UNIVERSIDADE FEDERAL DO PARANÁ

RAQUEL MACIEL DE SOUSA

INFLAMMATION AND LATE-ONSET ALZHEIMER'S DISEASE: A CASE-CONTROL ASSOCIATION STUDY

INFLAMAÇÃO E DOENÇA DE ALZHEIMER ESPORÁDICA: UM ESTUDO DE ASSOCIAÇÃO

CURITIBA

2017

RAQUEL MACIEL DE SOUSA

INFLAMMATION AND LATE-ONSET ALZHEIMER'S DISEASE: A CASE-CONTROL ASSOCIATION STUDY

INFLAMAÇÃO E DOENÇA DE ALZHEIMER ESPORÁDICA: UM ESTUDO DE ASSOCIAÇÃO

Dissertação apresentada como requisito à obtenção do grau de Mestre ao Programa de Pós Graduação em Genética, Departamento de Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientador: Profº. Dr. Ricardo L. R. de Souza

CURITIBA

2017

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
Setor CIÊNCIAS BIOLÓGICAS
Programa de Pós-Graduação GENÉTICA

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em GENÉTICA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de RAQUEL MACIEL DE SOUSA intitulada: Inflamação e doença de Alzheimer esporádica: um estudo de associação, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 21 de Julho de 2017.

RICARDO LEHTONEN RODRIGUES DE SOUZA
Presidente da Banca Examinadora (UFPR)

MEIRE SILVA BATISTELA
Avaliador Externo (UFPR)

LIYA REGINA MIKAMI
Avaliador Externo (UFPR)

ABSTRACT

Alzheimer's disease (AD) is the world's most common form of dementia, affecting over 44 million people worldwide. AD is a neurodegenerative disease, characterized by extracellular deposition of amyloid-β peptide and the intracellular formation of neurofibrillary tangles. During the last few years, neuroinflammation has been pointed out as an important component of AD pathology and several inflammation-related genes have already been associated with AD. Elevated levels of inflammatory cytokines and interleukins have been found in AD patients. Overexpression of these mediators is critical for the onset of the inflammatory process and mediates the expression of inflammation-related genes. Here we performed a case-control association study to evaluate the association between *TLR6*, *TLR2*, *NEK7*, *IL-18*, *IL-1B*, *NLRP10*, and *COL4A1* genetic polymorphisms, and AD susceptibility, and association with other AD cognitive variables. A total of 152 AD patients and 120 controls were included in the study. All the polymorphisms were genotyped using the Sequenom MassARRAY iPLEX Platform. rs6531669 (*TLR6*) was found to be associated with AD susceptibility, age of onset, and MMSE; rs13105517 (*TLR2*) was found to be associated with AD susceptibility, and MMSE; rs9919613 (*NLRP10*) was found to be associated with AD susceptibility, age of onset, and disease severity; rs1143643 (*Il-1B*) was found to be associated with age of onset, and disease severity; rs613430 (*COL4A1*) was found to be associated with disease severity. In conclusion, our study suggests that *TLR6*, *TLR2* and *NLRP10* are candidate genes for AD pathogenesis, and that these genes and *IL-1B* and *COL4A1* are associated with AD onset and other cognitive variables.

Key-words: Alzheimer's disease, inflammation, *TLR2*, *TLR6*, *IL-1B*, *NLRP10, COL4A1*.

RESUMO

A doença de Alzheimer (DA) é a forma de demência mais comum, afetando cerca de 44 milhões de pessoas em todo o mundo. A DA é uma doença neurodegenerativa, caracterizada por deposição extracelular do peptídeo β- amilóide e a formação intracelular de emaranhados neurofibrilares. Recentemente, a neuroinflamação surgiu como um componente importante da patologia da DA e diversos genes inflamatórios já foram associados a DA. Níveis elevados de citocinas e interleucinas inflamatórias foram encontrados em pacientes com DA. A superexpressão desses mediadores é crítica para o início do processo inflamatório e medeia a expressão de genes varios genes inflamatórios. O principal objetivo do estudo foi analisar associação entre polimorfismos dos genes *TLR6*, *TLR2*, *NEK7*, *IL-18*, *IL-1B*, *NLRP10* e *COL4A1*, e susceptibilidade à DA, e associação com outras variáveis cognitivas da DA, atraves estudo de associação caso-controle. Um total de 152 pacientes com DA e 120 controles foram incluídos no estudo. Todos os polimorfismos foram genotipados usando a plataforma Sequenom MassARRAY iPLEX. Foram encontradas associações entre o polimorfismo rs6531669 (*TLR6*) e susceptibilidade à DA, idade de início e MMSE; o polimorfismo rs13105517 (*TLR2*) encontrou-se associado com susceptibilidade à DA, e MMSE; rs9919613 (*NLRP10*) foi associado com a susceptibilidade à DA, idade de início e gravidade da doença; foram encontradas associações entre o polimorfismo rs1143643 (*Il-1B*) e idade de início e gravidade da doença; o polimorfismo rs613430 (*COL4A1*) encontrou-se associado à gravidade da doença. Em conclusão, o nosso estudo sugere que o *TLR6*, *TLR2* e *NLRP10* são genes candidatos para a patogênese da DA, e que esses genes e os genes *IL-1B* e *COL4A1*, estão associados à idade de início e outras variáveis cognitivas da doença.

Palavras-chave: Doença de Alzheimer, inflamação, *TLR2*, *TLR6*, *IL-1B*, *NLRP10, COL4A1*.

TABLE OF FIGURES

LIST OF TABLES

SUMMARY

1 INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative and progressive disease and is the leading cause of dementia (FREEMAN; TING, 2016). The majority of cases are sporadic and late-onset with no proven evidence for a Mendelian pattern of inheritance (HEPPNER *et al.*, 2015). There are no treatments to cure or halt the progression of AD, and there are no validated biomarkers for early diagnosis of the disease (HEPPNER *et al.*, 2015). AD pathology is characterised by extracellular deposition of amyloid-β (Aβ) plaques, intracellular accumulation of hyperphosphorylated tau protein, known as neurofibrillary tangles (NFTs) (HUANG; MUCKE, 2012), and an inflammatory response, which escalates with disease progression (HEPPNER *et al.*, 2015). Only very recently, the importance of inflammation in AD pathogenesis has been appreciated, and it is now thought to contribute to and exacerbate AD pathology (COUTURIER *et al.*, 2016; HENEKA *et al.*, 2015; HENEKA *et al.*, 2014; HEPPNER *et al.*, 2015; HICKMAN; EL KHOURY, 2014; MARCHESI, 2016; MINTER *et al.*, 2016; PERRY; HOLMES, 2014; SHICHITA; YOSHIMURA, 2016).

Elevated levels of inflammatory cytokines and interleukins, in particular IL-1β and IL-18, have been found in the brain and peripheral blood of AD patients (JOHNSTON *et al.*, 2011). Overexpression of these interleukins was shown to be critical for the onset of the inflammatory process (RUBIO-PEREZ; MORILLAS-RUIZ, 2012) and both mediate the expression of a vast range of inflammation-related genes (WEBER *et al.*, 2010). Inflammasomes are high-molecular-weight complexes that mediate the auto-activation of caspase-1, which cleaves the pro-forms of IL-1β and IL-18 to active forms (FRIEDLANDER *et al.*, 1997; HERX; YONG, 2001). Inflammasomes have been acknowledged for their crucial role in host defence against pathogens (VON MOLTKE *et al.*, 2013), but it's dysregulated activation is linked to the development of cancer and autoimmune, metabolic and neurodegenerative diseases (BROZ; DIXIT, 2016).

Several genes have been identified as having a key role in the inflammatory process and being associated with AD (GUERREIRO *et al.*, 2013; HOLLINGWORTH *et al.*, 2011). We propose to assess the association of some particular inflammation-related gene polymorphisms, such as *TLR6, TLR2, NEK7*, *IL-1B*, *IL-18*, *NLRP10*, and *COL4A1*, that might also be associated with AD. These analyses will help to better understand the role of inflammation in the pathogenesis of AD, thus contributing to the

discovery of new therapeutic targets, improved care and to advance the treatment of patients, and deliver new biomarkers for diagnosis.

The overall aim of this study is to verify the existence of association between inflammation-related gene polymorphisms and the risk of developing late-onset AD and evaluate the relationship between these genes and clinical and cognitive variables of the patients.

2 LITERATURE REVIEW

2.1 ALZHEIMER'S DISEASE

Dementia is one of the greatest public health challenges affecting over 46.8 million people worldwide, with a global estimated financial cost of US$818 billion in 2015 (The Global Voice on Dementia, 2016). AD, a fatal neurodegenerative disorder, is the most prevalent cause of dementia (HEPPENER *et al.*, 2015).

Over the last few years, there has been considerable progress in unravelling the genetic influences of AD. About 5% of AD cases, known as familial AD or early-onset AD (EOAD), are inherited as an autosomal dominant trait and are the result of full penetrant mutations in genes that encode the amyloid beta precursor protein (*APP*) (GOATE *et al.*, 1991), and presenilins 1 and 2 (*PSEN1* and *PSEN2*) (LEVY-LAHAD *et al.*, 1995; ROGAEV *et al.*, 1995). 95% of the AD cases are late-onset and sporadic (LOAD). LOAD is a non-familial, complex disease, and is likely the result of the interaction between environmental factors and highly prevalent genetic variants with low penetrance (TANZI, 1999). A major genetic risk factor for LOAD is the presence of the ε4 allele of apolipoprotein E (ApoE) (SAUNDERS *et al.*, 1993). However, the presence of ε4 is neither necessary nor sufficient for the development of AD.

So far, there is no cure or validated biomarkers for early diagnosis of LOAD. At present, only four currently available drugs have been approved for treating AD. They belong to 2 groups: the acetylcholinesterase (AChE) inhibitors and the N-Methyl-D-aspartate (NMDA) receptors (CHIANG; KOO, 2014; FRANCIS *et al*., 2005; HUANG; MUCKE, 2012). Nonetheless, it has been shown that these drugs are only a palliative measure, and their effectiveness decreases over time (FOLCH *et al*., 2017). Recently, a group of researchers conducted a clinical trial with aducanumab, an anti–β amyloid antibody drug that selectively targets aggregated Aβ. So far, this antibody has shown

to reduce soluble and insoluble Aβ in a transgenic mouse model of AD, and improve cognitive function in people diagnosed with early AD. Nevertheless, the trial is still too small to be conclusive (SEVIGNY *et al.*, 2016).

The early stages of AD are mainly characterized by mild cognitive and functional decline. However, with the disease development the intensity of memory loss and impairment of other intellectual abilities progresses, and at the late stages patients alter their personality and lose their bodily functions (HUANG; MUCKE, 2012). The prevalence of the disease increases with life expectancy, and it affects more than one-third of people over the age of 90 (HENRY *et al.*, 2010). Even though most patients with AD are 65 and older, evidence suggests that the pathological processes underlying the disease start years before a clinical diagnosis can be made (JACK *et al.*, 2013).

The primary pathological hallmarks of AD are the senile plaques, which result from the extracellular deposition of Aβ peptides (CAMPION *et al.*, 2016), and NFTs, intracellular aggregates composed of hyperphosphorylated forms of the microtubule-associated protein tau (CAMPION *et al.*, 2016). The Aβ peptide results from the sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases (FIGURE 1) (CAMPION *et al.*, 2016; HENRY *et al.*, 2010). In AD, the aberrant processing of APP or the dysfunctional clearance of the Aβ peptide results in the formation of the Aβ plaques.

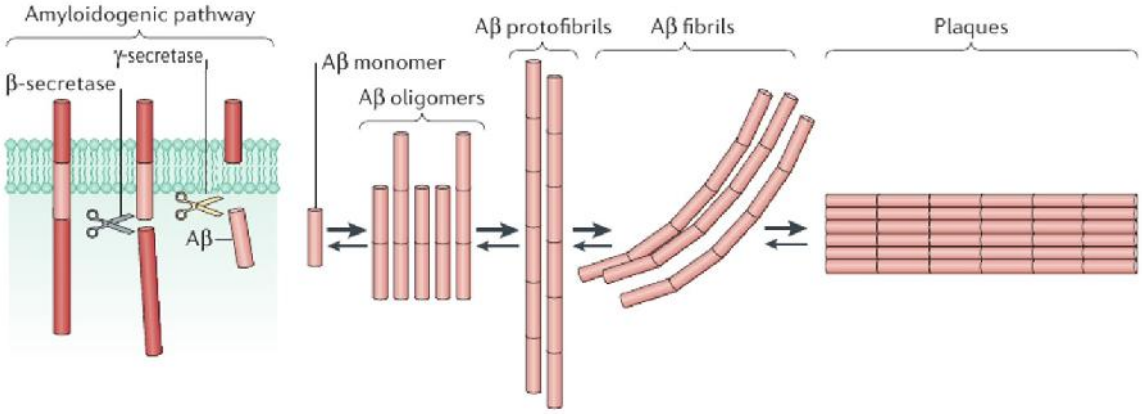


FIGURE 1 – FORMATION OF Aβ PLAQUES.
SOURCE: adapted from HEPPNER *et al.*, 2015
NOTE: Cleavage of APP by β- and γ-secretases results in the production and release of Aβ into the extracellular compartment. Aβ monomers may then go on to form oligomers which can, eventually, turn into fibrils and plaques.

There are several hypotheses for the pathogenesis of AD, but the most widely accepted are the amyloid cascade hypothesis and the tau hypothesis. According to the amyloid cascade hypothesis, Aβ accumulation and deposition in the brain are the initiating events in AD (HEPPNER *et al.*, 2015). The tau hypothesis postulates that tau protein abnormalities are the fundamental causes of the disease (LIU; CHAN, 2014).

However, neuroimaging studies and biomarkers have established that amyloid alterations occur prior to tau pathology, supporting the amyloid cascade hypothesis (PERRIN *et al*., 2009). Genetic evidence also supports this hypothesis, since mutations in the *APP*, *PSEN1*, and *PSEN2* genes are the only known causes of EOAD (BATEMAN *et al*., 2011) and, so far, no mutations in *tau* have been found to cause AD.

Nonetheless, Aβ deposition, tau phosphorylation, and subsequent NFT formation, are not sufficient to explain all the features of AD. Elderly individuals have shown abnormal levels of Aβ plaques but no signs of AD (AIZENSTEIN *et al.*, 2008). Also, animal models of AD expressing high levels of Aβ or tau protein did not show significant neurodegenerative changes (JOHNSTON *et al.*, 2011). Moreover, most clinical trials with immune-therapeutics showed clearance of Aβ plaques but no cognitive improvement in AD patients (HOLMES *et al.*, 2008).

It remains unclear how Aβ is linked to the NFT, but there is growing evidence that neuroinflammation could represent one of the critical linking factors. It has been shown that Aβ induces sustained inflammation which causes and propagates phosphorylated and aggregated tau species, substantially contributing to neuronal death in AD (ASAI *et al*., 2015; VENEGAS; HENEKA, 2017). These findings suggest that other factors might also be involved in the pathogenesis of AD (LIU; CHAN, 2014).

2.2 THE ROLE OF INFLAMMATION IN AD

Increasing evidence suggests that neurodegeneration is accompanied by an inflammatory process that is likely to interfere and contribute to the degenerative mechanisms involved in LOAD (ZHANG *et al.*, 2013).

The association between AD and mutations in genes encoding immune receptors of myeloid cells, such as triggering receptor expressed in myeloid cells 2 (*TREM2*), and the anti-inflammatory/phagocytosis receptor (*CD33*) (BRADSHAW *et al.*, 2013; GUERREIRO *et al.*, 2013; JONSSON *et al.*, 2013), and inflammation modulating cytokines, such as interleukin-10 (*IL-10*) and tumour necrosis factor α

(*TNFα*) (RAMOS *et al*., 2006), links immune alterations and AD pathogenesis. Additionally, several inflammatory cytokines, chemokines and other immune mediators are elevated in the brain and cerebro-spinal fluid (CSF) of AD patients and mild cognitive impairment (MCI) patients, a condition that precedes AD, indicating that inflammatory processes are involved in the pathology of AD (BROSSERON *et al.*, 2014; JOHNSTON *et al.*, 2011; TARKOWSKI *et al.*, 2003). The presence of inflammatory changes in MCI patients suggests that the immune system activation precedes Aβ deposition (BROSSERON *et al.*, 2014; TARKOWSKI *et al.*, 2003).

Epidemiologic evidence also suggests that inflammation is an important contributor to AD since prolonged treatment with nonsteroidal anti-inflammatory drugs is associated with delayed onset or slowed progression of AD (IN'T VELD *et al.*, 2001; SASTRE *et al.*, 2003; WEGGEN *et al.*, 2001), and histopathology studies show increased numbers of activated astro- and microglial cells surrounding Aβ deposits (WYSS-CORAY, 2006).

These observations imply that inflammatory processes may induce AD pathology, independently of Aβ deposition, and sustain increased Aβ levels, thus exacerbating the pathology (HEPPNER *et al.*, 2015).

2.3 MICROGLIA AND AD

Microglia are the principal innate immune cells of the central nervous system (CNS) and constitute the first line of defence against many pathological events (FALSIG *et al*., 2008). Activation of these cells has been associated with the pathogenesis of several neurodegenerative diseases, including AD (JANA *et al*., 2008).

It has been shown that microglia can act in two different ways. In one hand, activated microglia search for dead cells from the CNS and secrete several neurotrophic factors essential for neuronal survival (JANA *et al.*, 2008). This type of activation is associated with neuronal protection and regeneration. Additionally, these cells are responsible for the uptake and degradation of Aβ, thereby contributing to the clearance of Aβ (HENEKA *et al*., 2015). On the other hand, when activated by Aβ, microglia may misread the signal and interpret it as bacterial presence (HENEKA, 2017). Since there is a constant production of Aβ in the brain, microglia may never fully

succeed in removing the Aβ, thus contributing to the establishment of a chronic, long lasting, type of sterile inflammation in AD. During this type of activation, microglia release inflammatory mediators, including cytokines and reactive oxygen and nitrogen species (LUE *et al*., 2001; MURPHY *et al*., 1998; SZCZEPANIK *et al*., 2001; WALKER *et al*., 2006; YATES *et al*., 2000). Excessive production of these pro-inflammatory molecules is believed to play a significant role in enhancing the degenerative process in AD patients (JANA *et al.*, 2008).

Microglia are equipped with several cell membrane and cytosolic pattern recognition receptors (PRRs) which initiate the inflammatory phenotype (HEPPNER *et al.*, 2015). A few cell surface toll-like receptors (TLRs), such as TLR2, 4 and 6, and their co-receptors, such as oxidized low-density lipoprotein receptor (CD36), can be triggered by fibrillary and aggregated Aβ forms, to prime the cell (LIU *et al*., 2012; WEGGEN *et al*., 2001). Aβ makes microglia cells susceptible to a secondary stimulus and promotes their activation. It has been demonstrated that stimulation of the immune system in response to Aβ and pro-inflammatory cytokines impairs microglial clearance of Aβ and neuronal debris (HENEKA *et al*., 2013).

The gene encoding TLR6, a protein composed of 796 aa with a molecular mass of 91.9 kDa, is located on chromosome 4p14 (FIGURE 2), compromises 34,008 bases and is composed of 6 exons (GENECARDS, 2017; NCBI, 2017).

The *TLR2* gene is located on chromosome 4q31.3 (FIGURE 2), compromises 21,836 bases and 5 exons and it encodes a 784 aa protein with 89.8 kDa (GENECARDS, 2017; NCBI, 2017).

FIGURE 2 – LOCATION OF *TLR6 and TLR2* GENES ON CHROMOSOME 4.
SOURCE: Ensembl in 04/2017.
NOTE: The red rectangles represents the location of the *TLR6* and *TLR2* genes.

Recently, a number of studies have suggested that TLRs may be intimately associated with LOAD (BSIBSI *et al*., 2002; REED-GEAGHAN *et al*., 2009; LIU *et al*., 2012; YU; YE, 2015; MCDONALD *et al.*, 2016).

There is evidence that expression of *TLR2* is increased in brain tissue of AD patients and animal models of AD (YU; YE, 2015), and increased levels of *TLR2* mRNA have also been found in microglia from AD patients (BSIBSI *et al*., 2002). Studies show that inhibition of TLR2 results in a decrease of the inflammatory response and elevated

plaque clearance by microglia (LIU *et al*., 2012; MCDONALD *et al.*, 2016). Contrarily, some studies have reported that both TLR2 and TLR4 are necessary for fibrillar Aβ phagocytosis (REED-GEAGHAN *et al*., 2009).

Unlike other TLRs, which are functionally active as homomers, TLR2 can form heterodimers with TLR1 or TLR6, allowing detection of even more ligands (FARHAT *et al*., 2008). TLR4 and TLR6 can also form heterodimers, and CD36 serves as a co-receptor for TLR2-TLR6, as well as TLR4-TLR6 heterodimers (STEWART *et al*., 2010).

In AD, Aβ sustains chronic activation of primed microglia (due to the peptide's accumulation), resulting in a continuous production of inflammatory cytokines and chemokines by these cells, such as IL-1β and IL-18; in turn, the cytokines and chemokines sustain activation of the primed microglial cells. This process leads to a vicious cycle, which ultimately impairs microglial causing neurodegeneration and neurone loss (FIGURE 3) (HEPPNER *et al.*, 2015).

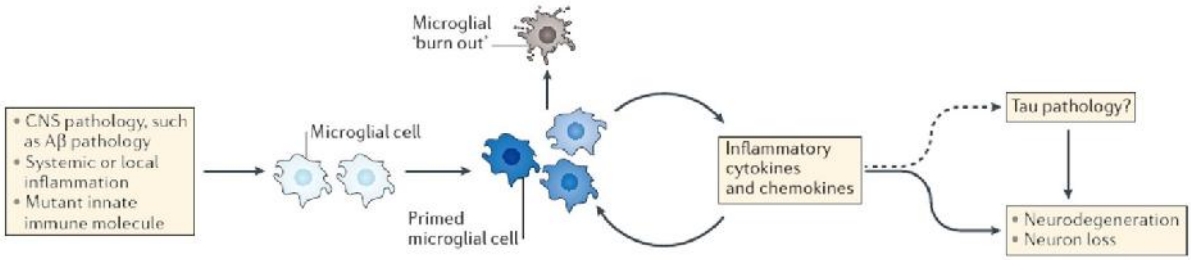


FIGURE 3 – MICROGLIAL PRIMING.
SOURCE: adapted from HEPPNER *et al.*, 2015.
NOTE: The presence of Aβ sustains chronic activation of primed microglial cells resulting in a constant production of inflammatory cytokines and chemokines, which, in turn maintain activation of the primed microglial cells. This process leads to a vicious cycle, which ultimately impairs microglia causing neurodegeneration and neuron loss.

TLR4-TLR6 heterodimers regulate the expression of pro-inflammatory mediators, including chemokines, and reactive oxygen and nitrogen species, and activation of these heterodimers primes microglia for IL-1β production.

Moreover, a common *TLR6* polymorphism has been associated with a reduced susceptibility to coronary artery disease (HAMANN *et al*., 2013), an inflammatory disease associated with the eventual development of AD in APOE ε4 allele carriers (BEERI *et al*., 2006).

The microglial cells activation is also intimately associated with the activation of the NLRP3 inflammasome (HENEKA *et al.*, 2013; SHEEDY *et al.*, 2013).

2.4 NLRP3 INFLAMMASOME AND AD

Inflammasomes are high-molecular-weight complexes that mediate the activation of inflammatory caspases. There are two families of inflammasomes: the NLR (Nod-like receptor) family and the PYHIN (pyrin and HIN domain-containing) family (HENEKA *et al.*, 2015). The assembly of each inflammasome is conducted by a PRR in response to pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) during tissue-based injury (DAVIS *et al.*, 2011). The NLRP3 inflammasome has probably attracted the most interest, because of its role in AD (LAMKANFI; DIXIT, 2014). Several genetic, behavioural phenotype and biochemical studies have shown that this inflammasome is activated in AD and contributes to the pathology of the disease (HALLE *et al.* 2008; HENEKA *et al.*, 2013; MURPHY *et al.* 2014; SALMINEN *et al.* 2008; SARESELLA *et al.*, 2016; TAN *et al.* 2013).

The NLRP3 inflammasome, from the NLR family, consists of a NLRP3 sensor protein, an adaptor protein known as apoptosis-associated speck-like protein (ASC) formed by two domains: a pyrin domain (PYD) and a caspase recruitment domain (CARD), and a pro-caspase-1. ASC's domains allow ASC to bridge the NLRP3 sensor protein to pro-caspase-1 (BROZ; DIXIT, 2016).

Activation of the NLRP3 inflammasome is a tightly regulated process that comprises two consecutive steps. The first step is provided by pro-inflammatory stimuli, such as Aβ, that upregulates the cellular expression of NLRP3, pro-IL-1β and pro-IL-18, through nuclear translocation of NF-κB (LAMKANFI; DIXIT, 2014). While this step is clear and well defined, the nature of the second step is more ambiguous, though it is known to require the oligomerization of NLRP3 and the assembly with ASC and pro-caspase-1 (GOLD; EL KHOURY, 2015), but the mechanisms behind this oligomerization are still being studied.

It was recently discovered that the oligomerization of the NLRP3 inflammasome requires the NIMA-related kinase 7 (NEK7), which binds to the NLRP3 leucine-rich repeats (LRRs) (HE *et al.*, 2016; SCHMID-BURGK *et al.*, 2016; SHI *et al.*, 2015). This kinase has been identified as an essential upstream regulator of NLRP3 (SCHMID-BURGK *et al*., 2016; SHI *et al*., 2015). NEK7 may be a novel promising therapeutic target for a variety of inflammatory diseases via direct targeting of the NLRP3 inflammasome (XU *et al*., 2016).

The gene encoding NEK7, a 302 aa protein with 34.6 kDa, is located on chromosome 1q31.3 (FIGURE 4). *NEK7* is composed of 165,458 bases and compromises 12 exons (GENECARDS, 2017; NCBI, 2017).

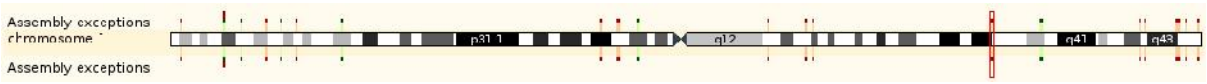


FIGURE 4 – LOCATION OF *NEK7* GENE ON CHROMOSOME 1.
SOURCE: Ensembl in 04/2017.
NOTE: The red rectangle represents the location of the *NEK7* gene.

Assembly and activation of this complex leads to the auto-activation of pro-caspase-1 to active caspase-1, which cleaves the pro-forms of IL-1β and IL-18 to their active forms (FIGURE 5) (SHAW *et al.*, 2011).

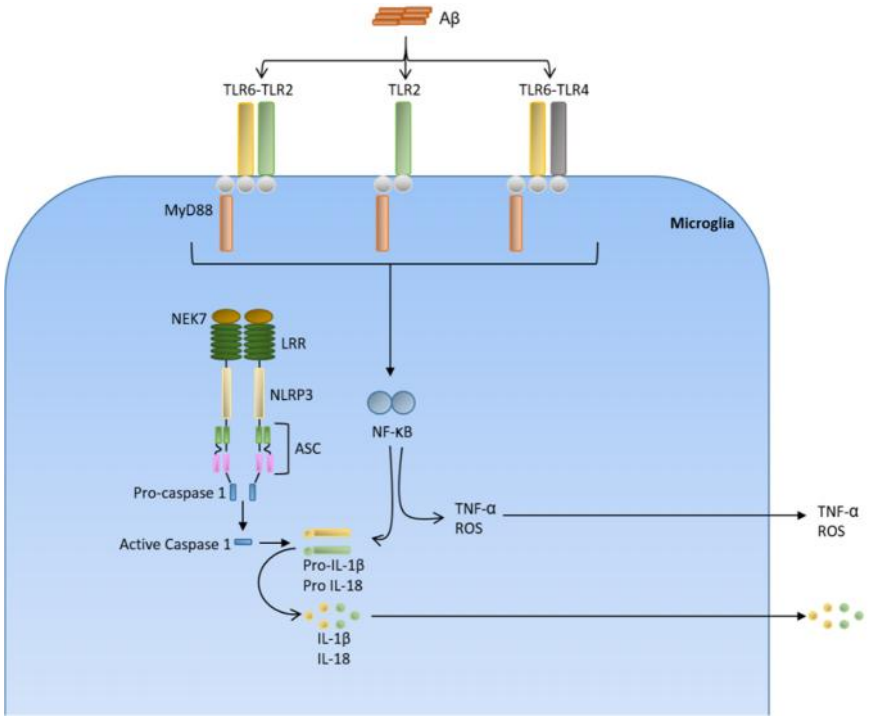


FIGURE 5 – FORMATION OF IL-1β AND IL-18 THROUGH ACTIVATION OF THE NLRP3 INFLAMMASOME.
SOURCE: the author.
NOTE: Toll-like receptors and their co-receptors can be triggered by Aβ to induce signalling via the innate immune system. This pro-inflammatory stimulus induces NLRP3 activation. Oligomerization and assembly of the NLRP3 inflammasome complex is regulated by NEK7 which binds to the NLRP3 leucine-rich repeats (LRRs). The assembly and activation of this complex lead to the auto-activation of pro-caspase-1 to active caspase-1 and subsequent processing of pro IL-1β and pro-IL-18 into its biologically active forms. The red boxes represent some of the genes which polymorphisms will be genotyped in the study.

2.5 IL-1B, IL-18 AND AD

Hyperactivation of IL-1β and IL-18 may lead to the overproduction of these, and other cytokines, which will result in excessive proliferation and recruitment of microglia cells and, eventually, lead to neurodegeneration and neurone loss (FREEMAN; TING, 2016; HEPPNER *et al.*, 2015).

IL-1β is a key mediator in the innate immune response in AD and elevated levels of these interleukins have been found in the brain and peripheral blood of AD patients, especially near Aβ plaques (JOHNSTON *et al.*, 2011; OJALA *et al*., 2009). Previous studies have identified an *IL-1B* polymorphism as a risk factor for AD (LIO *et al*., 2006; WAN *et al*., 2008). IL-1β has also been shown to induce nitric oxide synthase activity to produce the free radical NO, leading to neurotoxicity (ROSSI; BIANCHINI, 1996; RUBIO-PEREZ; MORILLAS-RUIZ, 2012). IL-1β secreted from astrocytes has been shown to enhance the production of APP and Aβ from the neurones (BLASKO *et al.*, 2000; BONIFATI; KISHORE, 2007; LI *et al.*, 2011). Studies have also demonstrated that IL-1β can induce the phosphorylation of tau protein and hence mediate the formation of NFT (GRIFFIN *et al.*, 2006; SALMINEN *et al.*, 2008). Blocking IL-1β signalling in the brain of a mouse model of AD resulted in an alteration of the inflammatory responses of the brain, rescuing cognition, attenuating tau pathology, and reducing fibrillar Aβ levels (KITAZAWA *et al.*, 2011). Heneka *et al*. (2013) demonstrated that knockout of *NLRP3* reduced IL-1β production which resulted in a reduction of amyloid plaque burden in mice. Conversely, knocking out the IL-1β receptor antagonist in mice increased the neuronal damage induced by Aβ (CRAFT *et al.*, 2005). These studies implicate a pro-inflammatory role of IL-1β in the pathogenesis of AD.

The *IL-1B* gene is located on chromosome 2q14.1 (FIGURE 6) and is composed of 7,153 bases with 7 exons, encoding a 269 aa protein with 30.7 kDa (GENECARDS, 2017; NCBI, 2017).

FIGURE 6 – LOCATION OF *IL-1B* GENE ON CHROMOSOME 2.
SOURCE: Ensembl in 04/2017.
NOTE: The red rectangle represents the location of the *IL-1B* gene.

IL-18 polymorphisms (rs1946518 and rs187238) have been shown to increase the risk of developing LOAD in Han Chinese and Italian populations (BOSSÙ *et al.*, 2007; YU *et al.*, 2009). On the other hand, other investigators have found that *IL-18* gene polymorphisms may decrease the risk of AD, especially among Asians and those with the *APOE* ε4 allele (LUO *et al*., 2016). High levels of this interleukin are present in the blood of patients with ischemic heart disease, type-2 diabetes, and obesity, which are risk factors for AD (SUTINEN *et al.*, 2012). Levels of IL-18 in AD patients have been shown to decrease as the disease progresses, and no significant upregulation is observed in severe AD patients as compared to age-matched control subjects (MOTTA *et al.*, 2007). This gradual decline suggests that IL-18 could be an initiator factor of AD pathogenesis. Sutinen *et al.* (2012) demonstrate that elevated protein levels of APP, BACE1, and the N-terminal fragment of PSEN1 and PSEN enhancer 2, which are components of the γ-secretase complex, are triggered by IL-18, suggesting IL-18 accelerates Aβ genesis. Preclinical studies have also demonstrated a link between IL-18 and tau pathology (OJALA *et al*., 2008).

The gene encoding IL-18, a 193 aa protein with 22.3 kDa, is located on chromosome 11q23.1 (FIGURE 7), has 20,867 bases and is composed of 6 exons (GENECARDS, 2017; NCBI, 2017).

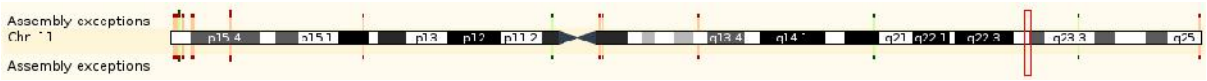


FIGURE 7 – LOCATION OF *IL-18* GENE ON CHROMOSOME 11.
SOURCE: Ensembl in 04/2017.
NOTE: The red rectangle represents the location of the *IL-18* gene.

Regulation of IL-1β and IL-18 may play a role in attenuating and/or balancing the innate immune response during neuroinflammation.

2.6 NLRP10 INFLAMMASOME AND AD

Another inflammasome, the NLRP10, has also been associated with the inflammatory processes underlying AD (MURPHY *et al.*, 2014). This inflammasome, also belonging to the NLR family, differs from NLRP3 because it does not contain LRRs (WANG *et al*., 2004). It is thought that the lack of LRRs prevents ASC, when bound to NLRP10, from binding to the NLRP3 sensor protein, hence preventing activation of the NLRP3 inflammasome (GOLD; EL KHOURY, 2015) and activating caspase-1 (WANG

et al., 2004). A study in a mouse model of AD showed that inhibition of the NLRP3 inflammasome leads to a reduction of the size of Aβ plaques (MURPHY *et al.*, 2014). These findings suggest that NLRP10 may act as a negative regulator of NLRP3 inflammasome activation.

The gene encoding NLRP10, a 655 aa protein with 75.0 kDa, is located on chromosome 11p15.4 (FIGURE 8), and is composed of 6,404 bases compromising 3 exons (GENECARDS, 2017; NCBI, 2017).

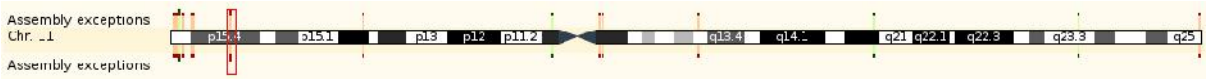


FIGURE 8 – LOCATION OF THE *NLRP10* GENE ON CHROMOSOME 11.
SOURCE: Ensembl in 04/2017.
NOTE: The red rectangle represents the location of the *NLRP10* gene.

2.7 *COL4A1* AND AD

Gain-of-function somatic mutations in the *COL4A1* gene have been shown to provoke inflammatory reactions and damage the brain in a broad range of diseases (GEORGE *et al.*, 1993; PÖSCHL *et al.*, 2004; GOULD *et al.*, 2005; GOULD *et al*., 2006; ALAMOWITCH *et al*., 2009). This gene encodes a type IV collagen alpha protein and its involved in brain and neuromuscular junction development (MARCHESI, 2016).

Marchesi (2016) proposed that low abundant somatic mutations of this and other genes, such as *NLRP3*, *APP*, *TREX1*, and *NOTCH3*, might promote localised inflammation and blood vessel damage in the brain. *COL4A1* mutations have been identified as a monogenic cause of cerebral small-vessel disease (CSVD) (GOULD *et al*., 2006; SIBON *et al*., 2007; ALAMOWITCH *et al*., 2009). Age-associated CSVD shares multiple risk factors and overlaps neuropathologically with AD (LOVE; MINERS, 2016). There is an additive component to the clinical and pathological effects of CSVD and AD, and growing evidence suggests that the disease processes also interact mechanistically at a cellular level. Cerebral microbleeds (CMBs), a kind of CSVD (BATH; WARDLAW, 2015), can be found in a large number of patients with AD (IKRAM *et al*., 2012; POELS *et al*., 2012), and high levels of IL-18 were observed in CMB patients.

Additionally, the presence of vascular risk factors has been reported to predict the development of AD or the conversion from MCI to AD (LUCHSINGER *et al*., 2005; HELZNER *et al*., 2009; LI *et al*., 2011; DE BRUIJN; IKRAM, 2014).

The *COL4A1* gene is located on chromosome 13q34 (FIGURE 9). This gene compromises 158,200 bases and is composed of 54 exons, and encodes a 1669 aa protein with 16.1 kDa (GENECARDS, 2017; NCBI, 2017).

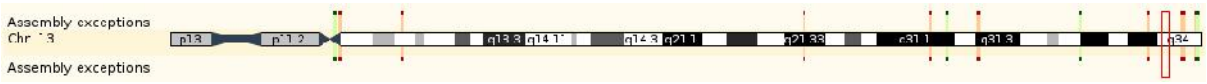


FIGURE 9 – LOCATION OF THE *COL4A1* GENE ON CHROMOSOME 13.
SOURCE: Ensembl in 04/2017.
NOTE: The red rectangle represents the location of the *COL4A1* gene.

Genetic variants and trait scores can be associated with the mechanisms mentioned above. One way to recognize genes involved in human disease is to identify polymorphic sites associated with the presence of the disease. Some genetic polymorphisms can contribute to the disease while others are simply useful markers. The most common type of polymorphism is a single nucleotide polymorphism (SNP) where a base is simply replaced by another. The primary goal of this study is to verify the existence of association between inflammation-related gene polymorphisms and the risk of developing AD.

3 OBJECTIVES

3.1 GENERAL OBJECTIVE

- To verify the existence of association between polymorphisms of inflammation-related genes and LOAD and evaluate the relationship between these genes and clinical and cognitive variables of the patients.

3.2 SPECIFIC OBJECTIVES

- Determine the genotypic and allelic frequencies, both in patients and the control group, of the following polymorphisms: rs6531669 (*TLR6*), rs13105517 (*TLR2*), rs4915274 of (*NEK7*), rs1143643 (*IL-1B*), rs187238 and rs1946518 (*IL-18*), rs9919613 (*NLRP10*), and rs9515185, rs4773142, rs9301441, rs613430 and rs649104 (*COL4A1*);

- Evaluate the relationship between the frequency of the polymorphisms and clinical and cognitive variables, such as cognitive performance, age of onset, duration of the disease, and severity of the disease;
- Evaluate the effect of gender and *APOE* status on AD susceptibility, severity of the disease, disease progression, duration of the disease, and age of onset.

4 JUSTIFICATION

As the world population lives longer, the prevalence of AD is increasing. However, at the moment, there are only symptom modifying drugs for AD, making the discovery of new molecular pathways and genes involved in AD development and progression a priority for medical research.

The goal of case-control association studies is to identify patterns of polymorphisms that vary systematically between individuals with different disease states and thus identify risk-enhancing or protective alleles.

Identifying genotype–disease correlations will help us to identify genetic risk factors for LOAD, deliver new therapeutic targets, improve care and advance treatment of patients, and deliver attractive biomarkers for this disease that are relevant for diagnostics. Plus, these studies are one of the main thrusts of the drive towards personalized medicine.

5 MATERIALS AND METHODS

5.1 PARTICIPANTS

AD patients (n=152) were recruited at Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR) and Instituto de Neurologia de Curitiba (INC). The diagnosis was based on the patient's medical history and cognitive tests. Patients under 60 were excluded from the sample to prevent possible cases of early-onset AD. Individuals aged 60 or over (n=120) were recruited as part of the control group (TABLE 1). The selection was based on the result of a mini mental status examination (MMSE) exam (APPENDIX 1). Exclusion criteria were the presence of infectious disease, both in the patients and control group; contracting any other type of dementia, having alcohol addiction or having an inconclusive diagnosis (in the patient's group).

Written informed consent was obtained from all participants and approved by the ethics committee of Universidade Federal do Paraná (APPENDIX 2). This study is part of a larger project.

TABLE 1 – DESCRIPTIVE DATA FOR ASSOCIATION SAMPLE.

	Cases	Controls
n	152	120
Basic descriptives		
% Female	64.90%	74.17%
% Male	35.10%	25.83%
Mean age (SD)	79.33 (6.82)	70.15 (7.18)
Mean Age of Onset (SD)	75.14 (6.05)	n/a
Mean Disease Duration (SD)	4.10 (2.77)	n/a
Mean MMSE Score (SD)	14.17 (7.46)	27.49 (2.10)
APOE genotyped (%)		
ε2/ε2	0%	0.84%
ε2/ε3	8.11%	8.40%
ε3/ε3	43.24%	71.42%
ε2/ε4	2.70%	0%
ε3/ε4	36.49%	19.33%
ε4/ε4	9.46%	0%

5.2 VARIABLES

Variables in this study included MMSE results; age of onset of the disease; duration of the disease, calculated from the age of onset and the patients age when the information was collected; and severity of the disease, obtained through Clinical Dementia Rating (CDR) values. The CDR is a scale used to characterize six domains of cognitive and functional performance. The level of dementia in AD is given by three different scores: 1 = Mild Dementia, 2 = Moderate Dementia, and 3 = Severe Dementia. In our study, the association between CDR results and the polymorphisms was tested in three different ways: we tested 3 against 2 (CDR 3:2), 3 against 2+1 (CDR 3:2+1), and 3+2 against 1 (CDR 3+2:1). This information was obtained from medical reports.

5.3 SNP TAGGING

NCBI has made information available from completely sequenced *TLR6, TLR2, NEK7*, *IL-1B*, *IL-18*, *NLRP10* and *COL4A1* genes in different populations. Linkage

Disequilibrium (LD) estimates based on D′ and r^2 are available for these SNPs on Haploview (BARRETT *et al*., 2004) and can be used to select a minimal number of SNPs that retain as much as possible of the genetic variation of the full SNP set. For *TLR6, TLR2, NEK7*, *IL-1B*, and *NLRP10* genes one SNP was representative of the whole gene. For the *IL-18* gene, SNPs were chosen according to publicly available data, LD and frequency in the population, and for *COL4A1*, five SNPs were selected according to their LD and frequency in the population. For each gene, only SNPs with a global Minor Allele Frequency (MAF) greater or equal to 0.15 were selected, to minimize the chances of selecting a rare variant. We selected a total of 12 polymorphisms: 5 polymorphisms from *COL4A1*, 2 polymorphisms from *IL-18*, and 1 polymorphism for each of the following genes: *IL-1B*, *NEK7*, *NLRP10*, *TLR2* and *TLR6.* Table 2 shows the SNPs genotyped from each gene.

TABLE 2 – LIST OF THE POLYMORPHISMS SELECTED FOR THE STUDY.

Gene	Locus	SNP	Functional Consequence	Global MAF/Minor Allele Count	Variants	Function of the protein
TLR6	4p14	rs6531669	intron variant	G=0.4363/2185	G/T	Fundamental role in pathogen recognition and activation of innate immunity
TLR2	4q31.3	rs13105517	intron variant, utr variant 5'	A=0.3594/1800	A/G	Fundamental role in pathogen recognition and activation of innate immunity
NEK7	1q31.3	rs4915274	intron variant	A=0.2945/1475	A/C	Essential in NLRP3 Inflammasome activation
IL-18	11q23.1	rs187238	upstream variant 2KB	G=0.2127/1065	C/G	Proinflammatory cytokine;
		rs1946518	upstream variant 2KB	T=0.4079/2043	G/T	↑ cytokine expression
IL-1B	2q14.1	rs1143643	intron variant	T=0.2877/1441	C/T	Proinflammatory cytokine; ↑ cytokine expression
NLRP10	11p15.4	rs9919613	intron variant	C=0.3490/1748	C/G	Inhibits formation of the NLRP3 Inflammasome
COL4A1	13q34	rs9515185	missense, upstream variant 2KB	G=0.4241/2124	C/G	Provokes inflammatory reactions and damages the brain in a wide variety of diseases
		rs4773142	intron variant, utr variant 5'	A=0.4052/2029	A/G	
		rs9301441	intron variant	T=0.4393/2200	C/T	
		rs613430	intron variant	G=0.1683/843	C/G	
		rs649104	intron variant	G=0.4056/2031	A/G	

5.4 DNA EXTRACTION AND QUANTIFICATION

Approximately 4 mL of peripheral blood from each participant was collected in a tube with EDTA. The samples were processed, and the DNA extraction followed an adapted version of the salting out method of Lahiri and Nurnberger's protocol (LAHIRI; NURNBERGER JR, 1991). The DNA was quantified and stored at -20ºC for posterior genotyping.

5.5 GENOTYPING

Polymorphisms were genotyped at the University of Auckland (New Zealand) using Sequenom MassARRAY iPLEX Platform (FIGURE 10). This method is based on primer extension to generate allele-specific products with distinct masses. It starts with a locus-specific PCR reaction, followed by a locus-specific primer extension reaction in which an oligonucleotide primer anneals immediately upstream of the polymorphic site being genotyped. The primer extension is made according to the sequence of the variant site and is a single complementary mass-modified base. Mass-modified dideoxynucleotide terminators are incubated in the primer and amplified target DNA. These terminators do not have a 3' hydroxyl group. The absence of this group prevents any other nucleotide of being added as no phosphodiester bond can be created.

Using mass spectrometry, the mass of the extended primer is determined. The primer's mass indicates the sequence and, therefore, the alleles present at the polymorphic site of interest. The mass of the observed primers is automatically translated into a genotype for each reaction through Sequenom supplies software (SpectroTYPER).

One of the key strengths of the MassARRAY iPLEX assay lies in its multiplexing capabilities. This technique allows us to analyse many individual loci of DNA in one-well reaction. Another significant advantage of this method is the extremely rapid turn-around time that is allowed due to the multiplexing capabilities, convenient assay design tools, and the use of simple reagents. Moreover, the iPLEX assay makes it possible to obtain highly precise, quantitative results.

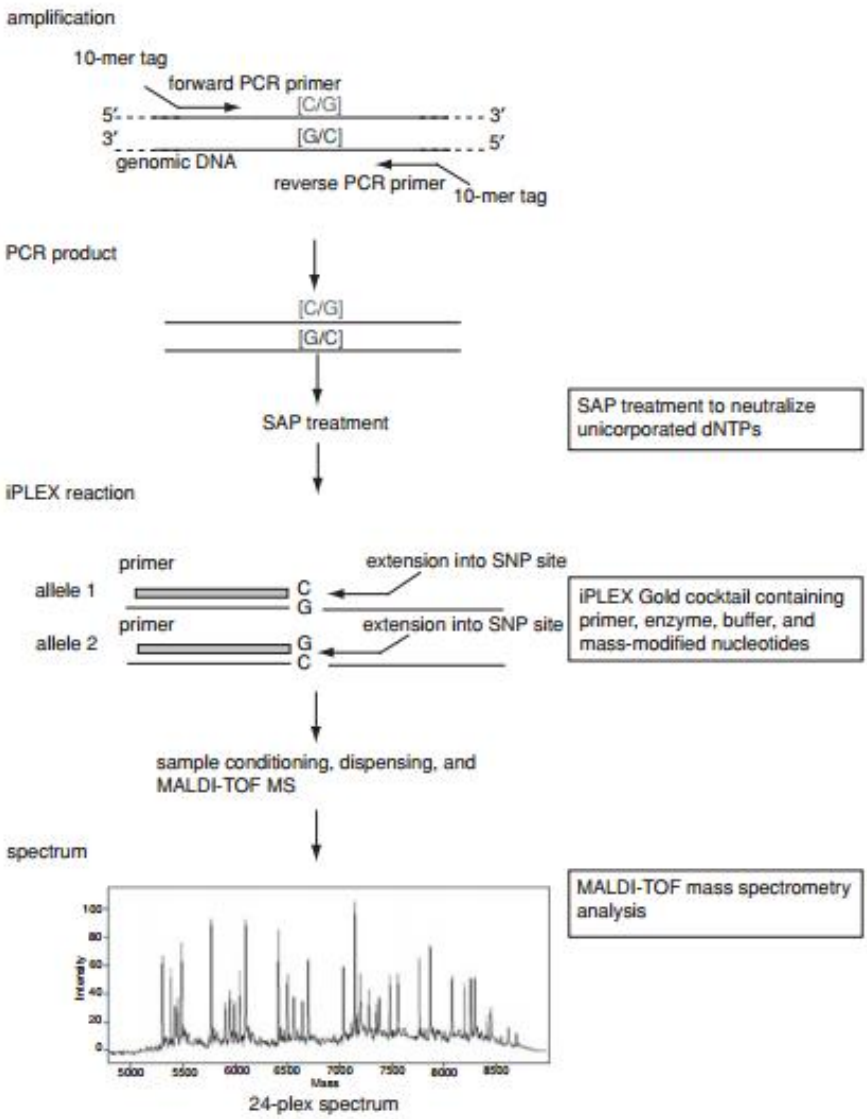


FIGURE 10 – THE MASSARRAY IPLEX REACTION.
SOURCE: GABRIEL *et al*., 2009.
NOTE: A schematic of the genotype reaction of a C-to-G SNP.

rs9515185 of the *COL4A1* gene could not be genotyped, possibly due to the presence of proximal SNPs and the inability of designing SNP-specific primers.

rs7412 and rs429358 from the *APOE* gene were previously genotyped, as part of another study, using real-time PCR, performed using a standard TaqMan® PCR kit protocol (Applied Biosystems).

5.6 STATISTICAL ANALYSIS

Normality, and homogeneity tests were performed for all variables. The $\chi 2$ test was used to test for Hardy-Weinberg equilibrium (HWE). Allele and genotype frequencies were obtained by direct counting. The $\chi 2$ test was used to compare allele

and genotype frequencies in cases and controls and subgroups defined by gender, and *APOE* status when appropriated. The relationship between genotypes and the other study variables was assessed by logistic regression analysis. All quantitative variables were converted to binary variables using a median split. The R package PredictABEL (KUNDU *et al*., 2011) was used for prediction of the disease risk, which was calculated based on logistic regression values, and the R package pROC (ROBIN *et al.*, 2011) to obtain the receiver operating characteristic (ROC) curves, and the area under the curve (AUC). All statistical analysis was performed using R software, and statistical test significance was evaluated using a P-value of 0.05 (5%).

6 RESULTS

Descriptive statistics for both sample groups are presented in Table 2. All SNPs followed Hardy-Weinberg equilibrium (HWE) in both groups ($P > 0.05$).

6.1 LOAD SUSCEPTABILITY

Of the eleven SNPs analysed in this study SNPs rs6531669 (*TLR6*), rs13105517 (*TLR2*), and rs9919613 (*NLRP10*) showed association with LOAD susceptibility (TABLE 3).

The X^2 test for rs6531669 showed a significant difference in the frequency of TLR6 genotypes between AD cases and controls (**P = 0.0173**, TABLE 3). In a recessive genetic model, individuals carrying two copies of the minor allele for rs6531669 of the *TLR6* gene (G/G), showed a consistently lower risk of LOAD (**P = 0.0065**, OR = 0.3310, 95 % CI = [0.1493, 0.7336], TABLE 4a). In addition, the X^2 test was also statistically significant for the recessive genetic model (**P = 0.0048**, TABLE 3). Moreover, the association remained statistically significant after adjusting for gender, and *APOE* status (**P = 0.0075**, OR = 0.3150, 95 % CI = [0.1352, 0.7343], TABLE 4b). When considering all the SNPs included in the study, and after adjusting for gender, and *APOE* status, the association of rs6531669 remained statistically significant (**P = 0.0278**, OR = 0.3817, 95 % CI = [0.1618, 0.9003], TABLE 4c), although, in this case, gender showed statistical significance as well (P = 0.0380, TABLE 4c). When using the X^2 test to analyse both genders separately, no significant association

was found in men (P > 0.05), but it remained statistically significant in women (P = 0.0118, SUPPLEMENTARY TABLE 1).

The X^2 test showed a significant difference in the frequency of *TLR2* alleles for rs13105517 between AD cases and control subjects (**P = 0.0351**, TABLE 3). The association between rs13105517 and LOAD susceptibility was not confirmed by the logistic regression model (P = 0.0516). However, considering a recessive genetic model, and after adjusting for gender, and *APOE* status, individuals carrying two copies of the minor allele (A/A) showed a higher risk of LOAD (**P = 0.0406**, OR = 2.3444, 95 % CI = [1.0369, 5.3004], TABLE 4b). In addition, the X^2 test was also statistically significant for the recessive genetic model (**P = 0.0477**, TABLE 3). When considering all the SNPs included in the study, as well as adjusting for gender, and *APOE* status, the association remained statistically significant (**P = 0.0395**, OR = 2.4346, 95 % CI = [1.0435, 5.6805], TABLE 4c) and gender showed statistical significance as well (P = 0.0380, TABLE 4c). When analysing both genders separately, no significant association was found in men (P > 0.05), but it remained statistically significant in women (P = 0.0434, SUPPLEMENTARY TABLE 1).

In a recessive genetic model, individuals carrying two copies of the minor allele for rs9919613 of the *NLRP10* gene (C/C), showed a lower risk of LOAD (**P = 0.0196**, OR = 0.407, 95 % CI = [0.1914, 0.8656], TABLE 4a). In addition, the X^2 test was also statistically significant for the recessive genetic model (**P = 0.0169**, TABLE 3). When adjusting for gender the association remained significant (**P = 0.0164**, TABLE 4b). However, when adjusting for gender, and *APOE* status, no statistically significant association was observed between cases and controls (P > 0.05). When analysing *NLRP10* C/T+T/T (absence of the protective genotype) together with the presence of ε4 (risk factor for AD), the risk of developing AD was higher than when considering the presence of ε4 alone (OR = 4,37; 95 % CI = [2.51, 7.60] for ε4, and OR = 4.44, 95 % CI = [2.49, 7.92] for ε4+C/T+T/T; data not shown). Additionally, when analysing the absence of ε4 together with the protective genotype C/C, the protective effect was greater than when considering the C/C genotype alone (OR = 0.25, 95 % CI = [0.14, 0.44]; data not shown).

No significant differences in the frequency of *NLRP10* alleles, and genotypes in the AD cases were detected compared with the controls.

For AD susceptibility, ROC curve for the 11 SNPs had an AUC of 0.6509 (FIGURE 11).

TABLE 3 – rs6531669, rs13105517, AND rs9919613 ALLELE AND GENOTYPE FREQUENCIES FOR AD PATIENTS (AD) AND CONTROL SUBJECTS (CRTL). GENOTYPE DISTRIBUTIONS IN A RECESSIVE AND DOMINANT GENETIC MODEL IN AD AND CTRL.

	rs6531669												
TLR6	**Groups**	**Allele Frequency (%)**			**Genotype Frequency (%)**				**Recessive Model**			**Dominant Model**		
		G	T	P	G/G	G/T	T/T	P	G/T + T/T (%)	G/G (%)	P	G/T + G/G (%)	T/T (%)	P
	AD (n=151)	32	68	0.0928	7	51	42	**0.0173**	93	7	**0.0048**	58	42	0.632
	Control (n=119)	39	61		18	43	39		82	18		61	39	
	rs13105517													
TLR2	**Groups**	**Allele Frequency (%)**			**Genotype Frequency (%)**				**Recessive Model**			**Dominant inhritance**		
		G	A	P	G/G	G/A	A/A	P	G/A + G/G (%)	A/A (%)	P	G/A + A/A (%)	G/G (%)	p
	AD (n=151)	62	38	**0.0351**	40	43	17	0.0958	83	17	**0.0477**	60	40	0.1317
	Control (n=119)	71	29		50	42	8		92	8		50	50	
	rs9919613													
NLRP10	**Groups**	**Allele Frequency (%)**			**Genotype Frequency (%)**				**Recessive Model**			**Dominant Model**		
		C	G	P	C/C	C/G	G/G	P	C/G + G/G (%)	C/C (%)	P	C/G + C/C (%)	G/G (%)	P
	AD (n=151)	32	68	0.136	8	48	44	0.0543	92	8	**0.0169**	56	44	0.6553
	Control (n=120)	38	62		18	41	42		83	18		59	42	

TABLE 4 – LOGISTIC REGRESSION ANALYSIS ODDS RATIO FOR ALZHEIMER'S DISEASE.

Gene	SNP	Model	OR	Lower CI	Upper CI	P
a.						
TLR6	rs6531669	G/T+T/T x G/G	0.3310	0.1493	0.7336	**0.0065**
NLRP10	rs9919613	C/G+G/G x C/C	0.4070	0.1914	0.8656	**0.0196**
b.						
TLR6	rs6531669	G/T+T/T x G/G	0.3150	0.1352	0.7343	**0.0075**
		Gender	1.6342	0.9298	2.8723	0.0878
		ε4	4.0719	2.2964	7.2203	**0.0000**
TLR2	rs13105517	G/A+G/G x A/A	2.3444	1.0369	5.3004	**0.0406**
		Gender	1.7180	0.9796	3.0129	0.0590
		ε4	3.9862	2.2627	7.0227	**0.0000**
NLRP10	rs9919613	C/G+G/G x C/C	0.3933	0.1836	0.8427	**0.0164**
		Gender	1.6324	0.9544	2.7920	0.0735
c.						
TLR6	rs6531669	G/T+T/T x G/G	0.3817	0.1618	0.9003	**0.0278**
TLR2	rs13105517	G/A+G/G x A/A	2.4346	1.0435	5.6805	**0.0395**
		Gender	1.8686	1.0352	3.3729	**0.0380**
		ε4	3.6416	1.9858	6.6779	**0.0000**

a. For each SNP;
b. In the presence of factors including gender (for *NLRP10*), and gender and *APOE* status (for *TLR6* and *TLR2*);
c. Considering all the SNPs, and in the presence of factors including gender and *APOE* status.

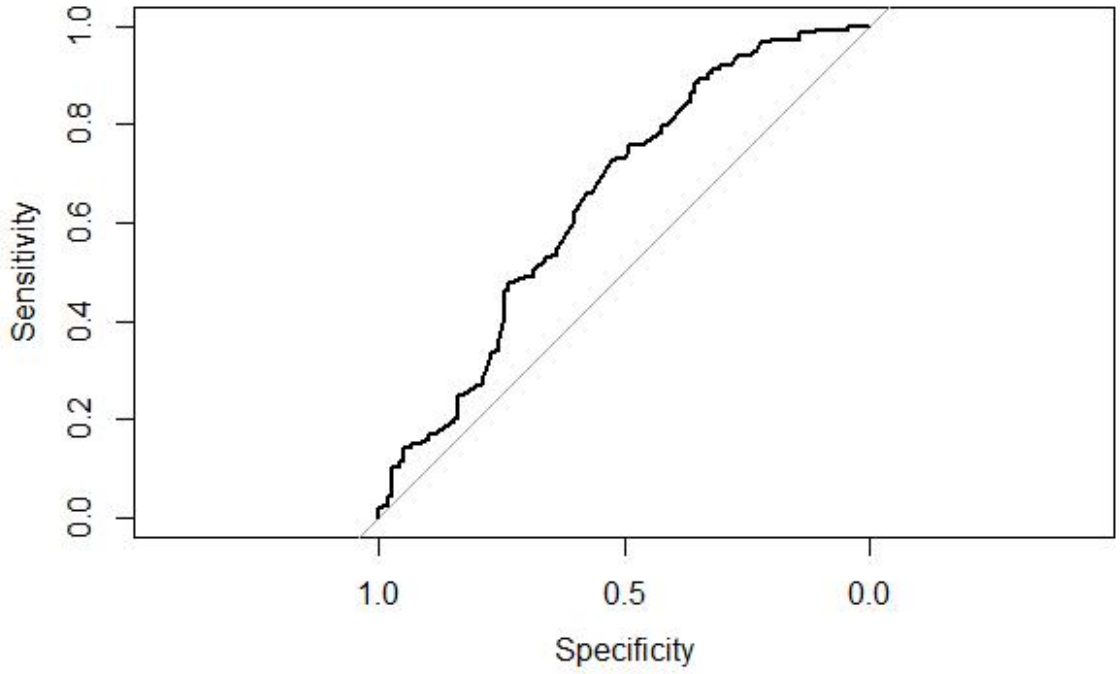


FIGURE 11 – ROC CURVE FOR AD SUCEPTIBILITY CONSIDERING THE RECESSIVE GENETIC MODEL OF THE 11 SNPs. AUC = 0.6509.

6.2 AGE OF ONSET

Of the eleven SNPs analysed in this study SNPs rs9919613 (*NLRP10*), and rs1143643 (*IL-1B*) showed association with the age of onset of the disease.

In a recessive genetic model for rs9919613 of the *NLRP10* gene, individuals carrying two copies of the minor allele (C/C) showed a higher risk of developing the disease earlier when compared to those carrying one or no copies of the allele (**P = 0.0125**, OR = 14.0877, 95 % CI = [1.7681, 112.2492], TABLE 6a). In addition, the X^2 test was also statistically significant for the recessive genetic model (**P = 0.0015**, TABLE 5). This association remained significant after adjusting for gender and *APOE* status (**P = 0.0084**, OR = 17.1105, 95 % CI = [2.0676, 141.5985], TABLE 6b). Gender showed statistical significance as well (P = 0.0098, TABLE 6b). When analysing both genders separately, no significant association was found in men (P > 0.05), but it remained statistically significant in women (P = 0.0089, SUPPLEMENTARY TABLE 2). Moreover, when considering all the SNPs included in the study, and after adjusting for gender and *APOE* status, the association of rs9919613 with the age of onset remained statistically significant (**P = 0.0021**, OR = 44.878, 95 % CI = [3.9571, 508.9622], TABLE 6c).

In a recessive genetic model for rs1143643 of the *IL-1B* gene, individuals carrying two copies of the minor allele (T/T) showed a lower risk of developing the disease earlier when compared to those carrying one or no copies of the allele (**P = 0.0471**, OR = 0.3348, 95 % CI = [0.1137, 0.9861], TABLE 6a). The X^2 test was also statistically significant for the recessive genetic model (**P = 0.0399**, TABLE 5). This association remained significant after adjusting for gender and *APOE* status (**P = 0.0479**, OR = 0.3278, 95 % CI = [0.1086, 0.9897], TABLE 6b). Gender showed statistical significance as well (P = 0.0164, TABLE 6b). When analysing both genders separately, no significant association was found neither in men or women (P > 0.05, SUPPLEMENTARY TABLE 2). When considering all the SNPs included in the study, and after adjusting for gender and *APOE* status, the association of the recessive genetic model of rs1143643 with the age of onset of the disease increased significantly (**P = 0.0099**, OR = 0.1208, 95 % CI = [0.0242, 0.6022], TABLE 6c), and the codominant genetic model for rs1143643 also showed association with the age of onset (P = **0.0238**, OR = 0.5212, 95 % CI = [0.2962, 0.9172], TABLE 6c).

For the age of onset, the ROC curve for the 11 SNPs had an AUC of 0.7379 (FIGURE 12).

TABLE 5 – GENOTYPE DISTRIBUTIONS OF rs9919613 OF THE NLRP10 GENE, AND rs1143643 OF THE IL-1B GENE, IN A RECESSIVE AND DOMINANT GENETIC MODEL, IN INDIVIDUALS WITH AN AGE OF ONSET < 75YO AND INDIVIDUALS WITH AN AGE OF ONSET > 75YO.

		rs9919613					
NLRP10	**Groups**	**Recessive Model**			**Dominant Model**		
		C/G + G/G (%)	C/C (%)	P	C/G + C/C (%)	G/G (%)	P
	AO < 75 (n=73)	84	16	**0.0015**	54	46	0.9054
	AO > 75 (n=69)	99	1		55	45	
		rs1143643					
IL-1β	**Groups**	**Recessive Model**			**Dominant Model**		
		C/T + C/C (%)	T/T (%)	P	C/T + T/T (%)	C/C (%)	P
	AO < 75 (n=74)	93	7	**0.0399**	52	48	0.5823
	AO > 75 (n=69)	81	19		57	43	

Abbreviations: AO, age of onset.

TABLE 6 – LOGISTIC REGRESSION ANALYSIS ODDS RATIO FOR THE AGE OF ONSET.

Gene	SNP	Model	OR	Lower CI	Upper CI	P
a.						
NLRP10	rs9919613	C/G+G/G x C/C	14.0877	1.7681	112.2492	**0.0125**
IL-1β	rs1143643	C/T+C/C x T/T	0.3348	0.1137	0.9861	**0.0471**
b.						
NLRP10	rs9919613	C/G+G/G x C/C	17.1105	2.0676	141.5985	**0.0084**
		Gender	2.6300	1.2629	5.4770	**0.0098**
		ε4	1.6195	0.7889	3.3248	0.1889
IL-1β	rs1143643	C/T+C/C x T/T	0.3278	0.1086	0.9897	**0.0479**
		Gender	2.3948	1.1736	4.8867	**0.0164**
		ε4	1.2184	0.6121	2.4254	0.5739
c.						
NLRP10	rs9919613	C/G+G/G x C/C	44.878	3.9571	508.9622	**0.0021**
IL-1β	rs1143643	C/T+C/C x T/T	0.1208	0.0242	0.6022	**0.0099**
		Gender	2.9155	1.2859	6.6106	**0.0104**
		ε4	1.6178	0.7235	3.6172	0.2413
IL-1β	rs1143643	C/C x C/T x T/T	0.5212	0.2962	0.9172	**0.0238**
		Gender	3.0693	1.4267	6.6031	**0.0041**
		ε4	1.2855	0.5996	2.7561	0.5186

a. For each SNP;
b. In the presence of factors including gender and *APOE* status;
c. Considering all the SNPs in a recessive (top) and codominant (bottom) genetic model, and in the presence of factors including gender and *APOE* status.

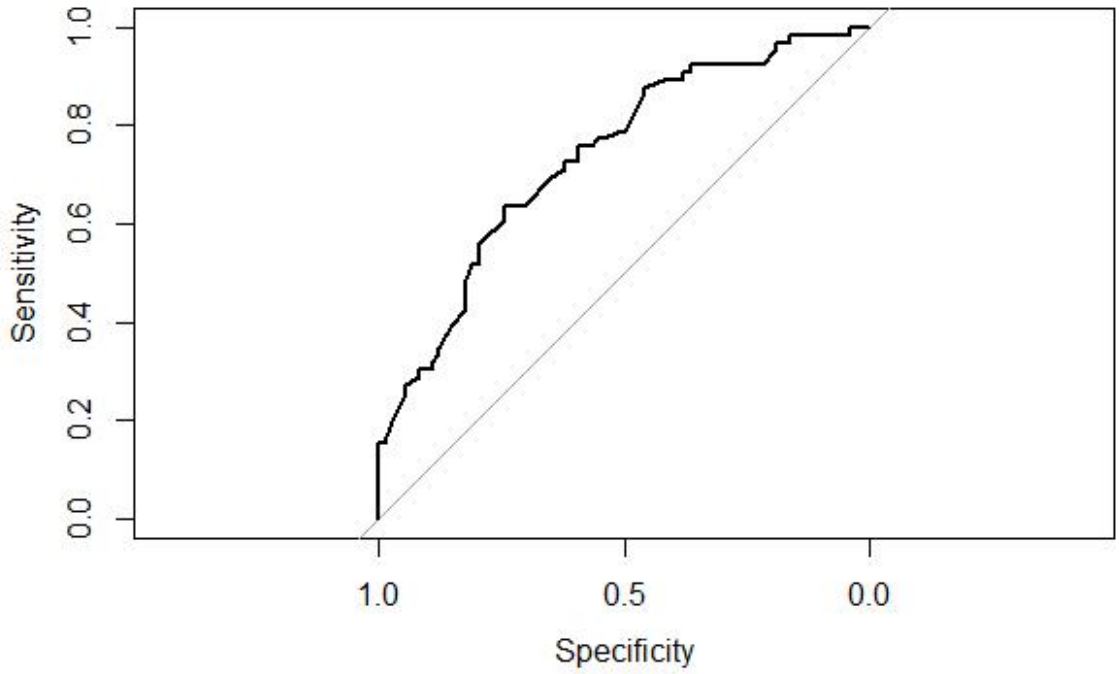


FIGURE 12 – ROC CURVE FOR AGE OF ONSET CONSIDERING THE RECESSIVE GENETIC MODEL OF THE 11 SNPs. AUC = 0.7379.

6.3 MINI MENTAL STATE EXAMINATION

No SNP showed any significant differences in allelic or genotypic frequencies for the MMSE results (data not shown). However, in a recessive genetic model, when considering all the SNPs included in the study, and after adjusting for gender and *APOE* status, individuals carrying two copies of the minor allele of rs6531669 of the *TLR6* gene (G/G), and rs13105517 of the *TLR2* gene (A/A), showed significant lower results when compared with those carrying one or no copies of these alleles (**P = 0.0445**, OR = 5.4781, 95 % CI = [1.0423, 28.7924], **P = 0.0350**, OR = 3.2392, 95 % CI = [1.0862, 9.6604], respectively, TABLE 7).

For the MMSE, the ROC curve for the 11 SNPs had an AUC of 0.6579 (FIGURE 13).

TABLE 7 – LOGISTIC REGRESSION ANALYSIS ODDS RATIO FOR MMSE.

Gene	SNP	Model	OR	Lower CI	Upper CI	P
TLR2	rs13105517	G/A+G/G x A/A	3.2392	1.0862	9.6604	**0.0350**
TLR6	rs6531669	G/T+T/T x G/G	5.4781	1.0423	28.7924	**0.0445**
		Gender	0.8038	0.3592	1.7988	0.5952
		ε4	1.3538	0.6262	2.9268	0.4413

NOTE: Considering all the SNPs, and in the presence of factors including gender and *APOE* status.

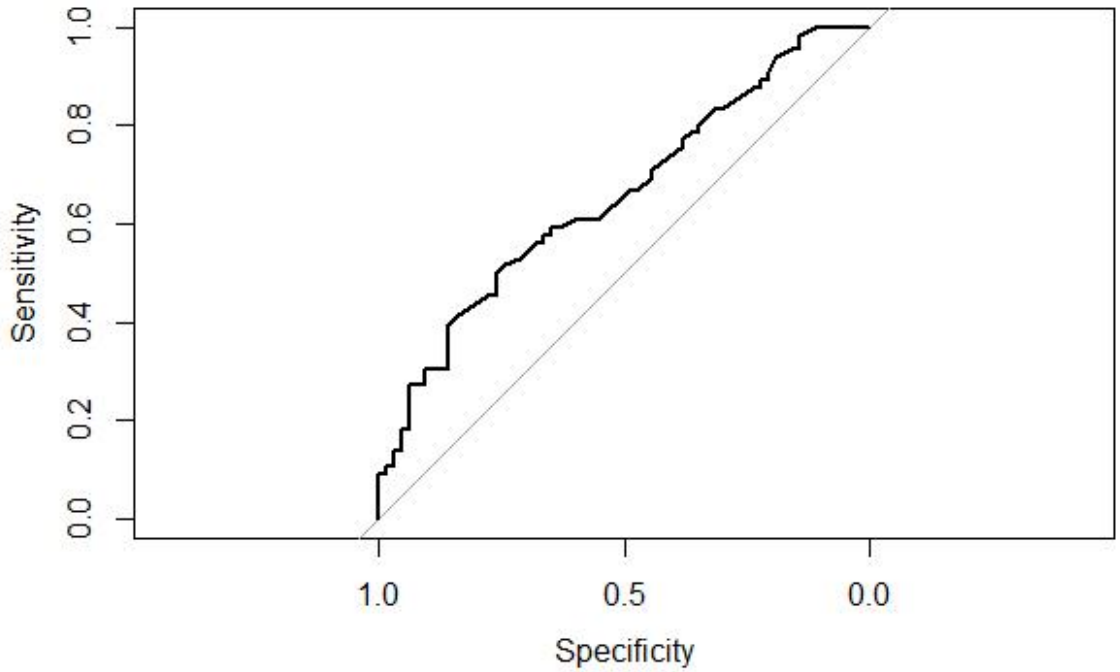


FIGURE 13 – ROC CURVE FOR MMSE CONSIDERING THE RECESSIVE GENETIC MODEL OF THE 11 SNPs. AUC = 0.6579.

6.4 DISEASE SEVERITY

6.4.1 CDR 3:2

No significant associations were found between the SNPs analysed in the study and CDR 3:2. Nevertheless, when adjusting for gender and *APOE* status, in a recessive genetic model for rs6531669 of the *TLR6* gene, individuals carrying two copies of the major allele (T/T) showed significant lower CDR results when compared with those with one or no copies of the allele (P = **0.0406**, OR = 0.3695, 95 % CI = [0.1424, 0.9583], TABLE 8a). Furthermore, when considering all the SNPs, and after adjusting for gender and *APOE* status, the association of the recessive model for rs6531669 and CRD 3:2 remained significant (P = **0.0406**, OR = 0.3695, 95 % CI = [0.1424, 0.9583], TABLE 8b).

In a recessive genetic model for rs1143643 of the *IL-1B* gene, when considering all the SNPs, and after adjusting for gender and *APOE* status, individuals carrying two copies of the major allele (C/C) showed higher disease severity when compared to

those with one or no copies of the allele (P = **0.0406**, OR = 0.3695, 95 % CI = [0.1424, 0.9583], TABLE 8b).

TABLE 8 – LOGISTIC REGRESSION ANALYSIS ODDS RATIO FOR CDR 3:2.

Gene	SNP	Model	OR	Lower CI	Upper CI	P
a.						
TLR6	rs6531669	G/T+G/G x T/T	0.3695	0.1424	0.9583	**0.0406**
		Gender	0.8198	0.3239	2.0748	0.6749
		ε4	1.5744	0.6501	3.8128	0.3145
b.						
TLR6	rs6531669	G/T+G/G x T/T	0.2976	0.0990	0.8945	**0.0309**
IL-1β	rs1143643	C/T+T/T x C/C	3.0152	1.0616	8.5645	**0.0383**
		Gender	0.5517	0.1801	1.6901	0.2978
		ε4	1.5363	0.5366	4.3986	0.4237

a. In the presence of factors including gender and *APOE* status;
b. Considering all the SNPs, and in the presence of factors including gender and *APOE* status.

6.4.2 CDR 3:2+1

In a codominant genetic model for rs6531669 of the *TLR6* gene, individuals carrying two copies of the major allele (T/T) showed lower disease severity when compared to the ones with one or no copies of this allele (P = **0.0325**, OR = 0.6336, 95 % CI = [0.4171, 0.9626], TABLE 10a). The association was more significant in a recessive genetic model for the same allele (P = **0.0268**, OR = 0.3905, 95 % CI = [0.1699, 0.8976], TABLE 10a). The recessive genetic model association was also confirmed in the X^2 test (P = **0.0245**, TABLE 9). The associations of the codominant and recessive models remained significant after adjusting for gender and *APOE* status (P = **0.0251**, OR = 0.6094, 95 % CI = [0.3952, 0.9399], P = **0.0208**, OR = 0.3609, 95 % CI = [0.1521, 0.8565], respectively, TABLE 10b). When including all the SNPs, and after adjusting for gender and *APOE* status, only the recessive model for rs6531669 remained significant (P = **0.0378**, OR = 0.3674, 95 % CI = [0.1428, 0.9453], TABLE 10c).

In a codominant genetic model for rs613430 of the *COL4A1* gene, individuals carrying two copies of the minor allele (G/G) showed greater disease severity when compared to those with one or no copies of this allele (P = **0.0244**, OR = 2.5397, 95 % CI = [1.1278, 5.7194], TABLE 10a). The association remained significant after adjusting for gender and *APOE* status (P = **0.0289**, OR = 2.4920, 95 % CI = [1.0983, 5.6545], TABLE 10b), and when considering all the SNPs, and after adjusting for

gender and *APOE* status (remained significant after adjusting for gender and *APOE* status (P = **0.0236**, OR = 2.8927, 95 % CI = [1.1532, 7.2559], TABLE 10c).

In a recessive genetic model for rs1143643 of the *IL-1B* gene, when considering all the SNPs, and after adjusting for gender and *APOE* status, individuals carrying two copies of the major allele (C/C) showed higher disease severity when compared to those with one or no copies of the allele (P = **0.0110**, OR = 3.2162, 95 % CI = [1.3075, 7.9113], TABLE 10c).

For the CDR 3:2+1, the ROC curve for the recessive genetic model of the 11 SNPs had an AUC of 0.7068 (FIGURE 14), and the ROC curve for the codominant genetic model of the 11 SNPs had an AUC of 0.7066 (FIGURE 15).

TABLE 9 – GENOTYPE DISTRIBUTIONS OF rs6531669 OF THE TLR6 GENE, IN A CODOMINANT, RECESSIVE AND DOMINANT GENETIC MODEL, IN INDIVIDUALS WITH A CDR VALUE OF 3 AND INDIVIDUALS WITH A CDR VALUE OF 1 OR 2.

		rs6531669									
TLR6	**Groups**	**Codominant Model**				**Dominant Model**			**Recessive Model**		
		G/G (%)	G/T (%)	T/T (%)	P	G/T + T/T (%)	G/G (%)	P	G/T + G/G (%)	T/T (%)	P
	CDR 3 (n=41)	10	66	24	0.0796	90	10	0.6131	76	24	**0.0245**
	CDR 2+1 (n=84)	7	48	45		93	7		55	45	

TABLE 10 – LOGISTIC REGRESSION ANALYSIS ODDS RATIO FOR CDR 3:2+1.

Gene	SNP	Model	OR	Lower CI	Upper CI	P
a.						
TLR6	rs6531669	G/G x G/T x T/T	0.6336	0.4171	0.9626	**0.0325**
TLR6	rs6531669	G/T+G/G x T/T	0.3905	0.1699	0.8976	**0.0268**
COL4A1	rs613430	C/C x C/G x G/G	2.5397	1.1278	5.7194	**0.0244**
b.						
TLR6	rs6531669	G/G x G/T x T/T	0.6094	0.3952	0.9399	**0.0251**
		Gender	0.8417	0.3714	1.9074	0.6797
		ε4	1.3723	0.6298	2.9902	0.4258
TLR6	rs6531669	G/T+G/G x T/T	0.3609	0.1521	0.8565	**0.0208**
		Gender	0.8373	0.3691	1.8994	0.6709
		ε4	1.3487	0.6187	2.9402	0.4518
COL4A1	rs613430	C/C x C/G x G/G	2.4920	1.0983	5.6545	**0.0289**
		Gender	0.8608	0.3791	1.9545	0.7202
		ε4	1.3257	0.6099	2.8813	0.4766
c.						
TLR6	rs6531669	G/T+G/G x T/T	0.3674	0.1428	0.9453	**0.0378**
IL-1B	rs1143643	C/T+T/T x C/C	3.2162	1.3075	7.9113	**0.0110**
		Gender	0.6585	0.2604	1.6652	0.3775
		ε4	1.2306	0.4927	3.0739	0.6568
COL4A1	rs613430	C/C x C/G x G/G	2.8927	1.1532	7.2559	**0.0236**
		Gender	0.9458	0.3891	2.2992	0.9021
		ε4	1.5699	0.6099	4.0408	0.3498

a. For each SNP;
b. In the presence of factors including gender and *APOE* status;
c. Considering all the SNPs in a recessive (top) and codominant (bottom) genetic model, and in the presence of factors including gender and *APOE* status.

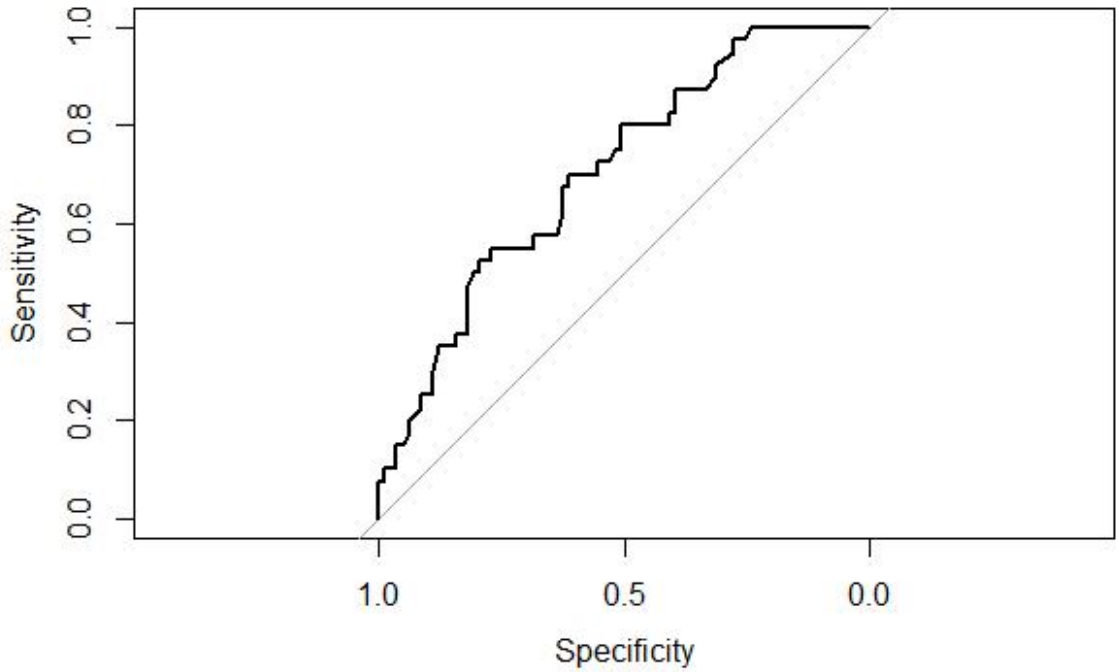


FIGURE 14 – ROC CURVE FOR CDR 3:2+1 CONSIDERING THE RECESSIVE GENETIC MODEL OF THE 11 SNPs. AUC = 0.7068.

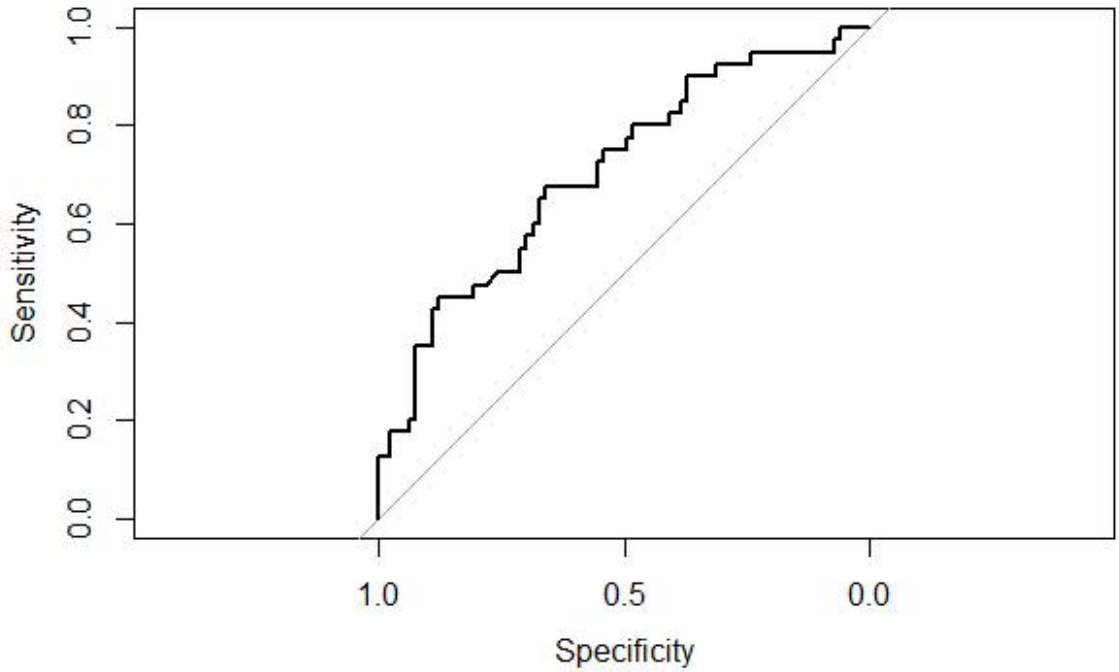


FIGURE 15 – ROC CURVE FOR CDR 3:2+1 CONSIDERING THE CODOMINANT GENETIC MODEL OF THE 11 SNPs. AUC = 0.7066.

6.4.3 CDR 3+2:1

In a recessive genetic model for rs9919613 of the *NLRP10* gene, individuals carrying two copies of the minor allele (C/C) showed a lower disease severity (P = **0.0195**, OR = 0.2134, 95 % CI = [0.0584, 0.7799], TABLE 12a). This association was also confirmed by the X2 test results (P = **0.0121**, TABLE 11). The association remained significant after adjusting for gender, and *APOE* status (P = **0.0108**, OR = 0.1723, 95 % CI = [0.0446, 0.6656], TABLE 12b), and when considering all the SNPs the association was even more significant (P = **0.0062**, OR = 0.1291, 95 % CI = [0.0298, 0.5587], TABLE 12c).

For the CDR 3+2:1, the ROC curve for the 11 SNPs had an AUC of 0.6653 (FIGURE 16).

TABLE 11 – GENOTYPE DISTRIBUTIONS OF rs9919613 OF THE NLRP10 GENE, IN A RECESSIVE AND DOMINANT GENETIC MODEL, IN INDIVIDUALS WITH A CDR VALUE OF 1 AND INDIVIDUALS WITH A CDR VALUE OF 2 OR 3.

		rs9919613					
NLRP10	**Groups**	**Recessive Model**			**Dominant Model**		
		C/G + G/G (%)	C/C (%)	P	C/G + C/C (%)	G/G (%)	P
	CDR 3+2 (n=87)	95	5	**0.0121**	43	57	0.5518
	CDR 1 (n=38)	82	18		37	63	

TABLE 12 – LOGISTIC REGRESSION ANALYSIS ODDS RATIO FOR CDR 3+2:1.

Gene	SNP	Model	OR	Lower CI	Upper CI	P
a.						
NLRP10	rs9919613	C/G+G/G x C/C	0.2134	0.0584	0.7799	**0.0195**
b.						
NLRP10	rs9919613	C/G+G/G x C/C	0.1723	0.0446	0.6656	**0.0108**
		Gender	0.7498	0.3304	1.7018	0.4911
		ε4	0.6179	0.2690	1.4190	0.2564
c.						
NLRP10	rs9919613	C/G+G/G x C/C	0.1291	0.0298	0.5587	**0.0062**
		Gender	0.9431	0.3915	2.2718	0.8960
		ε4	0.5619	0.2367	1.3340	0.1913

a. For *NLRP10*;
b. In the presence of factors including gender and *APOE* status;
c. Considering all the SNPs, and in the presence of factors including gender and *APOE* status.

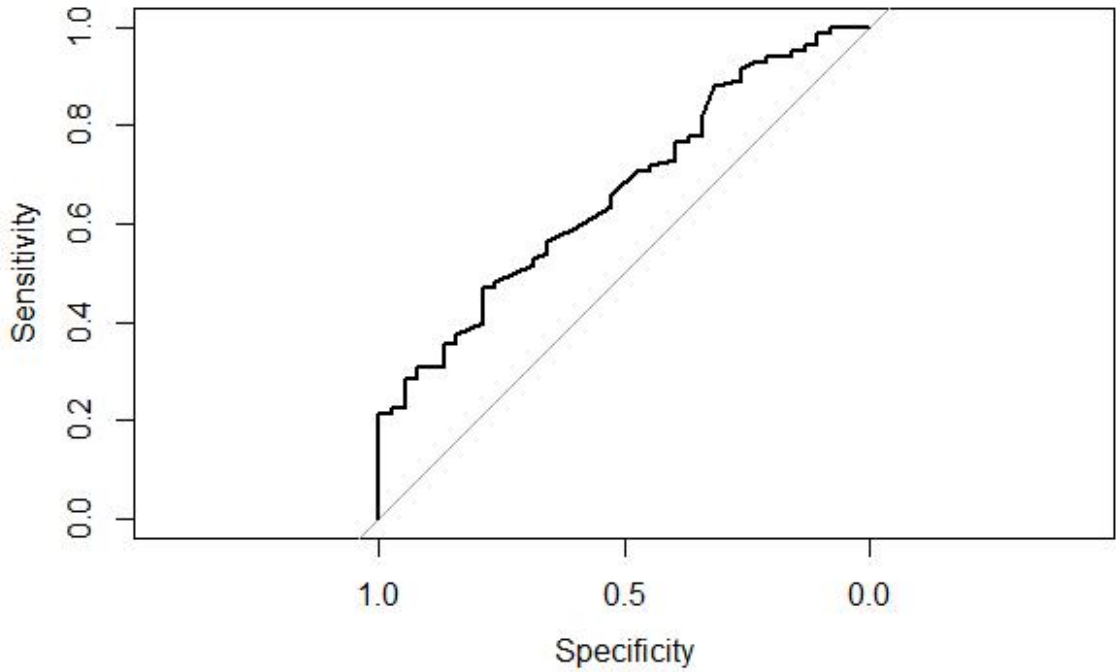


FIGURE 16 – ROC CURVE FOR CDR 3+2:1 CONSIDERING THE RECESSIVE GENETIC MODEL OF THE 11 SNPs. AUC = 0.6653.

6.5 DURATION OF DISEASE

No statistically significant associations were found for the duration of the disease.

6.6 NONSIGNIFICANT RELATIONSHIPS

No associations were found between any of the study variables and rs4915274 of the *NEK7* gene, rs187238 and rs1946518 of the *IL-18* gene, and rs4773142, rs9301441 and rs649104 of the *COL4A1* gene.

Figure 17 summarises the results found in the study.

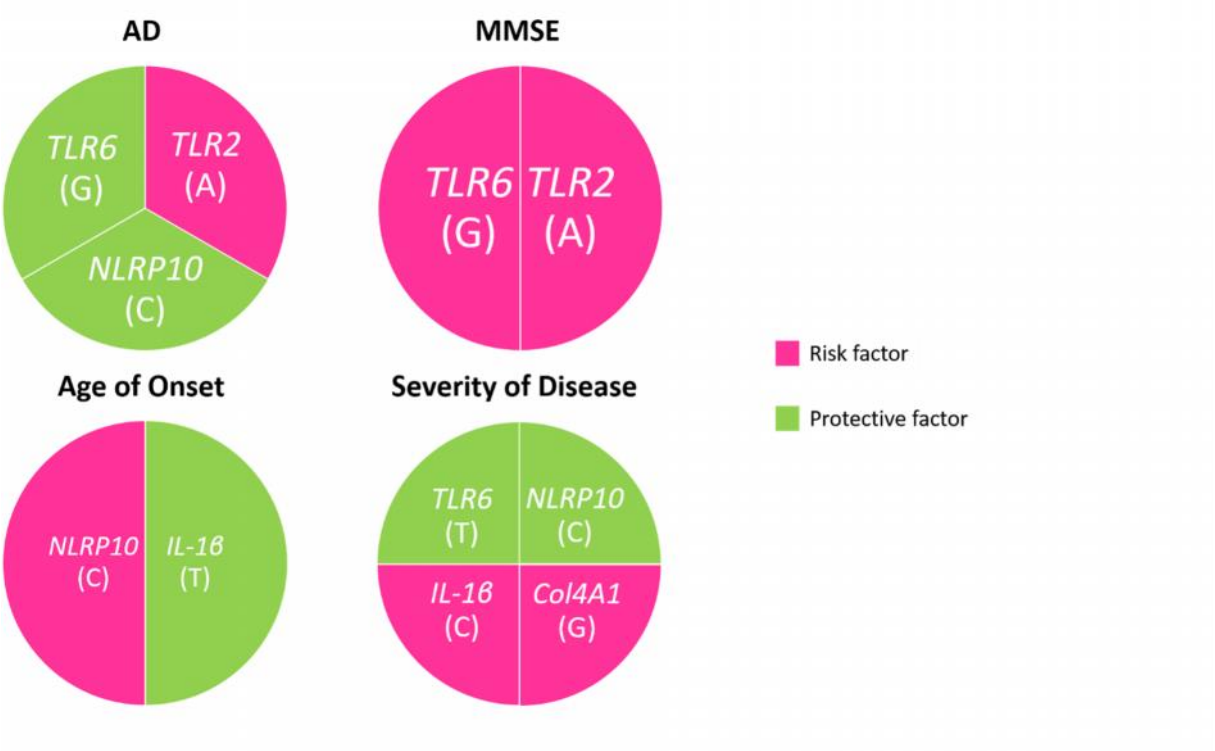


FIGURE 17 – GRAPHICAL RESUME OF ASSOCIATIONS FOUND IN THE STUDY, SEPARATED BY VARIABLES.
SOURCE: the author.
NOTE: AD – *TLR6* G/G genotype was associated with lower risk of developing AD, *TLR2* A/A genotype was associated with higher risk of developing AD, *NLRP10* C/C genotype was associated with lower risk of developing AD; MMSE – *TLR6* G/G and *TLR2* A/A genotypes were associated with lower MMSE results; Age of Onset – *NLRP10* C/C genotype was associated with early age of onset, *Il-1B* T/T genotype was associated with late age of onset; Disease Severity – *TLR6* T/T and *NLRP10* C/C genotypes were associated with lower disease severity, *Il-1B* C/C and *COL4A1* G/G genotypes were associated with greater disease severity.

7 DISCUSSION

AD is the world's most common form of dementia. It is estimated that the prevalence of the disease will be 100 million by 2050 due to the ageing population (BROOKMEYER *et al*., 2007). The pathogenesis of AD has not been fully elucidated yet, and over 100 compounds attempting to treat AD have failed (MULLANE; WILLIAMS, 2013). Therefore, current treatment options aim at pain and symptom management, such as acetylcholinesterase inhibitors, and often show little efficacy (KADUSZKIEWICZ *et al*., 2005; MULLANE; WILLIAMS, 2013; RAINA *et al*., 2008). Hence, research has focused on the discovery of genetic factors for LOAD.

The present study showed a significant association between rs6531669 of the *TLR6* gene, and AD. To our knowledge, this study was the first to find an association between *TLR6* polymorphisms and AD susceptibility.

TLR6 G/G genotype showed a consistent protective effect against the risk of developing LOAD. This association remained statistically significant after adjusting for gender and the presence of an *APOE* ε4 allele, the major genetic risk factor for sporadic, LOAD. This polymorphism was also associated with MMSE scores and CDR values. Individuals carrying two copies of the G allele had a lower score on the MMSE test than those with one or no copies of the G allele. Additionally, and consistent with this result, individuals carrying two copies of the T allele had lower CDR values than those with one or no copies of the T allele. A less significant association was found for the codominant genetic model of this polymorphism, where individuals carrying two copies of the T allele showed less severity of the disease.

It is possible to infer cognitive impairment trough the MMSE test results: lower MMSE results indicate severe cognitive impairment while higher results indicate mild cognitive impairment. These results show that the G/G genotype of the rs6531669 polymorphism is a genetic risk factor for cognitive impairment while the T/T genotype of the same polymorphism is a protective genetic factor for severity of the AD.

It is important to notice that *TLR6* G/G genotype was associated with lower risk of developing AD but, at the same time, with greater cognitive impairment. One plausible explanation is that the presence of this *TLR6* genotype is neither necessary nor sufficient for the development of AD. Although acting as a protective factor for AD, in the case of eventually developing the disease, individuals carrying two copies of the G allele have a higher risk of presenting severe cognitive impairment. Moreover, *TLR6* association with MMSE score was only significant when considering the effect of all the SNPs included in the study.

The TLR6 receptor has been previously associated with the innate immune activation of microglia. Stewart *et al.* (2010) showed that the TLR4-TLR6 heterodimer regulates the expression of pro-inflammatory mediators, such as chemokines, and reactive oxygen and nitrogen species, promoting microglial inflammatory responses associated with the pathology of AD. Additionally, CD36-TLR4-TLR6 signalling in microglia resulted in transcription of mRNA encoding pro-IL-1β, priming these cells for inflammasome activation and IL-1β secretion (STEWART *et al*., 2010). Interestingly, Reed-Geaghan *et al*. (2009) reported that TLR4 is necessary for binding fibrillar Aβ to the cell surface, and is required for activation of microglia and induction of phagocytosis, and a TLR4 polymorphism was shown to attenuate receptor signalling, thus being associated with decreased risk of AD (MINORETTI *et al*., 2006). It is

possible that the same is happening with TLR6 rs6531669, and that this polymorphism, or another in linkage disequilibrium with the first, attenuates receptor signalling, resulting in reduced microglia activation and, therefore, is associated with decreased risk of developing AD. Additionally, Liu and colleagues (LIU *et al*., 2012) demonstrated that TLR2-mediated Aβ-triggered inflammatory activation was suppressed by TLR6, which resulted in decreased production of interleukins, suggesting that TLR6 could be a potential therapeutic target AD.

Our study suggests that the *TLR6* rs6531669 is an important protective factor for the development of LOAD in a Brazilian population.

Although of lesser magnitude, rs13105517 of the *TLR2* gene showed association with the disease susceptibility. Inheritance of the *TLR2* A/A genotype increased the risk for AD, although this association was only significant after adjusting for gender and *APOE* status. When including all the SNPs in the analysis the association was stronger, showing that the genetic effect of rs13105517 is greater when combined with the influence of other polymorphisms. Additionally, *TLR2* A/A genotype was also associated with greater cognitive impairment than G/G+G/A genotypes.

Our results are consistent with other genetic studies that have also identified an association between *TLR2* and AD susceptibility (SOHRABIFAR *et al*., 2015; WANG *et al*., 2011; YU *et al*., 2011).

Direct interaction between TLR2 and Aβ has been shown to trigger inflammatory microglia activation (LIU *et al*., 2012; WALTER *et al*., 2007), and induce expression of pro-inflammatory cytokines (LIN *et al*., 2013). Several studies demonstrated that TLR2 deficiency is associated with reduced Aβ-triggered inflammatory activation, and increased Aβ phagocytosis (JANA *et al*., 2008; LIU *et al*., 2012; RAVARI *et al*., 2017). Additionally, inhibition of TLR2 has been found to be therapeutic in mouse models of AD (LIU *et al*., 2012; MCDONALD *et al.*, 2016). These findings suggest that inhibition of TLR2 in microglia could be beneficial in AD pathogenesis.

However, other studies have shown that TLR2 acts as a receptor to clear Aβ plaques, and a deficiency of TLR2 would lead to elevated Aβ plaque levels and intensified memory loss (HANKE; KIELIAN, 2011; RICHARD *et al*., 2008), showing that TLR2 might play a dual role in AD pathogenesis.

Our analysis suggests that the A/A genotype of rs13105517 is associated with a modest increase in AD risk and greater cognitive impairment in a Brazilian population.

rs9919613 of the *NLRP10* gene also showed association with AD susceptibility. *NLRP10* C/C genotype had a protective effect against AD when compared with C/G+G/G genotypes. This association remained significant when adjusting for gender. Interestingly, when adjusting for gender and *APOE* status, no statistically significant association was observed between cases and controls. We demonstrated that the presence of *APOE* ε4 allele and the absence of *NLRP10* C/C protective genotype confers a greater risk of developing AD than the presence of ε4 allele alone, and that the presence of the protective C/C genotype together with the absence of ε4 allele is a strong protective factor against the development of AD. One plausible interpretation is that the protective genetic effect of *NLRP10* is relevant in influencing AD susceptibility only in the absence of the *APOE* ε4 allele, while in ε4 carriers the genetic effect is essentially determined by this susceptibility factor. Moreover, the same genotype was associated with lower CDR scores, suggesting a protective effect against cognitive impairment, when compared with C/G+G/G genotypes.

This result is consistent with the findings that NLRP10 inflammasome has an anti-inflammatory effect since it inhibits the ASC-mediated activation of caspase 1 and consequent IL-1β release (WANG *et al*., 2004).

However, although associated with lower risk of AD and lower cognitive impairment, the *NLRP10* C/C genotype was also associated with early age of onset when compared with C/G+G/G genotypes.

Our data suggest that this NLRP10 polymorphism contributes to the susceptibility to AD, cognitive impairment, and age of onset of the disease, in a Brazilian population. To the best of our knowledge, this is the first time that an *NLRP10* polymorphism has been directly associated with AD susceptibility and age of onset.

Contrarily to *NLRP10*, rs1143643 of the *IL-1B* gene was associated with delayed age of onset. *IL-1B* T/T carriers showed a delay in onset of AD when compared with C/T+C/C carriers. In a lesser magnitude, and when considering all the SNPs in the study, the codominant genetic model for rs1143643 also showed a protective effect for the age of onset of AD. When analysing the X^2 test for the codominant genetic model, we conclude that inheritance of the T/T genotype is associated with delayed age of onset when compared with C/T and C/C genotypes.

Similar results were also observed in previous researches (GRIMALDI *et al.*, 2000). Grimaldi *et al*. (2010) reported an association of another *IL-1B* polymorphism with delayed age of onset in AD patients. Polymorphisms of other genes encoding inflammatory cytokines, such as IL-6 and IL-α, have been associated with a variable age at onset of AD (PAPASSOTIROPOULOS *et al*., 1999). These findings suggest that it is possible that genetic factors that regulate the immune response alter the course of AD. Hence, pharmacological control of cytokine-mediated chronic neuroinflammation in the initial stages of the disease might prove effective in controlling neurodegeneration and clinical progression of the disease.

When analysing all the SNPs together, *IL-1B* C/C genotype was found to be associated with higher CDR values, meaning individuals carrying two copies of the C allele showed greater disease severity than those with one or no copies of this allele.

IL-1β release from microglia has been shown to regulate activity and expression of β- and γ-secretases (SASTRE *et al*., 2008). One *IL-1B* polymorphism has already been associated with fourfold increase in production of IL-1β (NICOLL *et al*., 2000). It is possible that this polymorphism's genotype has a similar effect and is associated with up-regulation of *IL-1B* gene, resulting in higher production of IL-1β. This, in turn, may lead to greater β- and γ-secretases activity and expression, and consequently higher production of APP and deposition of Aβ, and microglia activation, which can ultimately result in greater severity of AD.

Our study shows that rs1143643 of the *IL-1B* is associated with age of onset, and severity of AD in a Brazilian population.

rs613430 of the *COL4A1* gene was also associated with CDR values. In a codominant genetic model, individuals carrying two copies of the G allele had a significantly higher disease severity than those with one or no copies of the allele.

Mutations in the *COL4A1* gene are associated with stress-induced haemorrhage, adult-onset stroke in humans and mice, and CMBs (GEORGE *et al.*, 1993; POSCHL *et al.*, 2004; GOULD *et al.*, 2006; ALAMOWITCH *et al.*, 2009). In recent years, CMBs have obtained much attention as an important cause of dementia and have been associated with MCI and AD (PETTERSEN *et al*., 2008). There is growing evidence that CMBs may confer increased risk of cognitive impairment, and future cognitive decline (UITERWIJK *et al*., 2014). Increased levels of cerebral Aβ have also been associated with CMBs (YATES *et al*., 2011). Rosidi *et al.* (2011) proposed that CMBs lead to a sustained local inflammatory response, characterized by initial

activation and persistent increase in microglia and macrophages, and that this inflammatory response leads to neuronal dysfunction and cell death. Additionally, Shoamanesh *et al.* (2015) demonstrated that CMBs patients showed high levels of various circulating markers of inflammation, such as the regulatory cytokine TNF-α.

Our results are consistent with these findings and suggest that the *COL4A1* polymorphism is associated with stronger disease severity in AD patients, resulting in greater cognitive impairment. Nevertheless, we believe this to be the first work that shows a direct association between a *COL4A1* gene polymorphism and severity of LOAD.

Our results show that gender is a significant factor for AD susceptibility and age of onset, but that might be due to the considerable difference between the number of woman and men in our sample.

In order to evaluate the clinical utility for both prognostic and diagnostic models, the ROC curve is typically employed and the AUC is used to measure the discrimination power of a classifier. It is assumed that classifiers with an AUC significantly greater than 0.5 have at least some ability to discriminate between cases and controls, however a more conservative approach is used for screening of individuals with an increased risk of disease, where is suggested that the AUC be 0.75, and for presymptomatic diagnosis of the general population, the AUC should be 0.99 (JANSSENS *et al.*, 2007). In our study, a classification model for the age of onset based on the 11 SNPs reached an AUC of 0.7379. An AUC of this magnitude suggests that this model has a fair discrimination power. The remaining variables showed an AUC under 0.7, which suggest poor discrimination power.

Our findings provide further evidence for a role of inflammation as a driving force in AD pathogenesis. The association between inflammation-related gene polymorphisms and AD susceptibility and cognitive variables further suggests that genetically determined alterations of the immune response can indeed alter the course of this disease. Moreover, this was the first study to suggest a direct association between *TLR6*, *NLRP10*, and *COL4A1* polymorphisms with susceptibility to AD, the age of onset, and disease severity.

More than potentially aiding the differential diagnosis of patients with dementia and assessing the degree of risk for the development of dementia, identification of genetic associations should foster the emphasis on the pursuit of anti-inflammatory therapies for the protection against and treatment of AD.

8 CONCLUSIONS

In conclusion, rs6531669 (*TLR6*) G/G genotype was associated with lower risk of developing AD and with lower MMSE results, and the T/T genotype was associated with lower disease severity. rs13105517 (*TLR2*) A/A genotype was associated with higher risk of developing AD, and with lower MMSE results. rs9919613 (*NLRP10*) C/C genotype was associated with lower risk of developing AD, and with lower disease severity. The same genotype was also associated with early age of onset. rs1143643 (*Il-1B*) T/T genotype was associated with late age of onset, and the C/C genotype was associated with greater disease severity. rs613430 (*COL4A1*) G/G genotype was associated with greater disease severity. Figure 18 summarises the associations found in this study.

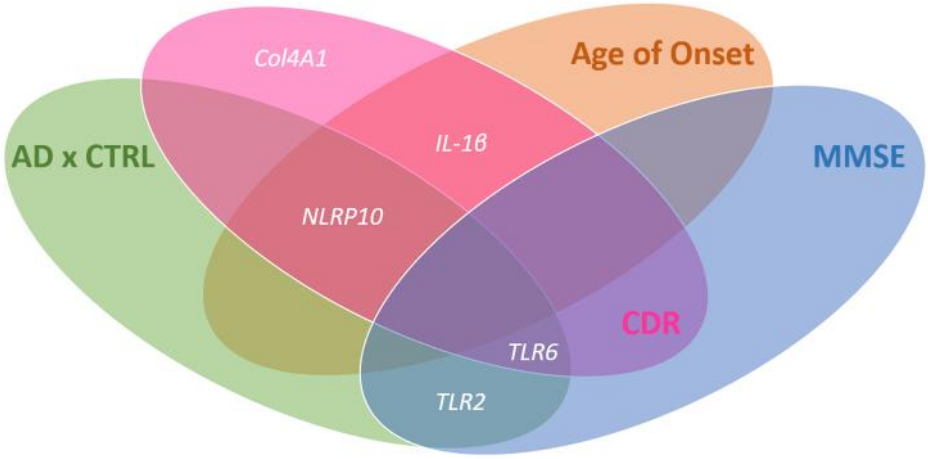


FIGURE 18 – GRAPHICAL RESUME OF ASSOCIATIONS FOUND IN THE STUDY.
SOURCE: the author.
NOTE: *TLR6* gene polymorphism was found to be associated with AD susceptibility, age of onset, and MMSE; *TLR2* gene polymorphism was found to be associated with AD susceptibility, and MMSE; *NLRP10* gene polymorphism was found to be associated with AD susceptibility, age of onset, and disease severity; *Il-1B* gene polymorphism was found to be associated with age of onset, and disease severity; *COL4A1* gene polymorphism was found to be associated with disease severity.

REFERENCES

AIZENSTEIN, H.J., NEBES, R.D., SAXTON, J.A., PRICE, J.C., MATHIS, C.A., TSOPELAS, N.D. *et al.* Frequent amyloid deposition without significant cognitive impairment among the elderly. **Archives of neurology**, v. 65, n. 11, p. 1509-1517, 2008.

ALAMOWITCH, S., PLAISIER, E., FAVROLE, P., PROST, C., CHEN, Z., VAN AGTMAEL, T.*, et al.* Cerebrovascular disease related to COL4A1 mutations in HANAC syndrome. **Neurology**, v. 73, n. 22, p. 1873-1882, 2009.

ASAI, H., IKEZU, S., TSUNODA, S., MEDALLA, M., LUEBKE, J., HAYDAR, T.*, et al.* Depletion of microglia and inhibition of exosome synthesis halt tau propagation. **Nature neuroscience**, v. 18, n. 11, p. 1584-1593, 2015.

BARRETT, J.C., FRY, B., MALLER, J. and DALY, M.J. Haploview: analysis and visualization of LD and haplotype maps. **Bioinformatics**, v. 21, n. 2, p. 263-265, 2004.

BATEMAN, R.J., AISEN, P.S., DE STROOPER, B., FOX, N.C., LEMERE, C.A., RINGMAN, J.M.*, et al.* Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease. **Alzheimer's research & therapy**, v. 3, n. 1, p. 1, 2011.

BATH, P.M. and WARDLAW, J.M. Pharmacological treatment and prevention of cerebral small vessel disease: a review of potential interventions. **International Journal of Stroke**, v. 10, n. 4, p. 469-478, 2015.

BEERI, M.S., RAPP, M., SILVERMAN, J., SCHMEIDLER, J., GROSSMAN, H., FALLON, J.*, et al.* Coronary artery disease is associated with Alzheimer disease neuropathology in APOE4 carriers. **Neurology**, v. 66, n. 9, p. 1399-1404, 2006.

BLASKO, I., VEERHUIS, R., STAMPFER-KOUNTCHEV, M., SAURWEIN-TEISSL, M., EIKELENBOOM, P. & GRUBECK-LOEBENSTEIN, B. Costimulatory effects of interferon-γ and interleukin-1β or tumor necrosis factor α on the synthesis of Aβ1-40 and Aβ1-42 by human astrocytes. **Neurobiology of disease**, v. 7, n. 6, p. 682-689, 2000.

BONIFATI, D.M. and KISHORE, U. Role of complement in neurodegeneration and neuroinflammation. **Molecular immunology**, v. 44, n. 5, p. 999-1010, 2007.

BOSSÙ, P., CIARAMELLA, A., MORO, M.L., BELLINCAMPI, L., BERNARDINI, S., FEDERICI, G. *et al*. Interleukin 18 gene polymorphisms predict risk and outcome of Alzheimer's disease. **Journal of Neurology, Neurosurgery & Psychiatry**, v. 78, n. 8, p. 807-811, 2007.

BROOKMEYER, R., JOHNSON, E., ZIEGLER-GRAHAM, K. and ARRIGHI, H.M. Forecasting the global burden of Alzheimer's disease. **Alzheimer's & dementia**, v. 3, n. 3, p. 186-191, 2007.

BRADSHAW, E.M., CHIBNIK, L.B., KEENAN, B.T., OTTOBONI, L., RAJ, T., TANG, A. *et al*. CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. **Nature neuroscience**, v. 16, n. 7, p. 848-850, 2013.

BROSSERON, F., KRAUTHAUSEN, M., KUMMER, M. and HENEKA, M.T. Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. **Molecular neurobiology**, v. 50, n. 2, p. 534-544, 2014.

BROZ, P. and DIXIT, V.M. Inflammasomes: mechanism of assembly, regulation and signalling. **Nature Reviews Immunology**, v. 16, n. 7, p. 407-420, 2016.

BSIBSI, M., RAVID, R., GVERIC, D. and VAN NOORT, J.M. Broad expression of Toll-like receptors in the human central nervous system. **Journal of Neuropathology & Experimental Neurology**, v. 61, n. 11, p. 1013-1021, 2002.

CAMPION, D., POTTIER, C., NICOLAS, G., LE GUENNEC, K. and ROVELET-LECRUX, A. Alzheimer disease: modeling an Aβ-centered biological network. **Molecular psychiatry**, v. 21, p. 861-871, 2016.

CHIANG, K. and KOO, E.H. Emerging therapeutics for Alzheimer's disease. **Annual review of pharmacology and toxicology**, v. 54, p. 381-405, 2014.

COUTURIER, J., STANCU, I.-C., SCHAKMAN, O., PIERROT, N., HUAUX, F., KIENLEN-CAMPARD, P. *et al.* Activation of phagocytic activity in astrocytes by reduced expression of the inflammasome component ASC and its implication in a mouse model of Alzheimer disease. **Journal of neuroinflammation**, v. 13, n. 1, p. 1, 2016.

CRAFT, J.M., WATTERSON, D.M., HIRSCH, E. and VAN ELDIK, L.J. Interleukin 1 receptor antagonist knockout mice show enhanced microglial activation and neuronal damage induced by intracerebroventricular infusion of human β-amyloid. **Journal of neuroinflammation**, v. 2, n. 1, p. 1, 2005.

DAVIS, B.K., WEN, H. and TING, J.P. The inflammasome NLRs in immunity, inflammation, and associated diseases. **Annual review of immunology**, v. 29, p. 707-735, 2011.

DE BRUIJN, R.F. and IKRAM, M.A. Cardiovascular risk factors and future risk of Alzheimer's disease. **BMC medicine**, v. 12, n. 1, p. 130, 2014.

FALSIG, J., VAN BEEK, J., HERMANN, C. and LEIST, M. Molecular basis for detection of invading pathogens in the brain. **Journal of neuroscience research**, v. 86, n. 7, p. 1434-1447, 2008.

FARHAT, K., RIEKENBERG, S., HEINE, H., DEBARRY, J., LANG, R., MAGES, J.*, et al.* Heterodimerization of TLR2 with TLR1 or TLR6 expands the ligand spectrum but does not lead to differential signaling. **Journal of leukocyte biology**, v. 83, n. 3, p. 692-701, 2008.

FOLCH, J., ETTCHETO, M., PETROV, D., ABAD, S., PEDRÓS, I., MARIN, M.*, et al.* Review of the advances in treatment for Alzheimer disease: Strategies for combating β-amyloid protein. **Neurologia (English Edition)**, 2017.

FRANCIS, P.T., NORDBERG, A. and ARNOLD, S.E. A preclinical view of cholinesterase inhibitors in neuroprotection: do they provide more than symptomatic benefits in Alzheimer's disease? **Trends in pharmacological sciences**, v. 26, n. 2, p. 104-111, 2005.

FREEMAN, L.C. and TING, J.P.Y. The pathogenic role of the inflammasome in neurodegenerative diseases. **Journal of neurochemistry**, v. 136, n. S1, p. 29-38, 2016.

FRIEDLANDER, R.M., GAGLIARDINI, V., HARA, H., FINK, K.B., LI, W., MACDONALD, G. *et al*. Expression of a dominant negative mutant of interleukin-1β converting enzyme in transgenic mice prevents neuronal cell death induced by trophic factor withdrawal and ischemic brain injury. **The Journal of experimental medicine**, v. 185, n. 5, p. 933-940, 1997.

GABRIEL, S., ZIAUGRA, L. and TABBAA, D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. **Current protocols in human genetics**, p. 2.12. 11-12.12. 16, 2009.

GENECARDS: HUMAN GENE DATABASE (GENECARDS). 2017. Available at: <http://www.genecards.org>. Access in: 05/09/2017.

GEORGE, E.L., GEORGES-LABOUESSE, E.N., PATEL-KING, R.S., RAYBURN, H. and HYNES, R.O. Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. **Development**, v. 119, n. 4, p. 1079-1091, 1993.

GOATE, A., CHARTIER-HARLIN, M.-C., MULLAN, M., BROWN, J., CRAWFORD, F., FIDANI, L. *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. **Nature**, v. 349, n. 6311, p. 704-706, 1991.

GOLD, M. and EL KHOURY, J. β-amyloid, microglia, and the inflammasome in Alzheimer's disease. β-amyloid, microglia, and the inflammasome in Alzheimer's disease. **Seminars in Immunopathology**, v. 37, n. 6, p. 607-611, 2015.

GOULD, D.B., PHALAN, F.C., BREEDVELD, G.J., VAN MIL, S.E., SMITH, R.S., SCHIMENTI, J.C.*, et al.* Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. **Science**, v. 308, n. 5725, p. 1167-1171, 2005.

GOULD, D.B., PHALAN, F.C., VAN MIL, S.E., SUNDBERG, J.P., VAHEDI, K., MASSIN, P.*, et al.* Role of COL4A1 in small-vessel disease and hemorrhagic stroke. **New England Journal of Medicine**, v. 354, n. 14, p. 1489-1496, 2006.

GRIFFIN, W.S.T., LIU, L., LI, Y., MRAK, R.E. and BARGER, S.W. Interleukin-1 mediates Alzheimer and Lewy body pathologies. **Journal of neuroinflammation**, v. 3, n. 1, p. 5, 2006.

GRIMALDI, L.M., CASADEI, V.M., FERRI, C., VEGLIA, F., LICASTRO, F., ANNONI, G.*, et al.* Association of Early-Onset Alzheimer's Disease with an Interieukin-1 x Gene Polymorphism. **Annals of neurology**, v. 47, n. 3, p. 361-364, 2000.

GUERREIRO, R., WOJTAS, A., BRAS, J., CARRASQUILLO, M., ROGAEVA, E., MAJOUNIE, E. *et al.* TREM2 variants in Alzheimer's disease. **New England Journal of Medicine**, v. 368, n. 2, p. 117-127, 2013.

HALLE, A., HORNUNG, V., PETZOLD, G.C., STEWART, C.R., MONKS, B.G., REINHECKEL, T. et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-β. **Nature immunology**, v. 9, n. 8, p. 857-865, 2008.

HAMANN, L., KOCH, A., SUR, S., HOEFER, N., GLAESER, C., SCHULZ, S.*, et al.* Association of a common TLR-6 polymorphism with coronary artery disease–implications for healthy ageing? **Immunity & Ageing**, v. 10, n. 1, p. 43, 2013.

HANKE, M.L. and KIELIAN, T. Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential. **Clinical Science**, v. 121, n. 9, p. 367-387, 2011.

HE, Y., ZENG, M.Y., YANG, D., MOTRO, B. and NÚÑEZ, G. NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. **Nature**, v. 530, n. 7590, p. 354-357, 2016.

HELZNER, E.P., LUCHSINGER, J.A., SCARMEAS, N., COSENTINO, S., BRICKMAN, A.M., GLYMOUR, M.M. et al. Contribution of vascular risk factors to the progression in Alzheimer disease. **Archives of neurology**, v. 66, n. 3, p. 343-348, 2009.

HENEKA, M.T. Inflammasome activation and innate immunity in Alzheimer's disease. **Brain Pathology**, v. 27, n. 2, p. 220-222, 2017.

HENEKA, M.T., GOLENBOCK, D.T. and LATZ, E. Innate immunity in Alzheimer's disease. **Nature immunology**, v. 16, n. 3, p. 229-236, 2015.

HENEKA, M.T., KUMMER, M.P. and LATZ, E. Innate immune activation in neurodegenerative disease. **Nature Reviews Immunology**, v. 14, n. 7, p. 463-477, 2014.

HENEKA, M.T., KUMMER, M.P., STUTZ, A., DELEKATE, A., SCHWARTZ, S., VIEIRA-SAECKER, A. *et al.* NLRP3 is activated in Alzheimer/'s disease and contributes to pathology in APP/PS1 mice. **Nature**, v. 493, n. 7434, p. 674-678, 2013.

HENRY, W., QUERFURTH, H. and LAFERLA, F. Mechanisms of disease Alzheimer's disease. **New Engl J Med**, v. 362, p. 329-344, 2010.

HEPPNER, F.L., RANSOHOFF, R.M. and BECHER, B. Immune attack: the role of inflammation in Alzheimer disease. **Nature Reviews Neuroscience**, v. 16, n. 6, p. 358-372, 2015.

HERX, L.M. and YONG, V.W. Interleukin-1β is required for the early evolution of reactive astrogliosis following CNS lesion. **Journal of Neuropathology & Experimental Neurology**, v. 60, n. 10, p. 961-971, 2001.

HICKMAN, S.E. and El KHOURY, J. TREM2 and the neuroimmunology of Alzheimer's disease. **Biochemical pharmacology**, v. 88, n. 4, p. 495-498, 2014.

HOLLINGWORTH, P., HAROLD, D., SIMS, R., GERRISH, A., LAMBERT, J.-C., CARRASQUILLO, M.M. *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, 2011.

HOLMES, C., BOCHE, D., WILKINSON, D., YADEGARFAR, G., HOPKINS, V., BAYER, A. *et al.* Long-term effects of Aβ 42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. **The Lancet**, v. 372, n. 9634, p. 216-223, 2008.

HUANG, Y. and MUCKE, L. Alzheimer mechanisms and therapeutic strategies. **Cell**, v. 148, n. 6, p. 1204-1222, 2012.

IN'T VELD, B.A., RUITENBERG, A., HOFMAN, A., LAUNER, L.J., VAN DUIJN, C.M., STIJNEN, T. *et al.* Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. **New England Journal of Medicine**, v. 345, n. 21, p. 1515-1521, 2001.

IKRAM, M.K., HILAL, S., CATINDING, J., TING, E., LEE, A., QIU, A.*, et al.* Cerebral microbleeds and cognitive impairment in community-dwelling elderly from Singapore. **Alzheimer's & Dementia: The Journal of the Alzheimer's Association**, v. 8, n. 4, p. P618, 2012.

JACK, C.R., KNOPMAN, D.S., JAGUST, W.J., PETERSEN, R.C., WEINER, M.W., AISEN, P.S. *et al.* Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. **The Lancet Neurology**, v. 12, n. 2, p. 207-216, 2013.

JANA, M., PALENCIA, C.A. and PAHAN, K. Fibrillar amyloid-β peptides activate microglia via TLR2: implications for Alzheimer's disease. **The Journal of Immunology**, v. 181, n. 10, p. 7254-7262, 2008.

JANSSENS, A.C.J., MOONESINGHE, R., YANG, Q., STEYERBERG, E.W., VAN DUIJN, C.M. and KHOURY, M.J. The impact of genotype frequencies on the clinical validity of genomic profiling for predicting common chronic diseases. **Genetics in Medicine**, v. 9, n. 8, p. 528-535, 2007.

JOHNSTON, H., BOUTIN, H. and ALLAN, S.M. Assessing the contribution of inflammation in models of Alzheimer's disease. **Biochemical Society Transactions**, v. 39, n. 4, p. 886-890, 2011.

JONSSON, T., STEFANSSON, H., STEINBERG, S., JONSDOTTIR, I., JONSSON, P.V., SNAEDAL, J. *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, 2013.

KADUSZKIEWICZ, H., ZIMMERMANN, T., BECK-BORNHOLDT, H.-P. and VAN DEN BUSSCHE, H. Cholinesterase inhibitors for patients with Alzheimer's disease: systematic review of randomised clinical trials. **Bmj**, v. 331, n. 7512, p. 321-327, 2005.

KITAZAWA, M., CHENG, D., TSUKAMOTO, M.R., KOIKE, M.A., WES, P.D., VASILEVKO, V. *et al*. Blocking IL-1 signaling rescues cognition, attenuates tau pathology, and restores neuronal β-catenin pathway function in an Alzheimer's disease model. **The Journal of Immunology**, v. 187, n. 12, p. 6539-6549, 2011.

KUNDU, S., AULCHENKO, Y.S., VAN DUIJN, C.M. and JANSSENS, A.C.J. PredictABEL: an R package for the assessment of risk prediction models. **European journal of epidemiology**, v. 26, n. 4, p. 261, 2011.

LAHIRI, D.K. and NURNBERGER JR, J.I. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. **Nucleic acids research**, v. 19, n. 19, p. 5444, 1991.

LAMKANFI, M. and DIXIT, V.M. Mechanisms and functions of inflammasomes. **Cell**, v. 157, n. 5, p. 1013-1022, 2014.

LEVY-LAHAD, E., WASCO, W., POORKAJ, P. and ROMANO, D.M. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. **Science**, v. 269, n. 5226, p. 973, 1995.

LI, C., ZHAO, R., GAO, K., WEI, Z., YAOYAO YIN, M., TING LAU, L. *et al.* Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. **Current Alzheimer Research**, v. 8, n. 1, p. 67-80, 2011.

LI, J., WANG, Y., ZHANG, M., XU, Z., GAO, C., FANG, C. et al. Vascular risk factors promote conversion from mild cognitive impairment to Alzheimer disease. **Neurology**, v. 76, n. 17, p. 1485-1491, 2011.

LIN, W., DING, M., XUE, J. and LENG, W. The role of TLR2/JNK/NF-κB pathway in amyloid β peptide-induced inflammatory response in mouse NG108-15 neural cells. **International immunopharmacology**, v. 17, n. 3, p. 880-884, 2013.

LIO, D., SCOLA, L., ROMANO, G.C., CANDORE, G. and CARUSO, C. Immunological and immunogenetic markers in sporadic Alzheimer's disease. **Aging clinical and experimental research**, v. 18, n. 2, p. 163-166, 2006.

LIU, L. and CHAN, C. The role of inflammasome in Alzheimer's disease. **Ageing research reviews**, v. 15, p. 6-15, 2014.

LIU, S., LIU, Y., HAO, W., WOLF, L., KILIAAN, A.J., PENKE, B. *et al.* TLR2 is a primary receptor for Alzheimer's amyloid β peptide to trigger neuroinflammatory activation. **The Journal of Immunology**, v. 188, n. 3, p. 1098-1107, 2012.

LOVE, S. and MINERS, J.S. Cerebrovascular disease in ageing and Alzheimer's disease. **Acta neuropathologica**, v. 131, n. 5, p. 645-658, 2016.

LUCHSINGER, J., REITZ, C., HONIG, L.S., TANG, M.-X., SHEA, S. and MAYEUX, R. Aggregation of vascular risk factors and risk of incident Alzheimer disease. **Neurology**, v. 65, n. 4, p. 545-551, 2005.

LUE, L.F., RYDEL, R., BRIGHAM, E.F., YANG, L.B., HAMPEL, H., MURPHY, G.M.*, et al.* Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. **Glia**, v. 35, n. 1, p. 72-79, 2001.

LUO, L., LI, K. and WANG, X. Relationship between the IL-18 gene polymorphisms and Alzheimer's disease: a meta-analysis. **International journal of clinical and experimental medicine**, v. 9, n. 11, p. 22720-22728, 2016.
MARCHESI, V.T. Gain-of-function somatic mutations contribute to inflammation and blood vessel damage that lead to Alzheimer dementia: a hypothesis. **The FASEB Journal**, v. 30, n. 2, p. 503-506, 2016.

MCDONALD, C.L., HENNESSY, E., RUBIO-ARAIZ, A., KEOGH, B., MCCORMACK, W., MCGUIRK, P. *et al.* Inhibiting TLR2 activation attenuates amyloid accumulation and glial activation in a mouse model of Alzheimer's disease. **Brain, Behavior, and Immunity**, 2016.

MINORETTI, P., GAZZARUSO, C., DI VITO, C., EMANUELE, E., BIANCHI, M., COEN, E.*, et al.* Effect of the functional toll-like receptor 4 Asp299Gly polymorphism on susceptibility to late-onset Alzheimer's disease. **Neuroscience letters**, v. 391, n. 3, p. 147-149, 2006.

MINTER, M.R., TAYLOR, J.M. and CRACK, P.J. The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. **Journal of neurochemistry**, v. 136, n. 3, p. 457-474, 2016.

MOTTA, M., IMBESI, R., DI ROSA, M., STIVALA, F. and MALAGUARNERA, L. Altered plasma cytokine levels in Alzheimer's disease: correlation with the disease progression. **Immunology letters**, v. 114, n. 1, p. 46-51, 2007.

MULLANE, K. and WILLIAMS, M. Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis—but what lies beyond? **Biochemical pharmacology**, v. 85, n. 3, p. 289-305, 2013.

MURPHY, G.M., YANG, L. and CORDELL, B. Macrophage colony-stimulating factor augments β-amyloid-induced interleukin-1, interleukin-6, and nitric oxide production by microglial cells. **Journal of Biological Chemistry**, v. 273, n. 33, p. 20967-20971, 1998.

MURPHY, N., GREHAN, B. and LYNCH, M.A. Glial uptake of amyloid beta induces NLRP3 inflammasome formation via cathepsin-dependent degradation of NLRP10. **Neuromolecular medicine**, v. 16, n. 1, p. 205-215, 2014.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION (NCBI). 2017. Available at: < https://www.ncbi.nlm.nih.gov>. Access in: 05/09/2017.

NICOLL, J.A., MRAK, R.E., GRAHAM, D.I., STEWART, J., WILCOCK, G., MACGOWAN, S.*, et al.* Association of interleukin-1 gene polymorphisms with Alzheimer's disease. **Annals of neurology**, v. 47, n. 3, p. 365, 2000.

OJALA, J., ALAFUZOFF, I., HERUKKA, S.-K., VAN GROEN, T., TANILA, H. and PIRTTILÄ, T. Expression of interleukin-18 is increased in the brains of Alzheimer's disease patients. **Neurobiology of aging**, v. 30, n. 2, p. 198-209, 2009.

OJALA, J.O., SUTINEN, E.M., SALMINEN, A. and PIRTTILÄ, T. Interleukin-18 increases expression of kinases involved in tau phosphorylation in SH-SY5Y neuroblastoma cells. **Journal of neuroimmunology**, v. 205, n. 1, p. 86-93, 2008.

PAPASSOTIROPOULOS, A., BAGLI, M., JESSEN, F., BAYER, T.A., MAIER, W., RAO, M.L.*, et al.* A genetic variation of the inflammatory cytokine interleukin-6 delays the initial onset and reduces the risk for sporadic Alzheimer's disease. **Annals of neurology**, v. 45, n. 5, p. 666-668, 1999.

PERRIN, R.J., FAGAN, A.M. and HOLTZMAN, D.M. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. **Nature**, v. 461, n. 7266, p. 916-922, 2009.

PERRY, V.H. and HOLMES, C. Microglial priming in neurodegenerative disease. **Nature reviews. Neurology**, v. 10, n. 4, p. 217, 2014.

PETTERSEN, J.A., SATHIYAMOORTHY, G., GAO, F.-Q., SZILAGYI, G., NADKARNI, N.K., ST GEORGE-HYSLOP, P.*, et al.* Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. **Archives of neurology**, v. 65, n. 6, p. 790-795, 2008.

POELS, M.M., IKRAM, M.A., VAN DER LUGT, A., HOFMAN, A., NIESSEN, W.J., KRESTIN, G.P.*, et al.* Cerebral microbleeds are associated with worse cognitive function The Rotterdam Scan Study. **Neurology**, v. 78, n. 5, p. 326-333, 2012.

PÖSCHL, E., SCHLÖTZER-SCHREHARDT, U., BRACHVOGEL, B., SAITO, K., NINOMIYA, Y. and MAYER, U. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. **Development**, v. 131, n. 7, p. 1619-1628, 2004.

RAINA, P., SANTAGUIDA, P., ISMAILA, A., PATTERSON, C., COWAN, D., LEVINE, M.*, et al.* Effectiveness of Cholinesterase Inhibitors and Memantine for Treating Dementia: Evidence Review for a Clinical Practice GuidelineCholinesterase Inhibitors and Memantine for Treating Dementia. **Annals of internal medicine**, v. 148, n. 5, p. 379-397, 2008.

RAMOS, E.M., LIN, M.-T., LARSON, E.B., MAEZAWA, I., TSENG, L.-H., EDWARDS, K.L.*, et al.* Tumor necrosis factor α and interleukin 10 promoter region polymorphisms and risk of late-onset Alzheimer disease. **Archives of neurology**, v. 63, n. 8, p. 1165-1169, 2006.

RAVARI, A., MIRZAEI, T., KENNEDY, D. and ARABABADI, M.K. Chronoinflammaging in Alzheimer; A systematic review on the roles of toll like receptor 2. **Life sciences**, v. 171, p. 16-20, 2017.

REED-GEAGHAN, E.G., SAVAGE, J.C., HISE, A.G. and LANDRETH, G.E. CD14 and toll-like receptors 2 and 4 are required for fibrillar Aβ-stimulated microglial activation. **Journal of Neuroscience**, v. 29, n. 38, p. 11982-11992, 2009.

RICHARD, K.L., FILALI, M., PRÉFONTAINE, P. and RIVEST, S. Toll-like receptor 2 acts as a natural innate immune receptor to clear amyloid β1–42 and delay the cognitive decline in a mouse model of Alzheimer's disease. **Journal of Neuroscience**, v. 28, n. 22, p. 5784-5793, 2008.

ROBIN, X., TURCK, N., HAINARD, A., TIBERTI, N., LISACEK, F., SANCHEZ, J.-C.*, et al.* pROC: an open-source package for R and S+ to analyze and compare ROC curves. **BMC bioinformatics**, v. 12, n. 1, p. 77, 2011.

ROGAEV, E., SHERRINGTON, R., ROGAEVA, E., LEVESQUE, G., IKEDA, M., LIANG, Y. *et al.* Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. **Nature**, v. 376, n. 6543, p. 775-778, 1995.

ROSIDI, N.L., ZHOU, J., PATTANAIK, S., WANG, P., JIN, W., BROPHY, M.*, et al.* Cortical microhemorrhages cause local inflammation but do not trigger widespread dendrite degeneration. **PLoS One**, v. 6, n. 10, p. e26612, 2011.

ROSSI, F. and BIANCHINI, E. Synergistic induction of nitric oxide by β-amyloid and cytokines in astrocytes. **Biochemical and biophysical research communications**, v. 225, n. 2, p. 474-478, 1996.

RUBIO-PEREZ, J.M. and MORILLAS-RUIZ, J.M. A review: inflammatory process in Alzheimer's disease, role of cytokines. **The Scientific World Journal**, v. 2012, Article ID 756357, 15 pages, 2012.

SALMINEN, A., OJALA, J., SUURONEN, T., KAARNIRANTA, K. and KAUPPINEN, A. Amyloid-β oligomers set fire to inflammasomes and induce Alzheimer's pathology. **Journal of cellular and molecular medicine**, v. 12, n. 6a, p. 2255-2262, 2008.

SARESELLA, M., LA ROSA, F., PIANCONE, F., ZOPPIS, M., MARVENTANO, I., CALABRESE, E. *et al.* The NLRP3 and NLRP1 inflammasomes are activated in Alzheimer's disease. **Molecular neurodegeneration**, v. 11, n. 1, p. 1, 2016.

SASTRE, M., DEWACHTER, I., LANDRETH, G.E., WILLSON, T.M., KLOCKGETHER, T., VAN LEUVEN, F. *et al*. Nonsteroidal anti-inflammatory drugs and peroxisome proliferator-activated receptor-γ agonists modulate immunostimulated processing of amyloid precursor protein through regulation of β-secretase. **The Journal of neuroscience**, v. 23, n. 30, p. 9796-9804, 2003.

SASTRE, M., WALTER, J. and GENTLEMAN, S.M. Interactions between APP secretases and inflammatory mediators. **Journal of neuroinflammation**, v. 5, n. 1, p. 25, 2008.

SAUNDERS, A.M., STRITTMATTER, W.J., SCHMECHEL, D., GEORGE-HYSLOP, P.S., PERICAK-VANCE, M., JOO, S.*, et al.* Association of apolipoprotein E allele ϵ4 with late-onset familial and sporadic Alzheimer's disease. **Neurology**, v. 43, n. 8, p. 1467-1467, 1993.

SCHMID-BURGK, J.L., CHAUHAN, D., SCHMIDT, T., EBERT, T.S., REINHARDT, J., ENDL, E. *et al*. A genome-wide CRISPR (clustered regularly interspaced short palindromic repeats) screen identifies NEK7 as an essential component of NLRP3 inflammasome activation. **Journal of Biological Chemistry**, v. 291, n. 1, p. 103-109, 2016.

SEVIGNY, J., CHIAO, P., BUSSIÈRE, T., WEINREB, P.H., WILLIAMS, L., MAIER, M. *et al*. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. **Nature**, v. 537, n. 7618, p. 50-56, 2016.

SHAW, P.J., MCDERMOTT, M.F. and KANNEGANTI, T.-D. Inflammasomes and autoimmunity. **Trends in molecular medicine**, v. 17, n. 2, p. 57-64, 2011.

SHEEDY, F.J., GREBE, A., RAYNER, K.J., KALANTARI, P., RAMKHELAWON, B., CARPENTER, S.B. *et al.* CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. **Nature immunology**, v. 14, n. 8, p. 812-820, 2013.

SHI, H., WANG, Y., LI, X., ZHAN, X., TANG, M., FINA, M. *et al*. NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. **Nature immunology**, v. 17, n. 3, p. 250-258, 2015.

SHICHITA, T. and YOSHIMURA, A. Inflammation in central nervous system diseases. **Clinical and Experimental Neuroimmunology**, v. 7, n. 1, p. 18-27, 2016.

SHOAMANESH, A., PREIS, S.R., BEISER, A.S., VASAN, R.S., BENJAMIN, E.J., KASE, C.S.*, et al.* Inflammatory biomarkers, cerebral microbleeds, and small vessel disease Framingham Heart Study. **Neurology**, v. 84, n. 8, p. 825-832, 2015.

SIBON, I., COUPRY, I., MENEGON, P., BOUCHET, J.P., GORRY, P., BURGELIN, I., et al. COL4A1 mutation in Axenfeld–Rieger anomaly with leukoencephalopathy and stroke. Annals of neurology, v. 62, n. 2, p. 177-184, 2007.

SOHRABIFAR, N., GHARESOURAN, J., TALEBI, M., GHOJAZADEH, M. and ARDEBILI, S.M.M. Association of CLU and TLR2 gene polymorphisms with late-onsetAlzheimer disease in a northwestern Iranian population. **Turkish journal of medical sciences**, v. 45, n. 5, p. 1082-1086, 2015.

STEWART, C.R., STUART, L.M., WILKINSON, K., VAN GILS, J.M., DENG, J., HALLE, A.*, et al.* CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. **Nature immunology**, v. 11, n. 2, p. 155-161, 2010.

SUTINEN, E.M., PIRTTILÄˆ, T., ANDERSON, G., SALMINEN, A. and OJALA, J.O. Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-beta production in human neuron-like cells. **J Neuroinflammation**, v. 9, n. 199.10, p. 1186, 2012.

SZCZEPANIK, A.M., FUNES, S., PETKO, W. and RINGHEIM, G.E. IL-4, IL-10 and IL-13 modulate Aβ (1–42)-induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line. **Journal of neuroimmunology**, v. 113, n. 1, p. 49-62, 2001.

TAN, M.-S., YU, J.-T., JIANG, T., ZHU, X.-C., WANG, H.-F., ZHANG, W. et al. NLRP3 polymorphisms are associated with late-onset Alzheimer's disease in Han Chinese. **Journal of neuroimmunology**, v. 265, n. 1, p. 91-95, 2013.

TANZI, R.E. A genetic dichotomy model for the inheritance of Alzheimer's disease and common age-related disorders. **The Journal of clinical investigation**, v. 104, n. 9, p. 1175-1179, 1999.

TARKOWSKI, E., ANDREASEN, N., TARKOWSKI, A. and BLENNOW, K. Intrathecal inflammation precedes development of Alzheimer's disease. **Journal of Neurology, Neurosurgery & Psychiatry**, v. 74, n. 9, p. 1200-1205, 2003.

THE GLOBAL VOICE ON DEMENTIA. Dementia statistics. 2016. Available at: <https://www.alz.co.uk/research/statistics >. Access in: 10/09/2016.

UITERWIJK, R., HUIJTS, M., STAALS, J., DUITS, A., GRONENSCHILD, E., KROON, A.A.*, et al.* Subjective Cognitive Failures in Patients With Hypertension Are Related to Cognitive Performance and Cerebral MicrobleedsNovelty and Significance. **Hypertension**, v. 64, n. 3, p. 653-657, 2014.

VAN HAUWERMEIREN, F. and LAMKANFI, M. The NEK-sus of the NLRP3 inflammasome. **Nature immunology**, v. 17, n. 3, p. 223-224, 2016.

VENEGAS, C. and HENEKA, M.T. Danger-associated molecular patterns in Alzheimer's disease. **Journal of leukocyte biology**, v. 101, n. 1, p. 87-98, 2017.

VON MOLTKE, J., AYRES, J.S., KOFOED, E.M., CHAVARRÍA-SMITH, J. and VANCE, R.E. Recognition of bacteria by inflammasomes. **Annual review of immunology**, v. 31, p. 73-106, 2013.

WALKER, D.G., LINK, J., LUE, L.-F., DALSING-HERNANDEZ, J.E. and BOYES, B.E. Gene expression changes by amyloid β peptide-stimulated human postmortem brain microglia identify activation of multiple inflammatory processes. **Journal of leukocyte biology**, v. 79, n. 3, p. 596-610, 2006.

WALTER, S., LETIEMBRE, M., LIU, Y., HEINE, H., PENKE, B., HAO, W.*, et al.* Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease. **Cellular Physiology and Biochemistry**, v. 20, n. 6, p. 947-956, 2007.

WAN, Y., WANG, G. and CHEN, S.-D. Genetic predisposition to inflammation: a new risk factor of Alzheimer's disease. **Neuroscience bulletin**, v. 24, n. 5, p. 314-322, 2008.

WANG, L.-Z., TIAN, Y., YU, J.-T., CHEN, W., WU, Z.-C., ZHANG, Q.*, et al.* Association between late-onset Alzheimer's disease and microsatellite polymorphisms in intron II of the human toll-like receptor 2 gene. **Neuroscience letters**, v. 489, n. 3, p. 164-167, 2011.

WANG, Y., HASEGAWA, M., IMAMURA, R., KINOSHITA, T., KONDO, C., KONAKA, K. et al. PYNOD, a novel Apaf-1/CED4-like protein is an inhibitor of ASC and caspase-1. **International immunology**, v. 16, n. 6, p. 777-786, 2004.

WEBER, A., WASILIEW, P. and KRACHT, M. Interleukin-1β (IL-1β) processing pathway. **Science signaling**, v. 3, n. 105, p. cm2-cm2, 2010.

WEGGEN, S., ERIKSEN, J.L., DAS, P., SAGI, S.A., WANG, R., PIETRZIK, C.U. *et al.* A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. **Nature**, v. 414, n. 6860, p. 212-216, 2001.

WYSS-CORAY, T. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? **Nature medicine**, v. 12, n. 9, p. 1005-1015, 2006.

XU, J., LU, L. and LI, L. NEK7: a novel promising therapy target for NLRP3-related inflammatory diseases. **Acta biochimica et biophysica Sinica**, v. 48, n. 10, p. 966-968, 2016.

YATES, P., SIRISRIRO, R., VILLEMAGNE, V.L., FARQUHARSON, S., MASTERS, C.L., ROWE, C.C.*, et al.* Cerebral microhemorrhage and brain β-amyloid in aging and Alzheimer disease. **Neurology**, v. 77, n. 1, p. 48-54, 2011.

YATES, S.L., BURGESS, L.H., KOCSIS-ANGLE, J., ANTAL, J.M., DORITY, M.D., EMBURY, P.B.*, et al.* Amyloid β and Amylin Fibrils Induce Increases in Proinflammatory Cytokine and Chemokine Production by THP-1 Cells and Murine Microglia. **Journal of neurochemistry**, v. 74, n. 3, p. 1017-1025, 2000.

YU, J.-T., MOU, S.-M., WANG, L.-Z., MAO, C.-X. and TAN, L. Toll-like receptor 2-196 to-174 del polymorphism influences the susceptibility of Han Chinese people to Alzheimer's disease. **Journal of neuroinflammation**, v. 8, n. 1, p. 136, 2011.

YU, J.-T., TAN, L., SONG, J.-H., SUN, Y.-P., CHEN, W., MIAO, D. *et al*. Interleukin-18 promoter polymorphisms and risk of late onset Alzheimer's disease. **Brain research**, v. 1253, p. 169-175, 2009.

YU, Y. and YE, R. Microglial Aβ receptors in Alzheimer's disease. **Cellular & Molecular Neurobiology**, v. 35, n. 1, p. 71-83, 2015.

ZHANG, B., GAITERI, C., BODEA, L.-G., WANG, Z., MCELWEE, J., PODTELEZHNIKOV, A.A. *et al*. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. **Cell**, v. 153, n. 3, p. 707-720, 2013.

APPENDIX

APPENDIX 1 – MINI MENTAL STATE EXAMINATION (MMSE) MODEL

MINI-EXAME DO ESTADO MENTAL
(Folstein, Folstein & McHugh, 1.975)

Paciente: _____

Data da Avaliação: ____/ ____/ ____ Avaliador: _____

ORIENTAÇÃO
- Dia da semana (1 ponto) .....( )
- Dia do mês (1 ponto) .....( )
- Mês (1 ponto) .....( )
- Ano (1 ponto) .....( )
- Hora aproximada (1 ponto) .....( )
- Local específico (aposento ou setor) (1 ponto) .....( )
- Instituição (residência, hospital, clínica) (1 ponto) .....( )
- Bairro ou rua próxima (1 ponto) .....( )
- Cidade (1 ponto) .....( )
- Estado (1 ponto) .....( )

MEMÓRIA IMEDIATA
- Fale 3 palavras não relacionadas. Posteriormente pergunte ao paciente pelas 3 palavras. Dê 1 ponto para cada resposta correta .....( )

  Depois repita as palavras e certifique-se de que o paciente as aprendeu, pois mais adiante você irá perguntá-las novamente.

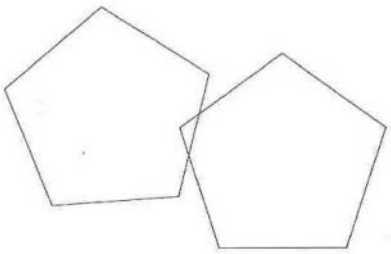
ATENÇÃO E CÁLCULO
- (100 - 7) sucessivos, 5 vezes sucessivamente (1 ponto para cada cálculo correto) .....( )

  (alternativamente, soletrar MUNDO de trás para frente)

EVOCAÇÃO
- Pergunte pelas 3 palavras ditas anteriormente (1 ponto por palavra) .....( )

LINGUAGEM
- Nomear um relógio e uma caneta (2 pontos) .....( )
- Repetir "nem aqui, nem ali, nem lá) (1 ponto) .....( )
- Comando: "pegue este papel com a mão direita dobre ao meio e coloque no chão (3 ptos) .....( )
- Ler e obedecer: "feche os olhos" (1 ponto) .....( )
- Escrever uma frase (1 ponto) .....( )
- Copiar um desenho (1 ponto) .....( )

ESCORE: (____/30)



APPENDIX 2 – CONSENT FORM MODEL

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE GENÉTICA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

TERMO DE CONSENTIMENTO LIVRE ESCLARECIDO

Eu .....ou responsável por.....
....., autorizo a participação na pesquisa: **"ASSOCIAÇÃO ENTRE VARIANTES DE GENES COMO BCHE, APOE, SLITRK3, NEP E GENES DA FAMÍLIA MLR HUMANA E A DOENÇA DE ALZHEIMER"**. Concordo com o armazenamento e guarda do material genético e utilização deste material para pesquisas científicas futuras nos termos citados na Carta de Informação. Tenho pleno conhecimento dos procedimentos que serão submetidos conforme descritos anteriormente. Assino o presente termo, após ter lido a Carta de Informação, entendido e não ter mais nenhuma dúvida.

Curitiba, ... de ..... de 2015

Ass.:.....
RG:.....

Data:.....Telefone:.....
☐ _____
☐ Controle idoso
☐ D. A.
Nome:.....
Local de Nascimento:.....
Data de nascimento:.....Idade:.....
Cor:.....Descendência .....
Medicamentos em uso:.....
.....
Fuma?.....
Consome álcool:.....
Doenças anteriores:.....
.....
Doenças na família (genéticas ou senis):.....
.....

Centro Politécnico - Jardim das Américas - Caixa Postal 19071 - CEP 81531-980 - Curitiba, Brasil
tel (+41) 33611587 / 33611684 – *fax* (+41) 33611793 – *e-mail* ppg-gen@ufpr.br

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE GENÉTICA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

Parentes com demência (qual):.....

Profissão:.....

Quando apareceram os primeiros sinais de demência:.....

Quais foram os sintomas:.....

.....

.....

Mudanças de comportamentos/personalidade (quais).....

.....

Donw na família?:.....

Exame clínico neurológico:.....

.....

Alimentação:.....

Sono:.....

Escolaridade.....

Responsável:.....

Resposta ao inibidor: .....

Horário tomou inibidor: .....

Efeito colateral ao inibidor:.....

Data da coleta de sangue:.....

OBS:.....

Peso:..... Altura:.....C.A.:.....

Dados laboratoriais:

Responsáveis:

Prontuário N. _____

Prof. Dr. Ricardo Lehtonen Rodrigues de Souza

Msc. Daiane Priscila Simão-Silva

Dr. Mauro Piovezan

Centro Politécnico - Jardim das Américas - Caixa Postal 19071 - CEP 81531-980 - Curitiba, Brasil
tel (+41) 33611587 / 33611684 – *fax* (+41) 33611793 – *e-mail* ppg-gen@ufpr.br

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE GENÉTICA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

CARTA DE INFORMAÇÃO AO PACIENTE E IDOSO CONTROLE NO ESTUDO DOS COMPONENTES GENÉTICOS DAS DEMÊNCIAS

Pesquisa:

ASSOCIAÇÃO ENTRE VARIANTES DE GENES COMO *BCHE*, *APOE*, *SLITRK3*, *NEP* E GENES DA FAMÍLIA *MLR* HUMANA E DEMÊNCIAS.

O presente trabalho tem por objetivo investigar os componentes genéticos das demências na tentativa de estabelecer ligações que possam ser utilizadas no diagnóstico, prognóstico e tratamento dos indivíduos acometidos pelas Demências.

Pouco se conhece da genética das Demências para aplicação na prática médica, mas os trabalhos científicos sugerem que os componentes genéticos são fundamentais para o aparecimento e desenvolvimento da doença.

O material utilizado nesse estudo será obtido através da simples e rápida coleta de 7ml de sangue, dos pacientes com Demências e idosos sadios. A coleta de sangue será realizada por profissionais especializados, ficando o voluntário isento de qualquer risco grave, sendo submetido apenas à coleta de sangue e uma rápida entrevista. O sangue coletado será levado ao laboratório onde os materiais genéticos (DNA/RNA) e proteínas serão retirados, estudados e comparados aos de outros pacientes e idosos saudáveis.

Por fim a pesquisa em questão apresenta riscos mínimos aos voluntários, sendo o benefício dos voluntários unicamente a contribuição científica que possa surgir com os resultados da pesquisa, no qual seu material foi fundamental para concretização, não havendo nenhum benefício de ordem financeira. O material poderá, no entanto, contribuir futuramente para a elaboração de novas formas de tratamento das demências.

O voluntário goza de total liberdade para se retirar do estudo a qualquer momento. Informamos também que haverá o armazenamento e guarda do material genético e utilização deste material para pesquisas científicas futuras.

É de responsabilidade do pesquisador sempre resguardar e manter dados e informações dos pacientes em sigilo absoluto. Os gastos relativos aos procedimentos laboratoriais serão absorvidos pelo orçamento da pesquisa sem qualquer gasto por parte do paciente ou familiar. Colocamo-nos à disposição dos pacientes ou responsáveis para elucidar qualquer dúvida relacionada à pesquisa.

Informações: Daiane P. Simão-Silva, email: dpscientist@gmail.com; fone: (042) 99594996.

SUPLEMMENTARY TABLES

SUPLEMMENTARY TABLE 1 – GENOTYPE DISTRIBUTIONS IN A RECESSIVE AND DOMINANT GENETIC MODEL IN AD AND CTRL, SEPARATED BY GENDER.

		rs6531669					
TLR6	**Groups**	**Recessive Model ♀**			**Recessive Model ♂**		
		G/T + T/T (%)	G/G (%)	P	G/T + T/T (%)	G/G (%)	P
	AD (n=151)	94	6	**0.01182**	92	8	0.2198
	Control (n=119)	82	18		84	16	
		rs13105517					
TLR2	**Groups**	**Recessive Model ♀**			**Recessive Model ♂**		
		G/A + G/G (%)	A/A (%)	P	G/A + G/G (%)	A/A (%)	P
	AD (n=151)	80	20	**0.0434**	89	11	0.4632
	Control (n=119)	91	9		94	6	

SUPLEMMENTARY TABLE 2 – GENOTYPE DISTRIBUTIONS OF rs9919613 OF THE *NLRP10* GENE, AND rs1143643 OF THE *IL-1B* GENE, IN A RECESSIVE, AND CODOMINANT GENETIC MODEL, IN INDIVIDUALS WITH AN AGE OF ONSET < 75YO AND INDIVIDUALS WITH AN AGE OF ONSET > 75YO, SEPARATED BY GENDER.

		rs9919613					
NLRP10	**Groups**	**Recessive Model ♀**			**Recessive Model ♂**		
		C/G + G/G (%)	C/C (%)	P	C/G + G/G (%)	C/C (%)	P
	AO < 75 (n=73)	85	17	**0.0089**	85	15	0.06737
	AO > 75 (n=69)	98	2		100	0	
		rs1143643					
IL-1β	**Groups**	**Recessive Model ♀**			**Recessive Model ♂**		
		C/T + C/C (%)	T/T (%)	P	C/T + C/C (%)	T/T (%)	P
	AO < 75 (n=74)	92	8	0.1781	94	6	0.1205
	AO > 75 (n=69)	81	19		80	20	
		rs1143643					
IL-1β	**Groups**	**Codominant Model ♀**			**Codominant Model ♂**		
		C/C	C/T	T/T	P	C/C	C/T	T/T	P
	AO < 75 (n=74)	45	47	8	0.4030	52	42	6	0.1998
	AO > 75 (n=69)	39	43	18		55	25	20	