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Systematic Review

Confirmed Synergy Between the ε4 Allele of Apolipoprotein E and the Variant K of Butyrylcholinesterase as a Risk Factor for Alzheimer's Disease: A Systematic Review and Meta-Analysis

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Abstract.
Background: Alzheimer's disease (AD) has several risk factors. *APOE4* is the main one, and it has been suggested that there may be a synergy between it and *BCHE-K* as a risk factor.
Objective: To investigate the association between *APOE4* and *BCHE-K* as a risk factor for AD.
Methods: We searched PubMed, Web of Science, Embase, and Scopus on August 8, 2021 for studies that analyzed the association of *APOE4* and *BCHE-K* with AD. The random effect model was performed in meta-analysis according to age group. A chi-square was performed with the meta-analysis data to verify if the effect found is not associated only with the E4 allele.
Results: Twenty-one studies with 6,853 subjects (3,528 AD and 3,325 Controls) were included in the meta-analysis. The quality of the evidence is moderate. There is a positive E4-K association for subjects with AD as shown by the odds ratio of 3.43. The chi-square meta test, which measures the probability that the E4-K association is due to chance, has an odds ratio of 6.155, indicating that the E4-K association is not a random event. The odds ratio of an E4-K association in subjects with AD increases to OR 4.46 for the 65- to 75-year-old group and OR 4.15 for subjects older than 75 years. The probability that the E4-K association is due to chance is ruled out by chi-square meta test values of OR 8.638 and OR 9.558.
Conclusion: The synergy between *APOE4* and *BCHE-K* is a risk factor for late-onset AD.

Keywords: Alzheimer's disease, *APOE*, BaβAC, cholinesterase, dementia, genetics, odds ratio

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative and irreversible disease, characterized by the presence of senile plaques with extracellular deposits of amyloid- β (Aβ) protein, called amyloid plaques and fibrillar tangles, composed of the hyperphosphorylated tau protein, causing damage to neurons [1]. Such neuronal damage results in brain atrophy, memory loss, neuromotor impairment, general brain degradation, and death [2].

There are several factors that can lead to the establishment of the clinical case of AD, being considered a multifactorial disease [3]. Among these factors, the main ones include impaired lifestyle (sedentary lifestyle, alcoholism, smoking, unhealthy diet), exposure to pesticides, depression, mild cognitive impairment, and genetic mutations [1, 3, 4].

Mutations in *APP, PSEN1, PSEN2, ABCA7*, and *SORL1* genes have been linked to early-onset and familial AD. The mutations in genes identified so far that are related to late-onset AD include: *ABCA7, BIN1, CASS4, CD33, CD2AP, CELF1, CLU, CR1, DSG2, EPHA1, FERMT2, HLA-DRB5-HLA-DRB1, INPP5D, MEF2 C, MS4A6A/MS4A4E, NME8, PICALM, PTK2B, SLC24A4, SORL1, ZCWPW1, TREM2*, and *APOE* [5]. Among these, the gene that encodes apolipoprotein E (apoE), located on chromosome 19, has an allele (E4) that has been the main one related to increased susceptibility to this disease [5–7].

A genetic association between *APOE4* and the variant K of butyrylcholinesterase (*BCHE-K*) has been suggested as a possible cause of increased chance of establishing AD [8]. Butyrylcholinesterase (BChE) is an enzyme synthesized from the *BCHE* gene and acts on the degradation of acetylcholine in the synaptic cleft, with greater prevalence in damaged regions of the brain by reaction to the accumulation of Aβ [9–11]. This association hypothesis had as its initial proposition the interaction observed between the enzyme BChE and neurofibrillary tangles, added to evidence that the K variant has less activity than its wild-type form [12–14].

Several studies have confirmed this hypothesis of association and others have not [8, 15, 16], for this reason the purpose of this study was to develop a systematic review and meta-analysis guided to answer the following question: Is there an association between *APOE4* and *BCHE-K* with AD?

Therefore, the PICO strategy is based on people with AD as a Population and for Intervention, it was the genotyping of the *BCHE-K* and *APOE4* alleles. For Comparison, a corresponding control group was analyzed and for Outcome, the number of events with or without association between the analyzed genes was verified.

MATERIALS AND METHODS

Information sources

This meta-analysis was performed according to the guidelines proposed by the PRISMA statement and Meta-Analysis of Observational Studies in Epidemiology [17]. The research protocol of this systematic review was registered in PROSPERO (Supplementary Material). The PubMed (MedLine), Web of Science, Embase, and Scopus databases were last consulted on August 16, 2021 by two authors of this study. No tools were used at this stage, nor any type of external assistance beyond the research group.

Search strategy

The search strategies were built manually because when they were performed using only terms registered on the platforms, such as MeSH terms, a lack of synonyms was found, specifically the lack of the term "Apolipoprotein E" in the singular. Therefore, it was decided to build search strategies manually and individually for each platform referring to its database, in order to increase research efficiency. These strategies can be found in the Supplementary Material. The central terms for the construction of the search strategies were: "Alzheimer's Disease", "Apolipoprotein E" and "Butyrylcholinesterase K". Studies in languages other than English were later excluded.

Inclusion and exclusion criteria

Inclusion criteria were: 1) people with probable or definitive diagnosis of AD, 2) compared with a group of cognitively healthy elderly, 3) genotyping of the *APOE* and *BCHE* genes in both groups; 4) articles in English. For exclusion: 1) systematic reviews, abstracts, meta-analyses, repeated studies; 2) not presenting the results of the genotyping by groups and only the association between both, 3) not presenting the ages or the compared groups have equidistant ages.

Selection process

The selection process was carried out by two independent reviewers, the first and second authors of this work. The duplicate removal step was assisted by the Mendeley reference manager (Version 1.19.4) [18] and the RAYYAN QCRI tool [19] was used to assist in the reading step by title and abstract. The conflicts between the reviewers that arose at this stage were resolved by the decision to include the article for full reading. The inclusion of studies that, after the integral reading stage, still had conflicts between the reviewers, was resolved from the decision of a third reviewer, the third author of this work (the list of decisions about each study can be found in the Supplementary Material).

Data extraction and bias risk assessment

The data extraction process was performed by the two independent reviewers, conflicts regarding data from the studies in this process were resolved in a meeting with the third reviewer from the research group. The following data were extracted: First author name, year of publication, type of study, ethnicity, genotyping, outcome, sample size of the experimental and control groups, age, gender, number of people carrying the alleles studied. All data were extracted in an integral way, that is, each carrier of the allelic combinations in both groups as a whole number.

To assess the risk of bias in the studies, the ROBINS-I tool [20] was used, which assesses the pre-intervention, intervention, and post-intervention criteria. The assessment for each item ranges from low risk to risk of critical bias. In the meta-analysis of this review, only studies with low risk and moderate risk of bias were included and can be found in the Supplementary Material.

Meta-analysis, statistical analysis, and quality of evidence

The meta-analysis and funnel plot was performed using the RevMan application [21]. Statistical heterogeneity between studies was assessed by the Cochran Q statistic (p values < 0.10 were considered indicative of statistically significant heterogeneity) and I^2 statistic (I^2 values less than 25% represented low heterogeneity, values between 50% represented moderate heterogeneity and values greater than 75% represented high heterogeneity) [22]. The random effect was used due to the different rates of heterogeneity found in the different forest plots. The chi-square test with Yates correction was used with meta-analysis data and presented in odds ratios (OR). The odds is the ratio of the probability that the event of interest occurs to the probability that it does not [23] and OR greater than 4 is very significant. The quality of evidence was evaluated using the GRADE tool [24].

Necessary adaptations performed

During the development of the work, exceptional needs were found that had to be circumvented due to some studies found to be old, before the 2000 s, with missing standardization.

Due to the non-standardized form of age presentation and to maintain the quality of the evidence, this work incorporated standards that caused other studies (Table 1) to be excluded in the secondary analyses, the incorporated standard was the separation of age groups: younger than 65 years (<65), between 65 to 75 years (65-75), and older than (>75).

The nomenclature adopted for this systematic review was: *APOE4*(+) when there is at least one E4 allele and *APOE4*(-), when not there, the same pattern was adopted for the K variant of the *BCHE* gene, with *BCHE-K*(+) being present and *BCHE-K* (-) when it is not [8].

In order to extract data from some studies, secondary calculations had to be made and the main one was the calculation of the frequency of the different *BCHE* allele multiplied by the frequency of *APOE4* presence and absence, calculated with the total number of study participants, this was a reason that made our quality of evidence will be penalized.

There was still the possibility that this effect was found to be a "hitchhiking effect", that is, the results obtained could only be the previously confirmed effect of the e4 allele in the establishment of AD [45] and to extinguish this possibility it was necessary to carry out more tests. These tests were all allelic combination Forests plots and the chi-square meta-test.

Data were compared in a chi-square meta-test (application of the chi-square test with the meta-analysis data) with Yates correction, assuming statistically significant data when $p < 0.05$. Normally this type of test is not used in meta-analyses, but this review makes its use possible due to: 1) need beyond the dichotomous analysis that is standardized in meta-analyses and 2) most of the studies used in this review used the chi-square with the presence of a negative

Table 1
Characteristics of the included studies in meta-analysis

Year/ Study	Country	Ethnicity	Sample Size AD	Sample Size control	Gender AD (M/W)	Gender Control (M/W)	Average age AD	Was there a separation by age group?	Test performed	Outcome of the association ε4, K, and AD
1997/ Lehmann [8]	UK*	Caucasian	110	172	–	–	65.9 and 81.4	Yes	χ^2 with Yates correction and OR	Increase risk
1998/ Crawford [25]	USA	Probably Caucasian	391	201	210/181	80/121	76.4 and 72.4**	No	χ^2 and OR through logistic regression	Presence of K increase risk in non-APOE4 carriers
1998/ Kehoe [26]	UK	Probably Caucasian	184	71	73/108	–	***	No	Pearson χ^2 and logistic regression	No association
1998/ Singleton [27]	UK*	Probably Caucasian	119	120	–	–	64.8 and 82.6	Yes	χ^2	No association
1999/ Grubber [28]	USA*	Caucasian	245	241	103/142	116/125	–	Yes	χ^2 and OR	Presence of K decrease risk in APOE4 carriers
1999/ Ki [29]	Korea*	Korean	86	106	62/24	46/60	73.2 and 77.9	Yes	χ^2 and OR	No association
1999/ Sodeyama [30, 31]	Japan*	Japanese	36	86	–	–	87.5	No	χ^2	No association
1999/ Tilley [32]	UK*	Caucasian	177	118	67/110	81/37	81.6	No	χ^2 and OR through logistic regression	Increase risk in people 75 years old and
1999/ Wiebusch [15]	Canada	Caucasian	135	70	63/72	45/25	–	Yes	χ^2 with Yates correction, Fisher's exact test and OR	Increase risk

1999/ Yamamoto [33]	Japan*	Probably Japanese	476	684	–	–	–	Yes	χ^2	No association
2000/Alvarez-Arcaya [34]	Spain	Caucasian	249	250	80/169	75/175	75.3	Yes	OR and chi-square or Fisher exact test	K variant is a protective factor in woman
2000/ Lee [35]	China	Chinese	87	101	42/45	56/45	69.9	Yes	χ^2 with Yates correction and OR	No association
2000/ Mattila [36]	Finland	Caucasian	80	67	29/51	37/30	73.5 and 80.4	No	χ^2 and multinomial logistic regression	No association
2000/ MciLroy [37]	Ireland	Probably Caucasian	175	187	62/113	58/129	77.7	Yes	χ^2 with Yates correction and OR	Increase risk
2004/ Raygani [38]	Iran*	Probably Persian	105	129	45/60	45/84	75	Yes	χ^2, OR and logistic regression	Increase risk
2005/ Beyer [39]	Spain*	Probably Caucasian	206	181	77/129	61/120	72.6	Yes	χ^2, OR and logistic regression	Unclear association
2007/ Piccardi [40]	Italy*	Probably Caucasian	158	118	42/116	42/116	76.8	No	χ^2	No association
2010/ Bizarro [41]	Italy*	Probably Caucasian	167	126	63/104	62/67	73.32	No	χ^2 with Yates correction	No association
2013/ Johansson [42]	Sweden	Caucasian	28	17	14/14	9/8	74	No	Spearman rank order correlation test	Unclear association
2016/ Vijayaraghavan [43]	Norway	Probably Caucasian	97	80	31/76	42/44	74.6	No	χ^2 and Fisher exact test	No association but marked cognitive decline over the years
2017/ Gabriel [44]	Portugal*	Probably Caucasian	217	200	100/117	89/111	70.6	No	χ^2	No association

*Country of the study was not available, therefore it was deduced from the data shown in the text or at the authors' address. **The study had two groups: clinic and community, both were used simultaneously in the general group (without age separation), this was the way found to include this article. ***The study presented several groups, we finally used the CAD group (confirmed Alzheimer's disease) that the authors made available, we used the data only in general classifications (without age separation). – Data not shown.

Table 2
Chi-square meta-test between allelic combinations of the *APOE* and *BCHE* genes of people with AD and control group in the general population

APOE4	*BCHE-K*	AD	Control	*p*	Odds	CI 95%	I^2*
–	–	1116	1954	reference	reference		68%
–	+	456	689	0.0415	1.159	1.007 to 1.330	63%
+	–	1379	545	<0.0001	4.427	3.913 to 5.008	64%
+	+	580	165	<0.0001	6.155	5.109 to 7.416	48%

+=presence; –=absence; *heterogeneity referring to the allelic comparison of the Alzheimer compared to the control group.

Table 3
Chi-square meta-test between allelic combinations of *APOE* and *BCHE* genes in people with AD and control group in younger than 65 years

APOE4	*BCHE-K*	AD	Control	*p*	Odds	CI 95%	I^2*
–	–	94	176	reference	reference		0%
–	+	25	38	0.5619	1.232	NS	38%
+	–	85	58	<0.0001	2.744	1.797 to 4.165	0%
+	+	26	19	0.0056	2.562	1.357 to 4.780	0%

+=presence; –=absence; *heterogeneity referring to the allelic comparison of the Alzheimer compared to the control group.

Table 4
Chi-square meta-test between allelic combinations of *APOE* and *BCHE* genes in people with AD and control group with people aged 65 to 75 years

APOE4	*BCHE-K*	AD	Control	*p*	Odds	CI 95%	I^2*
–	–	229	602	reference	reference		32%
–	+	81	171	0.1831	1.245	NS	1%
+	–	403	171	<0.0001	6.195	4.878 to 7.834	53%
+	+	138	42	<0.0001	8.638	5.898 to 12.650	38%

+=presence; –=absence; *heterogeneity referring to the allelic comparison of the Alzheimer compared to the group.

Table 5
Chi-square meta-test between allelic combinations of *APOE* and *BCHE* genes in people with AD and control group with people older than 75 years

APOE4	*BCHE-K*	AD	Control	*p*	Odds	CI 95%	I^2*
–	–	230	469	reference	reference		42%
–	+	151	153	<0.0001	2.012	1.529 to 2.652	73%
+	–	259	118	<0.0001	4.476	3.427 to 5.863	75%
+	+	150	32	<0.0001	9.558	6.372 to 14.570	3%

+=presence; –=absence; *heterogeneity referring to the allelic comparison of the Alzheimer compared to the control group.

reference, the Yates correction was applied due to the use by the first study on the subject (Table 1).

The negative reference in the chi-square test with Yates correction in this study is the use of a group with AD and the control, both without the presence of the E4 allele and the K variant, for comparison with the AD and Control groups, and the presence of alleles E4 and variant K occurs according to the calculation tested (Tables 2–5).

The use of a negative reference enables a more accurate answer than the dichotomous analysis of the meta-analysis in a study of genetic synergism (that is why most of the included studies used this negative reference, see the studies in Table 1). In other words, the use of a negative reference allows a better comparison in this study: The meta-analysis allows a comparison between two categories of groups, the control group and the group with the disease under analysis, a dichotomous perspective (e.g., first group, affected by AD and APOE4/BCHE-K carriers compared with, second group, control non-APOE4/BCHE-K carriers), but this study requires the analysis of four categories of groups analyzed simultaneously made possible by the chi-square test in order to exclude the aforementioned "hitchhike effect" (e.g., first group, affected by AD and carri-

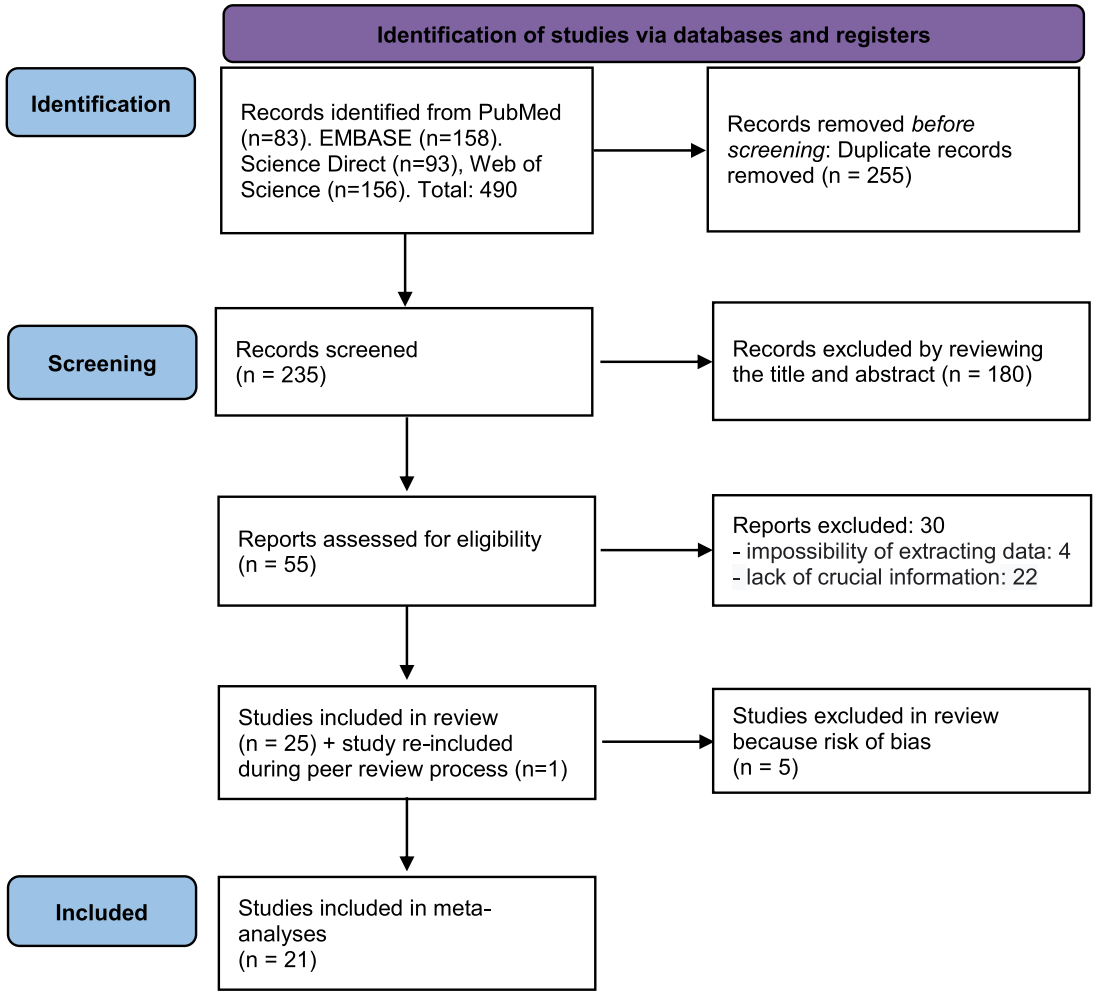


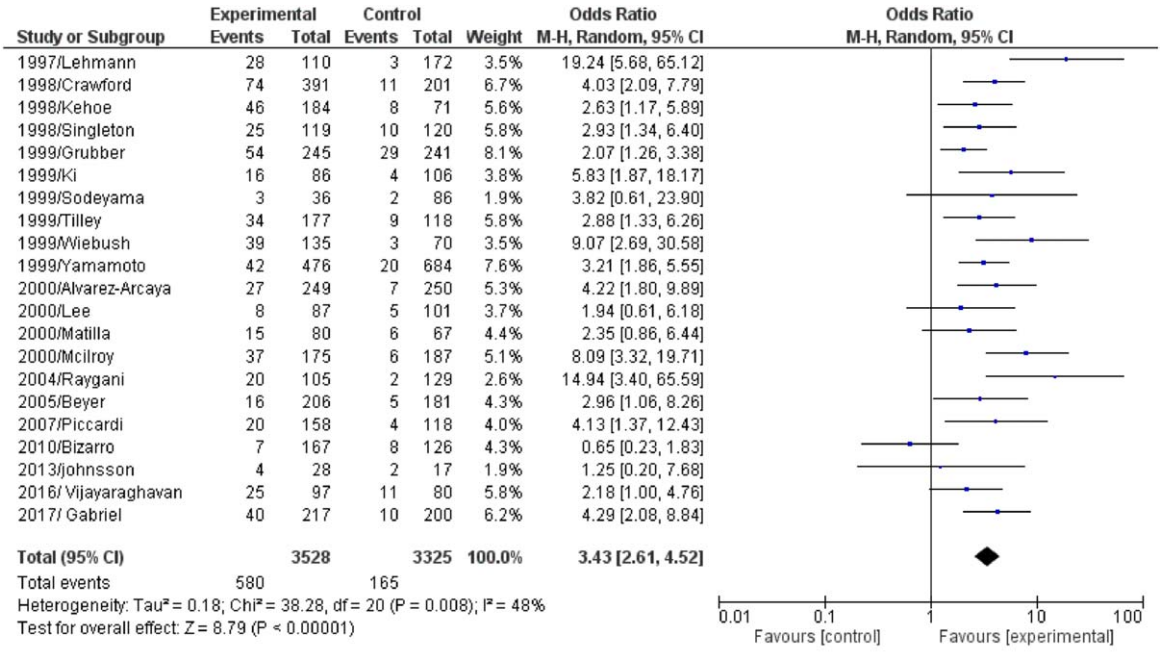
Fig. 1. Literature search flowchart.

ers APOE4/BCHE-K compared with, second group, affected by AD and APOE4/BCHE-K non-carriers, compared with, third group, APOE4/BCHE-K carrier controls and compared with, fourth group, APOE4/BCHE-K non-carrier controls).

RESULTS

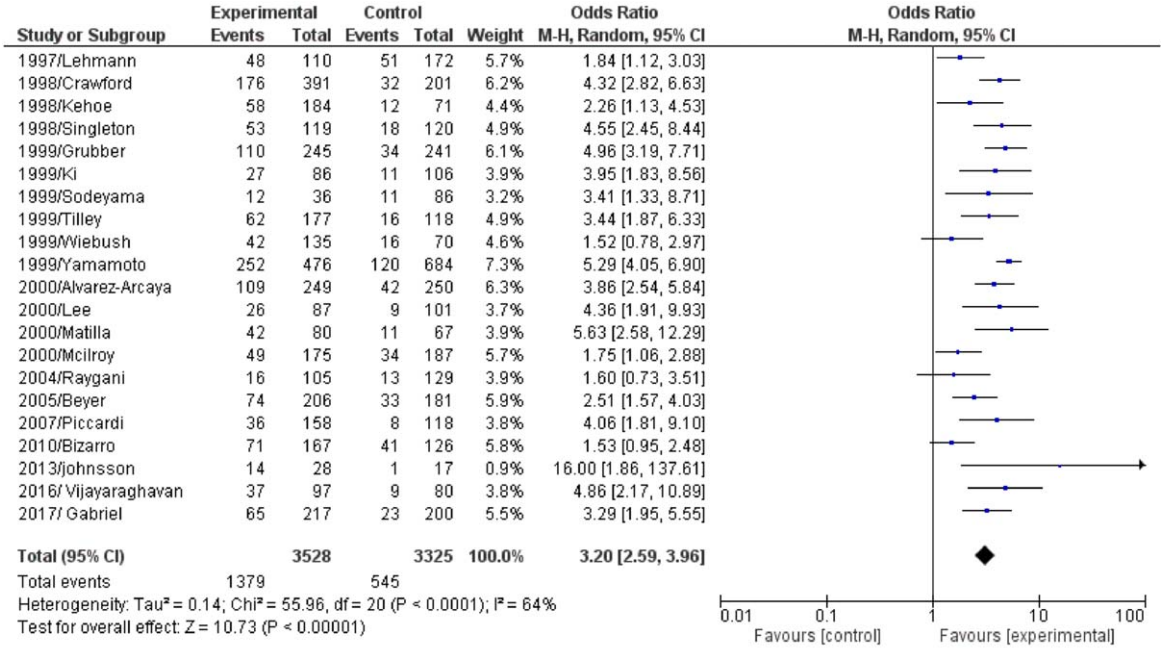
In total, 490 studies were found using the proposed terms. After removing duplicates, 235 articles were read by title and abstract. Of these 235, 55 were selected for full reading of the publication, where 30 articles were excluded for not meeting the inclusion criteria, thus leaving 25 articles for inclusion. During the peer review process 1 previously excluded article was requested to be re-included. However, it was decided to keep only articles with low and moderate risk of bias in the meta-analysis, which led to the exclusion of 5 articles, leaving 21 articles in the general meta-analysis (Fig. 1). The quality of the evidence, performed with the GRADE tool [24], was considered moderate (Supplementary Material).

Dichotomous comparisons (AD compared to Control) without age separation of *APOE4*(+)/*BCHE-K*(+) resulted in OR = 3.43 with CI = 2.61-4.52 ($p < 0.00001$) and *APOE4*(+)/*BCHE-K*(-) resulted in OR = 3.20 with CI = 2.59-3.96 ($p < 0.00001$) (Figs. 2 and 3). Figures 4–6 show the Forest plot's *APOE4*(+)/*BCHE-K*(+) in the AD group compared to control in the respective age groups: younger than 65 years old (<65), between 65 years old and 75 years old (65-75) and older than 75 years (>75). The other Forest plots and funnel plots can be found in the Supplementary Material.



APOE4(+), carrier of allele E4 of apolipoprotein E; *BCHE-K* (+), carrier of variant K of butyrylcholinesterase.

Fig. 2. Forest plot of the comparison between people *APOE4*(+)/*BCHE-K*(+) with Alzheimer's disease and control without age separation.



APOE4(+), carrier of allele E4 of apolipoprotein E; *BCHE-K* (-), non-carrier of variant K of butyrylcholinesterase.

Fig. 3. Forest plot of the comparison between people *APOE4*(+)/*BCHE-K*(-) with Alzheimer's disease and control without age separation.

Finally, the chi-square meta-tests with the data obtained, with Yates correction, comparing all the results, showed that the association *APOE4*(+)*/BCHE-K*(+) is a risk factor, and is superior the only presence of the E4 allele, evidencing the pathogenic role of the K variant (Tables 2–5).

The results found are related to the presence or absence of at least one allele of each of the genes studied (i.e., *APOE4*/e-+*BCHE-K*/-), considering that few studies showed genotyping in homozygosity for *APOE4*, and also few presented data referring to *APOE2*, preventing the calculation of the effect value of the E2-K interaction in relation to AD.

Still on the E2-K interaction, only the study by Wiebush (1999) [15] clearly showed the genotypes referring to the E2 allele and the K variant, and despite the reported low number of the E2 allele ($n = 19$, where 11 were AD), it is noteworthy that of these 11 E2 individuals, 6 had the K variant, and of these, 5 had an ε4 allele together (E2/E4, K/- in heterozygosity) and the individual who did not have the E4 allele had the K allele in homozygosity (E2/E3,K/K).

At first, the result of the association between the presence of *APOE4* and *BCHE-K* was positive for the general population, people aged between 65 and 75 years and older than 75 years (AD x Control - General population – Fig. 2: $p < 0.00001$, O.R.=3.43, 95% C.I.=2.61 to 4.52; 65 to 75 years – Fig. 5: $p < 0.00001$; O.R.=4.46, 95% C.I.=2.64 to 7.54, and; 75 Years or more – Fig. 6: $p < 0.00001$; O.R.=4.15 95% C.I.=2.71 to 6.36).

The chi-square meta-test used as a reference the data on the allelic combination both negative for E4 presence and K variant, confirmed that there is no hitchhiking effect and that the E4 allelic combination+K variant is 6.155 (O.R., 5.109 to 7.416) compared to 4.427 (O.R., 3.913 to 5.008) of the same combination without the K variant when compared to the benchmark in the general population (Table 2).

As expected, for early AD (younger than 65 years), the multifactorial risk alleles were not significant for AD origin, the OR was 2.562 (1.357 to 4.780) and was not significant ($p = 0.056$) with presence of the E4-K alleles compared to OR = 2.744 (1.797-4.165) when in the presence of only the E4 allele (Table 3).

The K variant allele synergistically with the ε4 allele evaluated in people aged 65 to 75 years has OR = 8.638 (5.898 to 12.650) compared to OR = 6.195 (4.878 to 7.834) when only the allele is present, E4 (Table 4). In the age group with people older than 75 years, the allele combination E4 and K has OR = 9.558 (6.372 to 14.570) compared to OR = 4.476 (3.427 to 5.863) in the presence of only the E4 allele (Table 5).

A secondary result was found to confirm that the K variant of the *BCHE* gene alone has an effect on the development of AD in the general population in relation to age ($p = 0.0415$, O.R.=1.159, 95% C.I.=1.007 to 1.330 – Table 2). Detailing this result in age groups, as expected it is not significant in people younger than 65 years ($p = 0.5619$ – Table 3) and is not significant in people aged between 65 and 75 years ($p = 0.1831$ – Table 4), but it is significant in people older than 75 years ($p < 0.001$, O.R.=2.012, 95% C.I.=1.529 to 2.652 – Table 5).

DISCUSSION

This study had a prevalence in the sample of Caucasian and Asian participants; none was carried out with a focus on African descents, Indigenous, and Latin patients, so the effects of the E4-K allelic combination for the previously cited ethnicities may be different from those found in this study due to ethnogenetic factors.

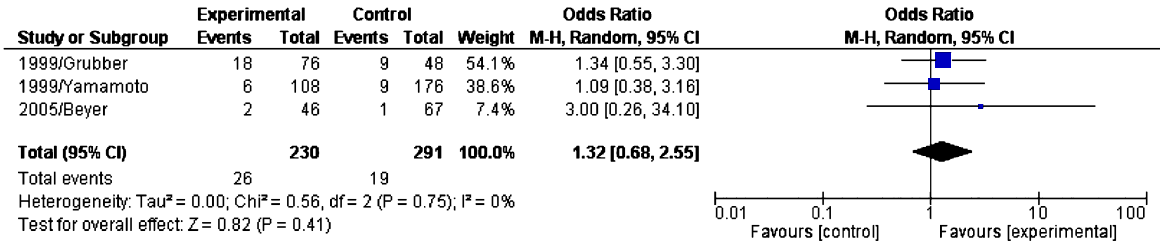
Studies on the possible association between *APOE4* and *BCHE-K* with the development of AD have shown conflicting results (Table 1). Our results are important because they help to demonstrate a positive effect of the association between *APOE4* and *BCHE-K* in LOAD, and that this association is not from a hitchhiking effect with the *APOE4* allele, but rather that the two alleles act synergistically, greatly increasing the chance development of AD, especially in people over 75 years of age.

Furthermore, the results suggest that the K variant of the *BCHE* gene alone may be a risk factor for AD in people older than 75 years. No positive influence was found on the development of early-onset AD, which may hypothesize that the role of *BCHE-K* alone or associated with certain *APOE* alleles in people younger than 65 years may be protective, but this hypothesis needs further investigation.

BChE has several functions, among them the role in cholinergic pathways in the degradation of neurotransmitters, such as acetylcholine [9] and in fat catabolism via the hydrolysis of octanoyl ghrelin into desacyl ghrelin and octanoic acid [9, 46]. The activity of the BChE-K in blood plasma has been reported to be reduced by 30% when compared to the wild-type allele [13, 14].

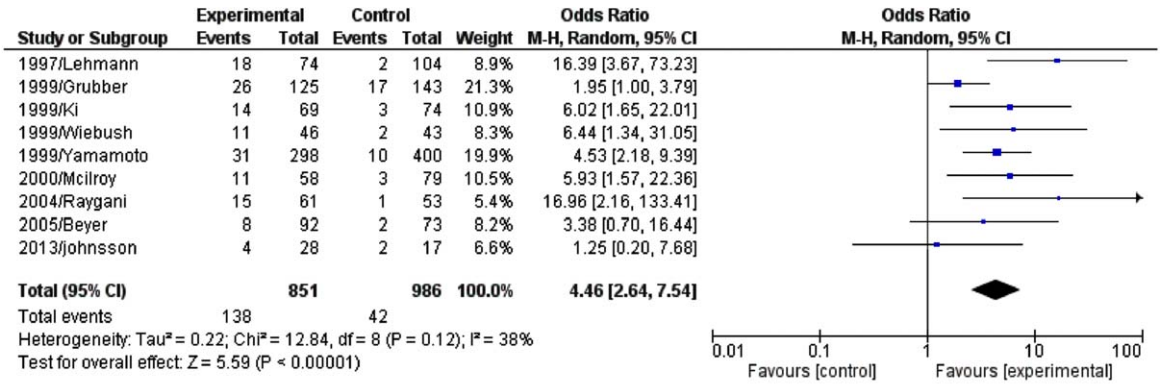
It is known that with aging there is naturally cognitive impairment [47], sarcopenia [48] and fat gain [49]. It is likely that the K variant activity deficit [9, 40, 41] is acting in synergy with these and other aging risk factors for the onset of AD.

The possible mechanism behind the results regarding the E4-K pathological association is unclear, but indirect evidence and probably the sum of these



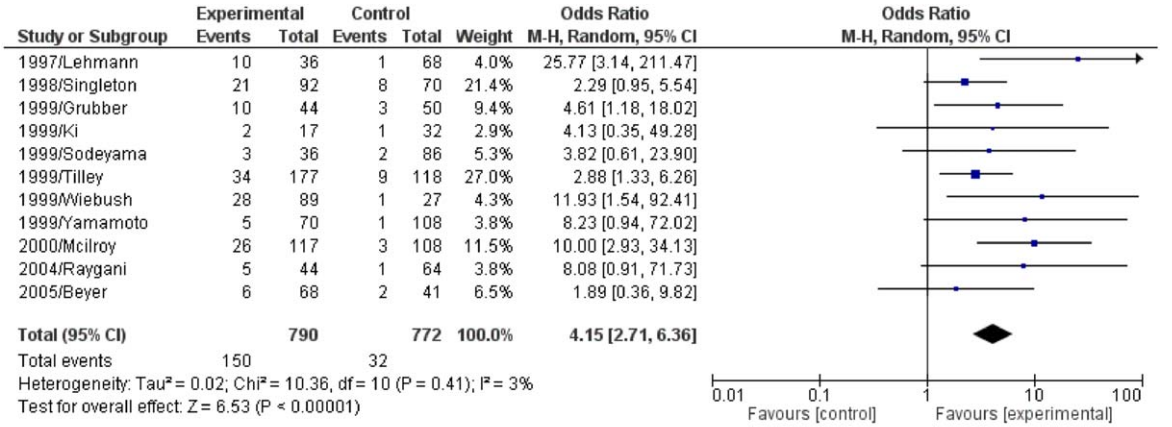
APOE4(+), carrier of allele E4 of apolipoprotein E; *BCHE-K* (+), carrier of variant K of butyrylcholinesterase.

Fig. 4. Forest plot of the comparison between people *APOE4*(+)/*BCHE-K*(+) with Alzheimer's disease and control in the population younger than 65 years old.



APOE4(+), carrier of allele E4 of apolipoprotein E; *BCHE-K* (+), carrier of variant K of butyrylcholinesterase.

Fig. 5. Forest plot of the comparison between people *APOE4*(+)/*BCHE-K*(+) with Alzheimer's disease and control in population between 65 and 75 years old.



APOE4(+), carrier of allele E4 of apolipoprotein E; *BCHE-K* (+), carrier of variant K of butyrylcholinesterase

Fig. 6. Forest plot of the comparison between people *APOE4*(+)/*BCHE-K*(+) with Alzheimer's disease and control in population older than 75 years.

BChE-K deficient factors [9, 13, 14, 50] are presented, along with the pathological role of E4 [5, 7, 51] which may provide an elucidation of these data obtained.

It has been found that *APOE4* individuals have dysregulated apoE expression, possibly by pericytes in the blood-brain barrier [52] and perhaps this dysregulation is related to Wiebush's initial evidence [15] in the E2-K interaction, where most AD individuals and with *BCHE-K* and *APOE2*, had an E4 allele together (E2/E4), indirectly suggesting, in an initial hypothesis, that the pathological role of the E4-K association is superior to the protective role of *APOE2*.

β-secretase cleaves amyloid-β protein precursor into Aβ peptide [53–55], increasing its concentration and activating secondary purification mechanisms. However, these mechanisms are deficient in E4 people [56–58], facilitating reactions such as neuroinflammation, which increases neurotoxicity.

The regions are compromised and when injured, the activity of butyrylcholinesterase increases in cholinergic degradation, replacing the primary action of acetylcholinesterase [9]. This dysregulation in the expression of E4 isoforms, accumulation of Aβ peptide and recruited BChE-K promotes the aggregation of these biochemical components in the Darreh-Shori hypothesis, which proposes the existence of a complex called BAβACs (BuChE/AChE-Aβ-ApoE Complex) [59, 60], corroborated later and reclassified into light, heavy, and ultra-heavy according to their molecular weight [60].

Our data contribute to the Darreh-Shorri theory [59–61], and it can be suggested that the pathological role of the K variant in this context is that its structural alteration in relation to wild type has a pathological synergy with E4 in the accumulation of BAβACs within the amyloid plaques by subtle molecular interaction.

This interaction may be in the sense of preventing the coupling of the E4 isoform in the initial form, since it was observed that people with AD and E4 have a lower accumulation of soluble BAβACs [59–61], explained by the inverse association between the genotype E4/E-, in which there is less accumulation of initial ApoE-Aβ complexes, resulting in high deposition of Aβ in the brain [60, 61] and there may be a positive E4-K interaction that facilitates the deposition of BAβACs within Aβ deposits.

This discrete molecular interaction is probably time-dependent, that is, over the years its effect is increasingly evident, gradually and silently interfering, mainly in the pathological accumulation of Aβ in the brain. This would explain why the E4-K association has a greater effect as a risk factor for sporadic AD in people older than 75 years than in people aged 65 to 75 years.

Conclusion

After more than two decades, the present study has brought evidence that contributes to a positive answer to the question "Is there an association between the ε4 allele of apolipoprotein E and the K variant of the *BCHE* gene with the development of AD?" raised by Lehmann, in 1997 [8], with the highest degree of scientific evidence available that the methodology of meta-analyses allows.

The authors of this study suggest that the next research involving this theme includes different ethnicities, since different statistical results in different ethnicities could indicate a possibility of investigation and, in the future, the chance of modulation of the effect of the E4-K association with AD.

If possible, there is also a suggestion to standardize the separation of groups into age groups (younger than 65 years old, between 65 to 75 and older than 75 years old), presentation of numbers between genders and *APOE4* (+) classifications: carriers of E4 allele of apolipoprotein E; *APOE4* (-): non-carriers of E4 allele of apolipoprotein E; *BCHE-K* (+): butyrylcholinesterase variant K carriers; *BCHE-K* (-): butyrylcholinesterase variant K non-carriers. The last suggestion would be to also investigate the E2 allele of apolipoprotein E with *BCHE-K* and AD.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

The data supporting the findings of this study are available within the article and its supplementary material.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/ADR-220084.

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