding properties[5]. Higher brain iron levels have been correlated with greater cognitive impairment in AD[6]. It has been proposed that excess iron in AD leads to oxidative damage[7].

Since an association between TF C2 and AD was first proposed in 1993[8], there have been 22 studies focused on this association[9-30]. However, contradicting data were published on the association between the TF gene rs1049296 polymorphism and AD. To get a clear picture, we performed a meta analysis on existing studies to examine allele frequencies at rs1049296 of the TF gene in patients with AD.

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Materials and Methods

Search strategies

We searched MEDLINE (1966 to April 2012), EMBASE (1966 to April 2012), and Cochrane Collaboration Registry for Randomized Controlled Trials (1966 to April 2012). As a search criterion, we used the following: "transferrin gene" or "TF gene" or "TF polymorphism" and "AD" or "Alzheimer's disease". No language restriction was applied.

Selection criteria

We limited our search to full text, published articles and human studies. Abstracts, case reports, editorials, and review articles were excluded. We also retrieved relevant references of included studies for our search. When a report overlapped with a more detailed publication, only the latter was used. All studies that investigate the association of the TF gene rs1049296 polymorphism with AD using a case-control design were considered.

Clinical diagnosis of probable AD were established according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM- IV)[31], the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) working group criteria[32] and the Consortium to Establish a Registry for Alzheimer's disease (CERAD) working group criteria[33]. Controls were defined as subjects not meeting the dementia criteria with intact cognitive functions. All populations except the sample in the Robson's study[18] were consistent with the Hardy-Weinberg equilibrium.

The study protocols for all populations were reviewed and approved by the appropriate institutional review boards in each country. Written informed consent to participation was provided by all subjects or, in cases of substantial cognitive impairment, a caregiver, legal guardian or other proxy. Genotyping methods for each data set were described in the original publications.

Data abstraction

Two researchers independently extracted the data and disagreements were resolved by discussion. Characteristics abstracted from the studies included the name of first author, publication date, country origin, ethnicity, control characteristics, genotyping methods, total number of cases and controls, and numbers of cases and controls with TF gene rs1049296 alleles and genotypes, respectively. Different ethnicity descents were categorized as Caucasian, Asian and African.

Data analysis methods

The primary analysis was conducted by comparing the C2 allele with the C1 allele. This meta analysis examined the contrasts of C2/C2 vs C2/C1+C1/C1 and C2/C2+C2/C1 vs C1/C1, corresponding to the recessive and dominant effects, respectively of the A allele. We also examined the association between C2 allele and AD risk compared with that for G allele (C2 vs C1). We used StataSE 12.0 statistical software packages to analyze our data. The odds ratio (OR) with 95% confidence interval (95% CI) was calculated to assess the association of the TF gene rs1049296 polymorphisms with AD risk. Heterogeneity between studies was assessed by using the chi-square-based Q-test and was considered statistically significant if $p < 0.1$[34]. Heterogeneity was quantified with the I^2 metric, which is determined by the formula $(Q - df)/Q$, where df is the number of degrees of freedom (1 less than the number of combined data sets). I^2 is considered large for values above 50% ($I^2 < 25\%$: no heterogeneity; $I^2 = 25\%–50\%$: moderate heterogeneity; $I^2 = 50\%–75\%$: large heterogeneity; $I^2 > 75\%$: extreme heterogeneity)[35]. The pooled OR was calculated by the fixed-effects model (the Mantel-Haenszel method) when there was no or moderate heterogeneity among studies[36]. Otherwise, the random-effects model (the DerSimonian-Laird method)[37] was used.

The Galbraith plot was used to spot the outliers as the possible major sources of heterogeneity[38]. Publication bias was assessed by visual inspection of Begger's funnel plots. Funnel plot asymmetry was assessed by the method of Egger's linear regression test, a linear regression approach to measure funnel plot asymmetry on the natural logarithm scale of the OR. The significance of the intercept was determined by the t-test ($p < 0.05$ was considered statistically significant publication bias)[39].

Results

Characteristics of included studies

The literature review identified 23 articles with detailed assessment, among which three articles[19-21] were excluded because they were derived from the same study population as other reports and another one was excluded because there was no detailed data provided[12].

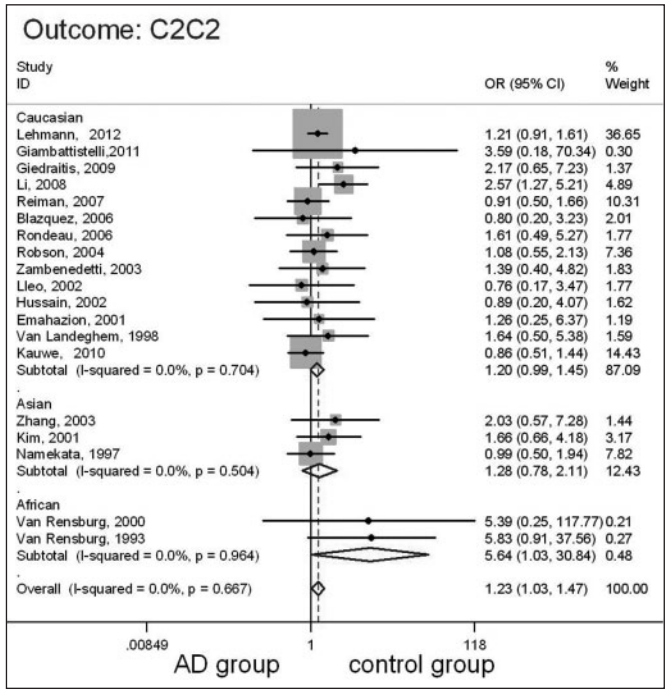

Figure 1: *Forest plot of AD risk associated with rs1049296 under C2C2 vs C2C1+C1C1 contrast in different ethnicity. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study specific weight. The diamond represents the pooled OR and 95% CI.*

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Table 1: Clinical characteristics of the populations from studies included in the meta analysis

Study	Country	AD		Control	
		N (% Female)	Mean age	N (% Female)	Mean age
Lehmann，2012	North Europe and North Spain	1666 (-)	79.0	6227	76.9
Giambattistelli，2011	Italy	160 (-)	75 ± 7.8 73 ± 7.6 (AAO)[b]	79	65 ± 10.3
Kauwe，2010	UK	1236（67%）	75.3	1390 (61%)	76.8
Giedraitis, 2009	Sweden (ULSAM)[a]	86 (0%)	80.2 (AAO)	404 (0%)	81.8
Li, 2008	Canada	753 (58%)	77.8 ±8.6	736 (64%)	73.4 ± 7.9
Reiman, 2007	USA, Netherlands	861 (-)	74.9 ± 6.6	550 (-)	77.4 ± 7.3
Blazquez, 2006	Spain	211 (58%)	72.2 ± 8.3 73.5 ± 7.7 AAO）	167 (58%)	72.1 ± 6.8
Rondeau, 2006	France	55 (-)	-	237 (-)	-
Robson, 2004	UK	191 (57%)	70.5 ± 9.2	269(-)	76.1 ± 8.9
Zambenedetti, 2003	Italy	132 (67%)	68 ± 0.6	318 (64%)	70 ± 0
Lleo, 2002	Spain	108 (74%)	78.8 61-93 (AAO)	110 (62%)	73.6 45-92 (AAO)
Hussain, 2002	UK	180 (-)	77.9± 8 46-93 (AAO)	121 (-)	77.9 ± 8.7 65-100 (AAO)
Emahazion, 2001	Scotland	121 (-)	-	152 (-)	-
Van Landeghem, 1998	Sweden	64 (-)	-	2133 (-)	-
Zhang, 2003	China	67 (60%)	80 ± 6.6	131 (31%)	69 ± 9.4
Kim, 2001a	Korea	164 (72%)	70.5 ± 8	239 (78%)	68.3 ± 4.6
Namekata, 1997	Japan	294	69 ± 5.9 38-93 (AAO)	291	70.9 ± 7.3 60-93 (AAO)
Van Rensburg, 2000	South Africa	27 (-)	-	27 (-)	-
Van Rensburg, 1993	South Africa	20 (-)	-	158 (-)	-

a: ULSAM, Uppsala Longitudinal Study of Adult Men; b: AAO, Age at onset.

Our final analysis included 19 case-control studies, enrolling 6,310 cases and 13,661 controls. Fourteen out of the 19 studies involved Caucasian populations, 3 studies involved Asian populations, and the other 2 were conducted in African populations. Genomic DNA was extracted from blood samples in all the studies, and depending on the centre, a broad range panel of technologies were used to genotype SNP. Detailed characteristics of the included studies are shown in Table 1.

Table 2 shows both genotype and allele frequencies of AD patients and controls in the selected studies. The allele frequencies are calculated from the corresponding genotype distributions.

Meta analysis results

The overall OR with its 95% CI showed statistical association between the TF gene rs1049296 polymorphism and the risk of

Table 2: Distribution of TF allele and frequencies among AD cases and controls in the included studies

Study	AD					Control				
	C2–Allele	C1-Allele	C2/C2 (frequency)	C2/C1 (frequency)	C1/C1 (frequency)	C2–Allele	C1-Allele	C2/C2 (frequency)	C2/C1 (frequency)	C1/C1 (frequency)
Lehmann , 2012	0.18	0.82	63 (0.037)	481 (0.288)	1122 (0.673)	0.17	0.83	196 (0.031)	1670 (0.268)	4361 (0.700)
Giambattistelli, 2011	0.30	0.70	3 (0.021)	81 (0.555)	62 (0.425)	0.27	0.73	0 (0.000)	39 (0.534)	34 (0.466)
Kauwe, 2010	0.38	0.62	26 (0.021)	897 (0.726)	313 (0.253)	0.38	0.62	34 (0.024)	993 (0.714)	363 (0.261)
Giedraitis, 2009	0.20	0.80	4 (0.048)	25 (0.298)	55 (0.655)	0.18	0.82	9 (0.023)	102 (0.255)	289 (0.723)
Li, 2008	0.16	0.84	28 (0.040)	171 (0.247)	493 (0.712)	0.15	0.85	11 (0.016)	177 (0.260)	494 (0.724)
Reiman, 2007	0.17	0.83	27 (0.032)	242 (0.283)	586 (0.685)	0.18	0.82	19 (0.035)	157 (0.285)	374 (0.680)
Blazquez, 2006	0.14	0.86	4 (0.019)	52 (0.248)	154 (0.733)	0.15	0.85	4 (0.024)	43 (0.256)	121 (0.720)
Rondeau, 2006	0.22	0.78	4 (0.073)	16 (0.291)	35 (0.636)	0.20	0.80	11 (0.046)	73 (0.308)	153 (0.646)
Robson, 2004	0.25	0.75	16 (0.084)	65 (0.340)	110 (0.576)	0.21	0.79	21 (0.078)	69 (0.257)	179 (0.665)
Zambenedetti, 2003	0.22	0.78	4 (0.030)	51 (0.386)	77 (0.583)	0.18	0.82	7 (0.022)	98 (0.308)	213 (0.670)
Lleo, 2002	0.17	0.83	3 (0.028)	31 (0.287)	74 (0.685)	0.18	0.82	4 (0.036)	32 (0.291)	74 (0.673)
Hussain, 2002	0.17	0.83	4 (0.022)	52 (0.289)	124 (0.689)	0.12	0.88	3 (0.025)	23 (0.190)	95 (0.785)
Emahazion, 2001	0.16	0.84	3 (0.025)	33 (0.273)	85 (0.702)	0.15	0.85	3 (0.020)	39 (0.257)	110 (0.724)
Van Landeghem, 1998	0.21	0.79	3 (0.047)	21 (0.328)	40 (0.625)	0.17	0.83	62 (0.029)	602 (0.282)	1469 (0.689)
Zhang, 2003	0.22	0.78	5 (0.075)	19 (0.284)	43 (0.642)	0.24	0.76	5 (0.038)	52 (0.397)	74 (0.565)
Kim, 2001a	0.23	0.77	10 (0.061)	57 (0.348)	97 (0.591)	0.23	0.77	9 (0.038)	92 (0.385)	138 (0.577)
Namekata, 1997	0.28	0.72	18 (0.061)	126 (0.429)	150 (0.510)	0.22	0.78	18 (0.062)	93 (0.320)	180 (0.619)
Van Rensburg, 2000	0.28	0.72	2 (0.074)	11 (0.407)	14 (0.519)	0.13	0.87	0 (0.000)	7 (0.259)	20 (0.741)
Van Rensburg, 1993	0.33	0.67	2 (0.111)	8 (0.444)	8 (0.444)	0.14	0.86	3 (0.021)	35 (0.245)	105 (0.734)

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Table 3: Main results of heterogeneity, pooled ORs, stratification analysis and Egger's test of the TF gene rs1049296 polymorphisms on AD risk in the meta analysis.

Genetic contrasts	Population	Heterogeneity p_h[a]; I^2		OR (95% CI) p_{OR}[b]	Egger's test t; p_E[c]; (95% CI)
C2C2 *vs* C2C1+C1C1	Caucasian	0.704	0.0%	1.20 (0.99-1.45) 0.060	1.77 0.094 (-0.12-1.39)
	Asian	0.504	0.0%	1.28 (0.78-2.11) 0.332	
	African	0.964	0.0%	5.64 (1.03-30.84) 0.046	
	Overall	0.667	0.0%	1.23 (1.03-1.47) 0.021	
C2C2+C2C1 *vs* C1C1	Caucasian	0.717	0.0%	1.12 (1.04-1.20) 0.003	1.55; 0.139; (-.025-1.66)
	Asian	0.041	68.6%	1.17 (0.93-1.48) 0.187	
	African	0.734	0.0%	3.06 (1.44-6.52) 0.004	
	Overall	0.184;	22.4%	1.13 (1.06-1.21) 0.001	
C2 *vs* C1	Caucasian	0.626;	0.0%	1.09 (1.03-1.160) 0.003	2.21; 0.041; (0.04-1.78)
	Asian	0.265	24.8%	1.15 (0.95-1.40) 0.146	
	African	0.818	0.0%	2.80 (1.52-5.19) 0.001	
	Overall	0.190	21.8%	1.11 (1.05- 1.17) 0.000	

ph, p-value of Q-test for heterogeneity test; pOR, p-value of Z-test for OR; pE, p-value of t-test for Egger's test.

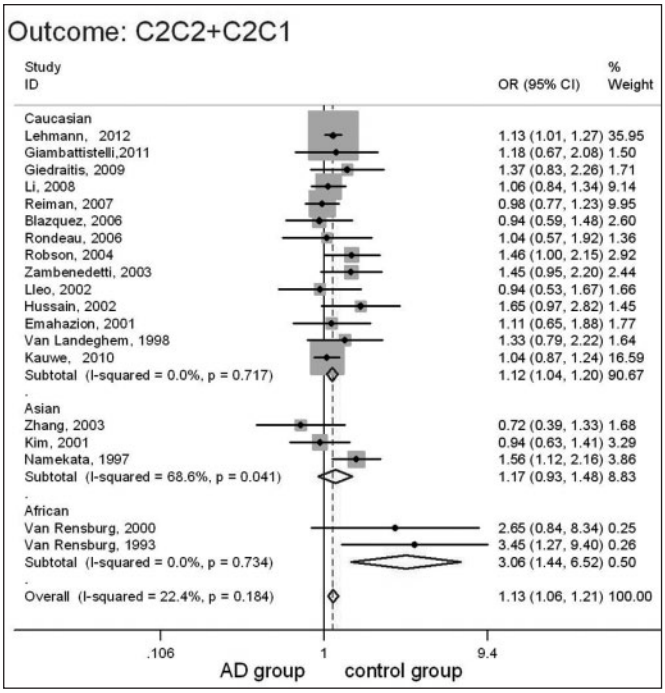

Figure 2: *Forest plot of AD risk associated with rs1049296 under C2C2+C2C1 vs C1C1 contrast in different ethnicity. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study specific weight. The diamond represents the pooled OR and 95% CI.*

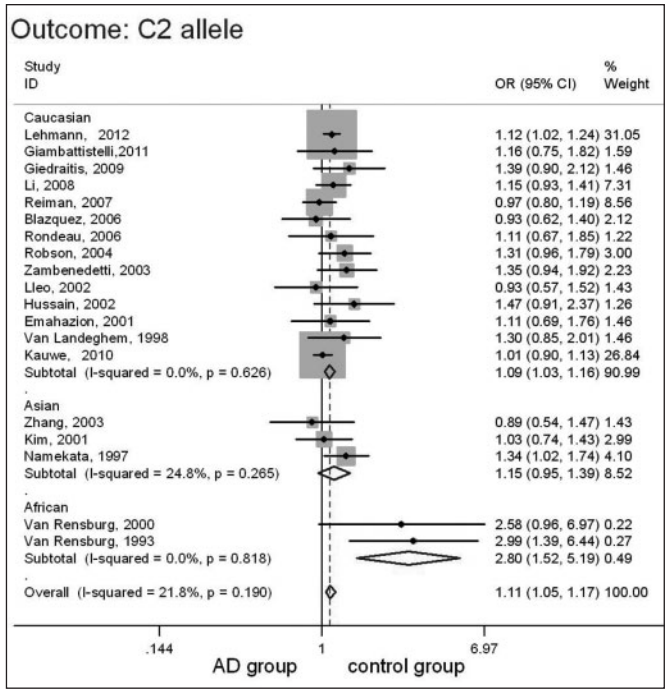

Figure 3: *Forest plot of AD risk associated with rs1049296 under allele contrast C2 vs C1 in different ethnicity. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study specific weight. The diamond represents the pooled OR and 95% CI.*

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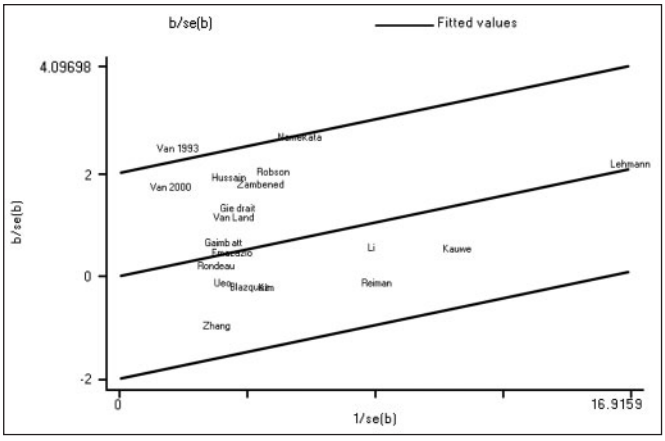

Figure 4: *Galbraith plot of associations between TF gene rs1049296 polymorphisms and AD risk, produced by first dividing each estimate by its standard error (se) to generate z-statistics (b/se(b)), which is then plotted versus 1/se(b) for each of studies. Inner line represents pooled effect and outer lines represent 95% limits. Each author represents the respective study included in the meta analysis (shown in Table 1) for the indicated association by C2C2 vs C2C1+C1C1.*

AD (Table 3). The overall OR for C2C2 vs C2C1+C1C1 was 1.23 by fixed-effects model with 95% CI of 1.03 to 1.47 (p=0.021) (Figure 1), 1.13 with 95% CI 1.06 to 1.21 (p=0.001) for C2C2+C2C1 vs C1C1 (Figure 2), 1.11 with 95% CI 1.05 to 1.17 (p<0.001) for C2 vs C1 (Figure 3). In the stratified analysis by race, p value was greater than 0.05 in all three genetic contrasts (Table 3).

Heterogeneity

In the contrast of C2C2 vs C2C1+C1C1, no heterogeneity among combined populations (p = 0.667, I^2= 0%), Caucasian subgroup (p = 0.704, I^2= 0%), Asian subgroup (p=0.504, I^2= 0%) or African subgroup (p = 0.964, I^2= 0%) was observed (Table 3). Neither was it observed in the contrast of C2 vs C1 (Table 3).

In the contrast of C2C2+C2C1 vs C1C1, large heterogeneity among Asian subgroup (p=0.041, I^2= 68.6%) was observed. And there was no heterogeneity among combined populations (p = 0.184, I^2= 22.4%), Caucasian subgroup (p = 0.717, I2= 0%) or African subgroup (p = 0.734, I^2= 0%) (Table 3).

The main cause of heterogeneity in the Asian subgroup was unclear, shown in the Galbraith plot for heterogeneity (Figure 4 and Figure 5).

The sample in the Robson's study[18] was not consistent with Hardy-Weinberg equilibrium. After excluding this study, the results were similar and still reached a positive association (data not shown).

Bias diagnostics

Begg's funnel plot and Egger's test were performed to assess the publication bias of the literature. The shapes of the funnel plot for the contrast of the C2C2 vs C2C1+C1C1 seemed approximately symmetrical (Figure 6), and Egger's test did not show any evidence of publication bias (t=1.77; p=0.094). This was also the case for the contrast of the C2C2+C2C1 vs C1C1 (t=1.55; p=0.139) and the contrast of C2 vs C1 2.21 (t=2.21; p=0.041) (Table 3).

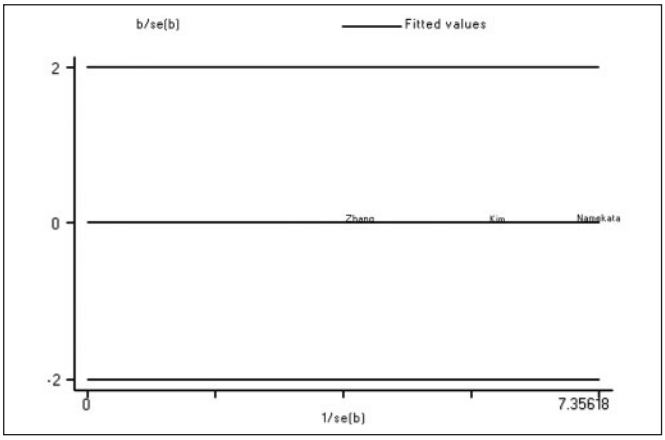

Figure 5: *Galbraith plot of associations between TF gene rs1049296 polymorphisms and AD risk, produced by first dividing each estimate by its standard error (se) to generate z-statistics (b/se(b)), which is then plotted versus 1/se(b) for each of studies. Inner line represents pooled effect and outer lines represent 95% limits. Each author represents the respective study included in the meta analysis (shown in Table 1) for the indicated association by C2 vs C1.*

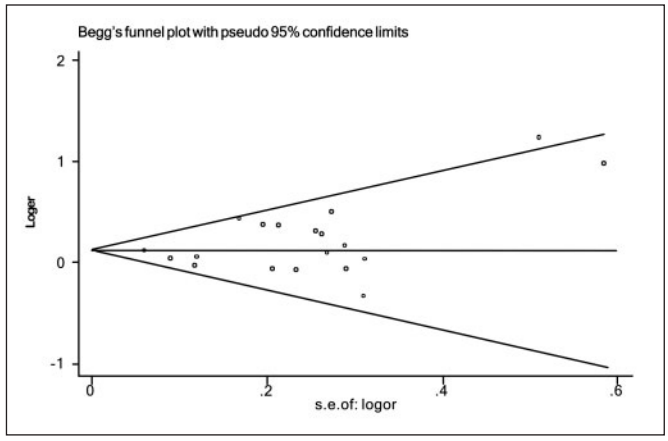

Figure 6: *Begg's funnel plot of publication bias in TF gene rs1049296 polymorphism studies. Log OR is plotted versus standard error for each of studies in this meta analysis. Each circle represents a separate study for the indicated association by C2C2 vs C2C1+C1C1.*

Discussion

It has been estimated that the APOE locus may account for 20% or less of LOAD risk. Thus additional genetic factors may act independently or in concert with the APOE ε4 allele in the manifestation of AD[40].

An increasing number of studies have suggested that oxidative stress may be at the basis of AD neurodegeneration[41,42]. Iron has been shown to be a key factor in

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biochemical reactions that produce free radicals, leading to peroxidation of cellular lipids and to neuronal damage or death[5,43]. Elevated iron concentrations are found in specific brain areas of AD patients[6]. Iron also interacts with Aβ in the extracellular space; induces aggregation of hyper-phosphorylated tau and neurofibrillary tangle formation[44]. Increased iron accumulation was found in the specific brain areas of the pre-clinical AD cases with increased glial accumulations of redox-active iron in the cerebellum[45], indicating that iron dysregulation and oxidative stress are the causes of AD pathogenesis.

Transferrin plays a major role in iron metabolism[46]. The original report showed TF as a candidate gene and TF C2 allele as a risk factor of AD[8]. However, subsequent studies have reported contrasting data on the association between TF C2 allele and AD[25-29]. While it was found associated with AD in both a Sweden population[25] and a Chinese population[26], TF C2 failed to show association with AD in a study with a Korean population[27,28]. Although most studies have been negative, the AlzGene meta-analysis of the allele (www.alzgene.org/)[47] has shown a significant, although low, odds ratio of AD: 1.2 (95% confidence interval, 1.06–1.3) (1 June 2010). Another large family-based study[48] also supported such association.

This meta analysis confirmed the association between TF gene rs1049296 polymorphism and AD with data pooled from 19 independent studies consisting of 6,310 AD patients and 13,661 normal controls. However, the association is significant but relatively weak with OR around 1.2 ($p<0.05$). This weak strength of association might explain the contradicting data from previous small independent studies that trend could be easily skewed by any bias in the sample. With more data being generated and incorporated into further analysis, the association or the lack of association between TF C2 and AD will be clearly confirmed.

During analysis of genetic contrast of C2C2+C2C1 vs C1C1, a significant heterogeneity was observed with the Asian subgroup. However, the heterogeneity was not observed in the overall population or in other genetic contrasts of the Asian subgroup. Whether there is heterogeneity requires further studies with much larger sample sizes to confirm.

In conclusion, this meta analysis confirmed that the rs1049296 in TF gene is associated with the risk of AD and TF C2 is a risk factor. Additional well-designed studies with larger sample sizes are warranted to validate these findings.

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