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JUCIMARA FERREIRA FIGUEIREDO ALMEIDA

BUSCA DE BIOMARCADORES PARA A DOENÇA DE ALZHEIMER

VITÓRIA

2023

JUCIMARA FERREIRA FIGUEIREDO ALMEIDA

BUSCA DE BIOMARCADORES PARA A DOENÇA DE ALZHEIMER

Exemplar de tese apresentada ao Programa de Pós-Graduação em Biotecnologia do Centro de Ciências da Saúde da Universidade Federal do Espírito Santo – RENORBIO, como requisito parcial para obtenção do título de doutora em Biotecnologia.

Orientadora: Profa. Dra. Flavia de Paula

VITÓRIA

2023

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Rede Nordeste de Biotecnologia

Ata da sessão da octagésima segunda defesa de Tese do Programa de Pós-Graduação em Biotecnologia da Rede Nordeste de Biotecnologia – RENORBIO, do Centro de Ciências da Saúde da Universidade Federal do Espírito Santo, da discente **JUCIMARA FERREIRA FIGUEIREDO ALMEIDA,** candidata ao título de Doutora em Biotecnologia. A defesa foi realizada às 14:00h do dia oito de fevereiro do ano dois mil e vinte e três, na Universidade Federal do Espírito Santo, por meio de videoconferência, conforme inciso 6º do Art. 51 Regulamento Geral da Pós-Graduação da UFES, aprovado na Resolução Nº 3-CEPE, de 28 de janeiro de 2022. A presidente da Banca, Profª. Drª. Flávia de Paula, apresentou os demais membros da comissão examinadora, Doutores: Adriana Madeira Alvares da Silva, da Universidade Federal do Espírito Santo, como membro titular interno; Flavia imbroisi Valle Errera, Universidade Federal do Espírito Santo, membro titular interno, Carlos Magno da Costa Maranduba, Universidade Federal de Juiz de Fora, membro titular externo; Agnes Lumi Nishimura, Queen Mary University of London, membro titular externo, Universidade Federal de Pernambuco, membro titular externo. Em seguida, cedeu a palavra à candidata que em 40 (quarenta) minutos apresentou sua Tese intitulada **"BUSCA DE BIOMARCADORES PARA A DOENÇA DE ALZHEIMER".** Terminada a apresentação, a presidente retomou a palavra e a cedeu aos membros da Comissão Examinadora, um a um, para procederem à arguição. A presidente convidou a Comissão Examinadora a se reunir em separado para deliberação. Ao final, a Comissão Examinadora retornou e a presidente informou aos presentes que a Tese havia sido **aprovada** e que a aluna deve providenciar dentro do período de 60 dias, a versão final da Tese. A Presidente, então, deu por encerrada a sessão, e eu, Breno Valentim Nogueira, Coordenador da Nucleadora UFES do Programa de Pós-Graduação em Biotecnologia, lavrei a presente ata, que é assinada pelos membros da Comissão Examinadora. Vitória, 08 de fevereiro de 2023.

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Profª. Drª Flávia de Paula.
Universidade Federal do Espírito Santo – Orientadora

_____
Profª. Drª. Adriana Madeira Alvares da Silva
Universidade Federal do Espírito Santo - Titular Interna

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Profª. Drª. Flavia imbroisi Valle Errera
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“O que está atrás de nós e o que está diante de nós são pequenas questões comparadas com o que está dentro”.

Ralph Waldo Emerson

RESUMO

ALMEIDA, J.F.F. **Busca de biomarcadores para a doença de Alzheimer.** 2023. 108f. Tese – Programa de Pós-Graduação em Biotecnologia, RENORBIO - UFES, Espírito Santo. Brasil.

A doença de Alzheimer (DA) é uma das formas mais comuns de demência na população e atualmente, no Brasil são mais de 1,2 milhões de indivíduos com DA. Na DA ocorre morte de neurônios e perda das sinapses, processos inflamatórios e atrofia cerebral causada pela formação de placas amilóides e emaranhados de neurofibrilas. Em 98% dos casos, a Doença de Alzheimer é esporádica, sendo o gene *APOE* considerado o maior fator de risco genético. O processo fisiopatológico da DA começa muitos anos antes dos sintomas clinicamente evidentes. Neste sentido, a identificação de biomarcadores genéticos permitiria um diagnóstico menos invasivo, mais preciso e com um valor mais acessível, da mesma forma poderia ser utilizado como marcador de progressão e resposta ao tratamento. Assim, este trabalho teve como objetivo principal buscar potenciais biomarcadores para a DA com o estudo dos polimorfismos nos genes *ABCA7* (rs3764650), *CR1* (rs6656401), *BIN1* (rs744373), *CLU* (rs11136000) e *MS4A6A* (rs610932) em uma meta-análise. Além disso, um estudo de associação das variantes dos genes *CASS4 (*rs911159), *TREM2* (rs75932628), *CD2AP* (rs9349407) e *MS4A4E*, (rs670139) e um estudo de risco combinado dos genes *APOE (*rs429358 e rs7412), *BIN1 (*rs744373), *ABCA7* (rs3764650) e *CLU* (rs11136000). Os resultados da meta-análise validaram o risco das variantes do gene *BIN1*, *CR1*, *ABCA7,* bem como o efeito protetor de *MS4A6A* e *CLU*. No estudo do risco combinado os resultados sugerem um efeito de risco para DA entre *APOE* com *CLU* e *APOE* com o gene *BIN1*. No estudo caso-controle houve associação positiva do polimorfismo rs911159 do gene *CASS4* com a doença de Alzheimer*.* Esses resultados além de validar estudos GWAS em uma população pouco estudada, como a população brasileira, foi importante para ampliar o entendimento da doença e aumentar a diversidade de populações estudadas no mundo em estudos genéticos para DA. Além disso, este trabalho abre caminhos para novos estudos em busca de biomarcadores de diagnóstico precoce para a doença de Alzheimer esporádica.

Palavras-chave: Doença de Alzheimer Esporádica, Biomarcadores, GWAS, Genética.

ABSTRACT

ALMEIDA, J.F.F. **Search for biomarkers for Alzheimer's disease.** 2023. 108f. Thesis – Programa de Pós-Graduação em Biotecnologia, RENORBIO - UFES, Espírito Santo. Brazil.

Alzheimer's disease (AD) is one of the most common forms of dementia in the population and currently in Brazil there are more than 1.2 million individuals with AD. In AD there is death of neurons and loss of synapses, inflammatory processes and brain atrophy caused by the formation of amyloid plaques and tangles of neurofibrils. In 98% of the cases, AD is sporadic, and the *APOE* gene acts as the most risk factor. The pathophysiological process of AD begins many years before clinically evident symptoms. In this sense, the identification of genetic biomarkers would allow for a less invasive, more accurate diagnosis with a more accessible value, in the same way it could be used as a marker of progression and response to treatment. Thus, the main objective of this work was to search for potential AD biomarkers by studying polymorphisms in the *ABCA7* (rs3764650), *CR1* (rs6656401), *BIN1* (rs744373), *CLU* (rs11136000) and *MS4A6A* (rs610932) genes in a meta-analysis. In addition, an association study of the *CASS4* (rs911159), *TREM2* (rs75932628), *CD2AP* (rs9349407) and *MS4A4E*, (rs670139) gene variants and a combined risk study of the *APOE* (rs429358 and rs7412), *BIN1* (rs744373) genes, *ABCA7* (rs3764650) and *CLU* (rs11136000). The results of the meta-analysis validated the risk of *BIN1*, *CR1*, *ABCA7* gene variants, as well as the protective effect of *MS4A6A* and *CLU*. In the combined risk study, the results suggest a risk effect for AD between *APOE* with *CLU* and *APOE* with the *BIN1* gene. In the case-control study, there was a positive association of the rs911159 polymorphism of the *CASS4* gene with Alzheimer's disease. These results, in addition to validating GWAS studies in a poorly studied population, such as the Brazilian population, were important to broaden the understanding of the disease and increase the diversity of populations studied in the world in genetic studies for AD. In addition, this work opens the way for new studies in search of early diagnostic biomarkers for sporadic Alzheimer's disease.

Keywords: Sporadic Alzheimer's Disease, Biomarkers, GWAS, Genetic.

LISTA DE FIGURAS

LISTA DE SIGLAS

Aβ42	Peptídeo β amilóide de aminoácido 42
Aβ40	Peptídio β amilóide de 40 aminoácidos
ACh	Acetilcolina
AChE	Acetilcolinesterase
ADRDA	*Alzheimer's Disease and Related Disorders Association*
APOE	*Apolipoproteina E*
APP	Proteína Precursora Amilóide
BACE1	*β site APP cleaving enzyme 1*
BIN1	*Brindging integrador 1*
CASS4	*Cas scaffold protein family member 4*
CD2AP	*CD2-associated protein*
CLU	*Clusterin*
CRAI	Centro de Referência de Atendimento ao Idoso
CT_{83}	Fragmento residual C-terminal de 83 aminoácidos
CT_{99}	Fragmento residual C-terminal de 99 aminoácidos
$CT_{57–59}$	Fragmento C-terminal de 57 à 59
DA	Doença de Alzheimer
DAE	Doença de Alzheimer Esporádica
DAF	Doença de Alzheimer Familial
DSM	*Diagnostic and Statistical Manual of Mental Disorders*
GWAS	*Genome-wide association study*
MAPT	Microtubule-associated protein tau
MS4A4E	*Membrane-spanning 4-domains subfamily A4E*
MS4A	*Membrane-spanning 4-domains subfamily A*
NINCDS	*National Institute of Neurological and Communicative Disorders and Stroke*
NMDA	Recetor N-metil-D-aspartato
PSNE1	*Presenilina 1*
PSEN2	*Presenilina 2*
PCR	*Polymerase Chain Reaction*
PET	*Positron Emission Tomography*

sAPPα	Peptídio solúvel APPα
sAPPβ	Peptídio solúvel APPβ aminoácidos
SNP	*Single Nucleotide Polymorphism*
TREM2	*Triggering receptor expressed on myeloid cells 2*

SUMÁRIO

1. INTRODUÇÃO

A população idosa mundial cresceu consideravelmente nas últimas décadas. Estima-se que 12% da população mundial tem mais de 60 anos de idade e esse número irá dobrar até 2050 (CUSTODIO et al., 2017). Consequentemente, uma maior ocorrência de doenças relacionadas com a idade, especialmente as demências (CUSTODIO et al., 2017). A doença de Alzheimer (DA) é uma das formas mais comuns de demência na população e atualmente, são mais de 46 milhões de pessoas com DA no mundo (CONDELLO; STOHR, 2016) (KADMIRI et al., 2017). Estima-se que o Brasil possua 1,2 milhões de indivíduos com DA e que a incidência seja de 100 mil novos casos por ano (MENDES, 2008).

Nos Estados Unidos estima-se que 5,4 milhões de americanos tenham a doença de Alzheimer. Além disso, espera-se que em 2050 seja quase 1 milhão de novos casos por ano (ALZHEIMER'S ASSOCIATION, 2016).

Regularmente, a perda de memória é o primeiro sintoma de DA. Com o desenvolvimento da doença, o paciente tem um declínio nas habilidades cognitivas e perda da autonomia (JIAO et al., 2015). Estudos mostram que na DA ocorre morte de neurônios e perda das sinapses, processos inflamatórios e atrofia cerebral causada pela formação de placas amilóides e emaranhados de neurofibrilas (HEINONEN et al., 1995; KANG; LEE; LEE, 2017; SCHEFF; SPARKS; PRICE, 1993).

A Doença de Alzheimer possui duas formas, a familial (DAF) e a esporádica (DAE).
A DAF tem início precoce, antes dos 65 anos de idade e corresponde a menos de 2% dos casos de DA. Esta forma da doença tem herança autossômica dominante, com mutações nos genes que codificam a proteína precursora amiloide (APP), a pré-senilina 1 (PS1) ou a pré-senilina 2 (PS2) (LAMBERT et al., 2009).

Já a DAE representa 98% dos casos e geralmente, com início dos sintomas, a partir dos 65 anos. Nestes casos, a doença possui herança multifatorial, com fatores ambientais e genéticos envolvidos na sua etiologia (YU; TAN; HARDY, 2014). Infelizmente, o diagnóstico atual da DAE ocorre quando a doença já está em estágios

avançados (JELLINGER et al., 2008). Nesse sentido, há uma necessidade para a detecção precisa na fase inicial da doença. Os estudos mostram que o processo fisiopatológico da DA começa muitos anos antes dos sintomas clinicamente evidentes (TAN; YU; TAN, 2014). Neste sentido, a identificação de biomarcadores genéticos permitiria um diagnóstico menos invasivo, mais preciso e com um valor mais acessível, da mesma forma poderia ser utilizado como marcador de progressão e resposta ao tratamento (LISTA et al., 2015).

Os estudos de associação amplo do genoma ou *Genome wide association studies* (GWAS) têm como objetivo detectar variações no genoma que estão associadas com características de interesse ou doenças. Este método busca identificar os genes que podem contribuir para o risco aumentado de desenvolver uma determinada doença complexa. Assim, estudos GWAS identificaram novos genes de suscetibilidade para DAE, como *TREM2*, *CD2AP*, *MS4A4E*, *ABCA7*, *CR1*, *BIN1* associados ao risco, e *CASS4*, *CLU* e *MS4A6A* associados a proteção. Aproximadamente 78% de todos os estudos genéticos do catálogo GWAS foram realizados em indivíduos de origem europeia (DEHGHANI; BRAS; GUERREIRO, 2021). Entretanto, muitas variantes identificadas por esses estudos não foram validadas em diferentes populações. Nesse sentido, estudar populações específicas pode sugerir um mecanismo genético diferente ou até mesmo diferentes genes envolvidos (DEHGHANI; BRAS; GUERREIRO, 2021).

Neste aspecto, tem ganhado destaque, a busca de variantes genéticas que possam ser usadas como biomarcadores para auxiliar no diagnóstico complementar da DA (FRIDMAN et al., 2004; KWOK; GU, 1999). Além disso, para melhorar a compreensão sobre os mecanismos genéticos na DA, numa população geral e em diferentes grupos étnicos é necessária uma atualização das informações através de um estudo de meta-análise. Os estudos de meta-análise são importantes, pois integram os resultados encontrados nos estudos de casos-controles e atualizam a compreensão geral das variantes relacionados a DA.

Assim, neste estudo objetivamos abordar essa questão por meio de uma grande meta-análise dos polimorfismos rs3764650 de *ABCA7*, rs6656401 de *CR1*, rs744373 de *BIN1*, rs11136000 de *CLU* and rs610932 *MS4A6A*. Além disso, um estudo de

associação das variantes rs911159 *CASS4*, rs75932628 *TREM2*, rs9349407 *CD2AP* e rs670139 *MS4A4E* na população da Grande Vitória-ES e um estudo de risco combinado das variantes rs429358 e rs7412 do gene *APOE*, rs744373 *BIN1*, rs3764650 *ABCA7* e rs11136000 *CLU*.

2. REVISÃO BIBLIOGRÁFICA

2.1 Características histopatológicas da Doença de Alzheimer

A Doença de Alzheimer foi inicialmente descrita pelo médico Aloysius Alzheimer que nasceu em 14 de junho de 1864 na Alemanha e morreu em 19 de dezembro de 1915 na Polônia (MAURER; VOLK; GERBALDO, 1997). Aloysius Alzheimer estudou medicina na Universidade de Berlin, se tornou médico psiquiatra e no ano de 1888 apresentou a Tese de doutorado em Würzburg, Alemanha (MAURER; VOLK; GERBALDO, 1997).

Em sua carreira, Aloysius investigava as doenças mentais. Aos 24 anos, Aloysius Alzheimer, foi trabalhar no Hospital Psiquiátrico de Frankfurt na Alemanha (MAURER; VOLK; GERBALDO, 1997). Em 1901, tratou uma paciente que foi diagnosticada com demência por exibir perda das faculdades mentais (MAURER; VOLK; GERBALDO, 1997). A paciente, Auguste D., tinha 51 anos de idade e apresentava mudanças no comportamento, delírios, agressividade e declínio das funções cognitivas (HIPPIUS; NEUNDÖRFER, 2003; THOMAS; FENECH, 2007). Em 1906, Auguste faleceu e Aloysius Alzheimer, após analisar o cérebro da paciente, verificou alterações histopatológicas que ainda não tinham sido descritas na literatura, como placas esféricas em todo o cérebro (THOMAS; FENECH, 2007).

Neste mesmo ano, Aloysius, na 37° Conferência de Psiquiatras da Alemanha, apresentou estas alterações cerebrais como sendo placas senis e emaranhados neurofibrilares (THOMAS; FENECH, 2007). Cerca de um ano depois, Aloysius, publicou um artigo intitulado de “*A characteristic serious disease of the cerebral cortex*” no qual relata as alterações cerebrais da paciente diagnosticada com demência (MAURER; VOLK; GERBALDO, 1997).

A grande contribuição de Aloysius Alzheimer para a medicina foi a descrição desta doença, que no ano de 1910, Emil Kraepelin, mencionou pela primeira vez a doença como Doença de Alzheimer, em seu livro livro "*The Handbook of Psychiatry*", como uma homenagem à Aloysius Alzheimer (THOMAS; FENECH, 2007).

2.2 Placas amilóides e proteína TAU

Na doença de Alzheimer há a formação das placas amilóides extracelulares e os emaranhados neurofibrilares e estas são as principais características histopatológicas cerebrais (THOMAS; FENECH, 2007).

As placas amilóides são formadas por agregados de peptídeos β amilóide de aminoácido 40 e 42 (Aβ40 e Aβ42) gerados pela processamento anormal do gene *Proteína Precursora Amilóide (APP)* (THOMAS; FENECH, 2007).

O gene *APP* codifica a proteína APP que tem papel em adesão celular, função sináptica e indução de apoptose (SUH; CHECLER, 2002). A proteína APP é abundante no sistema nervoso central e pertence a uma grande família de proteínas de membrana tipo I (NUSSBAUM; ELLIS, 2003). Esta proteína possui um longo domínio extracelular N-terminal e uma região citoplasmática C-terminal da qual origina-se por *splicing* de um mesmo transcrito de um único gene localizado no cromossomo 21q21 (NUSSBAUM; ELLIS, 2003). O processamento da APP depende de secretases distintas, e pode ocorrer pela via chamada de não-amiloidogênica e amiloidogênica (Figura 1).

A via normal, não-amiloidogênica, é realizada por um complexo enzimático de α-secretase e γ-secretase. A α-secretase cliva a proteína APP e forma um fragmento solúvel APPα (sAPPα) e um fragmento residual C-terminal de 83 aminoácidos (CT_{83}) que fica ancorado na membrana celular (AVRAMOPOULOS, 2009; THOMAS; FENECH, 2007). O fragmento C-terminal ancorado à membrana sofre uma segunda clivagem pela enzima γ-secretase que cliva o CT_{83}, e produz um peptídio chamado de p3 e um fragmento C-terminal de 57 à 59 aminoácidos ($CT_{57–59}$) (AVRAMOPOULOS, 2009; THOMAS; FENECH, 2007).

Alternativamente, pela via amiloidogênica, a APP pode ser clivada pela γ-secretase e β-secretase ou BACE-1 (β site APP cleaving enzyme). A enzima β-secretase cliva a APP formando um fragmento solúvel APPβ (sAPPβ) e um fragmento residual C-terminal de 99 aminoácidos (CT_{99}) (AVRAMOPOULOS, 2009; SUH; CHECLER, 2002;

THOMAS; FENECH, 2007). Em seguida, a γ-secretase cliva o CT_{99}, formando o fragmento de peptídeo Aβ e um fragmento C-terminal de 57 – 59 aminoácidos (CT_{57-59}) (AVRAMOPOULOS, 2009; SUH; CHECLER, 2002; THOMAS; FENECH, 2007). A γ-secretase forma dois tipos de peptídio β amilóide: Aβ40 e Aβ42 que estão presentes na placa Aβ (JARRETT; BERGER; LANSBURY, 1993). O Aβ40 é a forma não tóxica do peptídeo β amilóide enquanto que o Aβ42 é a forma neurotóxica pois tem a capacidade de se agregar formando placas, induzindo estresse oxidativo o que pode levar a apoptose celular (JARRETT; BERGER; LANSBURY, 1993).

Na doença de Alzheimer, o acúmulo de emaranhados de neurofibrilas nos neurônios também podem ser responsáveis pelo processo de neurodegeneração (KOSIK; JOACHIM; SELKOE, 1986; WOOD et al., 1986).

Os emaranhados neufibrilares são formados pela hiperfosforilação da proteína Tau que é codificada pelo gene *Microtubule-associated protein tau* (*MAPT*) e estão presentes principalmente em neurônios piramidais no hipocampo e no neocórtex cerebral (QUERFURTH; LAFERLA, 2010). A proteína Tau interage com a proteina Tubulina formando os microtúbulos (Figura 1). Os microtúbulos fazem parte do citoesqueleto e auxiliam na estabilidade e funcionamento neuronal normais (BRION, 1998; THOMAS; FENECH, 2007). Esta interação da proteína Tau é regulada por enzimas, como a Glicogênio sintase cinase 3 (GSK3) e a Quinase Dependente de Ciclina 5 (CDK5) (IQBAL et al., 2005). Na doença de Alzheimer os filamentos de proteína Tau ficam hiperfosforilados (PASTERNAK, 2007). A fosforilação anormal da proteína Tau provoca uma desagregação entre Tau e a Tubulina, desestruturando os microtúbulos. Esses eventos formam os emaranhados de neurofibrilas (figura 2) que se depositam intracelularmente nos neurônios (CASTELLANI; ROLSTON; SMITH, 2011). Com isso, os neurônios sofrem comprometimento do transporte axonal, perda sináptica e por conseguinte, morte neuronal (BRION, 1998; CASTELLANI; ROLSTON; SMITH, 2011).

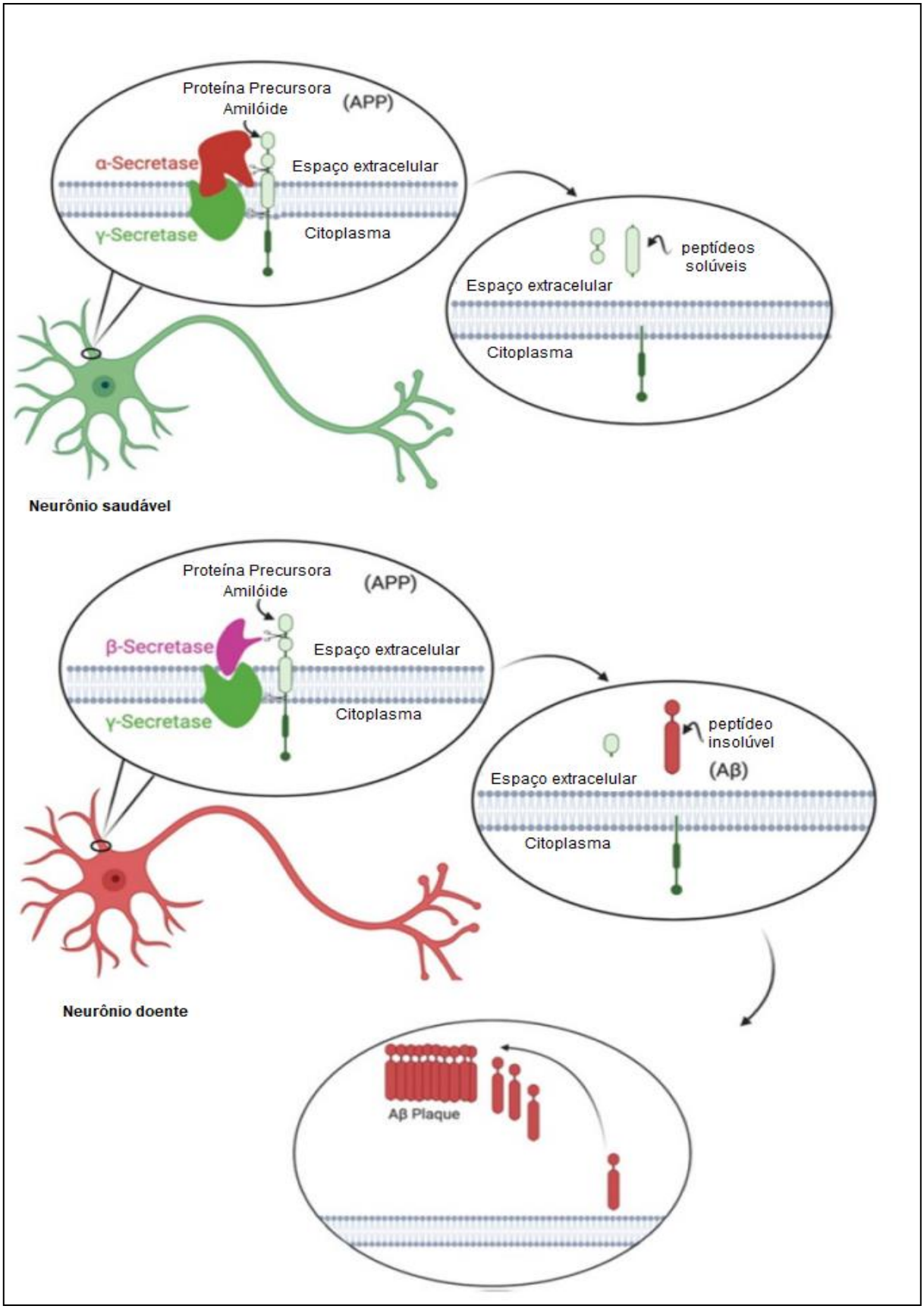


Figura 1: Imagem mostrando um neurônio saudável (superior) e degenerado (inferior) com formação de placas amilóides (Adaptado de ASHRAFIAN; ZADEH; KHAN, 2021).

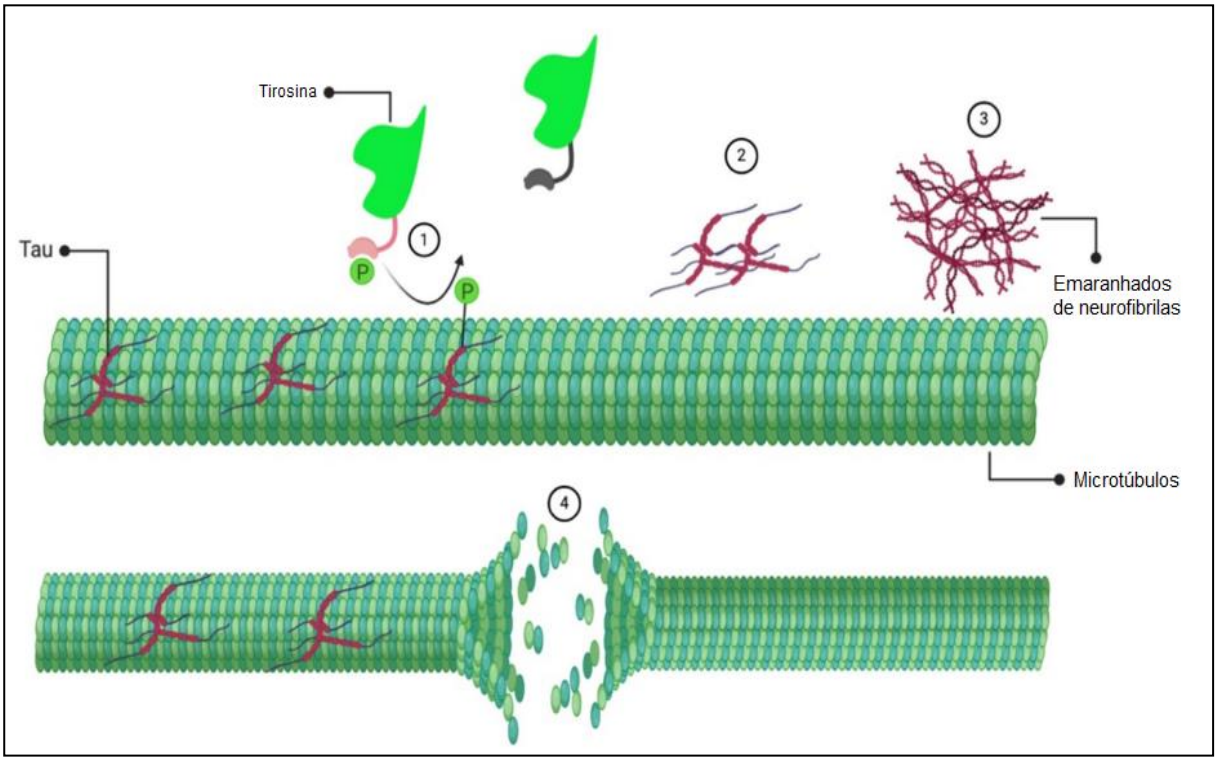


Figura 2: Imagem da formação de emaranhados de neurofibrilas: 1) A tirosina transfere um grupo fosfato para a proteína tau que está ligada aos microtúbulos. 2) As proteínas tau fosforiladas deixam os microtúbulos. 3) Depois de deixar os microtúbulos, as proteínas tau fosforiladas formam emaranhados neurofibrilares. 4) Na ausência de proteínas tau, ocorre a quebra dos microtúbulos (Adaptado de ASHRAFIAN; ZADEH; KHAN, 2021).

2.2 Genética da Doença de Alzheimer

A doença de Alzheimer possui duas formas de herança: a Doença de Alzheimer familial (DAF) e a Doença de Alzheimer esporádica (DAE).

A Doença de Alzheimer familial ou precoce ocorre, em geral, antes do 65 anos de idade e é uma desordem com padrão de herança autossômico dominante (BERTRAM; LILL; TANZI, 2010; HARPER, 1977; HOLTZMAN; MORRIS; GOATE, 2011). No início dos anos 1980, os pesquisadores que estudavam pacientes com síndrome de Down evidenciaram a associação entre a trissomia do 21 e DA (GOATE et al., 1991; KIDSON; CHEN, 1986). Entretanto, somente em 1991 a mutação no gene *APP* foi associada à doença de Alzheimer (GOATE et al., 1991). Além disso, mutações nos genes *Presenilina 1 (PSEN1)* localizado no cromossomo 14q24.3 e *Presenilina 2 (PSEN2) no* cromossomo 1q31-q42 (Figura 6) , também estão associadas à doença

de Alzheimer familial (NUSSBAUM; ELLIS, 2003; SCHELLENBERG et al., 1992).

Os genes *PSEN1* e *PSEN2* codificam duas proteínas pré-senilinas 1 e 2, respectivamente, que são componentes do complexo γ-secretase e que participam no processamento da proteína APP (SUH; CHECLER, 2002). As mutações nos genes *APP, PSEN1* e *PSEN2* estão relacionadas ao aumento da formação de $A\beta_{42}$ pois aumentam a atividade de proteólise de γ-secretase (CAI; GOLDE; YOUNKIN, 1993; CITRON et al., 1992; SUZUKI et al., 1994). Apesar de ser uma desordem rara, cerca de 2% dos casos, na hipótese da cascata amilóide, a mais aceita atualmente, sugere que as mutações nos genes *APP, PSEN1* e *PSEN2* e a fosforilação anormal da proteína Tau ocasionem o processo neurodegenerativo da Doença de Alzheimer (HARDY; HIGGINS, 1992).

Já a Doença de Alzheimer esporádica ou tardia, ocorre, em geral, após os 65 anos de idade e é mais frequente, atingindo cerca de 98% dos casos da doença. A DAE é uma desordem complexa ou multifatorial associado a influentes fatores genéticos de risco (YU; TAN; HARDY, 2014).

Atualmente, o alelo ε4 do gene *APOE* é considerado o maior fator de risco genético para DAE em diferentes populações (YU; TAN; HARDY, 2014). No ano de 1993, o gene *APOE* localizado no cromossomo 19q13.2, foi associado ao risco na doença de Alzheimer (CORDER et al., 1993; STRITTMATTER et al., 1993). Segundo Holtzman et al. (2000), o gene *APOE* possui duas variações genéticas (rs429358 e rs7412) levando a formação de três isoformas distintas: ApoE2 (ε2), formado pelos alelos T de rs7412 e T de rs429358; ApoE3 (ε3), formado pelos alelos C de rs7412 e T de rs429358; ApoE4 (ε4), formado pelos alelos C de rs7412 e C de rs429358 (HOLTZMAN et al., 2000; MORGAN; CARRASQUILLO, 2013).

O gene *APOE* codifica a proteína APOE que participa do transporte de colesterol no cérebro, reparo neuronal e na eliminação do peptídeo Aβ do sistema nervoso central (POIRIER, 1994; TOKUDA et al., 2000). O colesterol faz parte da estrutura das células nervosas, como a bainha de mielina dos neurônios (DONG et al., 2017). De acordo com o estudo de Dong et al. (2017) a desregulação da quantidade de colesterol nas células neuronais pode estar relacionada com o funcionamento anormal das enzimas

β-secretases e γ- secretases e, consequentemente, no desbalanço da formação dos peptídeos Aβ40 e Aβ42, relacionando assim, seu papel na DA. Segundo estudos, cada cópia do alelo ε4 causa uma diminuição na idade de aparecimento da doença em até dez anos (FARRER et al., 1997). Além disso, a presença de pelo menos um alelo ε4 pode aumentar em 3 vezes o risco para DAE (LAMBERT; AMOUYEL, 2011; STRITTMATTER et al., 1993).

2.3 Potenciais Biomarcadores para a DA:

Segundo Hye et al. (2006) os biomarcadores podem ser atributos morfológicos, de genes, transcritos, peptídeos ou metabólitos presentes no sistema biológico e são definidos pelo grupo *National Institute of Health Director's Iniciative on Biomarkers and Surrogate Endpoint* como sendo: indicadores de processos biológicos normais; ou patológicos; ou de respostas a intervenções farmacológicas e objetivamente mensuráveis (ATKINSON et al., 2001). Devido ao difícil diagnóstico da doença de Alzheimer e a capacidade limitada do sistema de saúde atual em encontrar e diagnosticar precocemente pacientes com a DA, o uso de biomarcadores pode ser utilizado como auxílio para o diagnóstico complementar e consequentemente uma condução eficaz de ensaios clínicos de novos candidatos a medicamentos contra a doença de Alzheimer (ZETTERBERG; BURNHAM, 2019).

Atualmente, a maioria das pesquisas sobre biomarcadores para a DA utilizam mecanismos de neuroimagem estrutural e functional, como por exemplo o Imagem por Ressonância Magnética (IRM) e o *Positron Emission Tomography* (PET), mas também há estudos potenciais sobre o uso de biomarcadores bioquímicos (CAVEDO et al., 2014)

A IRM possui boa resolução espacial com a capacidade de distinguir regiões do cérebro, estruturas e até mesmo diferenciar tecidos e camadas corticais, com isso pode ser utilizada para identificar atrofias cerebrais em sujeitos com DA. Já o IRM funcional alcança informações como o fluxo sanguíneo de determinada região e metabolismo de glicose. Este método adquire informações sobre o metabolismo do

cérebro e visualiza as atividades neuronais em locais específicos, sendo uma ferramenta importante no diagnóstico de DA possibilitando detectar alterações em regiões específicas do cérebro, como o hipocampo (CEDAZO-MINGUEZ; WINBLAD, 2010).

O PET já é utilizado como modalidade de diagnóstico em algumas doenças, como por exemplo o câncer, e produz imagens tridimensionais ou funcionais do organismo. É uma técnica que permite o mapeamento de diferentes substâncias químicas radioativas no organismo (PIKE et al., 2007).

Estudos têm sido realizados com o PIB-PET, uma sonda, marcada com um isótopo radioativo do carbono (11C) específica para se ligar ao peptídeo Aβ, o composto B de Pittsburgh (11C-PiB - *Pittsburgh compound B*). Os resultados mostraram que as regiões que mais apresentava absorção de PIB, estavam com maior atrofia, como o lobo medial temporal (JACK et al., 2008). Apesar do uso de neuroimagem auxiliar muito para o diagnóstico de doenças, possuem algumas limitações como o alto valor dos aparelhos e difícil interpretação dos dados (SONG et al., 2009).

Os marcadores bioquímicos, na doença de Alzheimer, têm ganhado destaque na última década. No líquido cefalorraquidiano (LCR) ou fluído cérebro espinhal os biomarcadores estudados são o nível da proteína Tau e de peptídeos Aβ (REIBER; PETER, 2001). Muitos pesquisadores têm realizado estudos com as proteínas APP, TAU e peptídeo Aβ. Os resultados ainda não são consensuais, mas tem sido observada uma diminuição de Aβ42 em pacientes com DA em relação aos controles. Esta relação sugere que devido a formação das placas de peptídeos Aβ na DA há uma redução de peptídeos Aβ solúveis circulantes no cérebro e no LCR (CRAIG-SCHAPIRO; FAGAN; HOLTZMAN, 2009).

No LCR No entanto, uma das limitações para os biomarcadores no LCR é que a coleta ocorre por punção lombar, e é considerada uma técnica invasiva e que requer cuidados para evitar contaminar o paciente (REIBER; PETER, 2001). Portanto, tem-se buscado biomarcadores, como plasma, sangue e soro pois são menos invasivos e portanto com um custo mais acessível (LISTA et al., 2015).

Nesse sentido, biomarcadores no plasma possuem limitações pois geralmente os biomarcadores derivados do cérebro estão em concentrações relativamente baixas no sangue devido à barreira hematoencefálica que impede a passagem livre de moléculas entre o SNC e os compartimentos sanguíneos (ZETTERBERG; BURNHAM, 2019).

Além disso, alguns dos biomarcadores são expressos em tecidos não cerebrais, o que pode confundir sua medição no sangue e no sangue pode haver anticorpos heterofílicos que podem alterar o resultado gerando falsos altos ou baixos. Esses tipos de anticorpos são muito menos problemáticos no LCR, onde os níveis de anticorpos são muito mais baixos. E finalmente, o biomarcador de interesse pode sofrer degradação por várias proteases no plasma (ZETTERBERG; BURNHAM, 2019).

Apesar de todas estas limitações, nas últimas décadas houve um aumento significativo de pesquisas sobre os mecanismos bioquímicos e moleculares da doença de Alzheimer na busca por biomarcadores. Nesse aspecto, tem ganhado destaque, a busca de polimorfismos de base única (SNP-*Single Nucleotide Polymorphism*) para identificar biomarcadores genéticos que possam ser úteis no diagnóstico complementar de doenças complexas como a DA. (FRIDMAN et al., 2004; KWOK; GU, 1999).

2.3.1 Gene *ABCA7*

O gene *ATP-binding cassette transporter A7 (ABCA7)* faz parte da família ABC e tem como função regular a homeostase de fosfolipídios e colesterol no sistema nervoso central e tecidos periféricos (RAMIREZ et al., 2016).

O gene ABCA7 está localizado no cromossomo 19p13.3 e é expresso em uma variedade de tecidos/órgãos, incluindo o cérebro e células sanguíneas (BERG; SINHA; GLUCK, 2019; REITZ et al., 2013). Além disso, níveis aumentados de expressão foram associados a casos mais graves de déficits cognitivos em indivíduos com DA (AIKAWA; HOLM; KANEKIYO, 2018).

Em estudos GWAS, o alelo G do polimorfismo rs3764650 no gene *ABCA7* apresenta foi relatado como fator de risco para DA e influencia os níveis de expressão do gene no cérebro (HOLLINGWORTH et al., 2011b; VASQUEZ; FARDO; ESTUS, 2013).

Estudos com indivíduos afro-americanos mostraram que ABCA7 é um forte preditor genético de (BERG; SINHA; GLUCK, 2019; REITZ et al., 2013). O polimorfismo rs3764650 do gene *ABCA7* pode gerar um risco indireto por meio de sua interação com outros fatores de risco, e isto explicaria a maior taxa de incidência de demência e DA na população afro-americana (BERG; SINHA; GLUCK, 2019).

Em estudos funcionais, a proteína ABCA7 foi altamente expressa em neurônios CA1 do hipocampo e células microgliais (BAO; WANG; MAO, 2016). Além disso, foi associado a placas amilóides em neurônios humanos, sugerindo um possível papel na patogênese da DA (SHULMAN et al., 2013).

Estudos funcionais mostraram que o alelo G do polimorfismo rs3764650 está associado à atrofia cortical e hipocampal e sua alta expressão está associada ao comprometimento cognitivo grave em pacientes com DA (HOLLINGWORTH et al., 2011b; LIU et al., 2014; RAMIREZ et al., 2016).

2.3.2 Gene *CR1*

O gene *Receptor do complemento 1 (CR1)* está localizado no cromossomo 1q32 e codifica a proteína CR1 que regula o sistema complemento na imunidade (ROGERS et al., 2006; WEIS et al., 1987).

O alelo A do polimorfismo rs6656401 no gene *CR1* foi associado a DAE como fator de risco em um estudo GWAS (LAMBERT et al., 2009). Estudos mostram que a proteína CR1 se liga ao peptídeo Aβ42 em seu sítio de ligação C3b, levando à depuração. No entanto, na doença de Alzheimer esse sistema pode ser afetado, o que pode resultar em aumento do acúmulo de peptídeo Aβ42 (MA et al., 2013). Além disso, o alelo A do polimorfismo rs6656401 no gene *CR1* pode estar relacionado com

a deposição de placas neuríticas em cérebros post-mortem, sugerindo uma relação entre o polimorfismo e a DAE (CHIBNIK et al., 2011; ROGERS et al., 2006).

2.3.3 Gene *BIN1*

O gene *Brindging integrador 1 (BIN1)* está localizado no cromossomo 2q14.3 e produz uma proteína BIN1 envolvida no processo de endocitose, apoptose celular e endocitose regulada por clatrina (PANT et al., 2009; REN et al., 2006).O gene *BIN1* foi associado à patogênese da DA por meio de estudos GWAS que relataram o alelo C no polimorfismo rs744373 em *BIN1* como fator de risco para a doença (HAROLD et al., 2009; LAMBERT et al., 2011; SESHADRI, 2010).

2.3.4 Gene *CLU*

O gene *Clusterin (CLU)* está localizado no cromossomo 8p21.1 e codifica as proteínas Apolipoprotein J (APOJ) ou CLU (RIZZI et al., 2009). A proteina CLU atua na interação celular, apoptose e regulação do sistema complemento (KARCH; GOATE, 2015a). Além disso, a proteína é uma apolipoproteína que transporta colesterol no cérebro e é responsável pela depuração de peptídeos Aβ , que está associada a um efeito neuroprotetor na DA (KARCH; GOATE, 2015a; RIZZI et al., 2009). O alelo T do polimorfismo rs11136000 do gene *CLU* foi considerado um fator protetor para DAE em estudos GWAS (HAROLD et al., 2009; NAJ et al., 2011b).

2.3.5 Gene *MS4A6A*

O gene *Membrane-Spanning 4-domains subfamília A6A* (*MS4A6A*), localizado no cromossomo 11q12.1, recebeu atenção recente em estudos GWAS. Isso se deve à descoberta de seu polimorfismo rs610932 com o alelo A como fator de proteção para DA (DENG et al., 2012; HOLLINGWORTH et al., 2011a; NAJ et al., 2011b).

A proteína MS4A6A teve sua expressão cerebral relacionada com emaranhados neurofibrilares e placas amilóides (HOLLINGWORTH et al., 2011a; KARCH et al., 2012).Além disso, o alelo A em rs610932 *MS4A6A* foi relacionado com um efeito

protetor no hipocampo contra a atrofia, o que poderia sugerir um papel de rs610932 *MS4A6A* na patogênese da DA (RAMIREZ et al., 2016).

2.3.5 Gene *TREM2*

O gene *Triggering receptor expressed on myeloid cells 2* (*TREM2*) está localizado no cromossomo 6p21.1 e faz parte da família *TREM*. *TREM2* é um membro do receptor imune inato e está relacionado com a via inflamátoria na doença de Alzheimer e em outras doenças neurodegenerativas (HU et al., 2013). A variante rs75932628 -T é rara e causa uma substituição p. R47H, há indícios de que esta variante aumente a suscetibilidade à DA, devido aos processos inflamatórios (WANG et al., 2018).

Estudos funcionais mostram que no cérebro o *TREM2* se correlaciona com a micróglias para a produção de citocinas pró-inflamatórias e com a estimulação de células do sistema imune (T CD4+). Além disso, em camundongos transgênicos APP a expressão de *TREM2* foi relacionada a formação de placas amilóides, sugerindo um papel no desenvolvimento da doença de Alzheimer (JONSSON et al., 2013; MELCHIOR et al., 2010).

O estudo de Jonsson et al.(2013) replicou a associação entre a variante rs75932628 e doença de Alzheimer em populações dos Estados Unidos, Alemanha, Holanda e Noruega e verificaram que o alelo T do polimorfismo rs75932628 conferiu um risco de doença de Alzheimer em todas as coortes de replicação. Além disso, estudos em uma população espanhola e Colombiana relataram associação positiva com o polimorfismo rs75932628 na doença de Alzheimer (ARBOLEDA-BUSTOS et al., 2018; BENITEZ et al., 2013). Estudos sugerem que *TREM2* está relacionado a doença de Alzheimer esporádica e o maior desafio para esta variante é realizar estudos com grande número amostral para conseguir detectá-la.

2.3.6 Gene *MS4A4E*

O gene *Membrane-spanning 4-domain subfamily A4E* (*MS4A4E)* está localizado no cromossomo 11q12.2 e a família de genes MS4A faz parte de componentes da

superfície celular (LIANG et al., 2001). O gene *MS4A4E* está implicado na modulação imunológica e está relacionado como sendo parte da via inflamatória na DA (KARCH; GOATE, 2015b). Neste aspecto, o estudo de Heneka et al. (2013) correlacionou a ativação de inflamassomas com a redução da fagocitose de placas Aβ. Além disso, as proteínas do gene *MS4A4E* são expressas em micróglias, mas seu mecanismo em DA ainda não está claro (VILLEGAS-LLERENA et al., 2016).

As funções do gene *MS4A4E* não estão totalmente caracterizadas, mas podem compartilhar algumas funções/propriedades de proteínas com sua família de genes (LIANG et al., 2001). Foi relatado que a família de genes *MS4A* podem regular a homeostase de cálcio dentro da célula (PAROLINI et al., 2012). Uma vez que altos níveis de cálcio intracelular podem facilitar a formação da placa amilóide e a hiperfosforilação de tau, os genes *MS4A* podem estar relacionados a doenças neurodegenerativas como a DA (LAFERLA, 2002).

2.3.7 Gene *CD2AP*

O gene *CD2-associated protein (CD2AP)* está localizado no cromossomo 6p12 e codifica a proteína CD2AP que regula a endocitose, estrutura do citoesqueleto, adesão de células e tráfico intracelular (LYNCH et al., 2003; PASCALE et al., 2005; TAO et al., 2017).

Vários estudos relatam um papel para o CD2AP na doença de Alzheimer. Por exemplo, CD2AP está implicado na via de endocitose o que leva a DA e pode afetar a endocitose da APP em neurônios (UBELMANN et al., 2017). Além disso, a proteína *CD2AP* foi encontrada expressa no cérebro de paciente com DA (SHULMAN et al., 2013) e pode controlar a degradação da APP nos neurônios (TAO et al., 2017). Em estudos GWAS o alelo C no polimorfismo rs9349407 no gene *CD2AP* foi considerado um fator de risco para DAE em populações caucasianas (HOLLINGWORTH et al., 2011a; NAJ et al., 2011a).

2.3.8 Gene *CASS4*

O gene *Cas scaffold protein family member 4 (CASS4)* está localizado no cromossomo 20q13.31 e foi associado para DAE em estudos GWAS (LAMBERT et al., 2013). O gene *CASS4* faz parte da família *CAS* que codifica uma proteína scaffold envolvida em processos de sinalização dependentes de integrina, essencial para a proliferação, sobrevivência, migração e motilidade celular (DENEKA; KOROBEYNIKOV; GOLEMIS, 2015).

Evidências indicam que *CASS4* desempenha papéis na patogênese da DA através da função citoesquelética, transporte axonal e metabolismo de APP e tau (LAMBERT et al., 2013). O estudo de Lin *et al.* (2017) analisaram as interações gene-estilo de vida e, neste estudo, o polimorfismo rs911159 do gene *CASS4* foi associado ao aumento do declínio cognitivo em uma população de Taiwan. Em contrapartida, no estudo clínico patológico Beechan et al. (2014) verificaram a associação entre o gene *CASS4* em resposta a lesão e DA com dados de autópsia cerebral e os dados sugerem que o polimorfismo rs7274581 *CASS4* é considerado um fator de proteção para DAE (BEECHAM et al., 2014).

2.4 Potenciais fatores de risco

A doença de Alzheimer é uma doença complexa e vários fatores como idade, sexo, raça e classe social são considerados fatores de risco. Nesse sentido, a idade é um dos fatores de risco mais importantes para declínio cognitivo na DA. A estimativa é que com o avançar da idade a prevalência de DA aumente 19% em indivíduos de 75-84 anos de idade e para 30-35%, possivelmente até 50% para aqueles com mais de 85 anos (IKEDA; YAMADA, 2010).

Embora o envelhecimento e a predisposição genética desempenhem papéis importantes no início da DA, o estilo de vida também parece ter um papel importante no desenvolvimento da doença. Nesse caso, estudos sugerem que o aumento do treinamento aeróbico aumenta o volume do hipocampo e o fluxo sanguíneo cerebral, sugerindo que a prática de atividade física pode ter efeitos neuroprotetores no cérebro (BERG; SINHA; GLUCK, 2019).

Além do físico, o estresse também pode ser um fator de risco para DA por meio de sua conhecida conexão de cortisol, sua toxicidade no hipocampo e no sistema límbico e danos epigenéticos, neste caso, sugerindo ser um fator causador do desenvolvimento do declínio cognitivo e da diminuição do bem-estar (KHALSA; NEWBERG, 2021).

2.5 Diagnóstico e tratamento

A doença de Alzheimer é diagnosticada através dos exames clínicos. Exames complementares são feitos para excluir outras causas de demência. Entretanto, o diagnóstico definitivo só é possível após falecimento do paciente, que é submetido a biópsia cerebral em exame *post-mortem*. Nesta biópsia é possível observar alterações histopatológicas características da doença como a presença de: as placas neuríticas e emaranhados neurofibrilares nas regiões de hipocampo e neocórtex (HYE et al., 2005). No cérebro destes pacientes também há atrofia cortical, alargamento dos sulcos corticais e dilatação das fissuras sylvianas (figura 3) (YAARI; COREY-BLOOM, 2007). Estas lesões cerebrais causam a diminuição de sinapses e morte dos neurônios (JELLINGER et al., 2008).

O método de diagnóstico mais utilizado para DA (FROTA et al., 2011) e o que apresenta maior sensibilidade e especificidade para a doença é feito segundo os critérios do *NINCDS-ADRDA (National Institute for Neurological and Communicative Disorders and Stroke –Alzheimer"s Disease and Related Disorders Association).* Neste critério, os pacientes são diagnosticados como DA provável ou DA possível (MCKHANN et al., 1984).

A doença de Alzheimer provável possui muitas características específicas, como por exemplo, o início insidioso (meses ou anos) e história clara ou observação de piora cognitiva (FROTA et al., 2011).

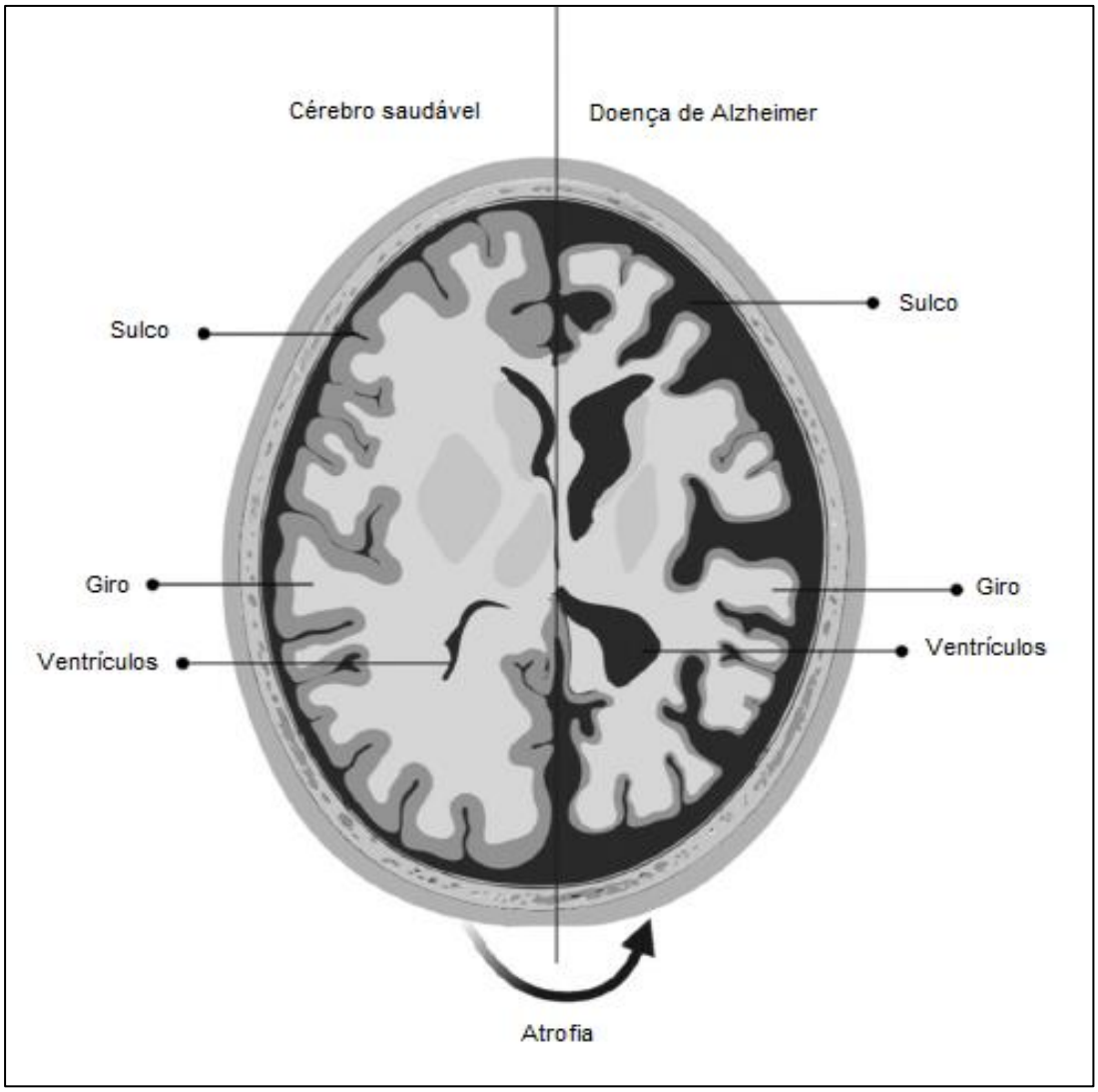


Figura 3: Imagem mostrando o fenômeno de atrofia que ocorre na doença de Alzheimer (Adaptado de ASHRAFIAN; ZADEH; KHAN, 2021).

Diferentemente, na DA possível o paciente preenche os critérios de diagnósticos clínicos porém apresenta algumas circunstâncias como, início abrupto e/ou padrão evolutivo distinto daquele observado usualmente, isto é lentamente progressivo, ou quando se tem evidência de outras etiologias (FROTA et al., 2011).

Como os sintomas são progressivos, geralmente, o diagnóstico é feito em estágios avançados da doença (JELLINGER et al., 2008). Portanto, tem sido crescente o estudo de SNPs associados com DAE como métodos no diagnóstico complementar da doença.

Até o momento, a DA é uma doença que não tem cura, mas que possui tratamento

paliativo que ameniza os sintomas e proporciona melhor qualidade de vida aos pacientes. Os medicamentos usados no tratamento são antipsicóticos, antidepressivos e ansiolíticos. Como opção terapêutica no tratamento sintomático da doença de Alzheimer para o déficit cognitivo são usados medicamentos inibidores da acetilcolinesterase (por exemplo: Donepezilo, Galantamina, Rivastigmina) e o antagonista dos recetores N-metil D-Aspartato (NMDA) (memantina) (FORLENZA, 2005).

Uma das causas da DA é baseada na hipótese da Teoria Neuroquímica, que se baseia na diminuição dos níveis de acetilcolina (ACh) nos pacientes (MORRISON; LYKETSOS, 2005). A acetilcolina é um hormônio neurotransmissor produzido pelo sistema nervoso e que está presente nas regiões cerebrais da memória e sua diminuição está correlacionada com o déficit cognitivo (MORRISON; LYKETSOS, 2005). Assim sendo, medicamentos inibidores da enzima acetilcolinesterase tem como objetivo diminuir a degradação de acetilcolina (FORLENZA, 2005). Atualmente, os inibidores das colinesterases são os principais medicamentos usados no tratamento da DA, contudo a resposta ao medicamento é heterogênea, sendo que alguns pacientes se beneficiam muito, enquanto outros, não apresentam melhora (FORLENZA, 2005). Além disso, destaca-se as reações adversas como fadiga, náuseas, vómitos, anorexia, insônia, agressividade, depressão e dores de cabeça (FORLENZA, 2005). No entanto, estudos mostram que a administração dos Inibidores da acetilcolinesterase em doentes com DA induz benefícios significativos, em relação ao grupo controle, sobre as capacidades funcionais e o déficit cognitivo (FORLENZA, 2005).

Já, o uso da memantina, um medicamento antagonista dos recetores NMDA, possui efeitos sobre neurotransmissão glutamatérgica (LI et al., 1997). O glutamato é um neurotransmissor cerebral associado às funções cognitivas e à memória. Assim, na DA, encontra-se níveis alterados de Glutamato e quando os níveis de glutamato ficam elevados por períodos prolongados podem causar morte dos neurônios (FORLENZA, 2005; LI et al., 1997). A memantina possui uma ação neuroprotetora contra a ativação elevada de receptores NMDA (MISZTAL; FRANKIEWICZ, 1996; PARSONS et al., 1993). Assim sendo, estudos clínicos sugerem que doses diárias de Memantina em portadores de demência leve ou moderada tiveram benefícios significativos sobre as

funções motoras e cognitivas (FORLENZA, 2005; PANTEV; RITTER; GORTELMEYER, 1993).

Os estudos de Wenk et al. (2000) e Tariot et al. (2004) sugerem que o tratamento combinado de Memantina e inibidores das colinesterases em pacientes com DA é seguro, bem tolerado e pode favorecer melhoras significativas nas funções cognitivas, funcionais e comportamentais.

Como atualmente o tratamento da DA é paliativo, há muitos estudos que buscam identificar estratégias terapêuticas para evitar a doença ou a fim de retardar o processo neurodegenerativo. Deste modo, as perspectivas futuras são as imunoterapias para a doença de Alzheimer.

Deste modo, o desenvolvimento de drogas para a DA baseou-se na hipótese da cascata amilóide (HARDY; HIGGINS, 1992). Essa hipótese propõe que a deposição de Aβ é o principal agente causador, resultando em alterações inflamatórias, emaranhados de neurofibrilas, morte dos neurônios e declínio cognitivo (figura 4). Os estudos buscam reduzir a produção de Aβ ou aumentar a depuração da proteína.

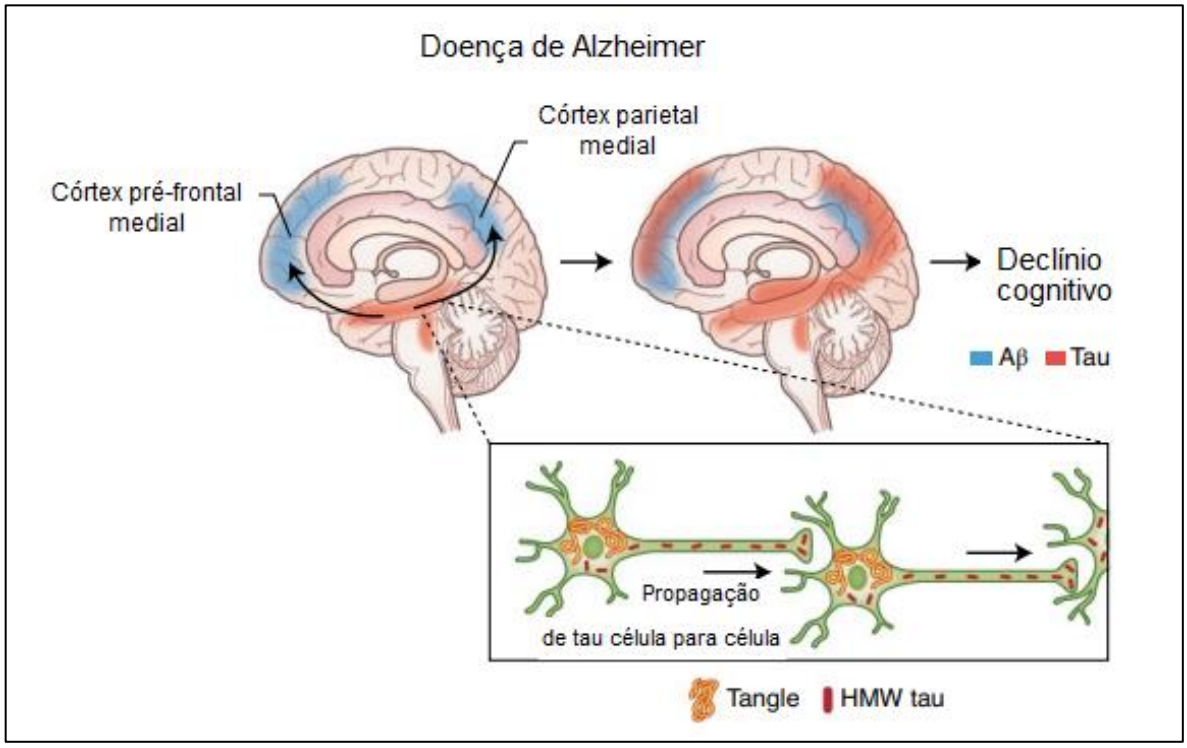


Figura 4: Imagem mostrando as placas Aβ com a disseminação da tau e o declínio cognitivo na DA (Modificado de (BUSCHE; HYMAN, 2020).

O estudo de Schenk et al. (1999) foi o pioneiro em descrever a imunização com o Aβ em modelos de camundongos transgênicos. Neste estudo, foi mostrado que a imunização com o Aβ42 poderia reduzir a deposição de placas amiloides em tecido cerebral. Este trabalho foi clinicamente relevante abrindo a possibilidade de novos estudos sobre imunoterapias com o Aβ.

Além disso, os testes com imunoterapias ativas mostraram bastante efeitos colaterais e os tratamentos por meio de anticorpos monoclonais mostraram-se promissores porém, até o momento os ensaios clínicos não obtiveram resultado satisfatório (CACABELOS, 2020; MORGAN, 2011).

3. RESULTADOS E DISCUSSÃO

Esta tese está estruturada em três estudos independentes, todos sobre biomarcadores na Doença de Alzheimer. Além disso, os estudos foram incluídos na íntegra e para o artigo em submissão, foi incluído o comprovante de submissão.

Capítulo 1

• *Updated Meta-Analysis of BIN1, CR1, MS4A6A, CLU, and ABCA7 Variants in Alzheimer's Disease* - publicado na revista Journal of Molecular Neuroscience.

Capítulo 2

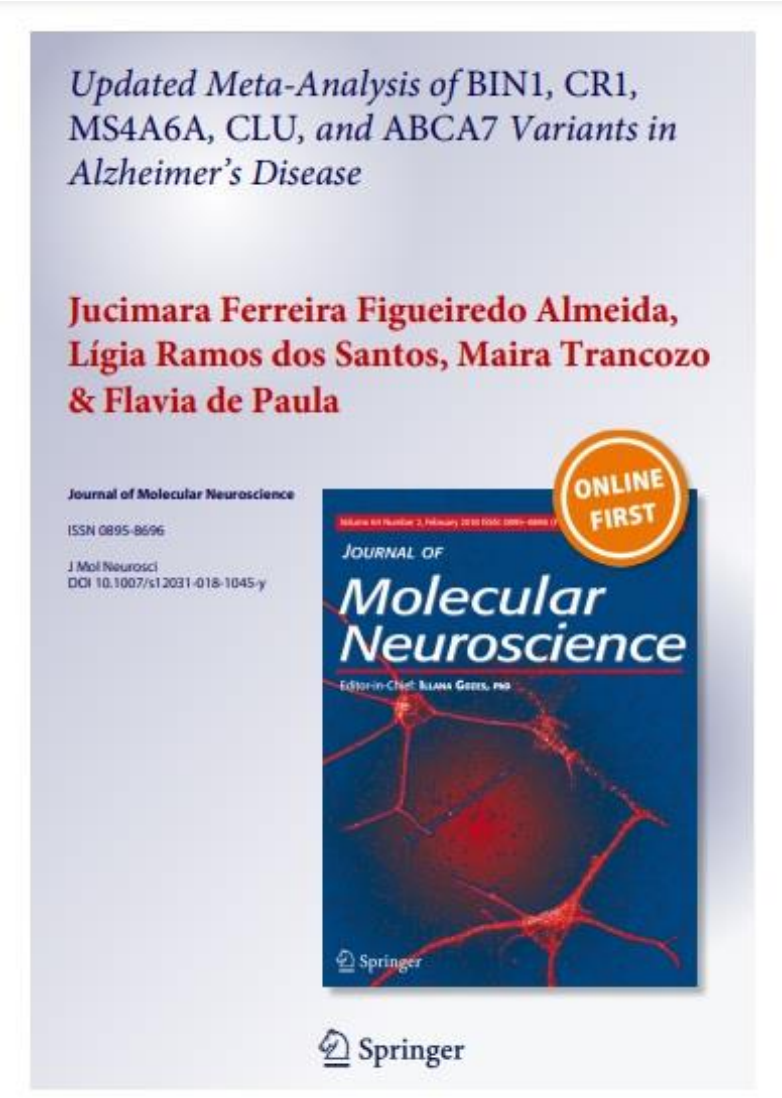
• *The combined risk effect among BIN1, CLU, and APOE genes in Alzheimer's Disease* - publicado na revista Genetics and Molecular Biology.

Capítulo 3

• *Risk assessment of CASS4, TREM2, CD2AP and MS4A4E variant's on alzheimer's disease in a Brazilian population* - manuscrito submetido na revista Neurological Sciences.

3.1 Capítulo 1

• *Updated Meta-Analysis of BIN1, CR1, MS4A6A, CLU, and ABCA7 Variants in Alzheimer's Disease* - publicado na revista *Journal of Molecular Neuroscience.*



Updated meta-analysis of *BIN1*, *CR1*, *MS4A6A*, *CLU* and *ABCA7* variants in Alzheimer's disease

Abstract

Genome-wide association studies (GWAS) have associated several genetic variants with late-onset Alzheimer's disease (LOAD), a neurodegenerative disease. Among those, rs3764650 *ABCA7*, rs6656401 *CR1* and rs744373 *BIN1* were associated as risk factors for LOAD, while rs11136000 *CLU* and rs610932 *MS4A6A* were protective. Recently, several case-control studies have investigated the association of these polymorphisms with AD. However, not all meta-analyses analyzed these variants across different ethnic groups. Therefore, we performed an updated meta-analysis of rs3764650 *ABCA7*, rs6656401 *CR1*, rs744373 *BIN1*, rs11136000 *CLU,* and rs610932 *MS4A6A* variants associated with LOAD, considering different ethnic populations. We utilized samples from 38 articles, comprising a total of 24,771 patients and 35,324 controls obtained through the PubMed database. Odds ratios (ORs) with 95% confidence intervals (CI) for polymorphisms were calculated by allelic comparison as an additive genetic model. We validated the risk for LOAD with *BIN1* (rs744373), *CR1* (rs6656401) and *ABCA7* (rs376465), as well as the protective association for *MS4A6A* (rs610932) and *CLU* (rs11136000) variants.

Keywords: Meta-analysis; Alzheimer’s disease; LOAD; GWAS SNPs.

1. Introduction

Alzheimer's disease (AD) is one of the most common forms of dementia (Kadmiri et al. 2017). More than 46 million elderly persons are affected worldwide by this neurodegenerative disorder (Condello and Stohr 2016). AD is characterized by brain atrophy caused by amyloid plaques and neurofibrillary tangle formation (Kang et al. 2017), two main hallmarks of AD that lead to neuronal death in the brain. Genetically, AD occurs in two forms: Family or Early onset Alzheimer's disease (EOAD), which affects individuals before 65 years, and Late-onset Alzheimer's disease (LOAD), which affects individuals older than 65 years. EOAD etiology arises from autosomal dominant inheritance with mutations in genes encoding the Amyloid precursor protein (APP), *Presenilin 1* (*PSEN1*) and *Presenilin 2* (*PSEN*2) (Lambert et al. 2009). However, the majority of AD cases are LOAD, comprising multifactorial characteristics (Yu et al. 2014). Currently, the e4 allele of *Apolipoprotein E* gene (*APOE*) is considered a major genetic risk factor for LOAD in different populations (Lambert and Amouyel 2011).

Genome-wide association studies (GWAS) have reported several variants associated with LOAD in terms of risk (rs3764650 *ABCA7*, rs6656401 *CR1* and rs744373 *BIN1*) and protection (rs11136000 *CLU* and rs610932 *MS4A6A*) (Harold et al. 2009; Lambert et al. 2009; Hollingworth et al. 2011; Naj et al. 2011). Several studies have performed meta-analyses to investigate the association between LOAD and these five variants. More recently, various case-controls studies have been published investigating the association of these variants with AD. However, a gap still exists in the current information, necessitating an update of the data through meta-analysis on those variants association with LOAD to better understand their roles in AD pathogenesis. Of note, few meta-analyses attempted to investigate the association of these variants across different ethnic groups. Therefore, we aimed to investigate the association of rs3764650 *ABCA7*, rs610932 *MS4A6A*, rs6656401 *CR1*, rs744373 *BIN1* and rs11136000 *CLU* with LOAD by performing an update meta-analysis in not only Caucasian populations but also in samples from different ethnic groups.

2. Material and Methods

Literature search

We searched for articles using the PubMed database (Sayers et al. 2009). To filter the results, we chose only articles written in English containing human samples that were published between January 1st of 2009 and August 28th of 2017. Prior to submission, we conducted another search of the PubMed database on November 24th to look for additional new studies. For the single nucleotide polymorphisms (SNPs) rs610932 *MS4A6A*, rs744373 *BIN1*, rs6656401 *CR1*, rs3764650 *ABCA7* and rs111136000 *CLU*, we searched using the following separate key words: "rs610932 MS4A6A", "MS4A6A", "rs744373 BIN1", "BIN1", "rs6656401 CR1", "CR1", "rs3764650 ABCA7", "ABCA7", "rs11136000 CLU" and "CLU". Studies that were referenced in articles discovered by the investigation were also used to search for additional relevant information.

Inclusion and exclusion criteria

Three authors evaluated all included studies by establishing five inclusion criteria for quality control of selected articles. The inclusion criteria are as follows: (1) the study investigates an association between LOAD and at least one of these SNPs: rs610932 *MS4A6A*, rs744373 *BIN1*, rs6656401 *CR1*, rs3764650 *ABCA7* and rs11136000 *CLU*; (2) the study has diagnostic criteria for LOAD patients; (3) the study provides genotype numbers or allele numbers of SNPs and total number of samples in controls and cases. When none of those data were provided, an email requesting the information was sent to the authors; (4) the study reported genotype frequencies in Hardy-Weinberg Equilibrium (HWE) for case and control groups; (5) the study specifies the genotyping method used. All studies that did not meet these five criteria, as well as duplicate publications, were excluded.

Data extraction

Three authors extracted the following information from articles selected for inclusion: (1) last name of first author; (2) year of publication; (3) country of sample; (4)

ethnicity of sample; (4) total number of controls and LOAD patients; (5) number of genotypes or alleles from each SNP in LOAD cases and controls; (6) genotyping method; (7) inclusion criteria for LOAD patients.

Statistical analysis

Statistical analyses were performed with R program software (R Development Core Team 2011) using the Meta package for meta-analysis (Schwarzer et al. 2007). We investigated association of SNPs with LOAD by meta-analysis in the overall sample, including all studies found of each individual SNP and in different ethnic groups. Odds ratio (ORs) with 95% confidence interval (CI) of polymorphisms were calculated by allelic comparison using an additive genetic model as follows: rs610932 *MS4A6A* (A versus C), rs744373 *BIN1* (C versus T), rs6656401 *CR1* (A versus G), rs3764650 *ABCA7* (G versus T) and rs11136000 *CLU* (T versus C). We assumed that minor allele frequencies from each polymorphism investigated were high-risk alleles. The fixed-effect model was used to calculate OR and the Z-test to determine its significance (p value ≤ 0.05 was considered statistically significant). Q test and I^2 tests were used to assess for heterogeneity. Levels of heterogeneity with a p value of Q test ≤ 0.05 (Rodrigues and Ziegelmann 2010) and I^2>50% (Higgins et al. 2003) were considered significant. In cases of significant heterogeneity, OR was calculated using a random-effect model. Otherwise, the fixed effect model was maintained. For publication bias assessment, we performed funnel plot, Begg's and Egger's test. Funnel plot, Begg's and Egger's test (Rodrigues and Ziegelmann 2010) were applied only in SNP meta-analyses with 10 or more studies. Funnel plot and Begg's and Egger's test is not recommended for use in fewer than 10 studies because the power of the test is usually too low to distinguish the true asymmetry (Macaskill et al. 2001; Sterne and Harbord 2004; Sterne et al. 2011). According to Sterne et al. (2011), there is a greater false positive risk the lower the power of test becomes.

3. Results

A flow chart with the articles included in this study is shown in Figure 1. Our search returned 1667 articles, 1629 of which were eliminated due to failure to meet our inclusion criteria, leaving 38 articles used in the meta-analysis. Some articles

contained more than one study that was used in our meta-analysis. Eight studies were obtained for rs610932 *MS4A6A*, 25 for rs744373 *BIN1*, 13 for rs6656401 *CR1*, 11 for rs3764650 *ABCA7* and 24 for rs11136000 *CLU.* Selected studies are indicated in Table S1.

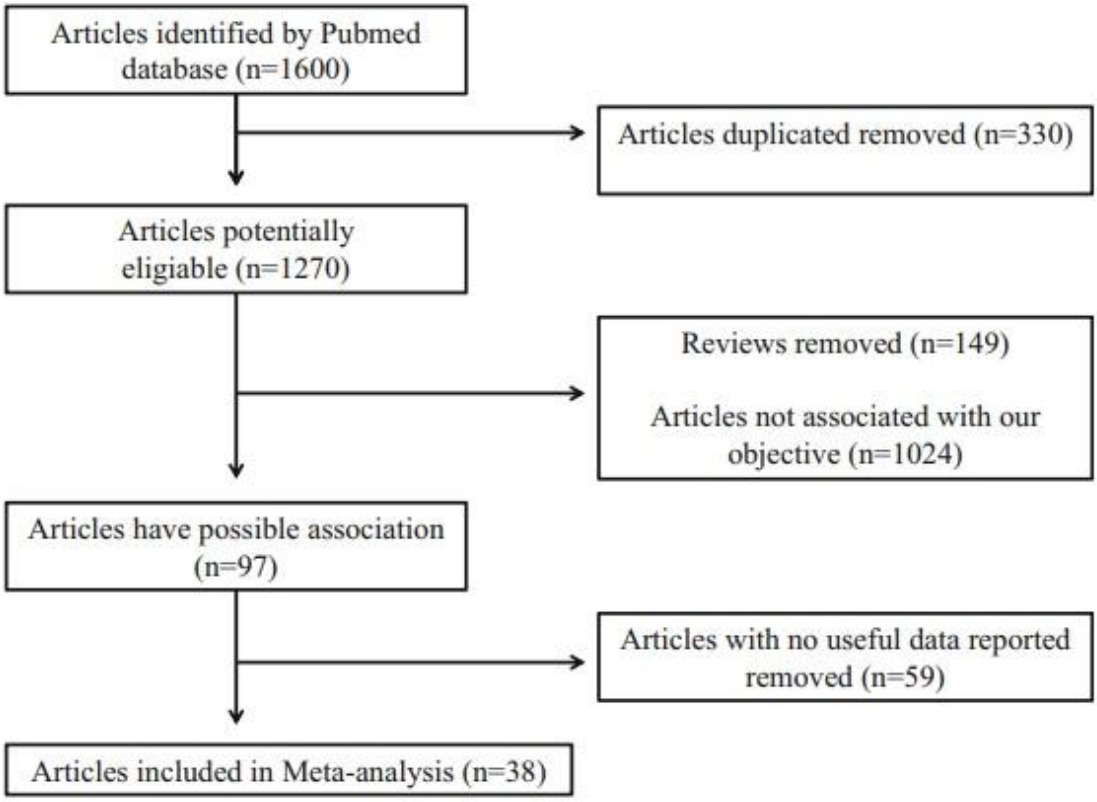


Fig. 1 Flow chart of articles found on PubMed database

The principal results of the meta-analysis are summarized in Table 1. The meta-analysis included 24,771 patients and 35,324 controls. We found a protective association for T allele rs11136000 *CLU* (OR=0.86, p<0.01) and A allele rs610932 *MS4A6A* (OR=0.88, p<0.01) for LOAD in the overall or total sample. The association with AD remained significant in Caucasian samples for both polymorphisms, in Asian samples for rs610932 *MS4A6A,* and in mixed sample for rs11136000 *CLU.* Forest plots are shown in Figure S1, S2, S3 and S4 for rs11136000 *CLU* and in Figure S5, S6 and S7 for rs610932 *MS4A6A*. We detected heterogeneity only among studies for rs11136000 *CLU* in the mixed sample (p=0.02; I^2=65%). The funnel plot (Figure S8, S9 and S10) was symmetric in both polymorphisms as is shown in the Begg's and Egger's test (Table 1).

Table 1 Meta-analyses results

Polymorphism	OR (95% CI)[a]	*p* value[b]	Heterogeneity[c]			Funnel symmetry	
			X^2	*p*	I^2	Begg's *p* value	Egger's *p* value
rs744373 *BIN1*							
Overall[d]	1.15 (1.11–1.20)	< 0.01	0.0073	< 0.01	44%	–	–
Overall[e]	1.14 (1.08–1.21)	< 0.01	0.0073	< 0.01	44%	0.06171	0.1931
Caucasian[d]	1.17 (1.12–1.22)	< 0.01	0.0084	0.01	47%	–	–
Caucasian[e]	1.16 (1.09–1.24)	< 0.01	0.0084	0.01	47%	0.06371	0.355
Mixed sample[d]	1.10 (1.02–1.19)	0.01	0.005	0.19	33%	–	–
rs6656401 *CR1*							
Overall[d]	1.14 (1.08–1.20)	< 0.01	0.0047	0.14	30%	0.2721	0.07082
Caucasian[d]	1.13 (1.07–1.20)	< 0.01	0.0044	0.14	33%	0.5312	0.2594
Asian[d]	1.26 (1.02–1.56)	0.04	0.0239	0.22	34%	–	–
rs11136000 *CLU*							
Overall[d]	0.86 (0.84–0.89)	< 0.01	0.0028	0.06	33%	0.3211	0.6008
Caucasian[d]	0.87 (0.84–0.89)	< 0.01	0.0012	0.23	19%	0.1001	0.1293
Mixed sample[d]	0.84 (0.76–0.92)	< 0.01	0.0306	0.02	65%	–	–
Mixed sample[e]	0.81 (0.66–0.98)	0.03	0.0306	0.02	65%	–	–
rs3764650 *ABCA7*							
Overall[d]	1.07 (0.98–1.16)	0.14	0.0089	0.17	30%	0.9287	0.6556
Caucasian[d]	1.25 (1.05–1.49)	0.01	0.0235	0.19	37%	–	–
Mixed sample[d]	1.02 (0.92–1.12)	0.76	0	0.57	0%	–	–
rs610932 *MS4A6A*							
Overall[d]	0.88 (0.84–0.92)	< 0.01	0	0.57	0%	0.8348	0.9285
Caucasian[d]	0.89 (0.84–0.93)	< 0.01	0	0.87	0%	–	–
Asian[d]	0.82 (0.73–0.93)	< 0.01	0.0103	0.16	45%	–	–

AD patients, Alzheimer's disease patients; *OR*, odds ratio; *CI*, confidence interval; *Overall*, all the studies related to that polymorphism

[a] OR and 95% IC were calculated by an additive genetic model

[b] *p* value of *Z* test

[c] Heterogeneity was calculated with *Q* test (x^2 test), *p* value (≤ 0.05 is considered significant) of *Q* test and I^2 (> 50% is considered significant)

[d] Calculated by fixed effect model

[e] Calculated by random effect model

We detected a risk association for C allele rs744373 *BIN1* (OR=1.15, p<0.01) and A allele rs6656401 *CR1* (OR=1.14, p<0.01) for LOAD in the overall sample. In rs6656401 *CR1*, the meta-analysis for Asian and Caucasian samples was associated with AD. No heterogeneity was observed in rs6656401 *CR1* in the overall sample, in neither Caucasian nor Asian samples. Their funnel plots (Figure S11 and S12) were symmetric. Forest plots for rs6656401 *CR1* are shown in Figure S13, S14 and S15. For rs744373 *BIN1*, association with AD was significant in Caucasian and mixed samples. Significant heterogeneity was detected in the rs744373 *BIN1* meta-analysis in the overall sample (p=<0.01) and in the Caucasian population (p=0.01). However, the funnel plot for both groups (Figure S16 and S17) was considered symmetric in

Egger's test and in Begg's test (Table 1). In addition, no heterogeneity was detected for the mixed sample. Forest plots of rs744373 *BIN1* are shown in Figures S18, S19, S20, S21 and S22.

We detected a risk association for G allele rs3764650 *ABCA7* (OR=1.25, p=0.01) for LOAD in the Caucasian sample. No association was found in either the total sample or the mixed sample. Furthermore, no heterogeneity was observed in any of the three groups. Forest plots and funnel plots are shown in Figure S23 to S26.

4. Discussion

In the present study, we investigated the association between GWAS variants rs610932 *MS4A6A*, rs744373 *BIN1*, rs6656401 *CR1*, rs3764650 *ABCA7* and rs11136000 *CLU* with late-onset AD using a meta-analysis. We observed risk association with AD for rs744373 *BIN1,* rs3764650 *ABCA7* and rs6656401 *CR1* and protection in rs11136000 *CLU* and rs610932 *MS4A6A.*

The *Bridging integrator 1* (*BIN1*) gene is located on chromosome 2q14.3 and produces a BIN1 protein involved in endocytosis, apoptosis and clathrin-regulated endocytosis (Ren et al. 2006; Pant et al. 2009). The *BIN1* gene has been associated with AD pathogenesis through GWAS that reported the C allele rs744373 polymorphism as a risk factor (Harold et al. 2009; Seshadri 2010; Lambert et al. 2011). In meta-analysis studies, the most current study by Zhu et al. (2016) found a risk association for rs744373 *BIN1* in LOAD. Our work also demonstrates association with LOAD for this variant in total sample analysis. We used some studies by Zhu et al. (2016) and added additional, new published studies. Unlike Zhu et al. (2016), we aimed to divide the sample into ethnic subgroups to investigate if the risk effect would be maintained. In the mixed subgroup, we found C allele rs744373 *BIN1* as a risk factor for AD but, surprisingly, no heterogeneity was observed in the subgroup formed by Asian and Turkish populations. In contrast, we identified some heterogeneity in the Caucasian subgroup and total samples, even though we had carefully chosen studies using similar methodologies according to our established criteria. Our results obtained by meta-analysis corroborate GWAS finding for BIN1 in AD. For instance, Chapuis et al. (2013) identified elevated BIN1 protein levels in AD patient brains. Additionally, Schmidt et al. (2012) reported a relationship between rs744373 *BIN1* and cognitive

decline and AD progression, suggesting participation of this gene in Alzheimer's disease pathogenesis.

The *complement receptor 1* (*CR1*) gene is located on chromosome 1q32 (Weis et al. 1987) and encodes the CR1 protein that regulates the complement system in immunity (Rogers et al. 2006). The A allele rs6656401 polymorphism in the *CR1* gene has been associated LOAD as a risk factor in a GWAS study (Lambert et al. 2009). In 2014, two meta-analyses (Luo et al. 2014; Shen et al. 2014) reported positive association of A allele rs6656401 with AD. Our meta-analysis also reports a risk for AD in the A allele rs6656401 *CR1*. In contrast to a study from Shen et al. (2014), we investigated the risk of rs6656401 in ethnic subgroups, as did Luo et al. (2014). Although our study and the Luo et al. (2014) study found association in the total sample and Caucasian and Asian subgroups, the study from Luo et al. (2014) has a sample limitation. Thus, we included new studies in this meta-analysis increase sample size and update provide and updated analysis of rs6656401 *CR1*. Our results sustain the role of *CR1* gene in AD pathway broadly discussed in many studies (Rogers et al. 2006; Ma et al. 2013). Rogers et al. (2006) reported that the CR1 protein binds to $A\beta_{42}$ peptide at its C3b ligation site, leading to clearance. However, this system may be affected in AD (Ma et al. 2013), which could result in increased $A\beta_{42}$ peptide accumulation. Interestingly, Chibnik et al. (2011) correlated the A allele r6656401 *CR1* with deposition of neuritic plaques in *post-mortem* brains, suggesting a relationship between r6656401 *CR1* and AD.

The *Clusterin (CLU)* gene is located on chromosome 8p21.1 and codes for Apolipoprotein J (APOJ) or CLU protein (Rizzi et al. 2009). CLU acts on cell interactions, apoptosis and regulates the complement system (Karch and Goate 2015). In addition, the protein is an apolipoprotein that transports cholesterol in the brain and is responsible for the clearance of Aβ peptides (Rizzi et al. 2009) and is associated with a neuroprotective effect in AD (Karch and Goate 2015). The T allele in the polymorphism rs11136000 *CLU* is considered a protective factor for LOAD by GWAS (Harold et al. 2009; Naj et al. 2011). In genetic studies, two recent meta-analyses found association of the T allele in rs11136000 *CLU* with LOAD (Liu et al. 2014b; Du et al. 2016), validating the neuroprotective effect observed in the GWAS (Harold et al. 2009; Lambert et al. 2009). Our meta-analysis corroborated this association and selected some studies used by both authors (Liu et al. 2014b; Du et al. 2016), along with newly added studies. In addition, we formed a mixed sample comprising populations from

China, Japan, India and Turkey since we could not form a unique set for other ethnicities as we did for Caucasian samples. Although we found T allele rs11136000 *CLU* to be a protective factor for LOAD in total samples and in both subgroups, heterogeneity was observed in the mixed sample. This could be due to the diverse ethnicity observed in this subgroup. More case-control studies are needed to better understand the involvement of ethnicity in these results.

The *Membrane-Spanning 4-domains subfamily A6A* (*MS4A6A*) gene, located on chromosome 11q12.1 (Deng et al. 2012), has received recent attention in GWAS due to the discovery of the rs610932 polymorphism of A allele being a protective factor for AD (Hollingworth et al. 2011; Naj et al. 2011). Meta-analyses of Mao et al. (2015) and Ji et al. (2015) found a protective association for AD with A allele in rs610932 *MS4A6A*. Our study confirmed this association. While Mao et al. (2015) performed the meta-analysis only in Asian studies, our study, similar to Ji et al. (2015), used some of the Asian patient samples while adding additional published studies, particularly those performed in Caucasian patients. Despite these findings, few case-control studies that investigate the role of this SNP in LOAD. A future meta-analysis containing more studies will be important to corroborate this finding. We believe the *MS4A6A* gene is involved in AD. As an example, MS4A6A protein expression in the brain has been associated with neurofibrillary tangles and amyloid plaques (Hollingworth et al. 2011; Karch et al. 2012). In addition, the A allele rs610932 *MS4A6A* was shown to be associated with a protective effect against hippocampal atrophy (Ramirez et al. 2016), suggesting a role for rs610932 *MS4A6A* in AD pathogenesis as well.

The *ATP-binding cassette transporter A7 (ABCA7)* gene is located on chromosome 19p13.3 and encodes ABCA7 transmembrane proteins that transports lipoprotein through the cell membrane (Ramirez et al. 2016). In functional studies, the ABCA7 protein was found to be highly expressed in hippocampal CA1 neurons and microglial cells (Bao et al. 2016). It has additionally been associated with amyloid plaques in human neurons (Shulman et al. 2013), suggesting a possible role in AD pathogenesis. In GWAS, G allele rs3764650, a polymorphism in the *ABCA7* gene, was reported to be a risk factor for AD (Hollingworth et al. 2011). So far, two subsequent meta-analysis studies have corroborated the GWAS finding, identifying AD risk in both total samples and ethnic subgroups (Liu et al. 2014a; Bao et al. 2016). Our meta-analysis used some samples in common with these studies and added new studies that had yet to be analyzed. Unfortunately, findings from a GWAS study containing

more than 10,000 samples (Hollingworth et al. 2011) did not have genotype numbers available. Still, we discovered association of the G allele rs3764650 *ABCA7* only in Caucasian samples. The negative results observed in total samples and non-Caucasian groups may be due to our small sample size in comparison to Liu et al. (2014a) and Bao et al. (2016) meta-analyses. Indeed, we believe rs3764650 *ABCA7* has a possible role associated with AD. For instance, functional studies have shown that the G allele rs3764650 is associated with cortical and hippocampal atrophy (Ramirez et al. 2016) and its high expression is associated with severe cognitive deficit in AD patients (Hollingworth et al. 2011; Liu et al. 2014a; Ramirez et al. 2016).

Our meta-analysis has some limitations. During the search for studies, some works were excluded because they did not provide sufficient data or did not meet our inclusion criteria, which are important to follow in order to avoid publication bias (Rodrigues and Ziegelmann 2010). For instance, we only included studies that had samples falling within Hardy-Weinberg Equilibrium. Since control samples that violate HWE may lead to a different conclusion for role of polymorphisms in this disease (Trikalinos et al. 2006; Liu et al. 2014b), they could also conceivably interfere in our meta-analysis results. Additionally, we were unable to perform recessive and dominant models for our meta-analysis since we did not have access to e genotype information for most studies. These factors all contributed to diminishing our sample size. However, our results still correspond with the association found in GWAS. Meta-analysis studies are important because they integrate results found in case-control studies, allowing for updates in the general understanding of AD-related SNPs. Ours findings will be very useful in aid in better prediction and diagnosis for Alzheimer's disease.

5. Conclusion

Our study suggests that rs610932 *MS4A6A*, rs3764650 *ABCA7,* rs744373 *BIN1*, rs6656401 *CR1* and rs11136000 *CLU* are associated with LOAD*.*

Appendix A. Supplementary data

The supplementary data in this article identified as Table S1 and Figures S1-S26 can be found in the online version.

Author's contributions

Jucimara Ferreira Figueiredo Almeida, Lígia Ramos dos Santos, Maira Trancozo and Flavia de Paula wrote the manuscript, and read and accepted the manuscript before submission. Jucimara Ferreira Figueiredo Almeida, Lígia Ramos dos Santos and Maira Trancozo performed the meta-analysis, the literature search, data extraction and evaluated the inclusion and exclusion criteria.

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de Pessoal de Nível Superior – CAPES.

Disclosure Statement

The authors declare no conflict of interest.

Reference

Bao J, Wang X, Mao Z (2016) Associations Between Genetic Variants in 19p13 and 19q13 Regions and Susceptibility to Alzheimer Disease: A Meta-Analysis. Medical Science Monitor 22:234–243 . doi: 10.12659/MSM.895622

Chapuis J, Hansmannel F, Gistelinck M, et al (2013) Increased expression of BIN1 mediates Alzheimer genetic risk by modulating tau pathology. Molecular Psychiatry 18:1225–1234 . doi: 10.1038/mp.2013.1

Chibnik LB, Shulman JM, Leurgans SE, et al (2011) CR1 is associated with amyloid plaque burden and age-related cognitive decline. Annals of Neurology 69:560–569 . doi: 10.1002/ana.22277

Condello C, Stohr J (2016) Aβ propagation and strains: Implications for the phenotypic diversity in Alzheimer's disease. Neurobiology of Disease. doi: 10.1016/j.nbd.2017.03.014

Deng YL, Liu LH, Wang Y, et al (2012) The prevalence of CD33 and MS4A6A variant in Chinese Han population with Alzheimer's disease. Human Genetics 131:1245–1249 . doi: 10.1007/s00439-012-1154-6

Du W, Tan J, Xu W, et al (2016) Association between clusterin gene polymorphism rs11136000 and late–onset alzheimer's disease susceptibility: A review and meta–analysis of case–control studies. Experimental and Therapeutic Medicine 12:2915–2927

Harold D, Abraham R, Hollingworth P, et al (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nature Genetics 41:1088–1093 . doi: 10.1038/ng.440

Higgins JPT, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ : British Medical Journal 327:557–560 . doi: 10.1136/bmj.327.7414.557

Hollingworth P, Harold D, Sims R, et al (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nature Genetics 43:429–435 . doi: 10.1038/ng.803

Ji W, Xu L, Zhou H, et al (2015) Meta-analysis of association between the genetic polymorphisms on chromosome 11q and Alzheimer's disease susceptibility. International Journal of Clinical and Experimental Medicine 8:18235–18244

Kadmiri N, Said N, Slassi I, et al (2017) Biomarkers for Alzheimer disease: Classical and novel candidates' review. Neuroscience. doi: 10.1016/j.neuroscience.2017.07.017

Kang S, Lee YH, Lee JE (2017) Metabolism-centric overview of the pathogenesis of Alzheimer's disease. Yonsei Medical Journal 58:479–488 . doi: 10.3349/ymj.2017.58.3.479

Karch CM, Goate AM (2015) Alzheimer's Disease Risk Genes and Mechanisms of Disease Pathogenesis. Biological Psychiatry 77:43–51 . doi: 10.1016/j.biopsych.2014.05.006

Karch CM, Jeng AT, Nowotny P, et al (2012) Expression of Novel Alzheimer ' s Disease Risk Genes in Control and Alzheimer ' s Disease Brains. 7: . doi: 10.1371/journal.pone.0050976

Lambert J-C, Heath S, Even G, et al (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nature Genetics 41:1094–1099 . doi: 10.1038/ng.439

Lambert JC, Amouyel P (2011) Genetics of Alzheimer's disease: New evidences for an old hypothesis? Current Opinion in Genetics and Development 21:295–301 . doi: 10.1016/j.gde.2011.02.002

Lambert JC, Zelenika D, Hiltunen M, et al (2011) Evidence of the association of BIN1 and PICALM with the AD risk in contrasting European populations. Neurobiology of Aging 32:756.e11-756.e15 . doi: 10.1016/j.neurobiolaging.2010.11.022

Liu G, Li F, Zhang S, et al (2014a) Analyzing Large-Scale Samples Confirms the Association Between the ABCA7 rs3764650 Polymorphism and Alzheimer's Disease Susceptibility. Molecular Neurobiology 50:757–764 . doi: 10.1007/s12035-014-8670-4

Liu G, Wang H, Liu J, et al (2014b) The CLU gene rs11136000 variant is significantly associated with Alzheimer's disease in Caucasian and Asian populations. NeuroMolecular Medicine 16:52–60 . doi: 10.1007/s12017-013-8250-1

Luo J, Li S, Qin X, et al (2014) Meta-analysis of the association between CR1 polymorphisms and risk of late-onset Alzheimer's disease. Neuroscience Letters 578:165–170 . doi: 10.1016/j.neulet.2014.06.055

Ma X-Y, Yu J-T, Tan M-S, et al (2013) Missense variants in CR1 are associated with increased risk of Alzheimer' disease in Han Chinese. Neurobiology of aging 35:443.e17-443.e21 . doi: 10.1016/j.neurobiolaging.2013.08.009

Macaskill P, Walter SD, Irwig L (2001) A comparison of methods to detect publication bias in meta-analysis. Statistics in Medicine 20:641–654 . doi: 10.1002/sim.698

Mao YF, Guo ZY, Pu JL, et al (2015) Association of CD33 and MS4A cluster variants with Alzheimer's disease in East Asian populations. Neuroscience Letters 609:235–239 . doi: 10.1016/j.neulet.2015.10.007

Naj AC, Jun G, Beecham GW, et al (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nature Genetics 43:436–441 . doi: 10.1038/ng.801

Pant S, Sharma M, Patel K, et al (2009) AMPH-1/Amphiphysin/Bin1 functions with RME-1/Ehd in endocytic recycling. Cell 11:1399–1410 . doi: 10.1038/ncb1986.AMPH-1/Amphiphysin/Bin1

R Development Core Team R (2011) R: A Language and Environment for Statistical Computing

Ramirez LM, Goukasian N, Porat S, et al (2016) Common variants in ABCA7 and MS4A6A are associated with cortical and hippocampal atrophy. Neurobiology of Aging 39:82–89 . doi: 10.1016/j.neurobiolaging.2015.10.037

Ren G, Vajjhala P, Lee JS, et al (2006) The BAR domain proteins: molding membranes in fission, fusion, and phagy. Microbiology and molecular biology reviews : MMBR 70:37–120 . doi: 10.1128/MMBR.70.1.37-120.2006

Rizzi F, Caccamo AE, Belloni L, Bettuzzi S (2009) Clusterin is a short half-life, poly-ubiquitinated protein, which controls the fate of prostate cancer cells. Journal of Cellular Physiology 219:314–323 . doi: 10.1002/jcp.21671

Rodrigues CL, Ziegelmann PK (2010) Metanálise: um guia prático. Rev HCPA 30:436–447

Rogers J, Li R, Mastroeni D, et al (2006) Peripheral clearance of amyloid beta peptide by complement C3-dependent adherence to erythrocytes. Neurobiology of Aging 27:1733–1739 . doi: 10.1016/j.neurobiolaging.2005.09.043

Sayers EW, Barrett T, Benson DA, et al (2009) Database resources of the National Center for Biotechnology Information. Nucleic Acids Research 37: . doi: 10.1093/nar/gkn741

Schmidt C, Wolff M, Von Ahsen N, Zerr I (2012) Alzheimer's disease: Genetic polymorphisms and rate of decline. Dementia and Geriatric Cognitive Disorders 33:84–89 . doi: 10.1159/000336790

Schwarzer G, Mair P, Hatzinger R (2007) meta : An R Package for Meta-Analysis. R Newsews 7:40–45

Seshadri S (2010) Genome-wide Analysis of Genetic Loci Associated With Alzheimer Disease. Jama 303:1832 . doi: 10.1001/jama.2010.574

Shen N, Chen B, Jiang Y, et al (2014) An Updated Analysis with 85,939 Samples Confirms the Association Between CR1 rs6656401 Polymorphism and Alzheimer's Disease. Molecular Neurobiology 51:1017–1023 . doi: 10.1007/s12035-014-8761-2

Shulman JM, Chen K, Keenan BT, et al (2013) Genetic susceptibility for Alzheimer disease neuritic plaque pathology. JAMA neurology 70:1150–7 . doi: 10.1001/jamaneurol.2013.2815

Sterne JAC, Harbord RM (2004) Funnel plots in meta-analysis. The Stata Journal 4:127–141 . doi: The Stata Journal

Sterne JAC, utton AJ, Ioannidis Jpa et a l (2011) Recommendations for examining and interpretingfunnel plot asymmetry in meta-analyses of randomisedcontrolled trials. Bmj 342:d4002–d4002

Trikalinos TA, Salanti G, Khoury MJ, Ioannidis JPA (2006) Impact of violations and deviations in Hardy-Weinberg equilibrium on postulated gene-disease associations. American Journal of Epidemiology 163:300–309 . doi: 10.1093/aje/kwj046

Weis JH, Morton CC, Bruns GAP, et al (1987) A complement receptor locus : genes encoding C3b / C4b receptor and C3d / Epstein-Barr virus receptor map to 1q32 . The Journal of Immunology 138:312–315

Yu JT, Tan L, Hardy J (2014) Apolipoprotein E in Alzheimer's Disease: An Update. Annual Review of Neuroscience 37:79–100 . doi: 10.1146/annurev-neuro-071013-014300

Zhu R, Liu X, He Z (2016) The Bridging Integrator 1 Gene Polymorphism rs744373 and the Risk of Alzheimer's Disease in Caucasian and Asian Populations: An Updated Meta-Analysis. Molecular Neurobiology 54:1419–1428 . doi: 10.1007/s12035-016-9760-2

Appendix A. Supplementary data

Tabela S1 disponível em:

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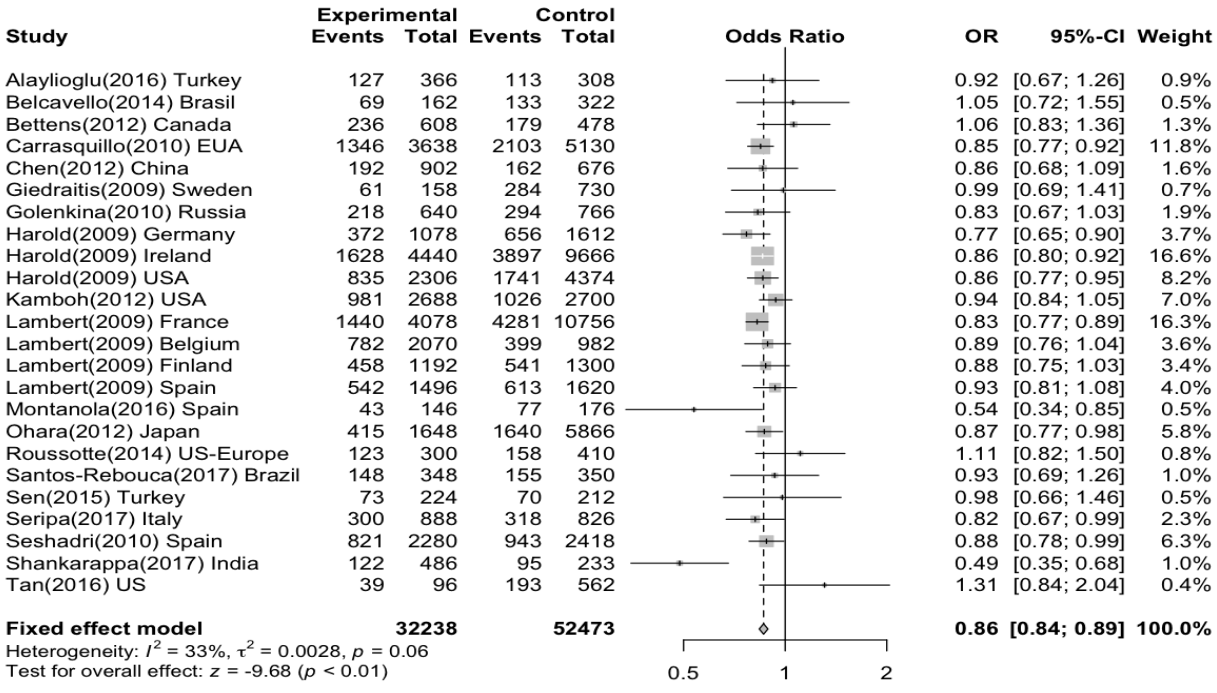

Figure S1. Forest plot on total sample of rs11136000 *CLU*.

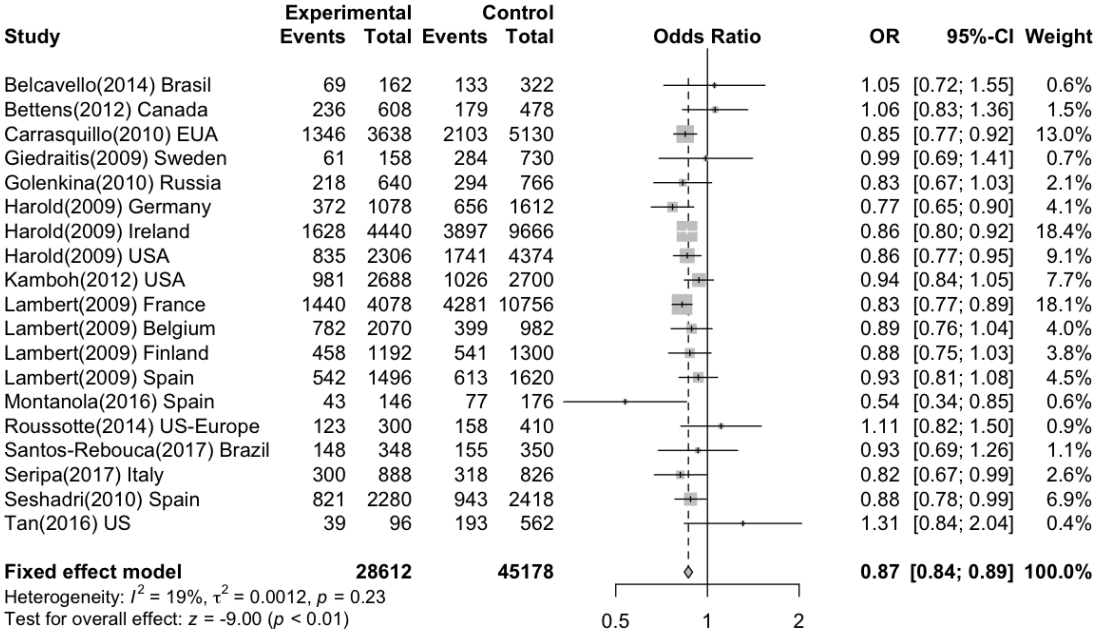

Figure S2. Forest plot on Caucasian population of rs11136000 *CLU*.

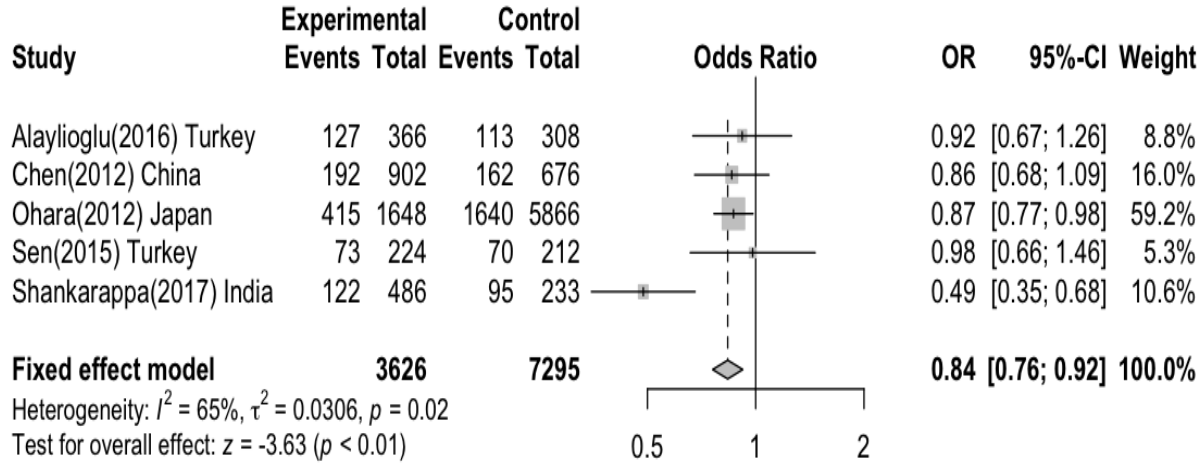

Figure S3. Forest plot using fixed effects model on Mixed population of rs11136000 *CLU*.

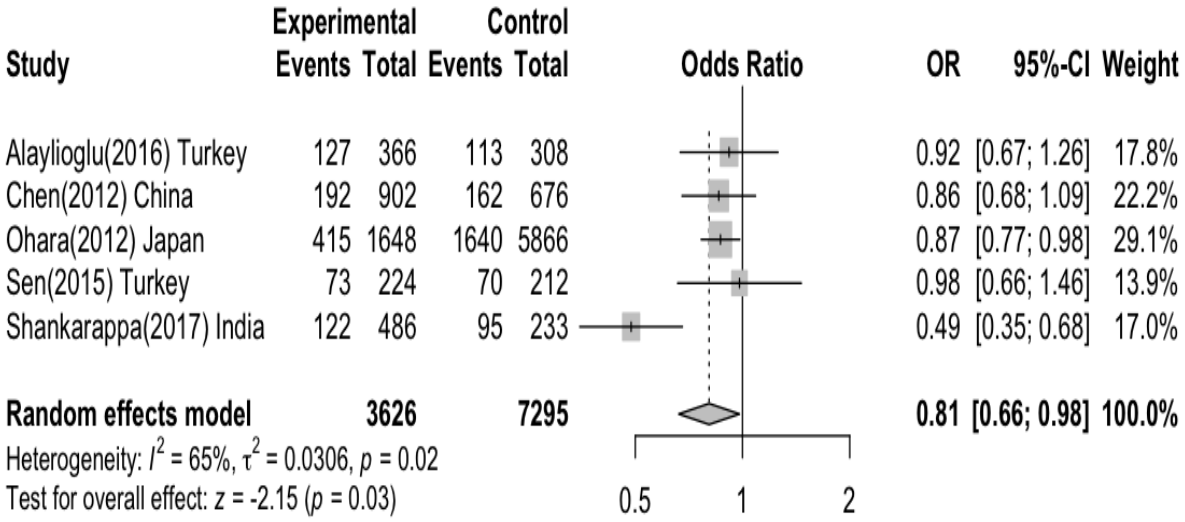

Figure S4. Forest plot using random effects model on Mixed population of rs11136000 *CLU*.

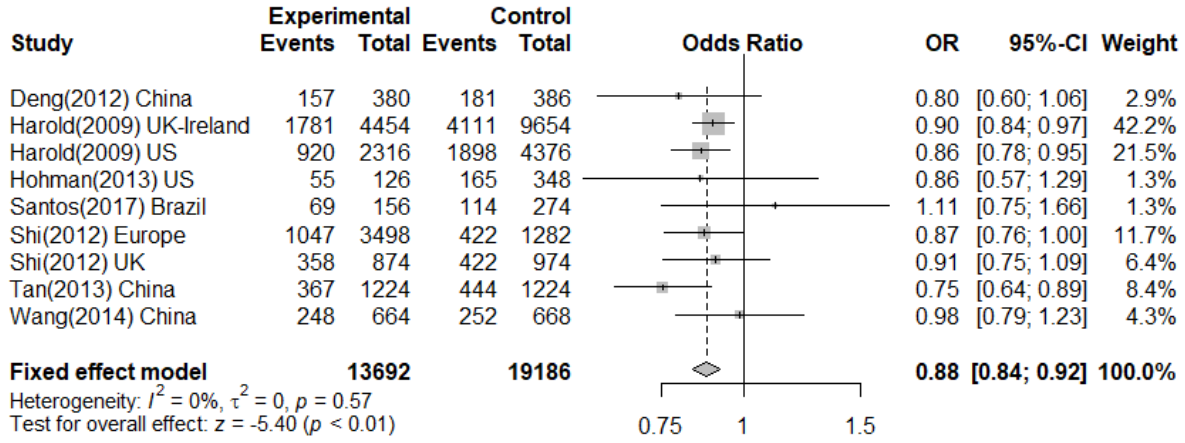

Figure S5. Forest plot on total sample of rs610932 *MS4A6A*.

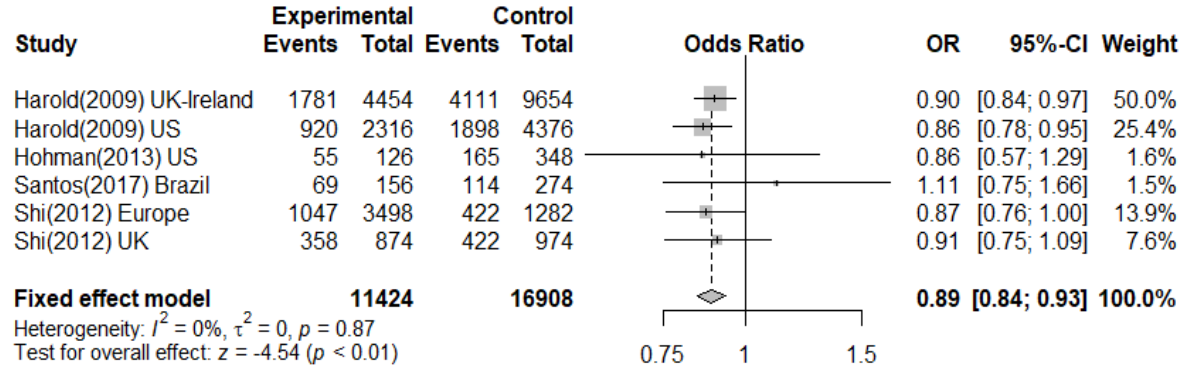

Figure S6. Forest plot on Caucasian population of rs610932 *MS4A6A*.

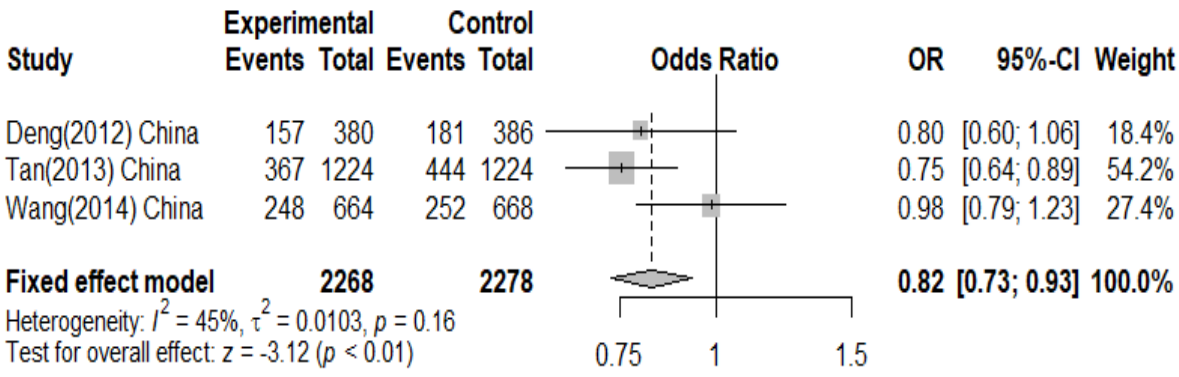

Figure S7. Forest plot on Asian population of rs610932 *MS4A6A*.

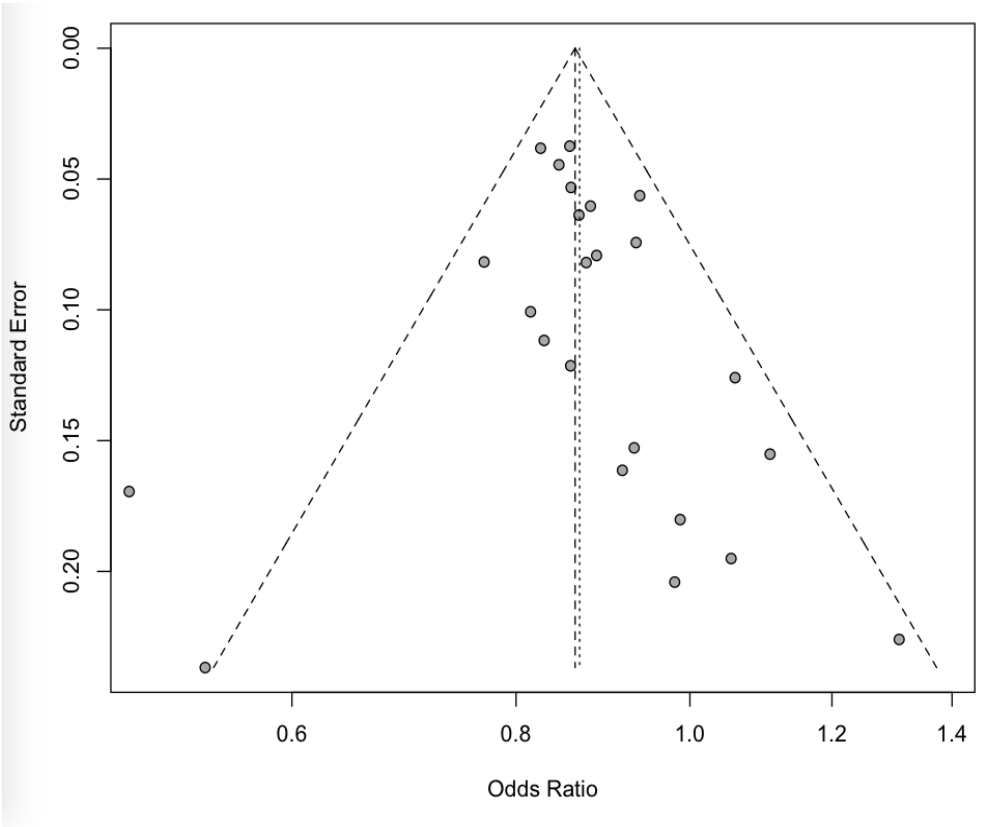

Figure S8. Funnel plot on total sample of rs11136000 *CLU.*

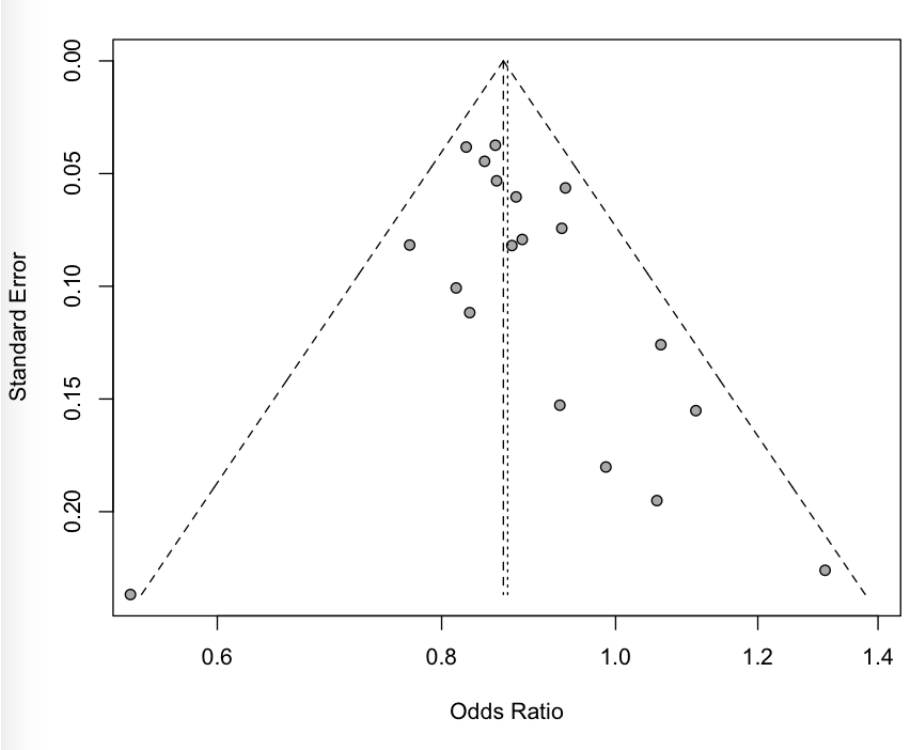

Figure S9. Funnel plot on Caucasian population of rs11136000 *CLU*.

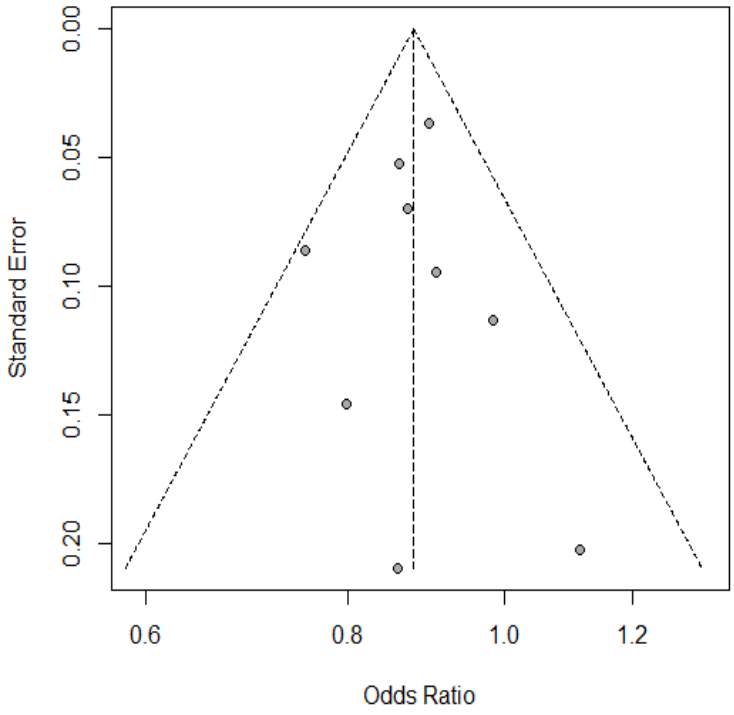

Figure S10. Funnel plot on total sample of rs610932 *MS4A6A*.

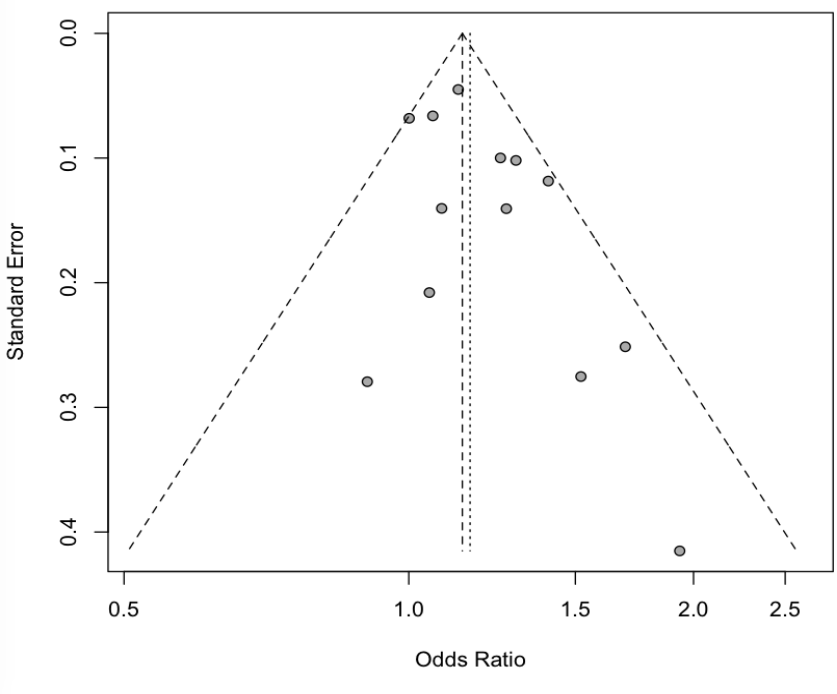

Figure S11. Funnel plot on total sample of rs6656401 *CR1*.

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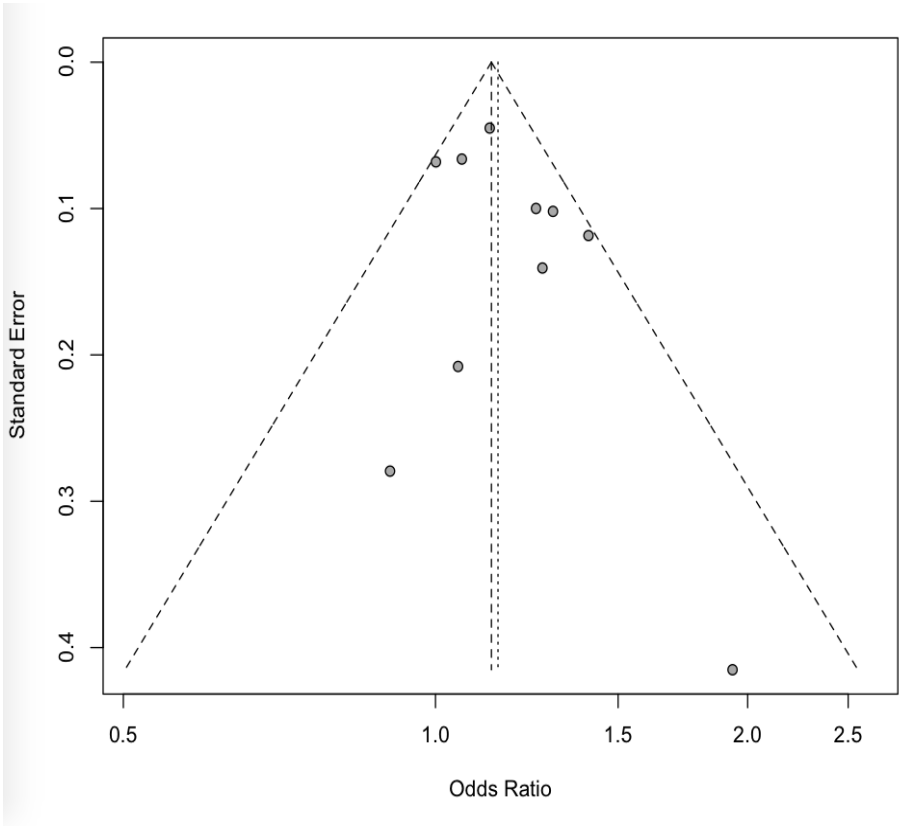

Figure S12. Funnel plot on Caucasian population of rs6656401 *CR1*.

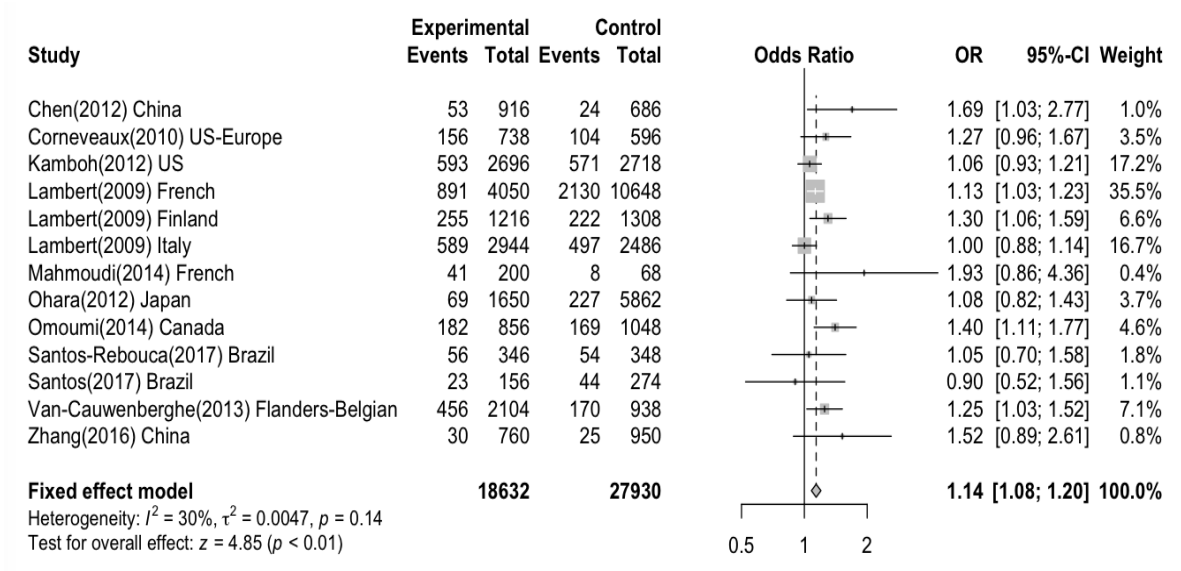

Figure S13. Forest plot using fixed effect model on total sample of rs6656401 *CR1*.

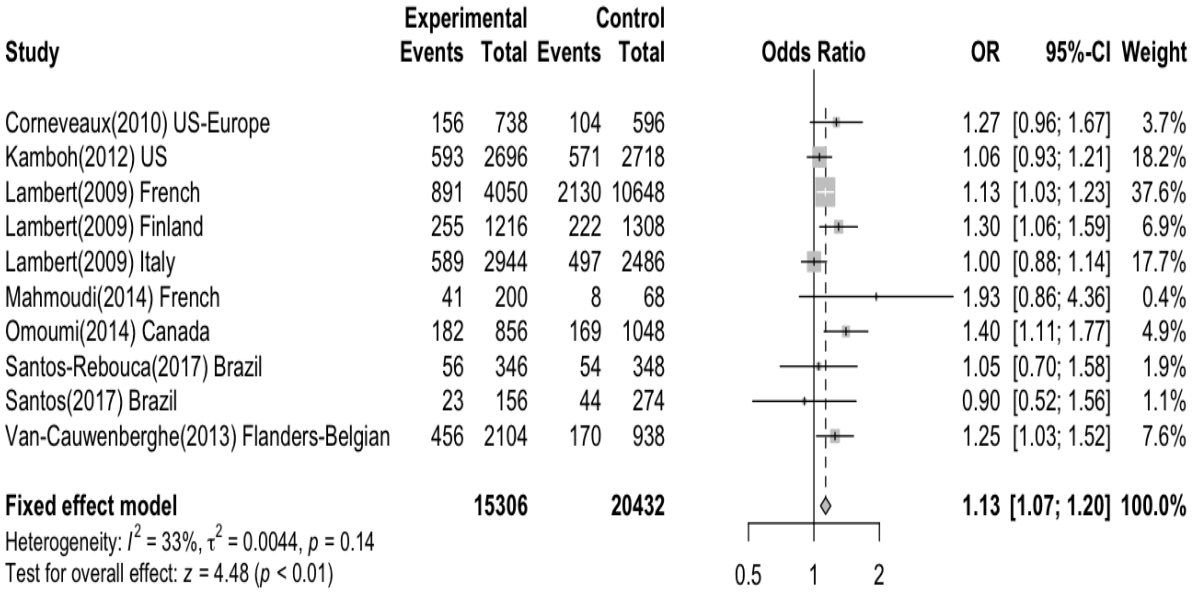

Figure S14. Forest plot using fixed effect model on Caucasian population of rs6656401 *CR1*.

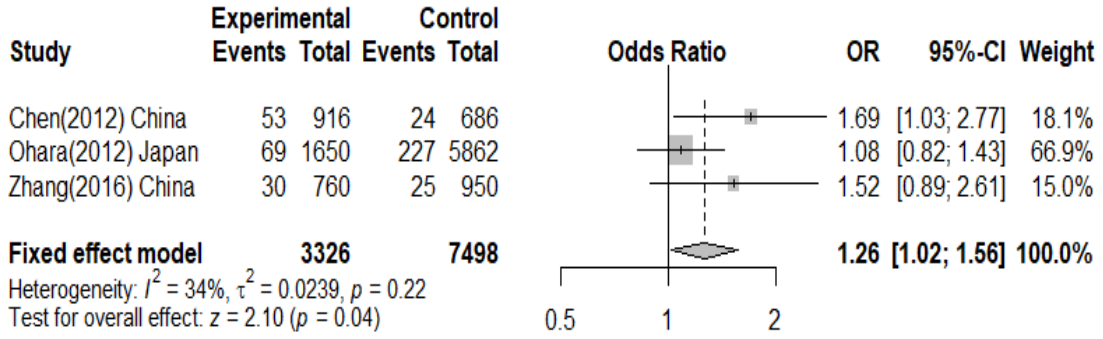

Figure S15. Forest plot using fixed effect model on Asian population of rs6656401 *CR1*.

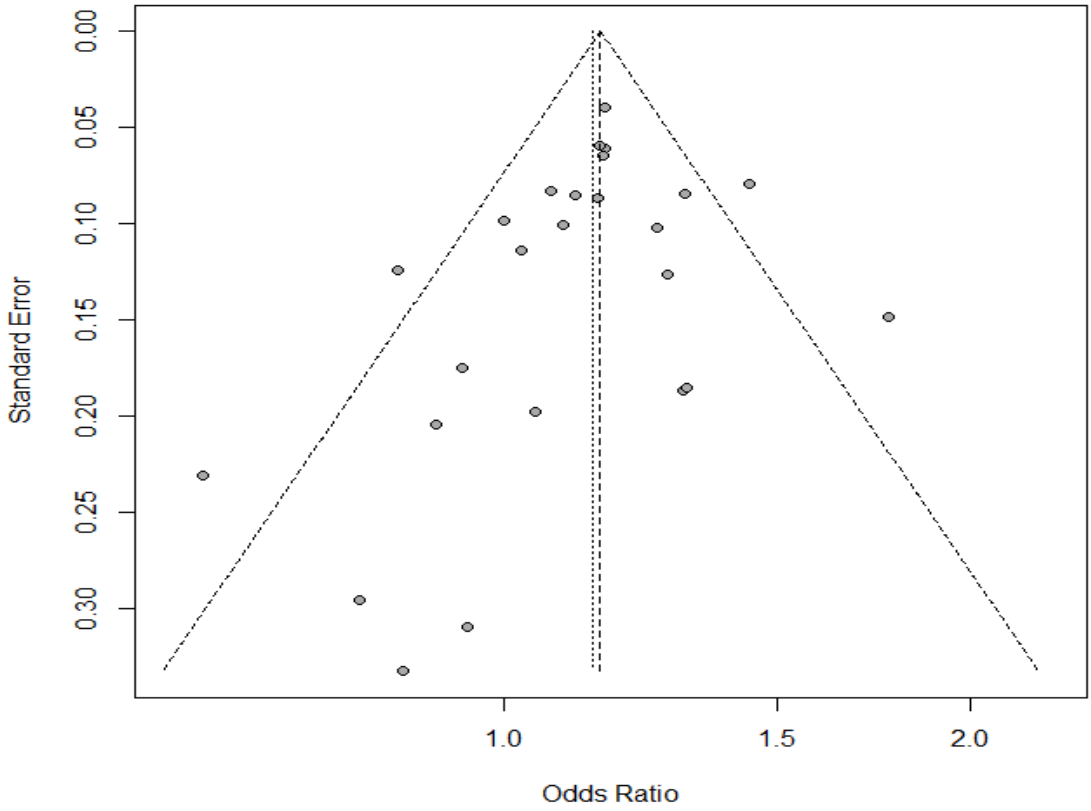

Figure S16. Funnel plot using random effects model on total sample of rs744373 *BIN1*.

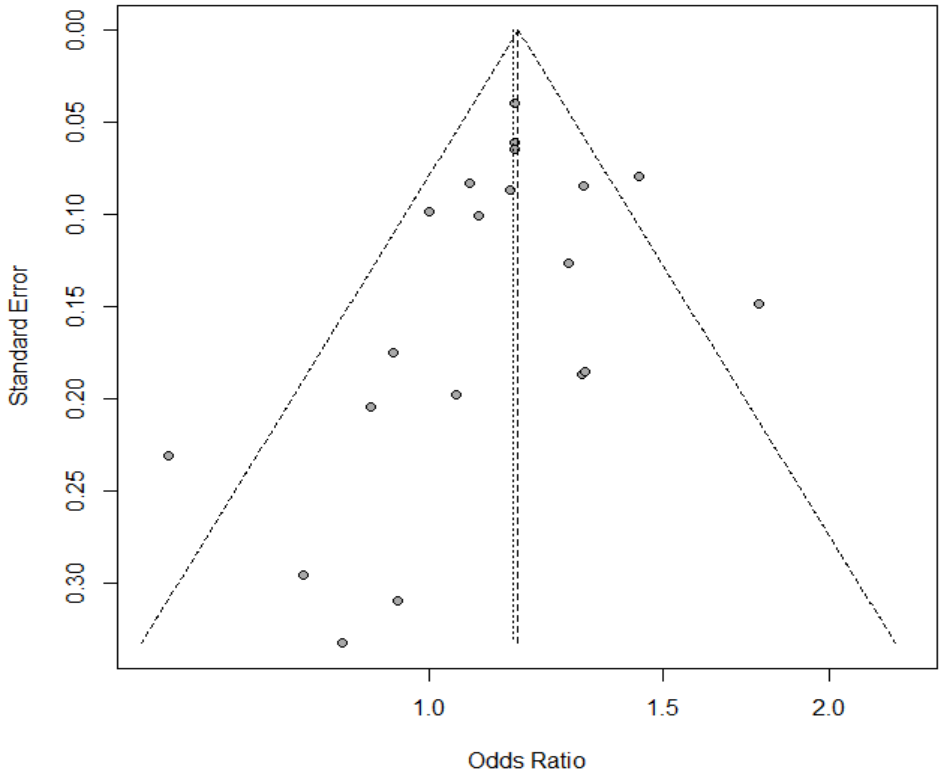

Figure S17. Funnel plot using random effects model on Caucasian population of rs744373 *BIN1*.

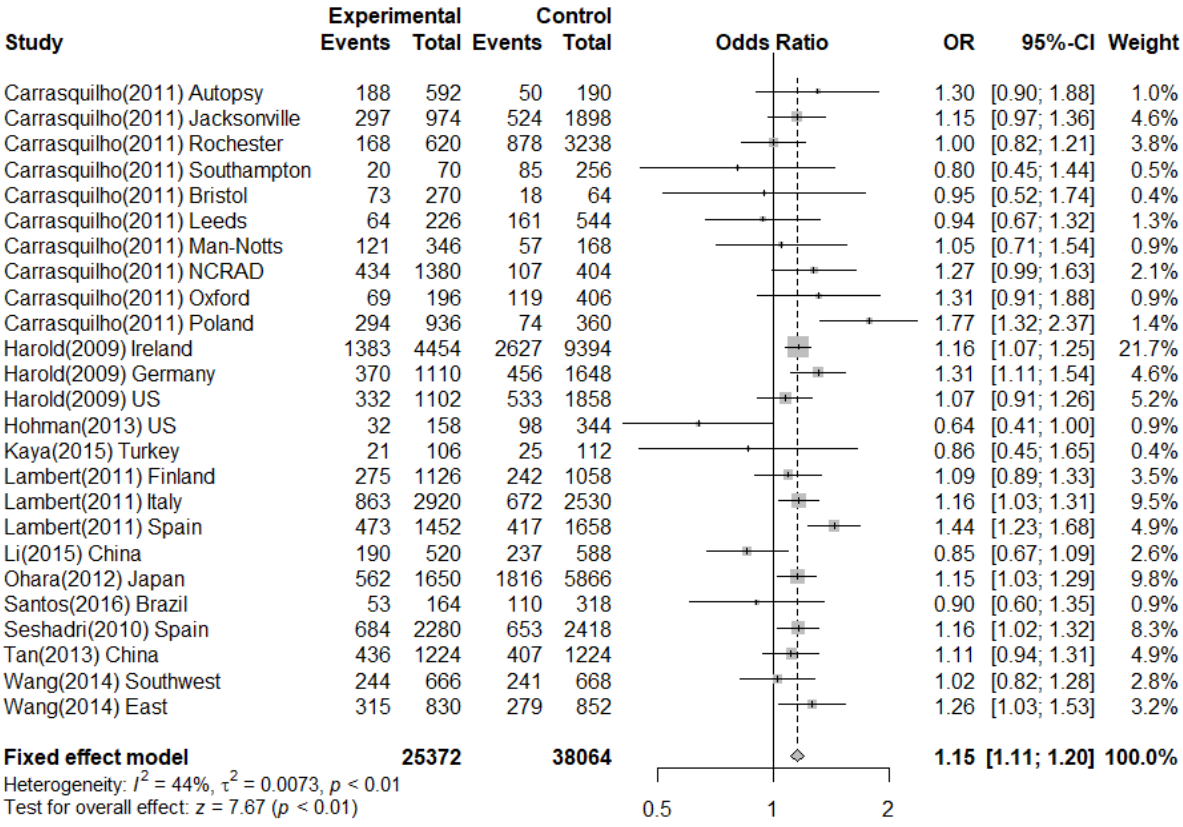

Figure S18. Forest plot using fixed effect model on total sample of rs744373 *BIN1*.

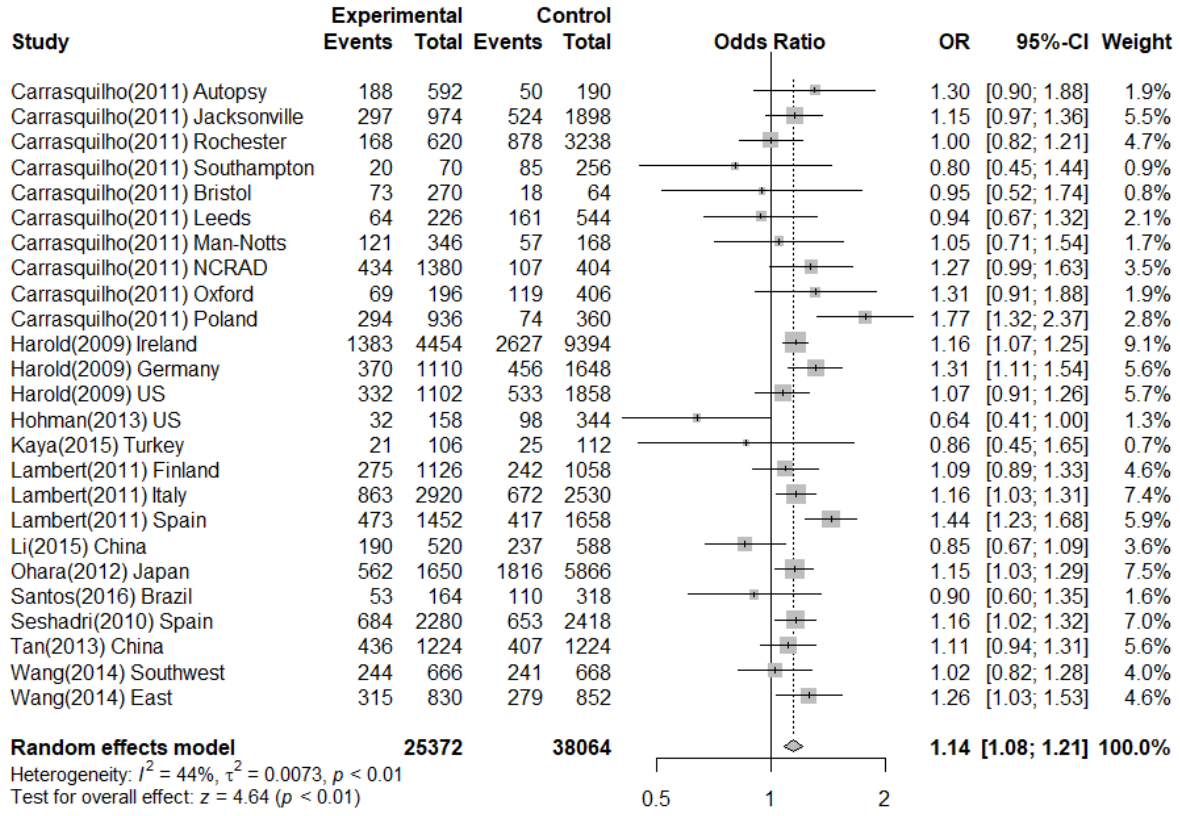

Figure S19. Forest plot using random effect model on total sample of rs744373 *BIN1*.

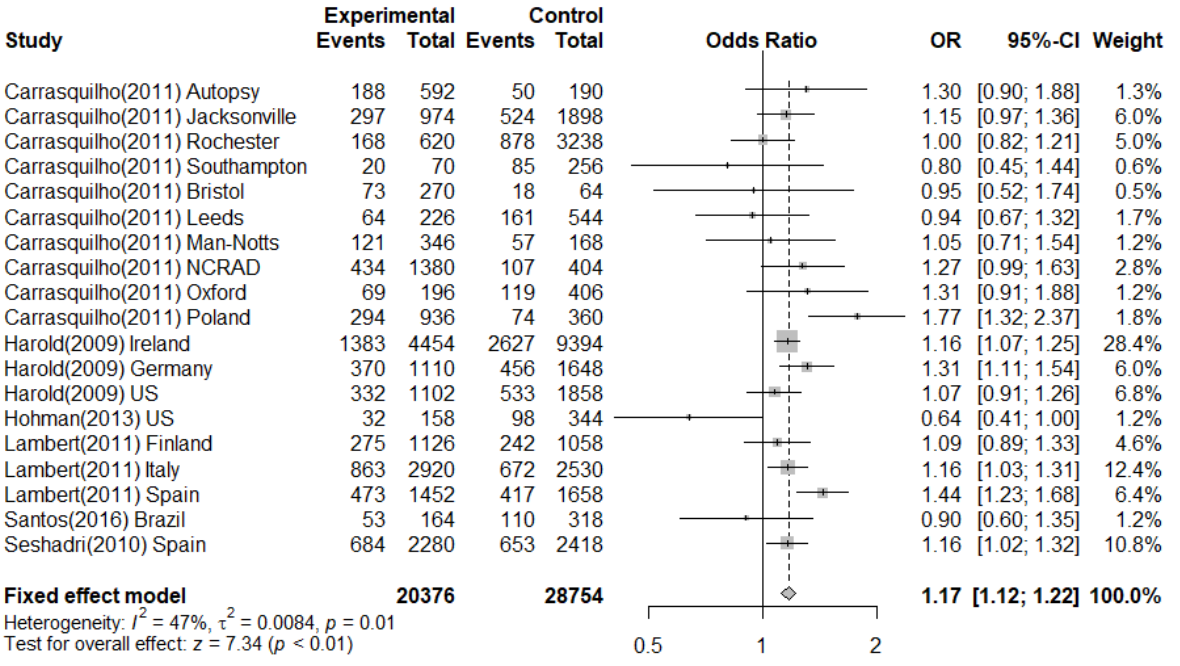

Figure S20. Forest plot using fixed effects model on Caucasian population of rs744373 *BIN1*.

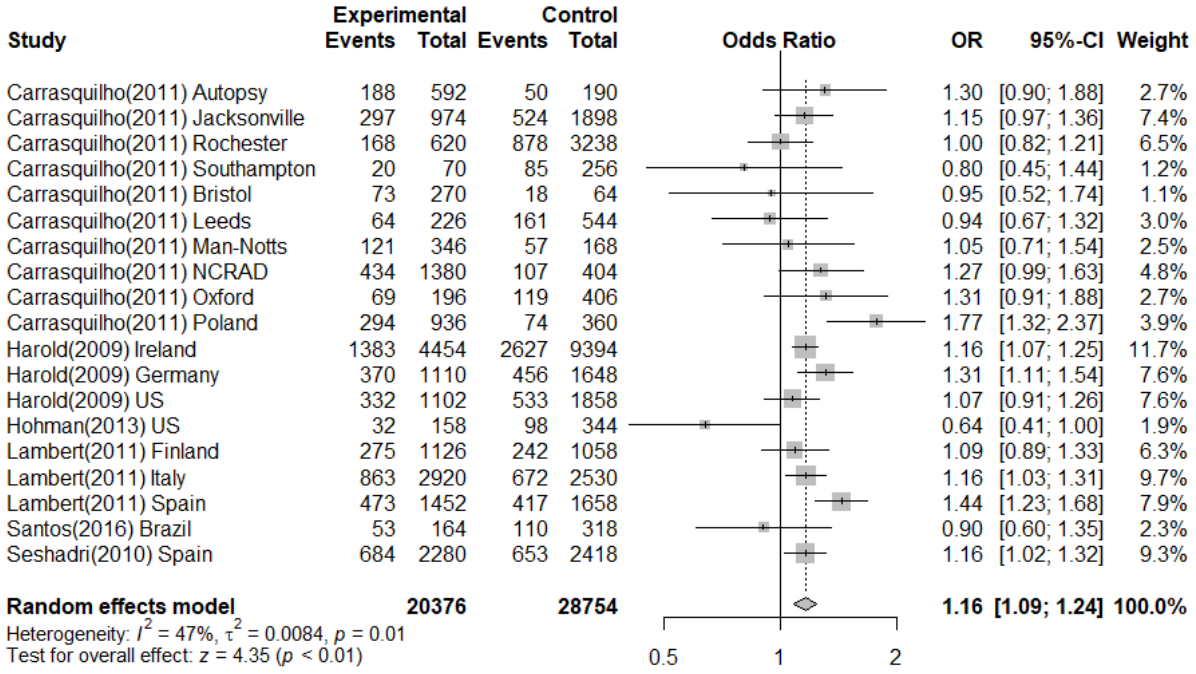

Figure S21. Forest plot using random effects model on Caucasian population of rs744373 *BIN1*.

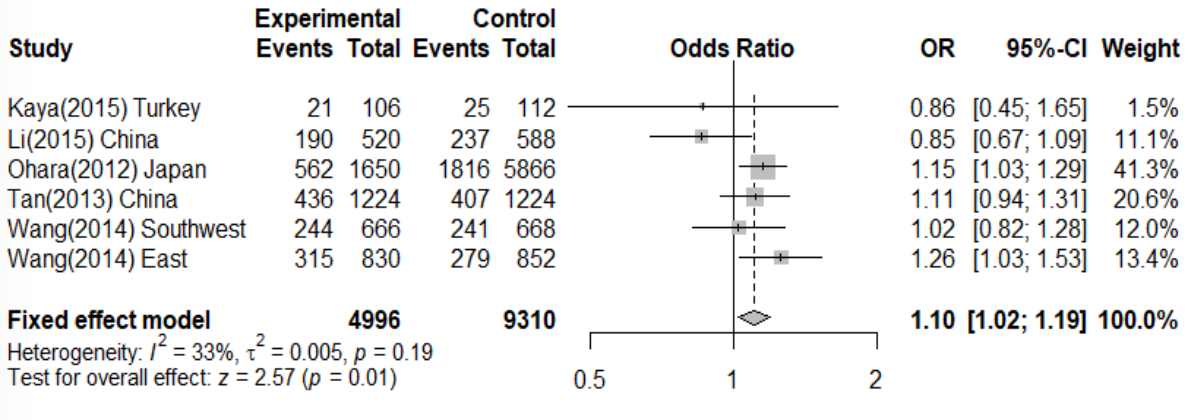

Figure S22. Forest plot using fixed effects model on Mixed population of rs744373 *BIN1*.

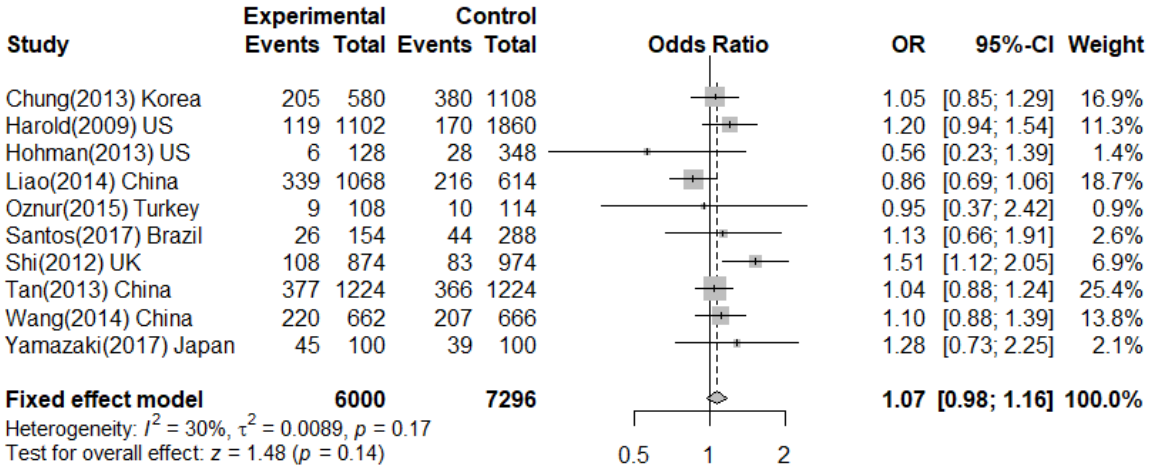

Figure S23. Forest plot using fixed effects model on total sample of rs3764650 *ABCA7*.

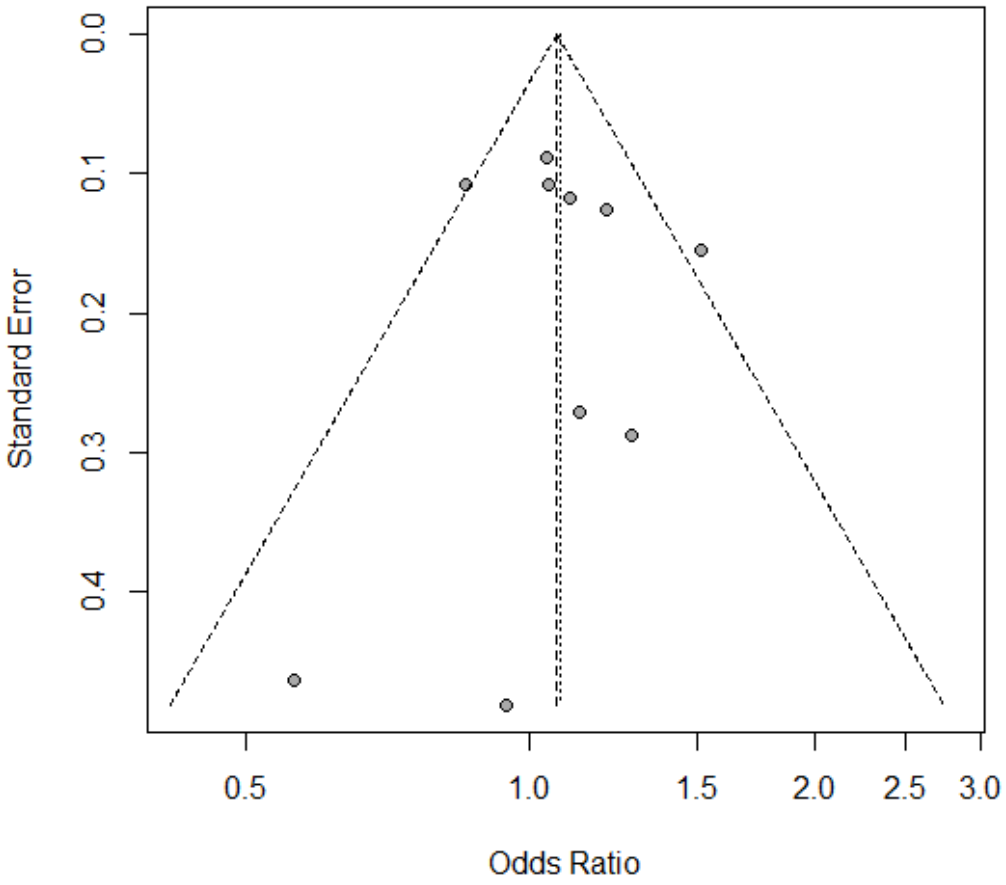

Figure S24. Funnel plot on total sample of rs3764650 *ABCA7*.

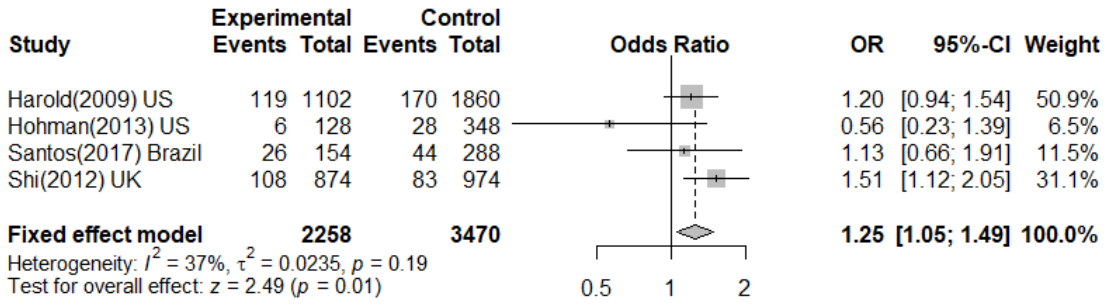

Figure S25. Forest plot using fixed effects model on Caucasian population of rs3764650 *ABCA7*.

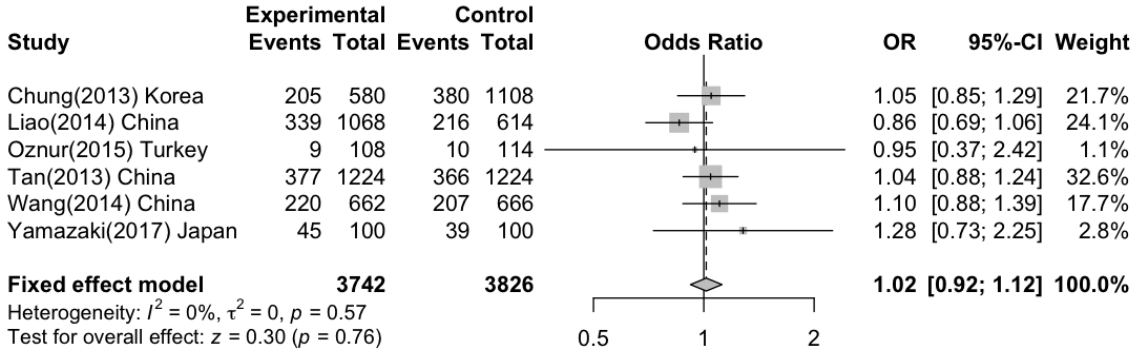

Figure S26. Forest plot using fixed effects model on Mixed population of rs3764650 *ABCA7*.

3.2 Capítulo 2

• *The combined risk effect among BIN1, CLU, and APOE genes in Alzheimer's Disease* - publicado na revista *Genetics and Molecular Biology.*

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Research Article
Human and Medical Genetics

The combined risk effect among *BIN1*, *CLU*, and *APOE* genes in Alzheimer's disease

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The combined risk effect among *BIN1*, *CLU,* and *APOE* genes in Alzheimer's disease

Abstract

Genome-wide associations studies (GWAS) are detecting new variants associated with late-onset of Alzheimer's disease (LOAD), a multifactorial neurodegenerative disorder. The variants rs744373 *BIN1,* rs11136000 *CLU* and rs3764650 *ABCA7* uncovered by GWAS led to different AD pathways, such as metabolism, trafficking and endocytosis of lipids and inflammation. However, most of the association studies did not replicate these variants with significance. This could be due to a small power effect evident when these variants are tested independently with LOAD. Therefore, we aimed to investigate whether the combination of different variants would additively modify the risk of association with LOAD that is observed in GWAS. We performed an association study testing pairwise variants in metabolism, trafficking and endocytosis of lipid (rs429358 and rs7412 *APOE*, rs744373 *BIN1*, rs3764650 *ABCA7* and rs11136000 *CLU)* pathways with LOAD in samples from southeastern Brazil. Our data suggest a risk effect for LOAD between *APOE* with *CLU* and *APOE* with *BIN1* genes.

Keywords: GWAS variants, APOE, CLU, BIN1, ABCA7.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that affects millions of elders globally (Prince *et al.*, 2016). Familial, or early-onset AD (EOAD), accounts for 2% of AD cases and occurs before 65 years. EOAD has Mendelian patterns of inheritance, with mutations in *APP* (amyloid precursor protein), *PSEN1* (presenilin 1) and *PSEN2* (presenilin 2) genes (Bertram *et al.*, 2010; Holtzman *et al.*, 2011). Unlike EOAD, late-onset AD (LOAD) has a multifactorial pattern, with influence of genetic and environmental factors. It occurs after 65 years and accounts for 98% of AD cases (Yu

et al., 2014). To date, the ε4 allele in the apolipoprotein E (*APOE*) gene is considered a major risk factor for LOAD worldwide (Lambert and Amouyel, 2011).

The main hypothesis regarding neurodegeneration in AD is that the amyloid cascade leads to amyloid plaque formation (Heppner *et al.*, 2015). This event occurs due to the impaired degradation of neurotoxic Aβ42 peptides. Both the increased formation and the decrease in the clearance of Aβ42 peptides, is considered to play a role in the development of AD. Recent studies suggest that cholesterol is a part of the regulation in the clearance of Aβ42 peptides formed in the brain (Kojro *et al.*, 2001; O'Brien *et al.*, 2011; Reitz, 2013). In neurons, cholesterol is vital for function and plasticity. Function and plasticity are important in the process of learning and memory formation, all of which are found to be impaired in AD (Pfrieger, 2003). Moreover, several genes beyond APOE have been implicated in alterations in cholesterol metabolism, trafficking and endocytosis, such as Clusterin *(CLU),* Bridging integrator 1 *(BIN1)* and the ATP-binding cassette transporter A7 (*ABCA7*) genes, all variants that have been identified in genome-wide associations studies (GWAS) (Harold *et al.*, 2009; Lambert *et al.,* 2009; Hollingworth *et al.*, 2011; Naj *et al.*, 2011; Karch and Goate, 2014). Most of the GWAS variants associated with LOAD have small effects individually (Ebbert *et al.*, 2015). In addition, the case-control studies that replicated those variants did not all reach significance. In this scenario, the nonsignificance may be due to a lack of the power effect of those variants when tested independently with LOAD. It is possible that a combination of different variants together would enhance the effect of association with LOAD that is observed in GWAS. Therefore, the main goal of this study was to test pairwise variants from metabolism, trafficking and endocytosis of lipid (rs429358 and rs7412 *APOE*, rs744373 *BIN1*, rs3764650 *ABCA7* and rs11136000 *CLU)* pathways with late-onset AD in a sample from southeastern Brazil.

Subjects and Methods

Subjects

This is an association study with 224 unrelated individuals. We selected 79 elderly patients diagnosed for probable AD with LOAD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer

disease and Related Disorders Association (NINCDS-ADRDA). These patients had a comprehensive diagnostic evaluation for dementia and fulfill other criteria, such as the Mini-Mental State Examination (MMSE). For controls, 145 healthy elderly patients were selected, and all were matched for sex and age. Demographic and clinical data of the sample composition is presented in Table 1. Our recent study (dos Santos *et al.*, 2017) demonstrated that our sample had no difference for variables, such as gender (p=0.536), ethnic background (p=0.641), schooling (p=0.281) and age (p=0.144), except for APOE status (p<0.001), among AD cases and controls.

All the participants in this research resided in the metropolitan region of Espírito Santo, Grande Vitória, in southeastern Brazil. They were assisted by a geriatrician at the Geriatric Unit of the Hospital Santa Casa de Misericórdia de Vitória (HSCMV) and Centro de Atendimento ao Idoso (CRAI), ES, Brazil. Additionally, the participants or their relatives gave written informed consent agreeing to participate in the research study. Information regarding age, gender, ethnic background composition and schooling was collected. The geriatrician meticulously selected only participants with no family history of Alzheimer's. This study was accepted by the Committee of Ethics in Human Research of Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória, Brazil.

Blood sampling and genotyping

Peripheral blood was collected into a 5 mL tube with 5% ethylene diamine tetraacetic acid (EDTA) at the Geriatric Unit of HSCMV and CRAI. The samples were stored at 4 °C prior to analyses. Genomic DNA was isolated according to previous methodology (Miller *et al.*, 1988). We calculated the adequate sample size using the proportion of the genes in the population based on the overall frequency of the minor alleles of each polymorphism. The estimate of adequate sample size for the *APOE* gene and *ABCA7* polymorphisms was 207, and for the *CLU* and *BIN1* genes it was 377 individuals. Therefore, our results are consistent since our sample contained 224 individuals.

Genotyping was performed by our previous coworkers (Almada *et al.*, 2012; Belcavello *et al.*, 2015; dos Santos *et al.*, 2016, 2017) using the Brazilian sample set of this study. The variant rs3764650 *ABCA7* was performed by Santos *et al.* (2017) through real time - polymerase chain reaction (qPCR), and the three standard

genotypes were confirmed by Sanger sequencing. Analysis of the variants rs744373 *BIN1,* rs11136000 *CLU* and (rs429358 and rs7412) *APOE* were performed, respectively, by Almada *et al.* (2012), Belcavello *et al.* (2015) and dos Santos *et al.* (2016) thought restriction fragment length polymorphism - polymerase chain reaction (RFLP-PCR).

Table 1 - Sample characteristics.

Variable	Controls 145 (100%)	AD Patients 79 (100%)
Gender		
Woman	106 (73.1%)	54 (68.4%)
Man	39 (26.9%)	25 (31.6%)
Ethnic background		
Caucasians	83 (57.2%)	45 (57.0%)
Afro-Brazilians	60 (41.4%)	34 (43.0%)
No identification	2 (1.4%)	0
Schooling		
Literate	94 (64.8%)	41 (51.9%)
Illiterate	45 (31.0%)	28 (35.4%)
No identification	6 (4.1%)	10 (12.7%)
APOE status		
ε4 -	102 (70.3%)	35 (44.3%)
ε4 +	43 (29.7%)	44 (55.7%)
Age (mean and SD)	80,1 ± 7,8	81,6 ± 7
MMSE	> 28	14-4

AD Patients = Alzheimer's disease patients; ε4 += ε4 carriers; ε4 -= ε4 non-carriers; SD= standard deviation; Mini-Mental State Examination (MMSE).

Statistical Analysis

All the statistical analyses were performed using SPSS (IBM) software v.23.0 for Windows. A *p*-value $\leq$ 0.05 was considered significant.

Logistic regression analysis was performed for each single nucleotide polymorphism (SNP) and for allelic combinations between two polymorphisms. The *p*-value was adjusted using *APOE* status, age, gender, school level and ethnic

background as variables. For education, was considered literate or illiterate. For *APOE* status, was considered an ε4 + for those that carried at least one ε4 allele; and ε4 -, for those that carried no ε4 allele. The *p*-value in *APOE* association with LOAD when pairwise with another variant, or not combined, was not adjusted for *APOE* status. The allelic combinations tested followed genes from lipid metabolism and the endocytosis pathway (*ABCA7, CLU, BIN1* and *APOE*). The allele frequencies of SNPs were inferred from the following studies: dos Santos *et al.* (2017) for rs3764650 *ABCA7;* dos Santos *et al.* (2016) for rs744373 *BIN1*, Belcavello *et al.* (2015) for rs11136000 *CLU,* and Almada *et al.* (2012) for rs429358 and rs7412 *APOE.*

Results

Results of the test of independent association for LOAD of *CLU* (rs11136000), *ABCA7* (rs3764650), *BIN1* (rs744373) and *APOE* (rs429358 and rs7412) are presented in Table 2. As expected, the ε4 allele in *APOE* was statistically significant. No association was observed for LOAD for the G allele in *ABCA7*, the T allele in *CLU* and the C allele in *BIN1* genes.

The data of combined allelic variants are presented in Table 3. A significant association was not observed between *CLU* and *ABCA7, CLU* and *BIN1* or *ABCA7* and *BIN1*. The presence of the ε4 allele in *APOE* alone was associated with LOAD in the absence of the minor G allele *ABCA7* ($p<0.001$), the absence of the minor C allele in *BIN1* ($p<0.001$) and the T allele in *CLU* ($p=0.030$), also after p-value adjustment. The presence of the C allele in *BIN1* and the ε4 allele in *APOE* showed risk for LOAD (OR = 3.489), even after p-value adjustment (OR = 3.678). The presence of both T alleles in *CLU* and the ε4 allele in *APOE* enhances the risk 3.911-fold for LOAD and after p-value adjustment (OR = 3.633). However, no association was found between the ε4 allele in *APOE* and the G allele in *ABCA7* ($p= 0.128$) and after *p*-value adjustment ($p=0.115$).

Table 2 - Test for independent interaction of SNPs with LOAD.

Polymorphism	AD Patients N (%)	Controls N (%)	OR (95% IC)	*p*-value[a]	OR (95% IC)	*p*-value[b]
ABCA7 (rs3764650)						
T	52 (68.4%)	105 (75.5%)	1 (Reference)	-	1 (Reference)	-
G	24 (31.6%)	34 (24.5%)	1.425 (0.767-2.648)	0.262	1.552 (0.794-3.037)	0.199
CLU (rs11136000)						
C	89 (57.1%)	170 (58.6%)	1 (Reference)	-	1 (Reference)	-
T	67 (42.9%)	120 (41.4%)	0.938 (0.632-1.390)	0.764	0.840 (0.537-1.314)	0.445
BIN1 (rs744373)						
T	106 (67.1%)	186 (65%)	1 (Reference)	-	1 (Reference)	-
C	52 (32.9%)	100 (35%)	0.912 (0.605-1.377)	0.662	0.960 (0.606-1.520)	0.861
APOE (rs429358, rs7412)						
ε4 -	103 (65.2%)	245 (84.5%)	1 (Reference)	-	1 (Reference)	-
ε4 +	55 (34.8%)	45 (15.5%)	2.907 (1.842-4.588)	< 0.001	3.029 (1.873-4.898)	< 0.001 [c]

ε4 - = ε4 non-carriers; ε4+ = ε4 carriers; AD Patients = Alzheimer's disease patients; OR = odds ratio; CI = confidential interval;*p*-value considerer ≤ 0.05; [a] = crude *p*-value; [b] = *p*-value adjusted by the variables age, gender, educational attainment, ethnic background and APOE ε4 status; [c] = *p*-value adjusted by the variables on [b] except for APOE ε4 status.

Table 3 - Combined allelic effect among variants in the study.

Genes		AD Patients N (%)	Controls N (%)	OR (95% IC)	*p*-value[a]	OR (95% IC)	*p*-value[b]
ABCA7 (G)	*APOE* (ε4)						
-	-	88 (57.1%)	213(73.9%)	1 (Reference)	-	1 (Reference)	-
-	+	40 (26%)	31 (10.8%)	3.123 (1.837-5.310)	<0.001	3.459 (1.977-6.052)	< 0.001
+	-	15 (9.8%)	30 (10.4%)	1.210 (0.621-2.360)	0.575	1.391 (0.702-2.755)	0.344
+	+	11 (7.1%)	14 (4.9%)	1.902 (0.831-4.352)	0.128	2.014 (0.844-4.806)	0.115
BIN1 (C)	*APOE* (ε4)						
-	-	61 (38.6%)	149(52.1%)	1 (Reference)	-	1 (Reference)	-
-	+	45 (28.5%)	37 (13.0%)	2.971 (1.753-5.033)	<0.001	3.376 (1.926-5.918)	< 0.001
+	-	42 (26.6%)	93 (32.5%)	1.103 (0.689-1.766)	0.683	1.313 (0.788-2.187)	0.297
+	+	10 (6.3%)	7 (2.4%)	3.489 (1.270-9.588)	0.015	3.678 (1.275-10.616)	0.016
CLU (T)	*APOE* (ε4)						
-	-	57 (36.5%)	138(47.6%)	1 (Reference)	-	1 (Reference)	-
-	+	32 (20.5%)	32 (11%)	2.421 (1.357-4.320)	0.030	2.664 (1.450-4.893)	0.020
+	-	46 (29.5%)	107(36.9%)	1.041 (0.655-1.654)	0.860	1.055 (0.640-1.741)	0.834
+	+	21 (13.5%)	13 (4.5%)	3.911 (1.834-8.341)	< 0.001	3.633 (1.628-8.107)	0.020
BIN1 (C)	*ABCA7* (G)						
-	-	79 (51.3)	148 (52.1)	1 (Reference)	-	1 (Reference)	-
-	+	24 (15.6)	36 (12.7)	1.249 (0.696-2.240)	0.456	1.474 (0.786-2.770)	0.226
+	-	49 (31.8)	94 (33.1)	0.977 (0.629-1.517)	0.916	1.077 (0.658-1.763)	0.768
+	+	2 (1.3)	6 (2.1)	0.624 (0.123-3.166)	0.570	0.824 (0.148-4.586)	0.825
BIN1 (C)	*CLU* (T)						
-	-	66 (42.3)	129 (45.1)	1 (Reference)	-	1 (Reference)	-
-	+	38 (24.4)	57 (19.9)	1.303 (0.785-2.162)	0.306	1.002 (0.565-1.775)	0.995
+	-	23 (14.7)	38 (13.3)	1.183 (0.651-2.149)	0.581	1.336 (0.691-2.584)	0.389
+	+	29 (18.6)	62 (21.7)	0.914 (0.517-1.555)	0.741	0.786 (0.431-1.435)	0.433
ABCA7 (G)	*CLU* (T)						
-	-	67 (43.5)	131 (45.5)	1 (Reference)	-	1 (Reference)	-
+	-	22 (14.3)	37 (12.9)	1.163 (0.635-2.127)	0.625	1.375 (0.715-2.641)	0.340
-	+	61 (39.6)	113 (39.2)	1.055 (0.688-1.620)	0.805	0.914 (0.562-1.486)	0.717
+	+	4 (2.6)	7 (2.4)	1.117 (0.316-3.952)	0.863	0.719 (0.190-2.719)	0.627

+ / - = allelic presence / allelic absence; AD Patients = Alzheimer's disease patients; OR = odds ratio; CI = confidential interval; p-value considerer ≤ 0.05; [a] = crude *p*-value; [b] = *p*-value adjusted by the variables age, gender, educational attainment and ethnic background.

Discussion

LOAD studies of additive combinations of genetic variants are scarce, and most of those published articles had non-GWAS variants. In this study, we aimed to investigate GWAS variants combined and with the *APOE* gene as well, for late-onset AD in samples from southeastern Brazil. Among the combination tested, we found a risk for LOAD between *CLU* and *APOE* and between *BIN1* and *APOE* genes.

The Apolipoprotein E (*APOE*) gene is localized at chromosome region 19q13.2 and encodes the APOE protein, an apolipoprotein (Morgan and Carrasquillo, 2013). Due to the SNPs rs429358 and rs7412 in the *APOE* gene, three haplotypes are formed: ε2 (T allele rs7412 and T allele rs429358), ε3 (C allele rs7412 and T allele rs429358), and the ε4 allele (C allele rs7412 and C allele rs429358) (Morgan and Carrasquillo, 2013). The APOE protein and Clusterin (CLU) protein, another apolipoprotein, carries cholesterol among brain cells (El Gaamouch *et al.*, 2016) and acts on the clearance of Aβ peptides (Rizzi *et al.*, 2009). The CLU or Apolipoprotein J (APOJ) protein (Rizzi *et al.*, 2009) is associated with a neuroprotective effect in AD (Schrijvers *et al.*, 2011). APOJ is encoded by the *Clusterin* (*CLU*) gene, located at chromosome 8p21.1 (Schrijvers *et al.* 2011). The *CLU* gene has the T allele from the polymorphism rs11136000 as a protective factor associated with LOAD in the later GWAS (Harold *et al.*, 2009; Lambert *et al.*, 2009). We found that the T allele in the rs11136000 *CLU* gene is not related to LOAD independently. Our result is corroborated with the new association studies (Tan *et al.*, 2016; Shankarappa *et al.*, 2017) of rs11136000 that did not find an association for LOAD in a population of 407 individuals from India (Shankarappa *et al.*, 2017), and 329 individuals from United States (Tan *et al.*, 2016). In combined variant tests, we found that the T allele in rs11136000 *CLU* in combination with the ε4 allele in *APOE,* enhances the odds of risk for LOAD. A possible explanation is that the rs11136000 variant may be underpowered alone in our sample and when in a combination with the ε4 allele in APOE. This variant may modulate the protectiveness aspect in carriers of the T allele in order to favor risk for AD. We believe that both genes may have functional implications in AD pathology. For instance, studies have shown that the lipidated APOE and CLU proteins can bind to Aβ peptides individually to direct them to clearance in the brain (Tokuda *et al.*, 2000). Additionally, another study demonstrated that in PDAPP transgenic mice, the absence of APOE

and CLU proteins affects the clearance of Aβ peptides (DeMattos *et al.*, 2004). This suggests that *APOE* and *CLU* genes may regulate this function together.

The *Bridging integrator 1 (BIN1)* gene is located at chromosome 2q14.3 and has the SNP rs744373 associated as a risk factor to LOAD (Harold *et al.*, 2009). The *BIN1* gene encodes the BIN1 protein, which is related to intracellular endosome trafficking of lipids and clathrin mediated endocytosis (Pant *et al.*, 2009). In AD, BIN1 may impact trafficking and endocytosis of cholesterol in the brain and the clearance of Aβ peptides, since it may not internalize with efficiency (Pant *et al.*, 2009; Dong *et al.*, 2017). In our data, the C allele in *BIN1* is not independently associated with LOAD. This result is also observed in the work of Hohman *et al.* (2013) in a population from United States (n=235) and in Li *et al.* (2015) in a study of the Han Chinese population (n=554). In our work, the combination of the C allele of rs744373 *BIN1* and the ε4 allele of APOE is risk association (p=0.015) for LOAD. We believe that *BIN1* and *APOE* may have a possible relation in AD. The study of Lazaris *et al.* (2015), for example, reported that the CC genotype in rs744373 *BIN1* modulates the association between plasma levels of APOE and brain amyloidosis, which implies evidence of the interaction between the *BIN1* and *APOE* genes.

The SNP rs3764650 in the *ABCA7* gene was reported by GWAS to be a risk factor for LOAD (Hollingworth *et al.*, 2011). The *ATP-binding cassette transporter A7* (*ABCA7*) gene is a member of the ABC transporters and is located at chromosome 19p13.3. This gene encodes the ABCA7 protein, which actively translocates lipids, such as cholesterol, through cell membranes to APOE and lipidated APOE (Abe-Dohmae *et al.*, 2004; Vasiliou *et al.*, 2009). In the present study, the G allele in rs3764650 *ABCA7* is not associated with LOAD, neither separately nor in combination with the ε4 allele in *APOE* genes. A study by Yazamaki *et al.* (2017) of 100 Japanese patients, and a study by Hohman *et al.* (2013) consisting of 238 American patients also did not find independent association with LOAD. Although our data found no relation of *APOE* with the *ABCA7* gene, studies support a role for ABCA7 in lipidation of the APOE protein with cholesterol and Aβ peptide clearance in the pathogenesis of AD (Kim *et al.*, 2008). For instance, an *in vitro* study by Chan *et al.* (2008) reported that *ABCA7* stimulates cholesterol efflux to *APOE*, and can suppresses Aβ production.

Our work found that the gene combinations of *CLU* with *APOE* and *BIN1* with *APOE* additively modify the risk of association with LOAD. However, we cannot ignore the possibility of a false positive result. This is due to the overpowering effect of ε4

APOE alone, disregarding the gene combination. Nevertheless, the possibility of a false-positive result is little plausible, since we did not find an association of the ε4 allele in *APOE* with the G allele in *ABCA7*. Regarding the data of combined variants that had no association for LOAD in our data, the Brazilian population is a combination of Iberian Caucasians, West Africans, and Native Americans (Lins *et al.*, 2010; Pena *et al.*, 2011). Such ethnic profiles might be responsible for different allele frequencies that may favor risk factors in each population. Moreover, late-onset AD is a complex disease with diverse components in its interactions, such as epigenetics, age, environment, sex, and genetics (Combarros *et al.*, 2009). With respect to genetic factors, no single polymorphism can fully explain the disease (Dong *et al.*, 2017). Rather, it is a combination of gene variants that may enlighten the concepts of susceptibility to AD (Vepsäläinen *et al.*, 2009). We believe our results are important to enhance the understanding in the underling etiology of the disease and establishment of novel therapeutic approaches for AD.

Conclusion

Our data suggest that combinations of variants in *CLU* with *APOE* and *BIN1* with *APOE* genes are associated with LOAD in the southeast Brazilian population.

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Conflict of interest

The authors declare no conflicts of interest.

Author Contributions

LRS, JFFA and FP wrote the manuscript. RLM was the physician in the study; he assisted with the clinical assessment of the recruited participants in the study. LHSP contributed to statistical analyses. All of the authors assisted, read and approved the manuscript before submission.

References

Abe-Dohmae S, Ikeda Y, Matsuo M, Hayashi M, Okuhira KI, Ueda K and Yokoyama S (2004) Human ABCA7 supports apolipoprotein-mediated release of cellular cholesterol and phospholipid to generate high density lipoprotein. J Biol Chem 279:604–611.

Almada BVP, De-Almeida LD, Camporez D, De-Moraes MVD, Morelato RL, Perrone AMS, Belcavello L, Louro ID and De-Paula F (2012) Protective effect of the APOE-e3 allele in Alzheimer's disease. Braz J Med Biol Res 45:8–12.

Belcavello L, Camporez D, Almeida LD, Morelato RL, Batitucci MCP and de Paula F (2015) Association of MTHFR and PICALM polymorphisms with Alzheimers disease. Mol Biol Rep 42:611–616.

Bertram L, Lill CM and Tanzi RE (2010) The genetics of Alzheimer disease: Back to the future. Neuron 68:270–281.

Chan SL, Kim WS, Kwok JB, Hill AF, Cappai R, Rye KA and Garner B (2008) ATP-binding cassette transporter A7 regulates processing of amyloid precursor protein *in vitro*. J Neurochem 106:793–804.

Combarros O, Cortina-Borja M, Smith AD and Lehmann DJ (2009) Epistasis in sporadic Alzheimer's disease. Neurobiol Aging 30:1333–1349.

DeMattos RB, Cirrito JR, Parsadanian M, May PC, O'Dell MA, Taylor JW, Harmony JAK, Aronow BJ, Bales KR, Paul SM *et al.* (2004) ApoE and clusterin cooperatively suppress Aβ Levels and deposition: evidence that ApoE regulates extracellular Aβ Metabolism In Vivo. Neuron 41:193–202.

Dong HK, Gim JA, Yeo SH and Kim HS (2017) Integrated late onset Alzheimer's disease (LOAD) susceptibility genes: Cholesterol metabolism and trafficking perspectives. Gene 597:10–16.

dos Santos LR, Belcavello L, Camporez D, Iamonde MMC, Zandonade E, Lírio MR, Imbroisi VEF, Drumond LI, Do Carmo PBM and de Paula F (2016) Association study of the BIN1 and IL-6 genes on Alzheimer's disease. Neurosci Lett 614:65–69.

dos Santos LR, Pimassoni LHS, Sena GGS, Camporez D, Belcavello L, Trancozo M, Morelato RL, Errera FIV, Bueno MRP and de Paula F (2017) Validating GWAS variants from microglial genes implicated in Alzheimer's disease. J Mol Neurosci 62:215–221.

Ebbert MTW, Ridge PG and Kauwe JSK (2015) Bridging the gap between statistical and biological epistasis in Alzheimer's disease. BioMed Res Int 2015:1–7.

El Gaamouch F, Jing P, Xia J and Cai D (2016) Alzheimer's disease risk genes and lipid regulators. J Alzheimers Dis 53:15–29.

Harold D, Abraham R, Hollingworth P, Sims R, Hamshere M, Pahwa JS, Moskvina V, Williams A, Jones N, Thomas C *et al.* (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease, and shows evidence for additional susceptibility genes. Nat Genet 41:1088–1093.

Heppner FL, Ransohoff RM and Becher B (2015) Immune attack: The role of inflammation in Alzheimer disease. Nat Rev Neurosci 16:358–372.

Hohman TJ, Koran ME and Thornton-Wells T (2013) Epistatic genetic effects among alzheimer's candidate genes. PLoS One 8:e80839.

Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V *et al.* (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43:429–435.

Holtzman DM, Morris JC and Goate AM (2011) Alzheimer's disease: The challenge of the second century. Sci Translat Med 3:77sr1.

Karch CM and Goate AM (2014) Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry 77:43-51.

Kim WS, Weickert CS and Garner B (2008) Role of ATP-binding cassette transporters in brain lipid transport and neurological disease. J Neurochem 104:1145–1166.

Kojro E, Gimpl G, Lammich S, Marz W and Fahrenholz F (2001) Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the -secretase ADAM 10. Proc Nartl Acad Sci U S A 98:5815–5820.

Lambert JC and Amouyel P (2011) Genetics of Alzheimer's disease: New evidences for an old hypothesis? Curr Opin Genet Dev 21:295–301.

Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B *et al.* (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41:1094–1099.

Lazaris A, Hwang KS, Goukasian N, Ramirez LM, Eastman J, Blanken AE, Teng E, Gylys K, Cole G, Saykin AJ *et al.* (2015) Alzheimer risk genes modulate the relationship between plasma apoE and cortical PiB binding. Neurol Genet 1:e22.

Li HL, Yang P, Liu ZJ, Sun YM, Lu SJ, Tao QQ, Guo QH and Wu ZY (2015) Common variants at Bin1 are associated with sporadic Alzheimer's disease in the Han Chinese population. Psychiatr Genet 25:21–25.

Lins TC, Vieira RG, Abreu BS, Grattapaglia D and Pereira RW (2010) Genetic composition of Brazilian population samples based on a set of twenty eight ancestry informative SNPs. Am J Hum Biol 22:187–192.

Miller SA, Dykes DD and Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215.

Morgan K and Carrasquillo MM (2013) Genetic variants in Alzheimer's Disease. Springer, New York, 213 p.

Naj AC, Jun G, Beecham GW, Wang L, Narayan B, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK *et al.* (2011) Common variants in MS4A4/MS4A6E, CD2AP, CD33, and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 43:436–441.

O'Brien RJ, Wong PC and Edu R (2011) Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci 34:185–204.

Pant S, Sharma M, Patel K, Caplan S, Carr CM and Grant BD (2009) AMPH-1/Amphiphysin/Bin1 functions with RME-1/Ehd1 in endocytic recycling. Nat Cell Biol 11:1399–410.

Pena SDJ, di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy F de SG, Kohlrausch F, Magno LAV, Montenegro RC, Moraes MO *et al.* (2011) The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS One 6:e17063.

Pfrieger FW (2003) Cholesterol homeostasis and function in neurons of the central nervous system. Cell Mol Life Sci 60:1158–1171.

Prince M, Comas-Herrera MA, Knapp M, Guerchet M and Karagiannidou MM (2016) World Alzheimer Report 2016: Improving healthcare for people living with dementia. Alzheimer's Disease International (ADI), London.

Reitz C (2013) Dyslipidemia and the risk of Alzheimer's disease. Curr Atheroscler Rep 15:307.

Rizzi F, Caccamo AE, Belloni L and Bettuzzi S (2009) Clusterin is a short half-life, poly-ubiquitinated protein, which controls the fate of prostate cancer cells. J Cell Physiol 219:314–323.

Schrijvers EMC, Koudstaal PJ, Hofman A and Breteler MMB (2011) Plasma clusterin and the risk of Alzheimer disease. JAMA 305:1322–1326.

Shankarappa BM, Kota LN, Purushottam M, Nagpal K, Mukherjee O, Viswanath B, Varghese M, Bharath S and Jain S (2017) Effect of CLU and PICALM polymorphisms on AD risk: A study from south India. Asian J Psychiatr 27:7–11.

Tan L, Wang HF, Tan MS, Tan CC, Zhu XC, Miao D, Yu WJ, Jiang T, Tan L, Yu JT *et al.* (2016) Effect of CLU genetic variants on cerebrospinal fluid and neuroimaging markers in healthy, mild cognitive impairment and Alzheimer's disease cohorts. Sci Rep 6:26027.

Tokuda T, Calero M, Matsubara E, Vidal R, Kumar A, Permanne B, Zlokovic B, Smith JD, Ladu MJ, Rostango A *et al.* (2000) Lipidation of apolipoprotein E influences its isoform-specific interaction with Alzheimer's amyloid β peptides. Biochem J 348:359–365.

Vasiliou V, Vasiliou K and Nebert DW (2009) Human ATP-binding cassette (ABC) transporter family. Hum Genomics 3:281–290.

Vepsäläinen S, Helisalmi S, Mannermaa A, Pirttilä T, Soininen H and Hiltunen M (2009) Topic collections combined risk effects of IDE and NEP gene variants on Alzheimer disease. J Neurol Neurosurg Psychiatry 80:1268–1270.

Yamazaki K, Yoshino Y, Mori T, Yoshida T, Ozaki Y, Sao T, Mori Y, Ochi S, Iga JI and Ueno SI (2017) Gene expression and methylation analysis of ABCA7 in patients with Alzheimer's disease. J Alzheimers Dis 57:171–181.

3.3 Capítulo 3

• *Risk assessment of CASS4, TREM2, CD2AP and MS4A4E variant's on alzheimer's disease in a Brazilian population* - manuscrito submetido na *revista Neurological Sciences.*

Este artigo foi enviado para correção do inglês e ainda está em análise (Janeiro de 2023).

RISK ASSESSMENT OF *CASS4*, *TREM2*, *CD2AP* AND *MS4A4E* VARIANT'S ON ALZHEIMER'S DISEASE IN A BRAZILIAN POPULATION

Abstract

Genome-wide association studies (GWAS) reported polymorphisms related with Late-onset of Alzheimer disease (LOAD), a neurodegenerative disorder. The variants rs911159 *CASS4*, rs75932628 *TREM2*, rs9349407 *CD2AP* and rs670139 *MS4A4E* are considered associated with LOAD on GWAS studies. Though, several association studies did not replicate those variants with impact. So, we aimed to address this issue by an association study of rs911159 *CASS4,* rs75932628 *TREM2,* rs9349407 *CD2AP* and rs670139 *MS4A4E* with LOAD in samples from southeastern Brazil, through RT-PCR and PCR-RFLP. The rs911159 *CASS4* showed a positive association with AD. In logistic regression, the rs911159 *CASS4* showed the highest significance [OR = 0.187 (95% CI: 0.059–0.59), P = 0.005]. In contrast, rs75932628 *TREM2*, rs9349407 *CD2AP* and rs670139 *MS4A4E* was not associated with the LOAD. We believe this work is important in the validation of GWAS variants with risk of AD and to increase the diversity of populations studied.

Keywords: Alzheimer's disease; Association study; Brazilian population; GWAS; CASS4.

1. Introduction

Alzheimer disease (AD) is a neurodegenerative disorder and the most common type of dementia, which affects 47 million elders worldwide [1]. Pathological changes occur inside peoples brain's AD, as neurofibrillary tangles, formed inside neurons by tau protein hyperphosphorylation and amyloid plaques whereas amyloid plaques accumulate outside neurons and are formed by Aβ42 peptides created via *Amyloid Precursor Protein* (APP) gene dysregulation [2]. Both changes are the main hallmarks of AD and are thought to lead the amyloid cascade hypothesis [2].

Most of the AD cases are late-onset AD (LOAD), have multifactorial pattern and occurs later than 65 years [3]. The ε4 allele in *Apolipoprotein E* (*APOE*) gene is considered a worldwide risk factor for LOAD [4]. Genome-wide associations studies

(GWAS) has identified several genes associated with LOAD as *CASS4, TREM2, CD2AP* and *MS4A4E* [5–7]. Functional studies indicated that rs911159 *CASS4,* rs75932628 *TREM2,* rs9349407 *CD2AP* and rs670139 *MS4A4E* variants are implicated on others pathways that leads to AD, as cytoskeletal function, axonal transport , inflammatory pathway, endocytosis pathway and immune pathway [6, 8–10]. Since it is important to corroborate GWAS variants in different population and to investigate their potential association with LOAD, case-control reports on Asians, Europeans and Americans attempt to replicate those variants. However, not all reached significance. Therefore, the main goal of this study was to investigate a possible association of rs911159 *CASS4,* rs75932628 *TREM2,* rs9349407 *CD2AP* and rs670139 *MS4A4E* with late-onset AD by association study in samples from southeastern Brazil.

2. Materials and Methods

Subjects

This association study has a total of 221 unrelated individual's participants. Healthy elders included 139 individuals matched by sex and age. Patients included 82 individuals diagnosed with probable AD with LOAD according to the criteria of National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA). Also, patients were diagnosed with a comprehensive diagnostic evaluation for dementia and fulfilled other criteria such as Mini-Mental State Examination (MMSE) with a score of 14-4 and Dementia Rating Scale (CDR) with score of 2. The healthy elders scored >25 on MMSE.

The participants were from Grande Vitória, a metropolitan region of Espírito Santo at the southeast of Brazil. The study was accepted by the Committee of Ethics in Human Research of Centro de Ciências da Saúde da Universidade Federal do Espírito Santo, Brazil. The elders were assisted and diagnosed by a geriatrician at Geriatric Unity of the Hospital Santa Casa de Misericórdia de Vitória (HSCMV) and Centro de Atendimento ao Idoso (CRAI), Espírito Santo, Brazil. The participants or their relatives provided a written informed consent before participation in the study as well as information regarding age, gender, ethnical background composition and schooling.

Blood sampling and genotyping

The peripheral blood was collected into a 5-ml tube with 5% of EDTA at Geriatric Unity of HSCMV and CRAI. After collection, it was stored at 4°C prior analysis. The genomic DNA was extracted according with previous methodology [11].

The rs75932628 polymorphism in *TREM2* gene (C>T RefSeq NM_018965.4) was determined by real-time polymerase chain reaction (qPCR). Genomic DNA (30 ηg/μl) was employed for qPCR according to the manufacturer's instructions (TaqMan SNP Genotyping Assay - Applied Biosystems, Carlsbad, California, USA) on a 7500 Fast Real-Time PCR System. Genotypes were analyzed using SDS v.2.0.5 software.

The rs670139 polymorphism in *MS4A4E* gene was determined by conventional polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers used were described by MAO *et al.* [12] study. PCR products were subjected to digestion at 55°C by 16 hours with *BSL1* enzyme (Thermo Scientific). The digestion products were electrophoresed on a 7% polyacrylamide gel stained with silver nitrate.

The rs911159 polymorphism in *CASS4* gene was determined by conventional polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The forward primer (5'-TGGGATTGGAGTAGCAGTCA-3') and the reverse primer (5'-AGCAAACACTTCCACCAACC-3') used was designed by Primer 3 v. 0.4.0 program. After amplification, the PCR products were subjected to digestion at 37°C by 15 minutes with *RsaI* enzyme. Digestion products followed the same protocol used on rs670139 *MS4A4E* for electrophoresis.

The rs9349407 polymorphism in *CD2AP* gene was determined by PCR-RFLP using degenerated primers. The forward primer (5'-AATCTATAGTAGTGTATACTAAG-3') was designed by *dCAPS Finder 2.0.* program with a G nucleotide degenerated in the end to form the restriction site (GGWCC) for the enzyme AVAII (Thermo Scientific).

The reverse primer (5'- TGTAGGCAACTGTAACACAATGG -3') was not degenerated and it was designed by Primer3 v. 0.4.0 program. After amplification, the PCR products were subjected to digestion at 37°C by 2 hours with *AVAII* enzyme. Digestion products followed the same protocol used on rs911159 *CASS4* and rs670139 *MS4A4E* for electrophoresis.

Statistical analysis

Statistical analysis was performed with SPSS (IBM) software v.23.0 for Windows. The $p \leq 0.05$ was considered statistically significant for all statistical analysis. Chi-square test with odds ratio (OR) and 95% confidence interval (95% CI) was performed to test the association between AD and the polymorphisms. Hardy-Weinberg Equilibrium (HWE) was calculated with 1 degree of freedom. Logistic regression was performed to associate AD and polymorphisms with at least one ε4 allele. Allele frequency of the *APOE* status was inferred in our previously study [13]. The p-value from logistic regression was adjusted using ethnic background, age, gender and school level as variables.

Differences in gender, schooling, APOE status and ethnic background composition among AD patients and non-dementia individuals were evaluated by Chi-square test (χ^2). Concerning education, participants were considered literate or illiterate. Apropos of APOE status, carries of at least one ε4 allele were considered ε4 +, whereas those who carried no ε4 allele were considered for ε4 -. Mann-Whitney test was performed comparing age among AD patients and healthy individuals.

3. Results

Information of ethnical background composition, schooling, age, gender and *APOE* status are shown in Table 1. As estimated, a significance difference was observed for APOE frequencies whereas no significance difference for schooling, age, gender and ethnical background composition. The rs911159 *CASS4,* rs9349407 *CD2AP* and rs670139 *MS4A4E* were in Hardy-Weinberg Equilibrium for patients and controls.

The genotype frequencies of rs911159 *CASS4,* rs75932628 *TREM2,* rs9349407 *CD2AP* and rs670139 *MS4A4E* in the sample are presented in Table 2. The logistic regression analysis is demonstrated at Table 2. The genotypes frequency of *CASS4* shows significance differences between healthy controls and AD patients, even after adjusting for schooling, ethnical background composition, age, gender and *APOE* status. The genotypes frequency of rs9349407 *CD2AP* and rs670139 *MS4A4E* variants did not show significance differences between healthy controls and AD patients. No significant difference between healthy controls and AD patients was observed for rs670139 *MS4A4E* and rs9349407 *CD2AP* genotypes. The T allele of

rs75932628 *TREM2* was not detected in our sample. In the present study, for rs75932628 *TREM2* all individuals had the CC genotype in our sample.

4. Discussion

GWAS studies published numerous genes associates with LOAD. Among them, *CASS4, TREM2, CD2AP* and *MS4A4E* genes have risk variants, rs911159*,* rs75932628*,* rs9349407 and rs670139, respectively, associated with Alzheimer's disease. In the present study, we evaluated the association of those variants with LOAD. Our data suggested that *CASS4* might play a role in LOAD susceptibility among Brazilian population.

The *CD2-associated protein (CD2AP)* gene is located on chromosome 6p12 and encodes a protein CD2AP that regulates endocytosis, cytoskeletal structure, adhesion of cells and intracellular trafficking [14]. Several studies report a role for CD2AP in Alzheimer's disease. For instance, CD2AP is implicated in the endocytosis pathway that leads to AD and may affect the endocytosis of APP in neurons [14]. Also, this protein was found expressed in AD patient’s brains and may controls the APP degradation in dendrites brain's [14].

The C allele in rs9349407 polymorphism in *CD2AP* gene was considered a risk factor for LOAD in GWAS studies [5]. In functional study, the rs9349407 *CD2AP* was correlated with the increase of neuritic plaques [15]. However, the replication of this variant was not validated by all genetic studies. The Asians studies of Xiao et al. [16] and Jiao et al. [17] reported risk association of rs9349407 *CD2AP* with LOAD in Han Chinese (n=1,210) and Mainland China population (n=547), respectively. Our study results are corroborated with others [18–21] studies that also did not find an association of this variant with LOAD. The Caucasians populations studies of Omoumi *et al.* [18] and Carrasquillo *et al.* [19] found no relation concerning rs9349407 *CD2AP* and LOAD in a sample formed by two Canadian cohorts (n= 2,864) and in six sets of sample from United States and Europeans populations (n= 6,835), respectively. Interestingly, the meta-analysis of Chen *et al.* [22] found a risk association of rs9349407 *CD2AP* with Late-Onset AD in a sample with Asians, Canadians, Americans and Europeans populations (n=54,936). It was noted that the only studies used by the authors [22] that were associated with LOAD were mainly Caucasian studies used by the GWAS studies. Hence, concerning the relation of rs9349407 *CD2AP* and LOAD, we observed

a positive association in large sample analysis with mixture ethnics population [22]. However, in separated ethnics populations, the association is not consensual [16–21]. This may be due different statistical analysis among studies that could lead to different results or the genetic characteristics of the population itself. For instance, Brazilian population is a mixture of Iberians Caucasians, Native Americans and West Africans [23, 24]. Since each population varies on allele frequency of each genetic marker, the variant could have different association across each ethnic population.

The *Membrane-spanning 4-domain subfamily A4E (MS4A4E)* gene is located on chromosome 11q12.2 and is part of the *MS4A* gene family of cell-surface proteins [25]. The *MS4A4E* gene is implicated in immune modulation and is related as being part of the immune pathway to AD [8]. Also, *MS4A4E* gene's proteins are expressed on microglial cells but its mechanism on AD is not clear yet [26]. The MS4A4E functions are not fully characterized but could share some proteins functions/proprieties with his gene family [25]. The *MS4A* gene was reported to regulate calcium homeostasis inside the cell [27]. Since high levels of intracellular calcium may facilitates amyloid plaque formation and hyperphosphorylation of tau [27], *MS4A* gene may be related to neurodegenerative diseases as AD.

The A allele in rs670139 polymorphism in *MS4A4E* gene was reported for risk for LOAD in GWAS studies in Caucasians populations [5]. The functional study of Karch *et al.* [28] assessed samples of parietal lobes from autopsied AD brains and found that A allele in rs670139 *MS4A4E* is associated with Clinical dementia rating (CDR), increased Braak tangle score and Braak plaque score in AD patients. This correlates a possible relation of rs670139 *MS4A4E* and LOAD. In genetic studies, so far, only four case-control studies investigated this loci variant, all in Asian population. Tan *et al.* [20] and Wang *et al.* [29] reported that A allele in rs670139 *MS4A4E* is associated with LOAD in a sample of of Northern Han Chinese and in a combined sample of East Han Chinese and Southwest Han Chinese population and East population. However, in both studies, results did not remain significant after Bonferroni correction. Our case-control study did not found association of this variant with AD as the study of Mao *et al.* [12] in an East Asian population and Miyashita *et al.* [30], in a Japanese population. In the investigation of meta-analysis of Mao *et al.* [12], no association was found for rs670139 with LOAD in a population formed by three Asians studies (n=3,214). We believe that perhaps the effect of rs670139 *MS4A4E* in LOAD is small and it is necessary a greater sample to detect his overall influence. Besides,

more case-control studies in different populations are important to better elucidate this variant relation with LOAD.

The gene *Triggering receptor expressed on myeloid cells 2 (TREM2)* is located on chromosome 6p21.1 and is part of the *TREM* family. *TREM2* is a member of the innate immune receptor and is involved as part of the immune system in Alzheimer's disease and other neurodegenerative diseases [10]. The rs75932628-T variant is rare and causes a replacement p. R47H. Studies show that this variant increases susceptibility to AD [31]. Due to inflammatory processes, R47H replacement may lead to an increased predisposition to Alzheimer's disease [32]. Functional studies show that in the brain *TREM2* correlates with microglia to produce pro-inflammatory cytokines and with the stimulation of immune system cells (CD4+ T cells). Furthermore, in APP transgenic mice the expression of *TREM2* was related to the formation of amyloid plaques, suggesting a role in the development of Alzheimer's disease [32, 33].

The study by Jonsson et al. [32] replicated the association between the rs75932628-T variant and Alzheimer's disease in populations from the United States, Germany, the Netherlands and Norway and found that the rs75932628-T polymorphism conferred a risk of Alzheimer's disease in all replication cohorts. Studies in a Spanish and Colombian population reported a positive association with the rs75932628 polymorphism in Alzheimer's disease [34, 35]. In an Iranian population, a study of 288 individuals the frequency of the T allele was about 0.86% but did not reach a statistically significant association with LOAD [36]. Our case-control study found no association for LOAD with this variant as well as the study by Wang et al. [31] (n=1589) and Ma et al. [37] (n=625) in the Chinese population. We believe that *TREM2* is related to sporadic Alzheimer's disease and the biggest challenge is to carry out studies with a large sample size to obtain sufficient statistical power to detect rare variants, such as rs75932628-T.

The *Cas scaffold protein family member 4 (CASS4)* gene is located on chromosome 20q13.31 and was reportad for LOAD in GWAS studies [6]. The *CASS4* gene is part of the CAS family that encodes a scaffold protein involved in integrin-dependent signaling processes, essential for cell proliferation, survival, migration and motility [38]. Evidence indicates that *CASS4* plays roles in AD pathogenesis through axonal transport and *APP* and tau metabolism [6].

In the clinic pathological GWAS study, Beechan et al. [39] verified the association between *CASS4* in response to injury and AD with brain autopsy data. The

study by Lin et al. [40] analyzed gene-lifestyle interactions and in this study polymorphism rs911159 *CASS4* was associated with increased cognitive decline in a Taiwanese population . In contrast, the polymorphism rs7274581 *CASS4* is considered a protective factor for LOAD by GWAS [39]. Our study corroborated this association and we report for the first time that variant rs911159 *CASS4* was associated with LOAD in a Brazilian population. Furthermore, in genetic studies of LOAD, *CASS4* data are scarce in terms of single nucleotide polymorphism (SNP) for different populations. More association studies are needed to better understand the involvement of ethnicity in these results.

In conclusion, our case-control study suggests that rs911159 *CASS4* are associated with LOAD and did not found association of rs75932628 *TREM2,* rs9349407 *CD2AP* and rs670139 *MS4A4E* with AD*.* We believe our study is important to help elucidate the molecular role of variants on the AD pathway. Also, the replication of GWAS variants in different populations is crucial to validate a risk marker discovery.

5. Conclusion

Our data of case-control study demonstrate that rs911159 *CASS4* are associated with LOAD in a southeastern Brazilian population.

Clinical significance.

First the first time, the current study provided evidence of rs911159 *CASS4,* rs75932628 *TREM2,* rs9349407 *CD2AP* and rs670139 *MS4A4E* with late-onset AD by association study in samples from southeastern Brazil. As such, it is important in the validation of GWAS variants with risk of AD in different populations for search an effective molecular biomarker system.

Author's contributions

Jucimara Ferreira Figueiredo Almeida and Flavia de Paula were the principal researchers and wrote the manuscript in assistance of Lígia Ramos dos Santos and Patricia Menêses Portela. The experimental procedures were performed by Jucimara Ferreira Figueiredo Almeida in assistance of Maíra Trancozo and Lucas Henrique

Gonzaga de Oliveira. Renato Lírio Morelato is the physician that gave support and helped with the clinical assessment of the recruited subjects. Lúcia Helena Sagrillo Pimassoni contributed to the statistical analyses. Flavia de Paula gave technical support for the experiment procedures. All of the authors contributed to the manuscript, read and accepted it before submission.

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Disclosure Statement

The authors declare no conflict of interest.

References

1. Prince M, Comas-Herrera A, Knapp M, et al (2016) World Alzheimer Report 2016 Improving healthcare for people living with dementia. Coverage, Quality and costs now and in the future. In: Alzheimer's Disease International (ADI). Alzheimer's Disease International (ADI), London, pp 1–140
2. Association A (2016) Alzheimer's Disease Facts and Figures. Alzheimer's Dement 12:. https://doi.org/10.1016/j.jalz.2016.03.001
3. Yu JT, Tan L, Hardy J (2014) Apolipoprotein E in Alzheimer's Disease: An Update. Annu Rev Neurosci 37:79–100. https://doi.org/10.1146/annurev-neuro-071013-014300
4. Lambert JC, Amouyel P (2011) Genetics of Alzheimer's disease: New evidences for an old hypothesis? Curr Opin Genet Dev 21:295–301. https://doi.org/10.1016/j.gde.2011.02.002

5. Hollingworth P, Harold D, Sims R, et al (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43:429–435. https://doi.org/10.1038/ng.803
6. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet 45:1452–8. https://doi.org/10.1038/ng.2802
7. Cruchaga C, Kauwe JSK, Harari O, et al (2013) GWAS of Cerebrospinal Fluid Tau Levels Identifies Risk Variants for Alzheimer's Disease. Neuron 78:256–268. https://doi.org/10.1016/j.neuron.2013.02.026
8. Karch CM, Goate AM (2015) Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol. Psychiatry 77:43–51
9. Ubelmann F, Burrinha T, Salavessa L, et al (2017) Bin1 and CD2AP polarise the endocytic generation of beta-amyloid. EMBO Rep 18:102–122. https://doi.org/10.15252/embr.201642738
10. Hu N, Tan M-S, Yu J-T, et al (2013) Increased Expression of TREM2 in Peripheral Blood of Alzheimer's Disease Patients. J Alzheimer's Dis 38:497–501. https://doi.org/10.3233/JAD-130854
11. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215. https://doi.org/10.1093/nar/16.3.1215
12. Mao YF, Guo ZY, Pu JL, et al (2015) Association of CD33 and MS4A cluster variants with Alzheimer's disease in East Asian populations. Neurosci Lett 609:235–239. https://doi.org/10.1016/j.neulet.2015.10.007
13. Almada BVP, De-Almeida LD, Camporez D, et al (2012) Protective effect of the APOE-e3 allele in Alzheimer's disease. Brazilian J. Med. Biol. Res. 45:8–12
14. Tao Q-Q, Liu Z-J, Sun Y, et al (2017) Decreased gene expression of CD2AP in Chinese patients with sporadic Alzheimer's disease. Neurobiol Aging 2–7. https://doi.org/10.1016/j.neurobiolaging.2017.03.013
15. Shulman JM, Chen K, Keenan BT, et al (2013) Genetic susceptibility for Alzheimer disease neuritic plaque pathology. JAMA Neurol 70:1150–1157. https://doi.org/10.1001/jamaneurol.2013.2815
16. Xiao Q, Liu Z, Tao S, et al (2015) Risk prediction for sporadic Alzheimer ' s disease using genetic risk score in the Han Chinese population. 6:

17. Jiao B, Liu X, Zhou L, et al (2015) Polygenic analysis of late-onset Alzheimer's disease from mainland China. PLoS One 10:1–10. https://doi.org/10.1371/journal.pone.0144898
18. Omoumi A, Fok A, Greenwood T, et al (2014) Evaluation of late-onset Alzheimer disease genetic susceptibility risks in a Canadian population. Neurobiol Aging 35:936.e5-936.e12. https://doi.org/10.1016/j.neurobiolaging.2013.09.025
19. Carrasquillo MM, Belbin O, Hunter T a, et al (2011) Replication of EPHA1 and CD33 associations with late-onset Alzheimer's disease: a multi-centre case-control study. Mol Neurodegener 6:54. https://doi.org/10.1186/1750-1326-6-54
20. Tan L, Yu JT, Zhang W, et al (2013) Association of GWAS-linked loci with late-onset Alzheimer's disease in a northern Han Chinese population. Alzheimer's Dement 9:546–553. https://doi.org/10.1016/j.jalz.2012.08.007
21. Wang HZ, Bi R, Hu QX, et al (2014) Validating GWAS-Identified Risk Loci for Alzheimer's Disease in Han Chinese Populations. Mol Neurobiol 53:379–390. https://doi.org/10.1007/s12035-014-9015-z
22. Chen H, Wu G, Jiang Y, et al (2015) Analyzing 54,936 Samples Supports the Association Between CD2AP rs9349407 Polymorphism and Alzheimer's Disease Susceptibility. Mol Neurobiol 52:1–7. https://doi.org/10.1007/s12035-014-8834-2
23. Lins TC, Vieira RG, Abreu BS, et al (2010) Genetic composition of Brazilian population samples based on a set of twenty eight ancestry informative SNPs. Am J Hum Biol 22:187–192. https://doi.org/10.1002/ajhb.20976
24. Pena SDJ, di Pietro G, Fuchshuber-Moraes M, et al (2011) The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS One 6:. https://doi.org/10.1371/journal.pone.0017063
25. Liang Y, Buckley TR, Tu L, et al (2001) Structural organization of the human MS4A gene cluster on Chromosome 11q12. Immunogenetics 53:357–68
26. Villegas-Llerena C, Phillips A, Garcia-Reitboeck P, et al (2016) Microglial genes regulating neuroinflammation in the progression of Alzheimer's disease. Curr Opin Neurobiol 36:74–81. https://doi.org/10.1016/j.conb.2015.10.004
27. LaFerla FM (2002) Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. Nat Rev Neurosci 3:862–872. https://doi.org/10.1038/nrn960
28. Karch CM, Jeng AT, Nowotny P, et al (2012) Expression of Novel Alzheimer's Disease Risk Genes in Control and Alzheimer's Disease Brains. PLoS One 7:e50976. https://doi.org/10.1371/journal.pone.0050976

29. Wang H, Bi R, Hu Q, et al (2014) Validating GWAS-Identified Risk Loci for Alzheimer ' s Disease in Han Chinese Populations. https://doi.org/10.1007/s12035-014-9015-z
30. Miyashita A, Koike A, Jun G, et al (2013) SORL1 Is Genetically Associated with Late-Onset Alzheimer's Disease in Japanese, Koreans and Caucasians. PLoS One 8:. https://doi.org/10.1371/journal.pone.0058618
31. Wang P, Guo Q, Zhou Y, et al (2018) Lack of association between triggering receptor expressed on myeloid cells 2 polymorphism rs75932628 and late-onset Alzheimer's disease in a Chinese Han population. Psychiatr Genet 28:16–18. https://doi.org/10.1097/YPG.0000000000000188
32. Jonsson T, Stefansson H, Steinberg S, et al (2013) Variant of TREM2 Associated with the Risk of Alzheimer's Disease. N Engl J Med 368:107–116. https://doi.org/10.1056/NEJMoa1211103
33. Melchior B, Garcia AE, Hsiung B-K, et al (2010) Dual Induction of TREM2 and Tolerance-Related Transcript, Tmem176b, in Amyloid Transgenic Mice: Implications for Vaccine-Based Therapies for Alzheimer's Disease. ASN Neuro 2:AN20100010. https://doi.org/10.1042/AN20100010
34. Benitez BA, Cooper B, Pastor P, et al (2013) TREM2 is associated with the risk of Alzheimer's disease in Spanish population. Neurobiol Aging 34:1711.e15-1711.e17. https://doi.org/10.1016/j.neurobiolaging.2012.12.018
35. Arboleda-Bustos CE, Ortega-Rojas J, Mahecha MF, et al (2018) The p.R47H Variant of TREM2 Gene is Associated With Late-onset Alzheimer Disease in Colombian Population. Alzheimer Dis Assoc Disord 32:305–308. https://doi.org/10.1097/WAD.0000000000000275
36. Mehrjoo Z, Najmabadi A, Abedini SS, et al (2015) Association Study of the TREM2 Gene and Identification of a Novel Variant in Exon 2 in Iranian Patients with Late-Onset Alzheimer's Disease. Med Princ Pract 24:351–354. https://doi.org/10.1159/000430842
37. Ma J, Zhou Y, Xu J, et al (2014) Association study of TREM2 polymorphism rs75932628 with late-onset Alzheimer's disease in Chinese Han population. Neurol Res 36:894–896. https://doi.org/10.1179/1743132814Y.0000000376
38. Deneka A, Korobeynikov V, Golemis EA (2015) Embryonal Fyn-associated substrate (EFS) and CASS4: The lesser-known CAS protein family members. Gene 570:25–35. https://doi.org/10.1016/j.gene.2015.06.062

39. Beecham GW, Hamilton K, Naj AC, et al (2014) Genome-Wide Association Meta-analysis of Neuropathologic Features of Alzheimer's Disease and Related Dementias. PLoS Genet 10:e1004606. https://doi.org/10.1371/journal.pgen.1004606

40. Lin E, Kuo P-H, Liu Y-L, et al (2017) Effects of circadian clock genes and environmental factors on cognitive aging in old adults in a Taiwanese population. Oncotarget 8:24088–24098. https://doi.org/10.18632/oncotarget.15493

Table 1. Characteristics of sample.

Variable	AD Patients 82 (100%)	Controls 139 (100%)	*p*-value
Gender			
Man	28 (34.1%)	36 (25.9%)	0.192 [a]
Woman	54 (65.9%)	103(74.1%)	
Schooling			
Literate	44 (53.7%)	89 (64.0%)	
Illiterate	29 (35.4%)	43 (30.9%)	0.154 [a]
No identification	9 (11.0%)	7 (5.0%)	
Ethnic background			
Caucasians	47 (57.3%)	82 (59.0%)	
Afro-Brazilians	30 (36.6%)	55 (39.6%)	0.159 [a]
No identification	5 (6.1%)	2 (1.4%)	
APOE status			
ε4 +	47 (57.3%)	43 (30.9%)	<0.001[a]
ε4 -	35 (42.7%)	96 (69.1%)	
Age (mean ± SD)	81 ± 7	80 ± 8	0.186 [b]
MMSE (mean ±SD)	13±6	25±5	<0.001
CDR	2	–	

AD Patients = Alzheimer's disease patients; ε4 += ε4 carriers; ε4 -= ε4 non-carriers; SD= standard deviation; [a] = AD patient versus control group by x^2 test; [b]= p-value of AD patient versus control group by Mann-Whitney test; p-value ≤ 0.05 considered significant. MMSE = Mini-Mental State Examination (value considering the schooling level and the average evolution time of the disease in AD patients); CDR = Dementia Rating Scale.

Table 2. Genotypes frequencies and logistic regression analyses.

	AD patients	Controls	*p* value[a]	OR (95%CI)[b]	*p* value[c]
MS4A4E					
CC	38 (46.3%)	56 (40.3%)		1 (reference)	-
CA	33 (40.2%)	63 (45.3%)	0.675	0.77 (0.42 – 1.39)	0.261
AA	11 (13.4%)	20 (14.4%)		0.81 (0.34 – 1.88)	0.489
Total	82 (100%)	139 (100%)			
CASS4					
GG	70 (94.6%)	51 (73.9%)		1 (reference)	-
GA	4 (5.4%)	18 (26,1.%)	0.001	0.187 (0.059 - 0,59)	0.005
AA	0	0			
Total	74 (100%)	69 (100%)			
CD2AP					
GG	27 (45.0 %)	47 (46.5%)		1 (reference)	-
GC	30 (50.0%)	46 (45.5%)	0.723	1.13 (0.58 – 2.19)	0.610
CC	03 (05.0%)	08 (07,9%)		0.65 (0.16 – 2.67)	0.629
Total	60 (100%)	101 (100%)			
TREM2					
CC	82 (100%)	139 (100%)	-	-	-
CT	0	0	-	-	-
TT	0	0	-	-	-
Total	82 (100%)	139 (100%)			

The numbers with percentages on parenthesis show the proportions of genotypes in AD patient and healthy control; AD Patients = Alzheimer's disease patients; p value considerer ≤0.05;[a]=AD patient versus control group by x^2 test; OR = Odds ratio; CI = Confidence interval;[b]= Logistic regression; [c] = p value adjusted by the variables age, gender, educational

4. CONSIDERAÇÕES FINAIS

O presente estudo teve como objetivo investigar possíveis biomarcadores para a doença de Alzheimer esporádica. Para se estudar esses biomarcadores genéticos, foi feito primeiramente um estudo, o primeiro manuscrito, de meta-análise atualizado sobre a associação das variantes de rs3764650 *ABCA7*, rs610932 *MS4A6A*, rs6656401 *CR1*, rs744373 *BIN1* e rs11136000 *CLU* com DAE. É importante ressaltar que esse estudo de meta-análise teve um número amostral de 24.771 pacientes e 35.324 controles, além disso a meta-análise investigou a associação destas variantes em populações caucasianas e em amostras de diferentes grupos étnicos.

Os resultados da meta-análise validaram o risco das variantes rs744373 do gene *BIN1*, rs6656401 do gene *CR1*, rs3764650 do gene *ABCA7,* bem como o efeito protetor de rs610932 do gene *MS4A6A* e rs11136000 de *CLU*. O presente trabalho reconhece algumas limitações metodológicas. Apesar dos nossos esforços para entrar em contato com alguns grupos de pesquisa, não conseguimos obter muitos dados de genótipos de grandes estudos. Consequentemente, os resultados negativos encontrados na pesquisa podem ter sido pelo menor número amostral. Apesar das limitações, este trabalho foi importante pois integrou resultados encontrados em estudos de associações de diferentes etnias, permitindo um entendimento geral dos polimorfismos em relação a DAE.

O próximo objetivo, que resultou no segundo manuscrito, foi realizar um estudo de associação combinando diferentes variantes de estudos caso-controle, de indivíduos da população de Vitória,ES, e verificar se modificaria aditivamente a suscetibilidade a DAE. As variantes testadas foram rs429358 e rs7412 do gene *APOE*, rs744373 *BIN1*, rs3764650 *ABCA7* e rs11136000 *CLU*. Nossos resultados mostraram um efeito de risco para DA entre *APOE* com *CLU* e *APOE* com o gene *BIN1*. Porém, este resultado pode ter sido fortemente influenciado pelo grande efeito do alelo ε4 do gene *APOE*. Entretanto, é importante ressaltar que nenhum polimorfismo isolado pode explicar completamente a doença de Alzheimer, pois são as combinações de genes relacionados a doença que juntos com fatores ambientais desencadeiam os sintomas. Acreditamos que esses resultados foram importantes para melhorar o entendimento

das variantes genéticas que causam a DA em uma população específica.

A população brasileira é interessante de ser estudada pois há alta prevalência de doença de Alzheimer e é composta por uma mistura de etnias como caucasianos ibéricos, africanos ocidentais e nativos sul-americanos. Nesse contexto, como a DA é uma doença complexa, os perfis étnicos podem evidenciar diferentes fatores de risco genéticos.

Diante disso, estudos GWAS relataram vários polimorfismos relacionados com a doença de Alzheimer, porém a maioria desses estudos de associação do genoma foram realizados usando amostras com uma ampla base genética europeia. É relevante aumentar a diversidade de populações estudadas.

À vista disso, o terceiro manuscrito teve como objetivo realizar um estudo de associação dos polimorfismos rs911159 do gene *CASS4*, rs75932628 do gene *TREM2*, rs9349407 do gene *CD2AP* e rs670139 do gene *MS4A4E* com DAE. Os resultados sugerem que não houve associação das variantes rs75932628 do gene *TREM2*, rs9349407 do gene *CD2AP* e rs670139 do gene *MS4A4E* com DAE, mas houve associação positiva com o polimorfismo rs911159 do gene *CASS4.* O grande desafio deste estudo foi o número amostral. É esperado grandes tamanhos de amostra para obter poder estatístico suficiente para detectar variantes com frequências de alelos menores e raras, como por exemplo a variante do gene *TREM2.* Apesar disso, este estudo amplia a compreensão da genética da doença de Alzheimer em uma população pouco estudada e valida resultados de estudos GWAS.

Diante disso, todos os estudos apresentados neste trabalho pretenderam encontrar biomarcadores em potencial para a doença de Alzheimer esporádica a fim de melhorar o diagnóstico precoce, compreender melhor a etiologia da DA e para novas abordagens terapêuticas.

5. REFERÊNCIAS

AIKAWA, T.; HOLM, M.-L.; KANEKIYO, T. ABCA7 and Pathogenic Pathways of Alzheimer's Disease. **Brain Sciences**, v. 8, n. 2, p. 27, 5 fev. 2018.

ALZHEIMER'S ASSOCIATION. 2016 Alzheimer's disease facts and figures. **Alzheimer's & Dementia**, v. 12, n. 4, p. 459–509, abr. 2016.

ARBOLEDA-BUSTOS, C. E. et al. The p.R47H Variant of TREM2 Gene is Associated With Late-onset Alzheimer Disease in Colombian Population. **Alzheimer Disease & Associated Disorders**, v. 32, n. 4, p. 305–308, out. 2018.

ASHRAFIAN, H.; ZADEH, E. H.; KHAN, R. H. Review on Alzheimer's disease: Inhibition of amyloid beta and tau tangle formation. **International Journal of Biological Macromolecules**, v. 167, p. 382–394, jan. 2021.

ATKINSON, A. J. et al. **Biomarkers and surrogate endpoints: Preferred definitions and conceptual frameworkClinical Pharmacology and Therapeutics**, 2001.

AVRAMOPOULOS, D. Genetics of Alzheimer's disease: recent advances. **Genome Medicine**, v. 1, n. 3, p. 34, 2009.

BAO, J.; WANG, X.; MAO, Z. Associations Between Genetic Variants in 19p13 and 19q13 Regions and Susceptibility to Alzheimer Disease: A Meta-Analysis. **Medical Science Monitor**, v. 22, p. 234–243, 22 jan. 2016.

BEECHAM, G. W. et al. Genome-Wide Association Meta-analysis of Neuropathologic Features of Alzheimer's Disease and Related Dementias. **PLoS Genetics**, v. 10, n. 9, p. e1004606, 4 set. 2014.

BENITEZ, B. A. et al. TREM2 is associated with the risk of Alzheimer's disease in Spanish population. **Neurobiology of Aging**, v. 34, n. 6, p. 1711.e15-1711.e17, jun. 2013.

BERG, C. N.; SINHA, N.; GLUCK, M. A. ABCA7 Risk Genotype Diminishes the Neuroprotective Value of Aerobic Fitness in Healthy Older African Americans. **Frontiers in Aging Neuroscience**, v. 11, n. APR, p. 1–9, 9 abr. 2019.

BERTRAM, L.; LILL, C. M.; TANZI, R. E. **The genetics of alzheimer disease: Back to the futureNeuron**, 2010.

BRION, J. P. **Neurofibrillary tangles and Alzheimer's diseaseEuropean Neurology**, 1998.

BUSCHE, M. A.; HYMAN, B. T. Synergy between amyloid-β and tau in Alzheimer's

disease. **Nature Neuroscience**, v. 23, n. 10, p. 1183–1193, 10 out. 2020.

CACABELOS, R. How plausible is an Alzheimer's disease vaccine? **Expert Opinion on Drug Discovery**, v. 15, n. 1, p. 1–6, 2 jan. 2020.

CAI, X. D.; GOLDE, T. E.; YOUNKIN, S. G. Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. **Science**, v. 259, n. 5094, p. 514–516, 1993.

CASTELLANI, R. J.; ROLSTON, R. K.; SMITH, M. Alzheimer Disease. **Disease a Month**, v. 56, n. 9, p. 1–60, 2011.

CAVEDO, E. et al. The Road Ahead to Cure Alzheimer's Disease: Development of Biological Markers and Neuroimaging Methods for Prevention Trials Across all Stages and Target Populations. **The journal of prevention of Alzheimer's disease**, v. 1, n. 3, p. 181–202, 2014.

CEDAZO-MINGUEZ, A.; WINBLAD, B. Biomarkers for Alzheimer's disease and other forms of dementia: Clinical needs, limitations and future aspects. **Experimental Gerontology**, v. 45, n. 1, p. 5–14, 2010.

CHIBNIK, L. B. et al. CR1 is associated with amyloid plaque burden and age-related cognitive decline. **Annals of Neurology**, v. 69, n. 3, p. 560–569, mar. 2011.

CITRON, M. et al. Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. **Nature**, v. 360, n. 6405, p. 672–674, 1992.

CONDELLO, C.; STOHR, J. Aβ propagation and strains: Implications for the phenotypic diversity in Alzheimer's disease. **Neurobiology of Disease**, 2016.

CORDER, E. et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. **Science**, v. 261, n. 5123, p. 921–923, 1993.

CRAIG-SCHAPIRO, R.; FAGAN, A. M.; HOLTZMAN, D. M. **Biomarkers of Alzheimer's diseaseNeurobiology of Disease**, 2009.

CUSTODIO, N. et al. Dementia in Latin America: Epidemiological evidence and implications for public policy. **Frontiers in Aging Neuroscience**, v. 9, n. JUL, p. 1–11, 2017.

DEHGHANI, N.; BRAS, J.; GUERREIRO, R. How understudied populations have contributed to our understanding of Alzheimer's disease genetics. **Brain**, v. 144, n. 4, p. 1067–1081, 7 maio 2021.

DENEKA, A.; KOROBEYNIKOV, V.; GOLEMIS, E. A. Embryonal Fyn-associated substrate (EFS) and CASS4: The lesser-known CAS protein family members. **Gene**, v. 570, n. 1, p. 25–35, out. 2015.

DENG, Y. L. et al. The prevalence of CD33 and MS4A6A variant in Chinese Han population with Alzheimer's disease. **Human Genetics**, v. 131, n. 7, p. 1245–1249, 2012.

DONG, H. K. et al. **Integrated late onset Alzheimer's disease (LOAD) susceptibility genes: Cholesterol metabolism and trafficking perspectivesGene**, 2017.

FARRER, L. A. et al. **Effects of Age, Sex, and Ethnicity on the Association Between Apolipoprotein E Genotype and Alzheimer Disease: A Meta-analysisJAMA: The Journal of the American Medical Association**, 1997.

FORLENZA, O. V. **Tratamento farmacológico da doença de AlzheimerRevista de Psiquiatria Clinica**, 2005.

FRIDMAN, C. et al. Alterações genéticas na doença de Alzheimer. **Archives of Clinical Psychiatry (São Paulo)**, v. 31, n. 1, p. 19–25, 2004.

FROTA, N. A. F. et al. Critérios para o diagnóstico de doença de Alzheimer. **Dement Neuropsychol**, v. 5, n. Suppl 1, p. 5–10, 2011.

GOATE, A. et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. **Nature**, v. 349, n. 6311, p. 704–6, 1991.

HARDY, J. A.; HIGGINS, G. A. Alzheimer's Disease: The Amyloid Cascade Hypothesis. **Science**, v. 256, n. 5054, p. 184–185, 10 abr. 1992.

HAROLD, D. et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. **Nature Genetics**, v. 41, n. 10, p. 1088–1093, 6 out. 2009.

HARPER, P. S. Mendelian inheritance or transmissible agent? The lesson Kuru and the Australia antigen. **Journal of medical genetics**, v. 14, n. 6, p. 389–98, dez. 1977.

HEINONEN, O. et al. Loss of synaptophysin-like immunoreactivity in the hippocampal formation is an early phenomenon in alzheimer's disease. **Neuroscience**, v. 64, n. 2, p. 375–384, jan. 1995.

HENEKA, M. T. et al. NIH Public Access. v. 493, n. 7434, p. 674–678, 2013.

HIPPIUS, H.; NEUNDÖRFER, G. The discovery of Alzheimer's disease. **Dialogues in Clinical Neuroscience**, v. 5, n. 1, p. 101–108, 2003.

HOLLINGWORTH, P. et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. **Nature Genetics**, v. 43, n. 5, p. 429–435, 3 abr. 2011a.

HOLLINGWORTH, P. et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1,

CD33 and CD2AP are associated with Alzheimer's disease. **Nature Genetics**, v. 43, n. 5, p. 429–435, 3 maio 2011b.

HOLTZMAN, D. M. et al. Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. **Annals of Neurology**, v. 47, n. 6, p. 739–747, 2000.

HOLTZMAN, D. M.; MORRIS, J. C.; GOATE, A. M. Alzheimer's Disease: The Challenge of the Second Century. **Science Translational Medicine**, v. 3, n. 77, p. 77sr1-77sr1, 2011.

HU, N. et al. Increased Expression of TREM2 in Peripheral Blood of Alzheimer's Disease Patients. **Journal of Alzheimer's Disease**, v. 38, n. 3, p. 497–501, 25 nov. 2013.

HYE, A. et al. Glycogen synthase kinase-3 is increased in white cells early in Alzheimer's disease. **Neuroscience Letters**, v. 373, n. 1, p. 1–4, 2005.

HYE, A. et al. Proteome-based plasma biomarkers for Alzheimer's disease. **Brain**, v. 129, n. 11, p. 3042–3050, 29 set. 2006.

IKEDA, T.; YAMADA, M. [Risk factors for Alzheimer's disease]. **Brain and nerve = Shinkei kenkyu no shinpo**, v. 62, n. 7, p. 679–90, jul. 2010.

IQBAL, K. et al. **Tau pathology in Alzheimer disease and other tauopathiesBiochimica et Biophysica Acta - Molecular Basis of Disease**, 2005.

JACK, C. R. et al. 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. **Brain**, v. 131, n. 3, p. 665–680, 2008.

JARRETT, J. T.; BERGER, E. P.; LANSBURY, P. T. The Carboxy Terminus of the β Amyloid Protein Is Critical for the Seeding of Amyloid Formation: Implications for the Pathogenesis of Alzheimer's Disease. **Biochemistry**, v. 32, n. 18, p. 4693–4697, 1993.

JELLINGER, K. A. et al. Biomarkers for early diagnosis of Alzheimer disease: 'ALZheimer ASsociated gene'- a new blood biomarker? **Journal of Cellular and Molecular Medicine**, v. 12, n. 4, p. 1094–1117, ago. 2008.

JIAO, B. et al. Polygenic analysis of late-onset Alzheimer's disease from mainland China. **PLoS ONE**, v. 10, n. 12, p. 1–10, 2015.

JONSSON, T. et al. Variant of TREM2 Associated with the Risk of Alzheimer's Disease. **New England Journal of Medicine**, v. 368, n. 2, p. 107–116, 10 jan. 2013.

KADMIRI, N. et al. Biomarkers for Alzheimer disease: Classical and novel candidates'

review. **Neuroscience**, 2017.

KANG, S.; LEE, Y. H.; LEE, J. E. Metabolism-centric overview of the pathogenesis of Alzheimer's disease. **Yonsei Medical Journal**, v. 58, n. 3, p. 479–488, 2017.

KARCH, C. M. et al. Expression of Novel Alzheimer's Disease Risk Genes in Control and Alzheimer's Disease Brains. **PLoS ONE**, v. 7, n. 11, p. e50976, 30 nov. 2012.

KARCH, C. M.; GOATE, A. M. Alzheimer's Disease Risk Genes and Mechanisms of Disease Pathogenesis. **Biological Psychiatry**, v. 77, n. 1, p. 43–51, jan. 2015a.

KARCH, C. M.; GOATE, A. M. Alzheimer's Disease Risk Genes and Mechanisms of Disease Pathogenesis. **Biological Psychiatry**, v. 77, n. 1, p. 43–51, jan. 2015b.

KHALSA, D. S.; NEWBERG, A. B. Spiritual Fitness: A New Dimension in Alzheimer's Disease Prevention. **Journal of Alzheimer's Disease**, v. 80, n. 2, p. 505–519, 23 mar. 2021.

KIDSON, C.; CHEN, P. DNA damage, DNA repair and the genetic basis of Alzheimer's disease. **Progress in brain research**, v. 70, n. 0079–6123, p. 291–301, 1986.

KOSIK, K. S.; JOACHIM, C. L.; SELKOE, D. J. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. **Proceedings of the National Academy of Sciences of the United States of America**, v. 83, n. 11, p. 4044–8, 1986.

KWOK, P. Y.; GU, Z. **Single nucleotide polymorphism libraries: Why and how are we building them?Molecular Medicine Today**, 1999.

LAFERLA, F. M. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. **Nature reviews Neuroscience**, v. 3, n. 11, p. 862–872, 2002.

LAMBERT, J.-C. et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. **Nature Genetics**, v. 41, n. 10, p. 1094–1099, 6 out. 2009.

LAMBERT, J.-C. et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. **Nature Genetics**, v. 45, n. 12, p. 1452–1458, 27 dez. 2013.

LAMBERT, J. C. et al. Evidence of the association of BIN1 and PICALM with the AD risk in contrasting European populations. **Neurobiology of Aging**, v. 32, n. 4, p. 756.e11-756.e15, 2011.

LAMBERT, J. C.; AMOUYEL, P. Genetics of Alzheimer's disease: New evidences for an old hypothesis? **Current Opinion in Genetics and Development**, v. 21, n. 3, p. 295–301, 2011.

LI, S. et al. Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. **Journal of neuropathology and experimental neurology**, v. 56, n. 8, p. 901–11, 1997.

LIANG, Y. et al. Structural organization of the human MS4A gene cluster on Chromosome 11q12. **Immunogenetics**, v. 53, n. 5, p. 357–68, jul. 2001.

LIN, E. et al. Effects of circadian clock genes and environmental factors on cognitive aging in old adults in a Taiwanese population. **Oncotarget**, v. 8, n. 15, p. 24088–24098, 11 abr. 2017.

LISTA, S. et al. **Biomarkers in Sporadic and Familial Alzheimer's DiseaseJournal of Alzheimer's Disease**, 2015.

LIU, G. et al. Analyzing Large-Scale Samples Confirms the Association Between the ABCA7 rs3764650 Polymorphism and Alzheimer's Disease Susceptibility. **Molecular Neurobiology**, v. 50, n. 3, p. 757–764, 19 dez. 2014.

LYNCH, D. K. et al. A cortactin-CD2-associated protein (CD2AP) complex provides a novel link between epidermal growth factor receptor endocytosis and the actin cytoskeleton. **Journal of Biological Chemistry**, v. 278, n. 24, p. 21805–21813, 2003.

MA, X.-Y. et al. Missense variants in CR1 are associated with increased risk of Alzheimer' disease in Han Chinese. **Neurobiology of aging**, v. 35, n. 2, p. 443.e17-443.e21, 2013.

MAURER, K.; VOLK, S.; GERBALDO, H. **Auguste D and Alzheimer's diseaseLancet**, 1997.

MCKHANN, G. et al. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. **Neurology**, v. 34, n. July 1984, p. 939–944, 1984.

MELCHIOR, B. et al. Dual Induction of TREM2 and Tolerance-Related Transcript, Tmem176b, in Amyloid Transgenic Mice: Implications for Vaccine-Based Therapies for Alzheimer's Disease. **ASN Neuro**, v. 2, n. 3, p. AN20100010, 14 jun. 2010.

MENDES, C. **Lítio e expressão gênica: implicações para a doença de Alzheimer.** [s.l.] Universidade de São Paulo, 2008.

MISZTAL, M.; FRANKIEWICZ, T. Learning deficits induced by chronic intraventricular infusion of quinolinic acid—protection by MK-801 and memantine. **European journal of pharmacology**, v. 296, n. 1, p. 1–8, 1996.

MORGAN, D. Immunotherapy for Alzheimer's disease. **Journal of Internal Medicine**,

v. 269, n. 1, p. 54–63, jan. 2011.

MORGAN, K.; CARRASQUILLO, M. M. **Genetic variants in Alzheimer's disease**. New York, NY: Springer New York, 2013.

MORRISON, A. S.; LYKETSOS, C. R Eview the Pathophysiology of Alzheimer ' S Disease. **Advanced Studies in Nursing**, v. 3, n. 8, p. 256–270, 2005.

NAJ, A. C. et al. Common variants in MS4A4/MS4A6E, CD2AP, CD33, and EPHA1 are associated with late-onset Alzheimer's disease. **Nature genetics**, v. 43, n. 5, p. 436–441, 2011a.

NAJ, A. C. et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. **Nature Genetics**, v. 43, n. 5, p. 436–441, 3 abr. 2011b.

NUSSBAUM, R. L.; ELLIS, C. E. Alzheimer's Disease and Parkinson's Disease. **New England Journal of Medicine**, v. 348, n. 14, p. 1356–1364, 3 abr. 2003.

PANT, S. et al. AMPH-1/Amphiphysin/Bin1 functions with RME-1/Ehd in endocytic recycling. **Cell**, v. 11, n. 12, p. 1399–1410, 2009.

PANTEV, M.; RITTER, R.; GORTELMEYER, R. **Clinical and behavioural evaluation in long-term care patients with mild to moderate dementia under Memantine treatment.Zeitschrift fur Gerontopsychologie und -psychiatrie**, 1993.

PAROLINI, D. et al. Expression of CD20 reveals a new store-operated calcium entry modulator in skeletal muscle. **The International Journal of Biochemistry & Cell Biology**, v. 44, n. 12, p. 2095–2105, dez. 2012.

PARSONS, C. G. et al. Patch clamp studies on the kinetics and selectivity of N-methyl-d-aspartate receptor antagonism by memantine (1-amino-3,5-dimethyladamantan). **Neuropharmacology**, v. 32, n. 12, p. 1337–1350, 1993.

PASCALE, M. et al. G Protein-coupled Receptor Kinase 2–mediated Phosphorylation of Ezrin Is Required for G Protein- coupled Receptor–dependent Reorganization of the Actin Cytoskeleton. **Molecular biology of the cell**, v. 16, n. 8, p. 1–13, 2005.

PASTERNAK, J. J. **Uma Introdução à Genética Molecular Humana: Mecanismos das Doenças Hereditárias.** 2ª ed. [s.l.] Guanabara Koogan, 2007.

PIKE, K. E. et al. β-amyloid imaging and memory in non-demented individuals: Evidence for preclinical Alzheimer's disease. **Brain**, v. 130, n. 11, p. 2837–2844, 2007.

POIRIER, J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. **Trends in neurosciences**, v. 17, n. 12, p. 525–530, 1994.

QUERFURTH, H. W.; LAFERLA, F. M. Alzheimer's Disease. **New England Journal**

of Medicine, v. 362, n. 4, p. 329–344, 28 jan. 2010.

RAMIREZ, L. M. et al. Common variants in ABCA7 and MS4A6A are associated with cortical and hippocampal atrophy. **Neurobiology of Aging**, v. 39, p. 82–89, 2016.

REIBER, H.; PETER, J. B. **Cerebrospinal fluid analysis: Disease-related data patterns and evaluation programsJournal of the Neurological Sciences**, 2001.

REITZ, C. et al. Variants in the ATP-Binding Cassette Transporter (ABCA7), Apolipoprotein E ϵ4, and the Risk of Late-Onset Alzheimer Disease in African Americans. **JAMA**, v. 309, n. 14, p. 1483, 10 abr. 2013.

REN, G. et al. The BAR Domain Proteins: Molding Membranes in Fission, Fusion, and Phagy. **Microbiology and Molecular Biology Reviews**, v. 70, n. 1, p. 37–120, mar. 2006.

RIZZI, F. et al. Clusterin is a short half-life, poly-ubiquitinated protein, which controls the fate of prostate cancer cells. **Journal of Cellular Physiology**, v. 219, n. 2, p. 314–323, 2009.

ROGERS, J. et al. Peripheral clearance of amyloid beta peptide by complement C3-dependent adherence to erythrocytes. **Neurobiology of Aging**, v. 27, n. 12, p. 1733–1739, 2006.

SCHEFF, S. W.; SPARKS, D. L.; PRICE, D. A. Quantitative assessment of synaptic density in the entorhinal cortex in Alzheimer's disease. **Annals of Neurology**, v. 34, n. 3, p. 356–361, 1993.

SCHELLENBERG, G. D. et al. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. **Science**, v. 258, n. 5082, p. 668–671, 1992.

SCHENK, D. et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. **Nature**, v. 400, n. 6740, p. 173–7, 1999.

SESHADRI, S. Genome-wide Analysis of Genetic Loci Associated With Alzheimer Disease. **Jama**, v. 303, n. 18, p. 1832, 2010.

SHULMAN, J. M. et al. Genetic susceptibility for Alzheimer disease neuritic plaque pathology. **JAMA Neurology**, v. 70, n. 9, p. 1150–1157, 1 set. 2013.

SONG, F. et al. **Plasma biomarkers for mild cognitive impairment and Alzheimer's diseaseBrain Research Reviews**, 2009.

STRITTMATTER, W. J. et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. **Proceedings of the National Academy of Sciences**, v. 90, n. 5, p. 1977–1981, 1 mar. 1993.

SUH, Y.-H.; CHECLER, F. Amyloid Precursor Protein, Presenilins, and alpha -Synuclein: Molecular Pathogenesis and Pharmacological Applications in Alzheimer's Disease. **Pharmacological Reviews**, v. 54, n. 3, p. 469–525, 1 set. 2002.

SUZUKI, N. et al. An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. **Science (New York, N.Y.)**, v. 264, n. 5163, p. 1336–40, 1994.

TAN, C.-C.; YU, J.-T.; TAN, L. Biomarkers for Preclinical Alzheimer's Disease. **Journal of Alzheimer's Disease**, v. 42, n. 4, p. 1051–1069, 10 out. 2014.

TAO, Q.-Q. et al. Decreased gene expression of CD2AP in Chinese patients with sporadic Alzheimer's disease. **Neurobiology of Aging**, p. 2–7, 2017.

TARIOT, P. N. et al. Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: a randomized controlled trial. **JAMA**, v. 291, n. 3, p. 317–324, 2004.

THOMAS, P.; FENECH, M. A review of genome mutation and Alzheimer's disease. **Mutagenesis**, v. 22, n. 1, p. 15–33, 2007.

TOKUDA, T. et al. Lipidation of apolipoprotein E influences its isoform-specific interaction with Alzheimer's amyloid β peptides. **Biochemical Journal**, v. 348, n. 2, p. 359–365, 2000.

UBELMANN, F. et al. Bin1 and CD2AP polarise the endocytic generation of beta-amyloid. **EMBO reports**, v. 18, n. 1, p. 102–122, 2017.

VASQUEZ, J. B.; FARDO, D. W.; ESTUS, S. ABCA7 expression is associated with Alzheimer's disease polymorphism and disease status. **Neuroscience Letters**, v. 556, n. 2, p. 58–62, nov. 2013.

VILLEGAS-LLERENA, C. et al. Microglial genes regulating neuroinflammation in the progression of Alzheimer's disease. **Current Opinion in Neurobiology**, v. 36, p. 74–81, fev. 2016.

WANG, P. et al. Lack of association between triggering receptor expressed on myeloid cells 2 polymorphism rs75932628 and late-onset Alzheimer's disease in a Chinese Han population. **Psychiatric Genetics**, v. 28, n. 1, p. 16–18, 2018.

WEIS, J. H. et al. A complement receptor locus : genes encoding C3b / C4b receptor and C3d / Epstein-Barr virus receptor map to 1q32 . **The Journal of Immunology**, v. 138, n. 1, p. 312–315, 1987.

WENK, G. L. et al. No interaction of memantine with acetylcholinesterase inhibitors approved for clinical use. **Life Sciences**, v. 66, n. 12, p. 1079–1083, 2000.

WOOD, J. G. et al. Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau). **Proceedings of the National Academy of Sciences of the United States of America**, v. 83, n. 11, p. 4040–3, 1986.

YAARI, R.; COREY-BLOOM, J. Alzheimer's disease. **Seminars in neurology**, v. 27, n. 1, p. 32–41, 2007.

YU, J.-T.; TAN, L.; HARDY, J. Apolipoprotein E in Alzheimer's Disease: An Update. **Annual Review of Neuroscience**, v. 37, n. 1, p. 79–100, 8 jul. 2014.

ZETTERBERG, H.; BURNHAM, S. C. Blood-based molecular biomarkers for Alzheimer's disease. **Molecular Brain**, v. 12, n. 1, p. 26, 28 dez. 2019.