J Neurol (2013) 260:1701–1708
DOI 10.1007/s00415-012-6690-4

REVIEW

CYP46A1 T/C polymorphism associated with the *APOEε4* allele increases the risk of Alzheimer's disease

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Received: 16 July 2012 / Revised: 24 September 2012 / Accepted: 25 September 2012 / Published online: 16 October 2012

Abstract Studies of the relationship between Alzheimer's disease (AD) and single nucleotide polymorphism (SNP) T/C in intron 2 of the cholesterol-24S-hydroxylase gene (*CYP46A1*) have reported inconsistent results. To confirm the association between the *CYP46A1* T/C polymorphism and AD risk, a meta-analysis containing 4,875 AD cases and 4,874 controls from 21 case–control studies was performed. There were 16 studies involving Europeans, four studies with Asians and one study with Africans. The combined results of overall analysis showed that the *CYP46A1* T/C polymorphism increased the risk of AD significantly in recessive model [CC versus CT + TT, odds ratio (OR) = 1.20, 95 % confidence interval (CI) = 1.04–1.38, $p = 0.01$]. On subgroup analysis by ethnicity, similarly significant differences in recessive model were also found in Europeans. Another analysis of the synergistic effect of the *CYP46A1* T/C polymorphism and the ε4 allele of the apolipoprotein E gene (*APOE ε4*) was performed in eight studies with available stratified information. The results revealed that the presence of *APOE ε4* allele could strengthen the effect of CC genotype on AD risk, and the reverse was also true. In conclusion, our meta-analysis has successfully proved that CC genotype of the *CYP46A1* T/C polymorphism could increase the risk of AD, and this effect would be weakened in *APOE ε4* non-carriers *and* strengthened in *APOE ε4* carriers.

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Keywords Cholesterol 24S-hydroxylase gene (*CYP46A1*) · Apolipoprotein E (*APOE*) · Alzheimer's disease (AD) · Meta-analysis · Polymorphism

Introduction

Alzheimer's disease (AD) is a common, complex genetic disorder with a prevalence rate of 5–10 % and an incidence that increases exponentially after the age of 65 years. Familial autosomal dominant AD, caused by mutations in the amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*) or presenilin-2 (*PSEN2*) genes [1], just accounts for ≤2 % of all cases. A majority of AD cases manifest as sporadic late onset form (LOAD), typically with onset above the age of 65 years. Many candidate genes have been studied in recent decades, the ε4 allele of the apolipoprotein E gene (*APOE ε4*) remains the one unquestionable risk factor for LOAD until 2009 [2]. In the last 5 years, an increasing number of genome-wide association studies (GWASs) in AD were performed; other novel genes for LOAD, such as clusterin (*CLU*), phosphatidylinositol-binding clathrin assembly protein (*PICALM*) and complement receptor 1 (*CR1*), have been identified [3–6]. However, these genes could not account for all the genetic components of AD, indicating that other genes are involved in the etiology of sporadic AD.

Recent evidence has suggested that cholesterol metabolism participates in the pathogenesis of Alzheimer's disease [7]. The mechanism mainly considered is that high intracellular cholesterol concentrations increase the amyloidogenic processing of amyloid precursor protein (*APP*), leading to greater amyloid-beta (Aβ) production [8, 9]. Brain cholesterol is synthesized locally and then transported into circulation through the blood brain-barrier

(BBB). The elimination of cerebral cholesterol involves two mechanisms, dependent of APOE and cholesterol 24-hydroxylase (CYP46). APOE is the main transporter of cholesterol in the neuronal cell membrane, and CYP46 is a key enzyme catalyzing formation of altered cholesterol which can pass the BBB.

CYP46A1 is located in 14q32.1, and this gene encodes CYP46, a member of the cytochrome P450 superfamily of enzymes. This enzyme is expressed in the brain, where it converts cholesterol to 24S-hydroxycholesterol. While cholesterol cannot pass the BBB, 24S-hydroxycholesterol is the major cholesterol elimination product of the brain because it is assumed to pass the BBB to the circulation via ATP-binding cassette transporters (ABCA1), becoming the origin of more than 90 % of plasma 24S-hydroxycholesterol [10].

A single nucleotide polymorphism (SNP) T/C (rs754203) in intron 2 of *CYP46A1* has been identified and reported to be significantly associated with increased risk for AD. According to the reports, the frequency of the *CYP46A1* T allele and TT genotype was significantly higher in AD patients from Switzerland, Greece, and Italy than in controls [11]. In contrast, another study reported that the *CYP46A1* C allele might act as a risk factor for AD in Italian patients [12]. Publications about this subject around the world are controversial. Among the studies about the association between the *CYP46A1* T/C polymorphism and AD risk, several studies have reported the synergistic effect of *CYP46A1* T/C and *APOE ε4* on AD risk [12, 13]. However, results in different studies have been inconsistent.

In view of the contradictory results previous reported, we conducted a meta-analysis by collecting and sorting the previous published studies, with the aim to report the association between the *CYP46A1* T/C polymorphism and AD. In addition, we also analyzed the synergistic effect of *CYP46A1* T/C and *APOE ε4* on AD risk in order to better understand the mechanism of effect of *APOE ε4* on AD risk.

Materials and methods

Literature search

We searched all published papers (before 2012) in the databases of PubMed, Medline and Embase. The keywords used were as follows: "polymorphism", "*CYP46*" or "Cholesterol 24S-hydroxylase", and "Alzheimer's disease" or "AD". Abstracts and unpublished reports were not included. Special consideration was dedicated to case–control studies. To avoid a possible loss of any relevant article, an additional control was performed through the references cited in identified articles, through the link ''related articles'' offered in the PubMed database, and through the references of review articles. Besides, we had referred to "Gene overview of all published AD-association studies for *CYP46A1*" in the AlzGene database [14, 15].

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) case–control study to evaluate the association between the *CYP46A1* T/C polymorphism and risk of AD, (2) useful data including genotype number or frequency given, and (3) genotype distribution of control population in Hardy–Weinberg equilibrium (HWE). The exclusion criteria were as follows: (1) studies with insufficient genotype number or frequency not included, (2) genotype distribution of control population not in HWE, and (3) if the same population was included as in previous study, only the most recent study or the study with larger sample size was included in our meta-analysis.

Data extraction

For each study, information was extracted including first author, year of publication, country of publication, number and ethnicity of study population, AD diagnosis criteria, and genotype number in cases and controls. In addition, when cases and controls were stratified by the *APOE ε4* carrier status, the distribution of *CYP46A1* T/C in both statuses would be extracted. Data were extracted independently by three investigators. Agreement was reached after discussion for conflicting data.

Statistical analysis

Odds ratio (OR) and 95 % CI were used to assess the association between the polymorphism of *CYP46A1* T/C and AD risk. Five different genetic models were used in our analysis: dominant model (CC + CT vs. TT), recessive model (CC vs. CT + TT), homozygote comparison (CC vs. TT), and heterozygote comparison (CT vs. CC; CT vs. TT). Heterogeneity was measured using the Q statistic. Pooled ORs were calculated using a fixed-effects model ($p > 0.1$) or a random-effects model ($p \leq 0.1$). To evaluate ethnicity-specific effects, subgroup analysis was performed based on the ethnicity of study population.

In the analysis of the synergistic effect of *CYP46A1* T/C and APOE ε4, the studies including stratification of the APOE ε4 allele carrier status would be conducted in another meta-analysis. Five genetic models were used again in both *APOE ε4* carrier statuses. Publication bias

was analyzed by Begg's funnel plot and Egger's test. All above statistical analysis were performed using Review Manager 5.0 and Stata 11.0.

Results

Study characteristics

A total of 86 references were identified in PubMed, Medline, Embase and AlzGene database, of which 42 were excluded for duplication. After careful reading of abstracts and titles, six reviews, two studies written in Chinese [17, 18] and 11 references not involving in the relationship between AD and *CYP46A1* were excluded. After careful reading of full text, two references not studying the SNP T/C were excluded [19, 20]. Moreover, one study was excluded because it was a prospective study and the population was divided into improved or stable group and deteriorated group according to the outcome of a two-year follow up [16]. In addition, distribution of the *CYP46A1* T/C polymorphism was unavailable in two studies [21, 22]. Finally, 21 studies of 20 references containing 4,875 AD cases and 4,874 controls were included in our meta-analysis. AD cases in these 21 studies were probable or possible. One study was only included in the recessive model calculation [23]. The populations came from different countries, including Italy, the USA, Germany, the UK, Zurich, Switzerland, Finland, Sweden, Scotland, France, Hungary, Poland, Spain and China. There were 16 studies involving Europeans, four studies involving Asians (only China) and one study involving Africans (African American) (Table 1). Besides, eight studies containing 1,721 AD cases and 1,454 controls were stratified by the *APOE ε4* carrier status and the stratified data was available. (Table 1).

Effect of *CYP46A1* T/C polymorphisms on AD risk

A meta-analysis of the *CYP46A1* T/C polymorphism association with AD risk was executed in all of included studies. The combined results of the overall analysis showed that there was significant positive association between the *CYP46A1* T/C polymorphism and AD risk in the recessive model (CC vs. CT + TT, OR = 1.20, 95 % CI = 1.04–1.38, $p = 0.01$; results shown in Table 2; forest plot shown in Fig. 1). However, there were no significant associations in other genetic models (results seen in Table 2, forest plots not shown, available on request).

On subgroup analysis by ethnicity of study population, increased AD risk among Europeans for CC genotype was just found in recessive model (CC versus CT + TT). In the other genetic models, there was no significant association between the *CYP46A1* T/C polymorphism and AD risk on subgroup analysis (Table 2).

In conclusion, only the CC genotype of the *CYP46A1* T/C polymorphism was found to increase the risk of AD significantly. But this phenomenon just appeared among Europeans, but not Asians and Africans.

Publication bias was analyzed by Begg's funnel plot, the shape of which appeared to be approximately symmetrical (CC vs. CT + TT), and Egger's test did not show any evidence of publication bias ($t = 0.02$, $p = 0.986$ for CC vs. CT + TT). Begg's funnel plot and Egger's test showed that there was no obvious publication bias in overall analysis (Fig. 2).

Synergistic effect of the *CYP46A1* T/C polymorphism and *APOE ε4* allele on AD risk

Distribution of the *CYP46A1* T/C polymorphism stratified by the *APOE ε4* carrier status was available in eight studies (Table 1), which were conducted into a meta-analysis in five different genetic models same as described above. CC genotype was proved to be an increased risk for AD in these eight studies once more (results of five genetic models shown in Table 3; forest plot of recessive model shown in Fig. 3a). In addition, promoted role of *APOE ε4* was found to play in AD patients in every study whose *APOEε4* is available.

We have made a further analysis of the synergistic effect of the *CYP46A1* T/C polymorphism and *APOE ε4* allele on AD risk and got three findings as follows: (1) there was no significant association between the *CYP46A1* T/C polymorphism and AD risk in every model when we just analyzed the *APOE* ε4 carriers (Table 3). Thus, we thought that the presence of the *APOE* ε4 allele was a larger risk factor for AD than the *CYP46A1* T/C polymorphism, even when considered as an independent risk factor. (2) The CC genotype could also increase the risk of AD in *APOE* ε4 non-carriers, but its effect was weakened significantly by the absence of the *APOE* ε4 allele (Table 3, and the result of genetic model *APOE* ε4 negative +CC versus CT + TT, shown in Fig. 3b). (3) Accordingly, the presence of the *APOE* ε4 allele was proved to strengthen the effect of CC genotype on AD risk in the model of *APOE* ε4 positive +CC versus CT + TT (Fig. 3b).

Discussion

Alzheimer's disease is one of the most disabling and burdensome health conditions worldwide. As the world's population ages, we face a looming epidemic of AD. It is predicted that by the year 2050, worldwide prevalence of AD could grow to 106.8 million. [40] Effective

Table 1 Characteristics of studies included in this meta-analysis

First author	Years	Country	Ethnicity	Stratified data available	AD diagnosis	AD				Control			
						TT	CT	CC	Total	TT	CT	CC	Total
Desai [24]	2002	USA	European	Yes	NINCDS-ADRDA criteria	215	174	45	434	192	168	41	401
Desai [24]	2002	USA	African	Yes		36	17	1	54	42	17	2	61
Kolsch [25]	2002	Germany	European	No	DSM-IV criteria and neuroimaging studies	63	42	10	115	72	56	16	144
Papassotiropoulos [11]	2003	Switzerland, etc.	European	No	NINCDS-ADRDA criteria	120	69	12	201	109	114	25	248
Borroni [12]	2004	Italy	European	No	NINCDS-ADRA criteria	65	68	10	143	86	38	10	134
Chalmers [26]	2004	UK	European	No	CERAD criteria	46	36	4	86	28	27	3	58
Combarros [27]	2004	Spain	European	Yes	NINCDS-ADRDA criteria	177	117	27	321	191	114	10	315
Ingelsson [28]	2004	USA	European	Yes	NINCDS-ADRDA criteria	90	69	19	178	49	51	5	105
Johansson [29]	2004	Sweden, Canada	European	No	NINCDS-ADRDA criteria	283[a]	302[a]	68[a]	653	301[a]	281[a]	67[a]	649
Kabbara [30]	2004	France	European	No	DSM-III-R and NINCDS-ADRDA criteria	292	252	57	601	305	269	57	631
Wang [31]	2004	China	Asian	Yes	NINCDS-ADRDA criteria	77	20	2	99	61	49	3	113
Golanska [32]	2005	Poland	European	Yes	NINCDS-ADRDA criteria	89	94	32	215	85	75	13	173
Juhasz [33]	2005	Hungary	European	No	NINCDS-ADRDA and DSM-IV criteria	54	66	5	125	50	49	3	102
Fernandez [34]	2006	Spain	European	No	NINCDS-ADRDA and DSM-IV criteria	65[a]	31[a]	4[a]	100	67[a]	46[a]	6[a]	119
Li [35]	2006	China	Asian	Yes	NINCDS-ADRDA criteria	57	57	10	124	63	38	8	109
Tedde [10]	2006	Italy	European	Yes	DSM-IV criteria	147	131	18	296	91	78	8	177
Helisalmi [23]	2006	Finland	European	No	NINCDS-ADRDA criteria	–	–	43[b]	422[b]	–	–	24[b]	469[b]
Ma [36]	2006	China	Asian	No	NINCDS-ADRDA criteria	78	73	23	174	76	81	21	178
Wang [37]	2007	China	Asian	No	NINCDS-ADRDA criteria	85	72	11	168	102	98	15	215
Golanska [38]	2009	Poland	European	No	NINCDS-ADRDA criteria	94[a]	92[a]	27[a]	213	84[a]	74[a]	13[a]	171
Ghebranious [39]	2011	USA	European	No	NINCDS-ADRDA criteria	68	67	18	153	130	137	35	302

[a] Calculated value

[b] For this study only presenting information for genotype of CC, only the recessive model was calculated

interventions may significantly reduce the prevalence and incidence of Alzheimer's disease and ultimately reduce the global burden of the disease. But unfortunately, no effective treatment is available for AD up to now, because of the limited understanding of the etiology of AD, especially the sporadic type. Many studies argue that the physiopathology of AD is complex and multifactorial, but mainly attributed to genetic and environmental factors.

The genetics of AD has taken impressive steps forwards in recent decades. To date, more than 600 genes have been linked to the disorder [41]. However, only a few of them are supported by a sufficient level of evidence. Meanwhile,

Table 2 Meta-analyses of the *CYP46A1* T/C polymorphism and AD risk in subgroups

Category	CT + CC vs. TT		CC vs. CT + TT		CC vs. TT		CT vs. CC		CT vs. TT	
	OR (95 % CI)	N[a]	OR (95 % CI)	N	OR (95 % CI)	N	OR (95 % CI)	N	OR (95 % CI)	N
Overall	0.99 (0.86, 1.14)	20	1.20 (1.04, 1.38)*	21	1.14 (0.97, 1.33)	20	0.88 (0.75, 1.03)	20	0.97 (0.84, 1.12)	20
Europeans	1.02 (0.89, 1.18)	15	1.22 (1.05, 1.43)*	16	1.16 (0.98, 1.37)	15	0.86 (0.73, 1.03)	15	1.00 (0.86, 1.15)	15
Asians	0.83 (0.48, 1.42)	4	1.05 (0.68, 1.60)	4	1.02 (0.65, 1.59)	4	0.92 (0.59, 1.45)	4	0.92 (0.59, 1.45)	4
Africans	1.11 (0.50, 2.42)	1	0.56 (0.05, 6.32)	1	0.58 (0.05, 6.70)	1	2.00 (0.17, 24.19)	1	1.17 (0.52, 2.61)	1

N number of studies, *OR* odds ratio, *CI* confidence interval, *vs.* versus

* Significant association between *CYP46A1* T/C polymorphism and AD risk; $p \leq 0.05$

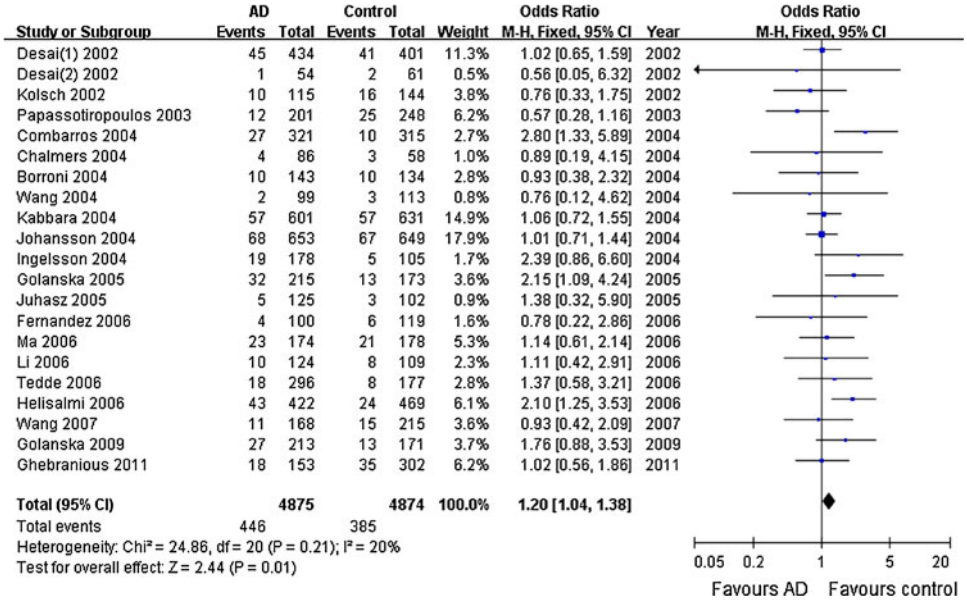

Fig. 1 Meta-analysis of 21 case–control studies about the *CYP46A1* T/C polymorphism and the risk for AD using the fixed-effects model. The genotype effect for CC vs. CT + TT was estimated for each study. The pooled OR for CC versus CT + TT was also calculated. The OR and 95 % CI for the effect for all comparisons are plotted on the graph. Studies are arranged chronologically based on the year of publication

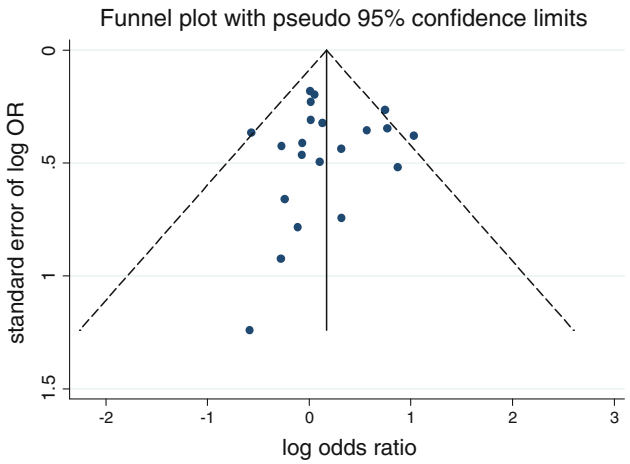

Fig. 2 Begg's funnel plot for *CYP46A1* T/C polymorphism (CC vs. CT + TT) and AD risk

cholesterol metabolism is believed to play a role in the development of AD according to some epidemiology research and biochemical studies [7, 42]. Thus, association between cholesterol related genes has drawn more attention in recent years, especially *CYP46A1*, whose encoding enzyme participates in one of the most important mechanisms for the elimination of excess brain cholesterol.

A SNP T/C (rs754203) in intron two of *CYP46A1* has been studied in various populations. However, the different results of various studies were contradictory. Llorca et al. [43] have recently reported the negative association between the *CYP46A1* T/C polymorphism and AD even stratified by *APOE ε4* status in a meta-analysis, containing 13 case–control studies (date of publication from 2002 to 2005), with 3,409 AD cases and 3,209 controls. We conducted this comprehensive genetic meta-analysis after 5 years because of more published studies and the importance of *CYP46A1* for AD risk.

Our results were completely contrary to previous report by Llorca et al. [43]. A total of 21 case–control studies containing 4,875 AD cases and 4,874 controls were included in our meta-analysis. The results confirm the association between *CYP46A1* T/C polymorphism and AD risk. Our results indicate that the CC genotype of *CYP46A1*

Table 3 Synergistic effect of the *CYP46A1* T/C polymorphism and the APOEε4 allele on AD risk

APOEε4 status	CT + CC vs. TT		CC vs. CT + TT		CC vs. TT		CT vs. CC		CT vs. TT	
	OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*
Overall	1.01 (0.78, 1.31)	0.93	1.48 (1.13, 1.95)	0.005*	1.49 (1.13, 1.98)	0.005*	0.68 (0.51, 0.90)	0.007*	0.96 (0.74, 1.24)	0.73
ε4 positive	1.10 (0.83, 1.44)	0.52	1.33 (0.79, 2.24)	0.28	1.40 (0.82, 2.38)	0.22	0.78 (0.44, 1.36)	0.38	1.03 (0.77, 1.37)	0.85
ε4 negative	1.03 (0.85, 1.23)	0.79	1.53 (1.07, 2.17)	0.02*	1.52 (1.05, 2.18)	0.03*	0.65 (0.45, 0.93)	0.02*	0.96 (0.74, 1.24)	0.68

[a] *OR* odds ratio, *CI* confidence interval, *vs.* versus

* Significant association between *CYP46A1* T/C polymorphism and AD risk; $p \leq 0.05$

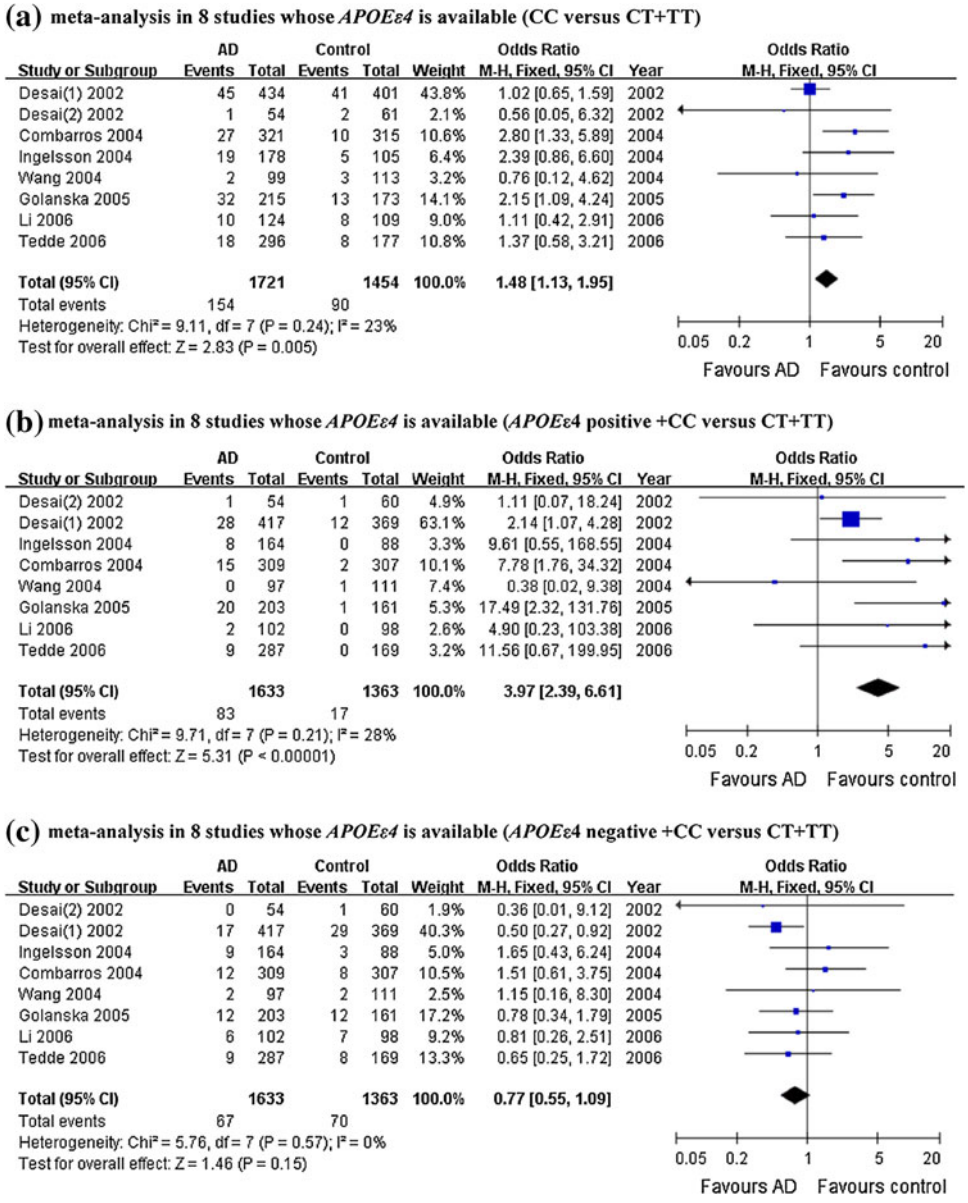

Fig. 3 Meta-analysis of eight case–control studies for which data of *APOEε4* is available about the *CYP46A1* T/C polymorphism and the risk for AD using the fixed-effects model. The genotype effect for CC versus CT + TT was estimated for each study. The pooled OR for CC versus CT + TT was also calculated. The OR and 95 % CI for the effect for all comparisons are plotted on the graph. Studies are arranged chronologically based on the year of publication. **a** Meta-analysis in eight studies whose *APOEε4* is available (CC vs. CT + TT), **b** meta-analysis in eight studies whose *APOEε4* is available (*APOEε4* positive +CC vs. CT + TT), **c** meta-analysis in eight studies whose *APOEε4* is available (*APOEε4* negative +CC vs. CT + TT)

T/C could increase the risk of AD in the recessive model (CC versus CT + TT, OR = 1.20, 95 % CI = 1.04–1.38, $p = 0.01$) on overall analysis. When stratifying by ethnicity, we obtained similar results just in Europeans in the recessive model (CC versus CT + TT, OR = 1.22, 95 % CI = 1.05–1.43, $p = 0.01$), but no association was found in either Asians or Africans at all. This result suggests that homozygous CC had a more obvious effect on an individual's phenotype than did CT and TT, so individuals with the CC genotype could have higher risk of AD. In spite of

negative results in non-Europeans, sufficient evidence could confirm the role of the CC genotype of *CYP46A1* T/C in clinical evaluation, because only four studies involving Asians and only one study involving Africans were included in our meta-analysis. To clarify this kind of association in Asians and Africans, more studies are required.

Most of the genetic association studies on AD have either evaluated single genetic variation at one single time or have analyzed multiple variations independently, rather than estimating the size of the effect associated with gene–gene interaction. As a result, little information as to whether the impact of genetic variants on AD risk is simply the sum of their individual effects or whether more complex interactive effects are available. To assess the synergistic effect of the *CYP46A1* T/C polymorphism and *APOE ε4* allele on AD Risk, we further conducted meta-analysis in the studies with available stratified information (eight studies, containing 1,721 AD cases and 1,454 controls). Three important findings were obtained: (1) the presence of the APOEε4 allele was a lager risk factor for AD than the *CYP46A1* T/C polymorphism, even likely to be an independent risk factor; (2) the absence of the *APOE ε4* allele could weaken the effect of the CC genotype on AD risk, but could not counteract it completely; (3) accordingly, the presence of the *APOE ε4* allele could strengthen the effect of the CC genotype on AD risk.

The *CYP46A1* T/C polymorphism could increase the risk of AD with the synergistic influence of the *APOE ε4* allele, mainly due to the same metabolic pathways of cholesterol in the brain. Cholesterol in the brain, containing approximately one-fourth of total body cholesterol, is a major component of myelin and of neuronal and glial membranes [44]. It is synthesized in situ and cannot be degraded locally. Excess cholesterol must be conversed by CYP46 into 24S-hydroxycholesterol which easily traverses the blood–brain barrier (BBB) first [10], and then transports on lipoproteins that resemble HDL particles, which can circulate in the cerebrospinal fluid and bring the 24S-hydroxycholesterol to BBB. By this process, approximately 6–7 mg of cholesterol leaves the human brain each day. During this process, *APOE* and several members of the adenosine triphosphate cassette transporter family, such as *ABCA1,* are believed to play major roles in regulating lipid and lipoprotein homeostasis in the central nervous system (CNS). But as one, both of the two cholesterol related genes play essential roles in elimination of cholesterol in the brain, finally modulating Aβ deposition and clearance.

Some limitations of our meta-analysis should be acknowledged. First, the sample sizes in most of the included studies were small, which could increase the probability of false positives or false negatives. Second, many factors participate in the progression of AD, and we did not carry out subgroup analyses based on other factors such as age, gender, sex, education attainment or blood cholesterol level due to a lack of sufficient sample size. Third, the influence of bias in this analysis could not be completely excluded, because studies with positive results are easier to publish. Moreover, we have not tested the publication bias of meta-analysis in the eight studies stratified by the *APOE ε4* carrier status, because Egger's test usually requires at least ten studies to detect it.

In conclusion, our meta-analysis provides evidence that the *CYP46A1* T/C polymorphism could increase the risk of AD in recessive model (CC versus CT + TT). The CC genotype of the *CYP46A1* T/C may be a predictor for use in clinical evaluation of AD. Besides, we also confirmed the synergistic effect of the *CYP46A1* T/C polymorphism and *APOE ε4* allele that the presence of the *APOE ε4* allele could strengthen the effect of CC genotype on AD risk, and the reverse was also true. Nevertheless, further studies with large sample size, especially in subgroup analysis, should be done to confirm our findings.

Conflicts of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. Six authors of this paper are willing to provide their autographs if necessary.

Ethical standard All human studies must state that they have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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