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Original Article

Association between *COX-2* G-765C polymorphism and the risk of Alzheimer's disease: a meta-analysis

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Abstract: Background and object: The morbidity rate of Alzheimer's disease (AD) has been increasing with the progression of population aging globally, so the burden of the disease is showing an upward tendency. During the research on AD, *COX-2* gene G-765C polymorphism has been explored its function in the susceptibility to this disease, but no consensus was reached. Therefore, we performed this meta-analysis with the hope of obtaining a clearer opinion on this topic. Methods: 7 eligible articles were retrieved from online databases. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated to assess the strength of the association between *COX-2* gene G-765C polymorphism and AD susceptibility. Inter-study heterogeneity was inspected with Q test, and sensitivity analysis was performed to verify the stability of pooled results. Additionally, publication bias between included studies was detected using both Begg's funnel plot and Egger's test. Results: *COX-2* gene G-765C polymorphism decreased the risk of AD under five genetic comparisons of CC vs. GG, CC+GC vs. GG, CC vs. GG+GC, C vs. G and GC vs. GG, and a similar trend was also revealed in Caucasian and other-ethnicity groups after stratification analysis of ethnicity under corresponding contrasts. Conclusion: The C allele of the G-765C polymorphism in the *COX-2* gene may provide protection against AD onset.

Keywords: Alzheimer's disease, prostaglandin-endoperoxide synthase 2, COX-2, polymorphism, susceptibility, prostaglandin

Introduction

Alzheimer's disease (AD) is a primary neurodegenerative disease common in presenile and senile people, with latent onset and slow progression [1]. Mainly damaging mentality, AD can show some distinct intracephalic pathological characteristics, such as neurofibrillary tangle (NFT), extracellular deposition of amyloid protein beta, the formation of senile plaques around nerve cells and capillaries, cytotoxic edema and neuron loss [2, 3]. Additionally, these damages primarily occur in basal forebrain (BF), hippocampus and cerebral cortex [4]. As one of the main health problems in developed countries, AD is featured by inflammatory lesions in nerve [5, 6], and can decrease the patients' capabilities of learning and memory [7-9]. Although the formation and pathogenesis of AD remain unclear, modern medicine has proposed some potentially relevant elements, like genetic factors, neurotransmitter, free radicals, immune system dysfunction, and the toxicity of amyloid protein beta and environmental factors. Consequently, these possible aspects, including genetic polymorphisms, have been discussed their associations with the susceptibility to AD at home and abroad.

Cyclooxygenase-2 (COX-2) enzyme is an important catalyst in the production of prostaglandin (PG), and quickly increases its expression affected by incitants like cytokines, growth factors, inflammatory mediators and hormones [10, 11]. Researches have demonstrated that COX-2 can promote angiogenesis and inhibit cell apoptosis due to the effect of long-term chronic inflammation or other factors [12]. Inducible COX-2 is the main active enzyme in cellular inflammatory responses, and expresses higher in people with cerebral ischemia, encephaledema, epilepsy and senile dementia [13]. The expression of COX-2 can accelerate the generation of amyloid plagues, and this

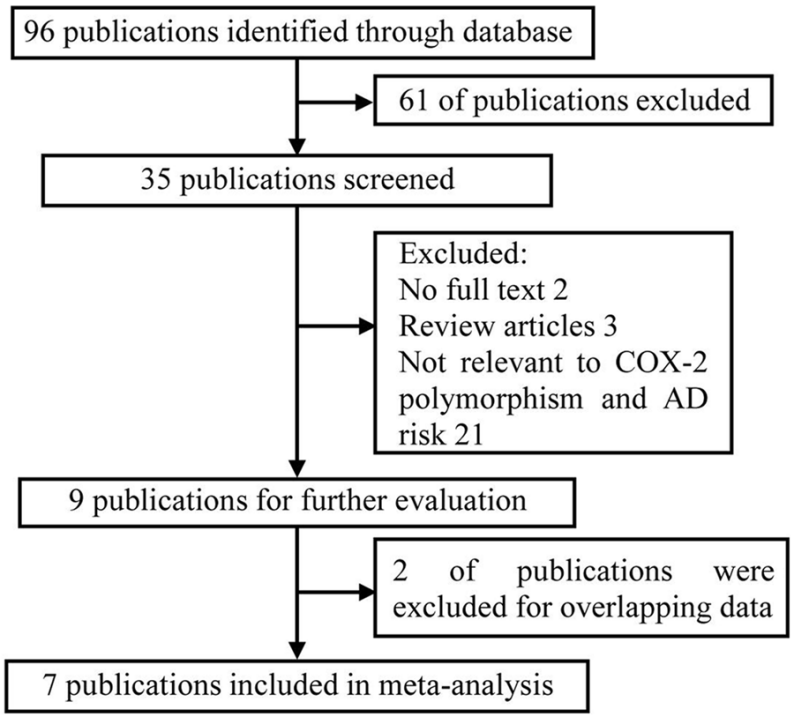

Figure 1. Flowchart for literature selection with specific exclusion reasons.

function leads to the initiation of chronic inflammatory responses and to consistently increase of inflammatory products, thus widely affecting neuron growth, plasticity, damage and degeneration [14]. Reportedly, the polymorphisms in the promoter region of *COX-2* gene can critically impact the expression of the gene [15], and the polymorphisms G-765C and G-1195A are the most commonly studied ones in this gene.

Studies have been carried out to ascertain the role of the G-765C polymorphisms of *COX-2* gene in AD susceptibility, but their findings did not reach a uniform conclusion. Hence, we combined previously relevant studies to achieve a more reliable perspective on this matter in this meta-analysis.

Materials and methods

Literature searching strategy

A systemic retrieval of relevant publications in English or Chinese language was carried out in the online databases of PubMed, EMBASE, Google Scholar Web, CNKI and Wanfang, using the combination of the keywords as follows: "Alzheimer's disease" or "Alzheimer disease" or "Alzheimer's", "prostaglandin-endoperoxide synthase 2" or "PTGS2" or "COX-2" or "PGHS-2", and "polymorphism" or "mutation" or "variant". Additionally, a manual search of the references in relevant papers was completed for other possibly eligible articles.

Selection criteria

The predefined criteria for all included studies consisted of the following aspects: (1) with a case-control design; (2) evaluating the relationship between *COX-2* gene G-765C polymorphism and AD susceptibility; (3) providing sufficient information about genotype distribution in both case and control groups; and (4) limited to human beings. Literature was excluded if they were letters, commentaries, case reports, conference abstracts or review articles. As for reports with overlapping data, the one would be ultimately incorporated into our study which covered the largest dataset.

Data extraction

Principal information of each eligible study was extracted separately by two investigators, and contained first author's name, publication year, original country, ethnicity, source of control, genotyping method, genotype frequencies in cases and controls as well as *P* value for Hardy-Weinberg equilibrium (HWE) in control group. Disagreements over abstracted data were settled through discussion between the two investigators, and a third investigator would be consulted if the discussion could not reach a consensus.

Statistical analysis

All data analyses were conducted with STATA 12.0 software (Stata Corporation, College Station, TX, USA). The intensity of the association between *COX-2* gene G-765C polymorphism and AD risk was assessed through calculating pooled odds ratios (ORs) with the corresponding 95% confidence intervals (95% CIs). Heterogeneity between included studies

Table 1. Essential information of included studies

First author-Year	Ethnicity (Country)	Control source	Sample size (Case/Control)	Genotyping method	Case			Control			HWE
					GG	GC	CC	GG	GC	CC	
Abdullah-2006	Caucasian (USA)	Population-based	168/161	PCR	122	42	4	96	57	8	0.902
Feber-2010	Caucasian (Hungary)	Population-based	237/245	PCR-RFLP	177	56	4	146	81	18	0.156
Michele-2014	Caucasian (Italy)	Population-based	84/80	PCR-pyrosequencing	55	26	3	49	24	7	0.124
Tang-2013	Asian (China)	Population-based	244/226	PCR-RFLP	229	15	0	210	16	0	0.581
Toral-Rios-2015	Mix (Mexico)	Population-based	94/100	TaqMan	80	13	1	65	32	3	0.692
Listi-2010	Caucasian (Italy)	Population-based	341/190	PCR	237	94	10	115	62	13	0.252
Juhasz-2008	Caucasian (Hungary)	Population-based	123/123	PCR	80	43		56	67		/

Notes: PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; TaqMan, TaqManSNP; HWE, Hardy-Weinberg equilibrium.

Table 2. *COX-2* gene G-765C polymorphism and Alzheimer's disease susceptibility

Genetic comparison	Group	OR (95% CI)	*P* value for heterogeneity	Model for analysis
CC vs. GG	Caucasian	0.31 (0.18, 0.53)	0.733	
	Other-ethnicity	0.27 (0.03, 2.67)	/	
	Total	0.31 (0.18, 0.52)	0.861	
CC+GC vs. GG	Caucasian	0.58 (0.47, 0.70)	0.493	
	Other-ethnicity	0.51 (0.31, 0.84)	0.059	
	Total	0.57 (0.47, 0.68)	0.312	
CC vs. GG+GC	Caucasian	0.35 (0.20, 0.59)	0.774	
	Other-ethnicity	0.35 (0.04, 3.40)	/	Fixed
	Total	0.35 (0.21, 0.58)	0.892	
C vs. G	Caucasian	0.60 (0.50, 0.72)	0.504	
	Other-ethnicity	0.53 (0.33, 0.84)	0.082	
	Total	0.59 (0.50, 0.70)	0.350	
GC vs. GG	Caucasian	0.66 (0.53, 0.83)	0.514	
	Other-ethnicity	0.52 (0.32, 0.87)	0.068	
	Total	0.64 (0.52, 0.78)	0.284	

was examined with chi-square-based Q test whose *P* value more or less than 0.05 represented the absence or presence of significant heterogeneity, thus determining the use of fixed- or random-effects model for calculating ORs. To test the stability of pooled results, sensitivity analysis was carried out through sequential omission of each included study to observe alteration in whole results before and after omission. Begg's funnel plot and Egger's regression test were adopted to visually and statistically inspect the significance of between-study publication bias [16, 17].

Results

Study characteristics

Initially, the literature searching strategy identified 96 potentially relevant publications, and 61 of them were excluded in primary check. Consequent screening further eliminated 28 more ineligible papers, and 7 articles were finally incorporated in this meta-analysis [18-24]. **Figure 1** displays the details of literature selection with specific exclusion reasons. Essential information of all included studies is listed in **Table 1**.

Quantitative data synthesis

As shown in **Table 2**, *COX-2* gene G-765C polymorphism reduced the risk of AD by 36% to 69% in overall analysis under CC vs. GG (**Figure 2**), CC+GC vs. GG, CC vs. GG+GC, C vs. G and GC vs. GG contrasts (OR=0.31, 95% CI=0.18-0.52; OR=0.57, 95% CI=0.47-0.68; OR=0.35, 95% CI=0.21-0.58; OR=0.59, 95% CI=0.50-0.70; OR=0.64, 95% CI=0.52-0.78). Additionally, after subgroup analysis by ethnicity, this polymorphism also exerted a similar effect in Caucasian group under the same five contrasts and in other-ethnicity group under CC+GC vs. GG, C vs. G and GC vs. GG comparisons. These results indicated the protective role of the C

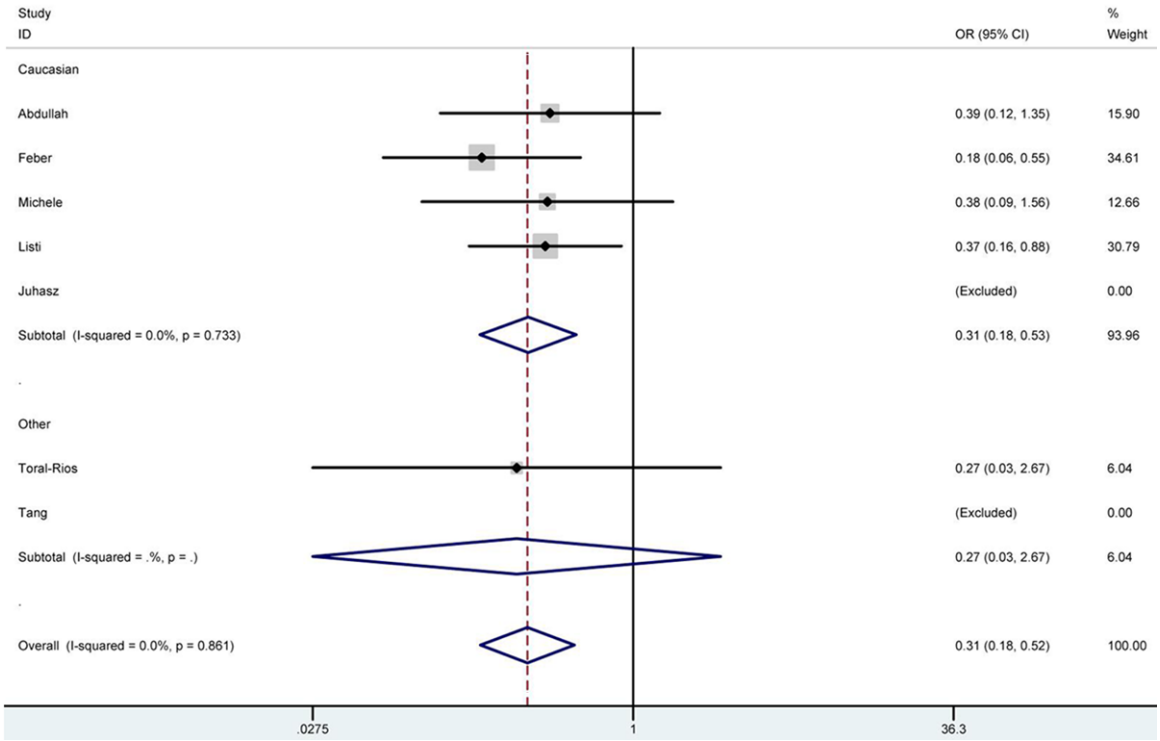

Figure 2. Forest plot for the association between *COX-2* gene G-765C polymorphism and Alzheimer's disease susceptibility under CC vs. GG contrast.

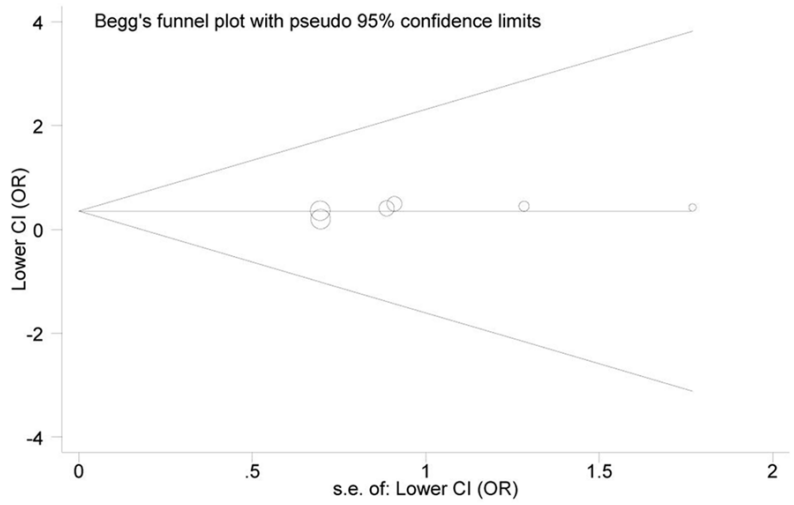

Figure 3. Begg's funnel plot for publication bias.

allele of *COX-2* gene G-765C polymorphism against AD occurrence.

Heterogeneity test

P value more than 0.05 in Q test declared there was no significant heterogeneity across selected studies under any one of the five genetic contrasts, so the fixed-effects model was employed to calculate pooled ORs.

Sensitivity analysis

In the whole procession of sensitivity analysis, no substantial alteration occurred between pooled results before and after single-study deletion (data not shown), showing the statistical robustness of the results.

Publication bias investigation

The shapes of Begg's funnel plots seemed symmetry (**Figure 3**), implying publication bias was negligible. Furthermore, these results were statistically supported by data from Egger's test (*P*=0.285).

Discussion

AD is a senile disease with progressive dementia as the main symptom. Amidst the progres-

sion of population aging all over the world, this disease has shown a rising tendency in its morbidity rate. AD clinical demonstrations mainly contain progressive dysmnesia, gradual degeneration of cognitive functions, the decrease of attention and judgment, personality changes and difficulties in language communication. Since these neurological and psychiatric symptoms severely undermine the health and life quality of AD patients, this disease has imposed heavy burden on social and families, becoming one of the hot topic of research in medicine. The precise etiology of AD has not been completely understood yet, but genetic polymorphisms have been manifested to play substantial roles in the initiation and progression of this disease.

Cyclooxygenase (COX) is an important rate-limiting enzyme in catalyzing arachidonic acid to generate PGs, and takes part in multiple pathological and physiological processes, especially in inflammation and tumors. As one of the three isozymes of COX in body, COX-2 has low expression only in particular organs such as brain and kidney under normal situations, and expresses higher in pathological states when induced by inflammatory cytokines or tumor promoters. Its close relationship with inflammatory process has been confirmed by numerous evidence from researches, and its increased expression has also been suggested to be closely correlated with the occurrence and development of multiple tumors, including colorectal cancer [25], gastric cancer [26], pancreatic cancer [27], lung cancer [28], prostate cancer [29] and osteosarcoma [30]. Constitutively expressing in brain cortex, hippocampus, amygdala, hypothalamus and spinal cord in adults [31], COX-2 participates not only in physiological processes, like synaptic transmission of excitatory neurons, inflammations, hyperpathia and various cerebral injuries, but also in pathological processes of AD and Parkinson's disease (PD). It has been found that the expression of COX-2 protein of hippocampal pyramidal neurons in regions CA1-CA4 was distinctly higher in AD patients than in controls, and that the content of COX-2 was closely related to the density of amyloid granules [32]. In addition, the expression of COX-2 increased in the frontal cortex of AD patients, with elevated level of PGs metabolized by COX in cerebrospinal fluid. Encoding COX-2 protein, human *COX-2* gene is located on the chromosome 1 with 10 exons and 9 introns. In the promoter regions of the gene, there are multiple binding sites regulating the expression of COX-2, therefore, the polymorphisms in these promoter regions could affect the bindings in these regions, and thus alter the expression and functions of COX-2 via changing the transcription of the gene, eventually producing great differences in susceptibility to diseases between different individuals.

Among the most studied polymorphisms of the *COX-2* gene, we determined to make the G-765C polymorphism as our target in this study. After statistical analysis, this polymorphism expressed a significantly reducing effect on AD susceptibility both in total analysis and in subgroup analysis by ethnicity. As shown in **Table 2**, this polymorphism overall decreased the risk of AD by 36% to 69% under the five genetic comparisons, indicating the protective role of C allele against this disease. However, among previous studies, two of them got a conclusion that this polymorphism might not significantly associated with AD risk in Sicilian population and Chinese population, respectively [22, 23]. Considering the sample size of the former was the smallest among our included studies while the latter was the only one focusing on Asian population, we hypothesized that the discrepancies between these results might be attributed to but not limited to the following aspects: small sample size and different genetic backgrounds.

Our findings were obtained on the basis of eligible studies through strict screening, so they had certain reliability and power. Additionally, a series of verification such as heterogeneity test, sensitivity analysis and publication bias investigation provided more evidence for the credibility of our results. Having said that, it is better to be cautious when interpreting these findings because of some inevitable shortcomings in the present meta-analysis. For example, the number of included studies was relatively small, which might result from the language and source limitations in literature searching strategy, and thus reduced the comprehensiveness of the results. Meanwhile, the majority of original data from included studies concerned Caucasian populations; as a result, the representative strength of the results in other ethnicities was weakened. Moreover, other poten-

tially relevant factors or possible interactions between our determined polymorphism and certain respects were not further discussed in this study due to limited data.

In summary, the results in our meta-analysis displayed a protective function of the C allele of the G-765C polymorphism in *COX-2* gene against AD susceptibility. In view of those limitations in this study, our findings need to be further verified in future by better-designed studies containing larger sample size and more considerations of other possible factors as well as gene-gene and gene-environmental interactions.

Disclosure of conflict of interest

None.

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