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Chapter 11

Genetics of Alzheimer´S Disease

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Additional information is available at the end of the chapter

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1. Introduction

Alzheimer's disease, which was first described in 1907 by Alois Alzheimer, is a progressive neurodegenerative disorder characterized by memory loss and other cognitive functions, and is the most common cause of dementia in old age. Histopathologically, AD is defined by the presence of two specific features: neuritic plaques (NP), containing beta amyloid (Aβ) deposits and neurofibrillary tangles (NTF), containing hyperphosphorylated tau protein [1-3] (Figure 1). The pathological changes observed in the brains of AD patients are not distributed uniformly over the cerebral cortex. Instead, these changes are located in specific cortical areas, indicating a relationship between disease progression and the connectivity of affected areas [2, 4-5]. These changes follow a pattern that correspond to the information transmission routes between cortical and subcortical areas of the brain, suggesting a direct correlation between anatomical damage and the clinical phases of the disease.

There are two subtypes of AD: 1) familial Alzheimer´s disease which is associated with mutations in three different genes and 2) sporadic Alzheimer´s disease, which is much more common and the causes for it, are not yet completely understood. In recent decades, numerous genome-wide association studies (GWAS) have been performed in an attempt to identify new risk loci related with the development of sporadic cases. In this regard, genetic association studies of cases and controls, have proven the existence of polymorphic variants in genes which could be interpreted as genetic susceptibility factors contributing to the development of LOAD. However, these results are not replicated in all populations, suggesting the importance of geographical and environmental factors in the phenotypic expression of the disease. For this purpose and in order to validate the data obtained, it is necessary to take in account confounding factors as genetic admixture in population-based genetic association studies. This review, describe the genetics of Alzheimer´s disease and some of the most relevant GWAS conducted to date.

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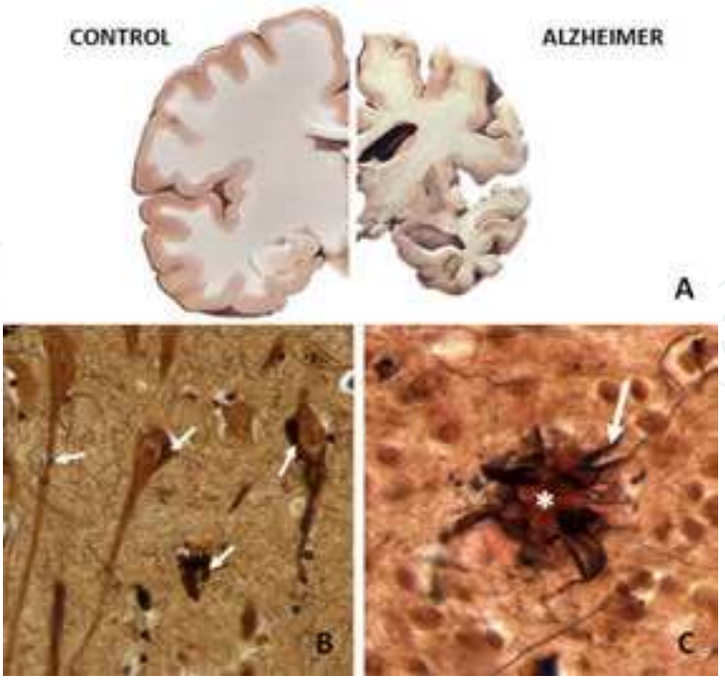


Figure 1. Pathological changes observed in AD patients brains. (A) Cross-section on the left represents a normal brain and the one on the right represents a brain with Alzheimer's disease. The picture shows the generalized brain atrophy in AD, characterized by widening in sulcus, ventricles dilatation and extensive cell loss. **(B)** Silver stain showing the presence of neurofibrillary tangles (NFT) the Tau protein aggregates are indicated by white arrows. We observe the formation of these deposits at different stages of neurodegeneration. **(C)** Double staining showing a neuritic plaque (NP), amyloid deposits are seen in red and marked with an asterisk; neurofibrillary aggregates surrounding the amyloid are marked with the arrow.

2. Diagnosis

There are currently several clinical tools for the diagnosis of AD, including the minimum mental state examination (MMSE) [6] and the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition [DSM-IV]). In general terms, these tools consist of a semi-structured interview with an appropriate reporter and the patient with damage being described as loss of two or more of the following cognitive areas: memory, orientation, calculation and language. Other aspects are similarly evaluated, such as problem solving, social relationships, work, hobbies and personal care. Another commonly used criterion for diagnosis is that of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer´s Disease and Related Disorders Association (NINCDSADRDA). Under this criterion, the state of dementia is clinically determined based on the loss of two cognitive areas and the absence of other systemic disorders, accompanied by a progressive loss of memory. These criteria are sufficient to determine *probable Alzheimer´s disease.* However, diagnosis of AD requires exclusion of other neurodegenerative diseases, such as frontotemporal dementia, Parkinson's disease and Lewy Body disease. Discrimination between AD and other types of dementia are usually achieved based on clinical history and through neurological examinations that require imaging studies. Nevertheless, definitive diagnosis of AD requires postmortem confirmation by histopathological examination to demonstrate the presence of NP and NFT (Figure 1).

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2. Neuritic plaques and the β-amyloid precursor protein

Neuritic plaques are extracellular deposits of 10-100 μm in diameter, contain an insoluble core consisting of a peptide known as amyloid-β (Aβ), surrounded by microglia, reactive astrocytes and dystrophic neurites [7]. Aβ is a peptide of 39-42 amino acids [8-9] that originates as a normal secretory product derived from amyloid-β precursor protein (AβPP) [10]. AβPP is an integral membrane protein that is widely expressed in epithelial cells of various organs, such as the thyroid gland, skin and the central nervous system. AβPP is a type I integral membrane glycoprotein that resembles a signal-transduction receptor [10]. This protein is conformed by a large extracellular domain, a hydrophobic transmembrane domain and a short cytoplasmic carboxyl terminus (Figure 2). The gene is located on chromosome 21q21 and consists of 18 exons. Alternative splicing generates several isoforms with lengths varying between 365 and 770 amino acid residues. In the central nervous system, four isoforms are expressed: APP695, APP714, APP751 and APP770. Amyloid-β is present only in APP695, APP751 and APP770 (Figure 3A, 3B). The APP695 isoform is mainly expressed in neuronal cells [11], while the APP751 and APP770 isoforms are expressed in glial cells [12-13]. To date, the primary function of the protein has not been defined yet, but it has been proposed that it could participate as a growth factor in cultured fibroblasts [14] and play role in cell adhesion [15], intraneuronal calcium regulation [16], neural plasticity [17] and act as a regulator of synapse formation [18]. AβPP, is posttranslationally modified by N-and O-glycosylation, phosphorylation and tyrosine sulphation and undergoes two types of proteolytic processing [19] through three

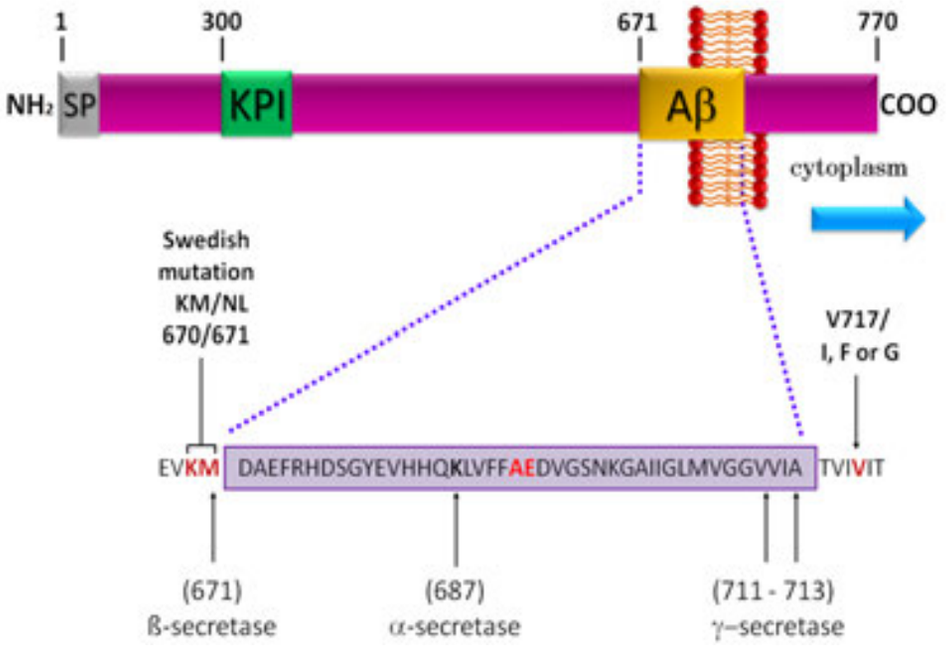


Figure 2. Schematic representation of AβPP. AβPP is a member of a family of conserved type I membrane proteins, consists of a large extracellular domain, a hydrophobic transmembrane domain, and a short cytoplasmic carboxyl terminus. Amyloid sequence contains 40-and 43-amino acid residues that extend from the ectodomain into the transmembrane domain of the protein. The Aβ sequence lies partially outside the cell membrane (amino acids 1–17 of Aβ) and the some identified mutations in the protein are indicated in bold.

enzyme activities α-secretase, β-secretase which cleave AβPP within the luminal domain, and a third activity, termed γ-secretase which cleaves APP within the transmembrane domain. All three AβPP secretases are transmembrane proteases.

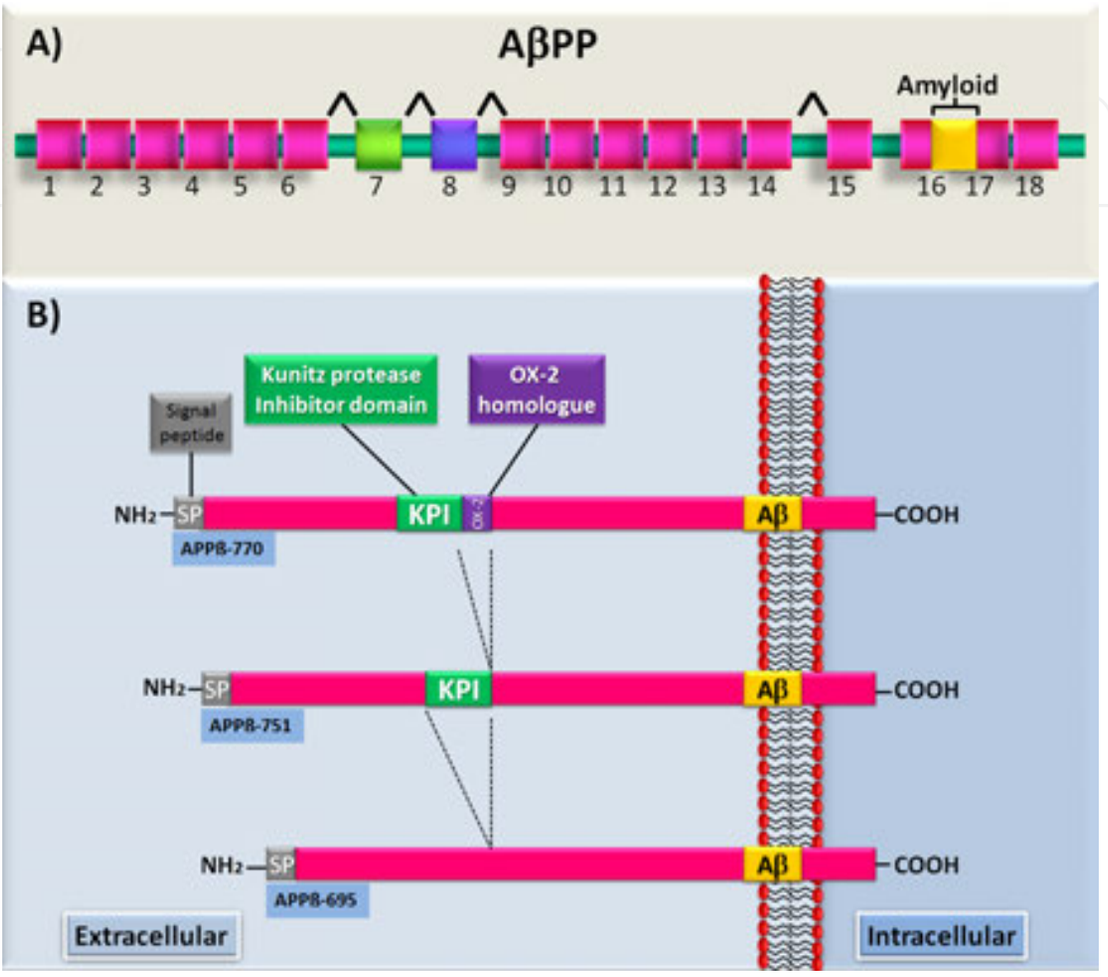


Figure 3. Human APP gene structure. (A) The APP gene consist of 18 exons, is located on chromosome 21 (21q21.2-3). The region encoding the amyloid sequence comprises part of exons 16 and 17 (yellow box). **(B)** APP is alternatively spliced into several products, named according to their length in amino acids (ie, APP695, APP714, APP751, APP770, and APP563) that are expressed differentially by tissue type. The major APP derivatives in the CNS are APP695, APP751 and APP770. Some isoforms contain a 57 amino acid KPI domain and a 19 aminoacid MRC OX-2 antigen in the extracellular sequences (pink box).

It is believed that the principal proteolytic cleavage of AβPP is non-amyloidogenic pathway, which is performed by the action of a protease known as alpha-secretase. This protease cleaves, at residues 612-613 corresponding to the middle portion Aβ (Lys16 and Leu17 in Aβ peptide), thereby preventing amyloid formation [20-21]. α-secretase (ADAM10) generates two products: a soluble fragment (sAPPα) that is released into the extracellular space and a carboxyl terminal membrane-anchored product, called C83. Finally, the C83 fragment is cut by γ-secretase generating a 6 KDa fragment, called AICD (APP intracellular domain-) and a ~3kDa peptide (p3) that is released into the extracellular space (Figure 4A).

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The first step of amyloidogenic processing is carried out by the action of β-secretase, (BACE1), which generates the formation of two products: 1) a soluble product (sAβPP) that is released into the extracellular space and 2) a carboxyl terminal membrane-anchored called C99. In the same way C99 is cut by the γ-secretase, generating the AICD fragment into the cytoplasm and the neurotoxic fragment amyloid beta (Aß) (Figure 4B) [22].

Although AβPP metabolism and amyloid peptide accumulation represent central events in the pathogenesis of AD, in animal models, it has not been possible to demonstrate that their occurrence *per se* is capable of generating the complete pathology of the disease.

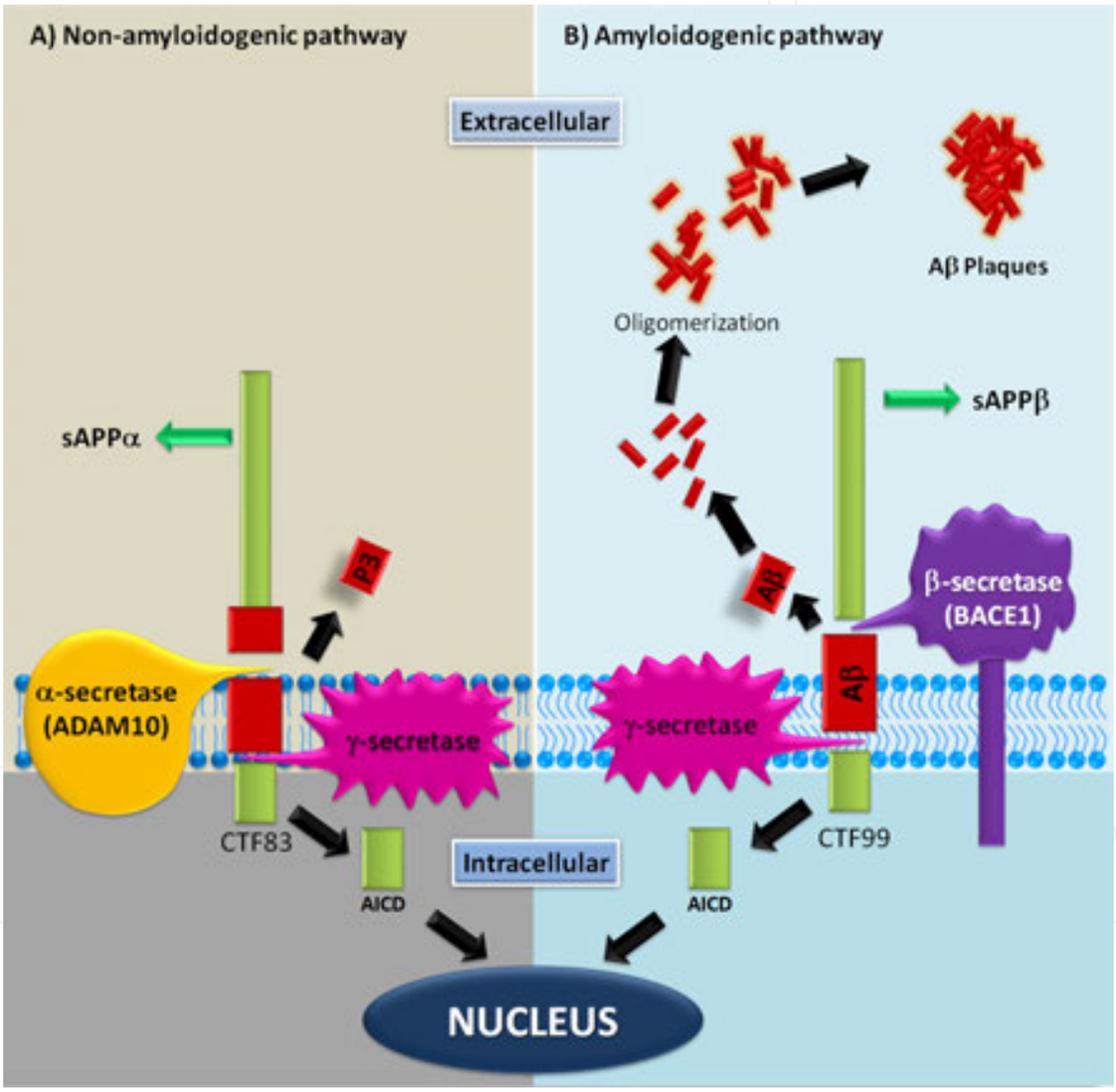


Figure 4. AβPP Processing. The AβPP is an integral membrane protein, is sequentially processed by the three proteases α-, β-, and γ-secretase. **(A)** The non amyloidogenic pathway involves the α-secretase, which made the cut at the middle portion of the fragment corresponding to the amyloid sequence; preventing the amyloid peptides generation. **(B)** The amyloidogenic pathway involves β-secretase, leading to the formation of C-terminal fragments (CTFs) that are subsequently cleaved by the "γ-secretase-complex" which is responsible for the formation of A*β* (40 or 42 amino acids in length) and the A*β*PP intracellular domain peptide (AICD) of 58 or 56 amino acids.

3. Neurofibrillary tangles and tau

Neurofibrillary tangles are simpler and yet more enigmatic than neuritic plaques. Unlike NP, the density of NFT in the brains of AD patients is closely related to the severity of dementia [23-24]. In particular, neurofibrillary degeneration is a prerequisite for the clinical expression of AD pathology, i.e., *dementia*, whereas amyloid accumulation in the absence of neurofibrillary tangles does not produce the AD pathology. The structural units of NFT are paired helical filaments (FHA), which are formed by the association of five to six6 fragments of the microtubule binding protein Tau. The gene that encodes this protein is located on chromosome 17 and comprises 16 exons of which-1 and 14 exons, can be transcribed but not translated [25-27]. Alternative RNAm splicing of exons 2, 3 and 10, of the *MAPT* gene generates the formation of six isoforms which are expressed in adult brain [28]. Each isoform differs from each other by the presence or absence of a 29-aminoacid or 58-aminoacid inserts in the amino-terminal half and by the inclusion or not in the carboxy-terminal half of the protein of a 31-aminoacid repeat encoded by exon 10 of *MAPT* [25, 29-30]. When exon 10 is excluded, the result is a protein with three repeats of the microtubule-binding domain (3RMBD). When exon 10 is included, a fourth microtubule binding domain is added to generate four-repeat tau (4RMBD) [28, 31-32] (Figure 5). Under normal conditions, Tau is a highly soluble protein, since it contains no apparent secondary structure [33-34]. However, in pathological conditions, Tau tends to self-assemble into the insoluble filament structures [32]. To date have been identified and MAPT gene mutations, however, none of these mutations have been associated with the development of AD. This type of mutations in the Tau gene cause frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) [35-39]

4. Genetics of AD

Conventionally, AD is divided into two forms depending on the age of onset: early onset Alzheimer´s disease (EOAD) and late onset Alzheimer´s disease (LOAD). EOAD or familial cases, which account for only 5-10% of all cases, exhibit an autosomal dominant mode of inheritance, high penetrance of clinical symptoms and onset before 55 years of age. LOAD or sporadic cases account for 90-95% of all AD cases, usually present a later onset age (≥ 65 years) and apparently do not show familial aggregation associated with the development of the disease. Twin studies provide insight into the relative contributions of genetic and environmental influences for Alzheimer´s disease and other types of dementia [40-42]. It has long been argued that a twin study design is advantageous for identifying risk and protective factors because this type of study has suggested the existence of a genetic component associated with the development of LOAD cases [43]. The results of these studies have shown that the pairwise concordance rate for Alzheimer´s disease is 78% (7/9) among monozygotic and 39% (9/23) among dizygotic twin pairs [40]. In 2006, Gatz adjusted their findings for age and also included like-and unlike-sex pairs, and the results showed that the age–adjusted heritability for AD was estimated to be 58-79%, and there were no significant differences between men and women regarding prevalence or heritability after controlling for age [41]. Nevertheless, it was observed

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that among patients who develop LOAD, approximately 40-65% present an indirect genetic agent in the form of the 4 allele of apolipoprotein E (ApoE/4) [44-47]. However, the effect of APOEe4 as a genetic risk factor is not sufficient or necessary for developing the disease [48-49].

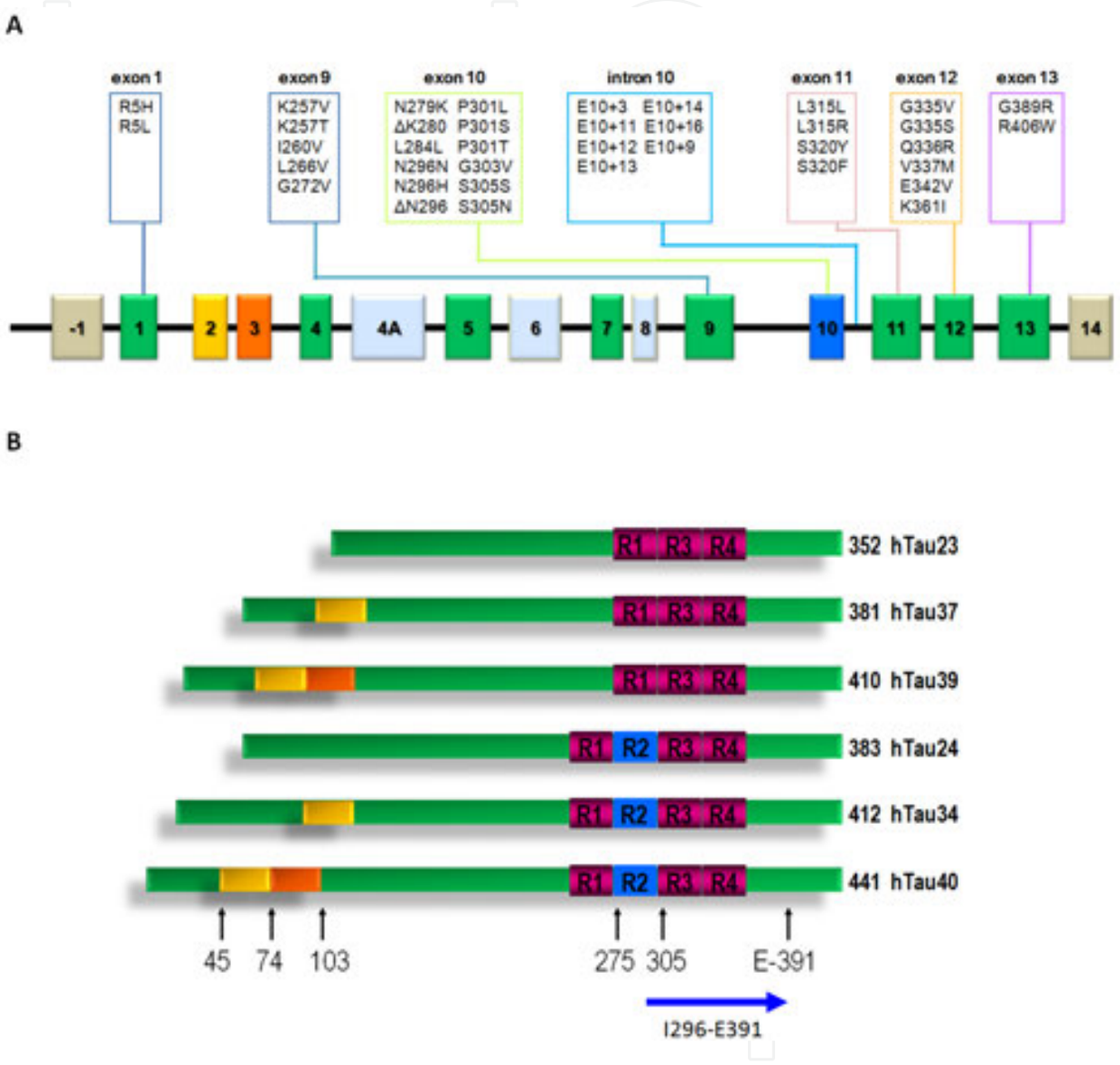


Figure 5. Human Tau-protein. (A) Schematic representation human tau gene (MAPT). The human gene comprises 16 exons of which exons-1 and 14, can be transcribed but not translated. In central nervous system are expressed 6 isoforms, which are obtained by alternative splicing of exons 2, 3 and 10; the exons 1, 4, 5, 7, 9, 11, 12 and 13 are expressed in all isoforms. In boxes, are indicated the mutations found in the gene which have been associated with FTDP-17. **(B)** Representation of the 6 Tau isoforms. The different isoforms differ from each other by the presence of one or two inserts located in the region N (yellow and orange boxes) and the presence of 3 or 4 repeated domains, located in C-terminus of the molecule (blue box) and termed microtubule binding domain (MTBD). The expression of the different isoforms is regulated during development; in fetal stages are expressed only isoforms containing 3 repeated whereas adult stages, all isoforms are expressed.

5. Early onset alzheimer´s disease

While the vast majority of cases of AD occur late in life and are sporadic, approximately 5–10% of cases exhibit an early onset. EOAD or Familial Alzheimer´s disease is associated with mutations in proteins such as presenilin 1 and 2 (PS1 and PS2) and amyloid precursor protein (APP) [50-58]. Currently, more than 200 distinct disease-causing mutations have been identified across these genes, which exhibit an autosomal dominant transmission pattern.

5.1. APP mutations

To date, approximately 36 different missense mutations in the APP gene have been identified among 85 families (Table 1). AβPP mutations account for 0.1% of AD patients, all missense mutations influence APP processing since they are positioned in or near the Aβ coding exons 16-17, in the transmembranal domain, where the sites recognized by the α, β and γ-secretases are found (Figure 2). This alters the APPβ processing and causes the accumulation of Aβ42 fragments [54-55]. The major mutations in APPβ include the Swedish double mutation (APPSW: APPK670N, APPM671L) [59] and the London mutation (V717I) [55]. The Swedish mutation is located just outside the N-terminus of the Aβ domain of APP, favors β-secretase cleavage and it is associated with increased levels and deposition of Aβ1-42 in the brains of AD patients [60-61]. London mutation is located in exon 17 and leads to a valine to isoleucine change at amino acid 717 (V717I) [55], corresponding to the transmembrane domain near the γ-secretase cleavage site. Other mutations in APP linked to EOAD include the Dutch (E693Q) [62], Indiana (V717F) [58], Florida (I716V) [63], Iowa (D694N) [64] and Arctic (E693G) [65] mutations. Besides the mutations identified in the APP gene is known that duplication of APP regions containing several genes [66-68] or APP [69] were clinically linked to EOAD.

The transgenic animal models developed to date that overexpress these mutations have the potential to develop extracellular deposits of amyloid beta and exhibit different types of neurological abnormalities [55, 70-73]. For example, transgenic mouse line APP/V717I displays deficits in the maintenance of long-term potentiation, premature death and cognitive impairment, which is directly correlated with amyloid plaque formation [74]. Another transgenic mouse line used to investigate the pathology of AD is Tg2576, which carries the Swedish mutation. These mice exhibit memory loss starting at 6 months of age, which coincides with the appearance of detergent-insoluble amyloid aggregates [73]. Overexpression of mutated AβPP in cell cultures induces DNA synthesis and apoptosis [75], suggesting that APPβ could induce the apoptotic events observed in Alzheimer´s disease patients via activation of specific pathways of neuronal signaling.

5.2. Presenilin mutation

Presenilin represent the catalytic component of the gamma-secretase complex, which also includes nicastrin, anterior pharynx-defective 1 (Aph-1) and presenilin enhancer 2 (Pen-2) [76]. Presenilins are expressed in several tissues and in the brain, where are mainly expressed in neurons [52]. Presenilins localize into the endoplasmic reticulum (ER), Golgi apparatus, endosomes, lysosomes, phagosome plasma membranes and mitochondria [77-79]. During

Mutation	Phenotype	Age of Onset	References
E665D	AD, but may not be pathogenic	86?	Peacock, et al., 1994
KM670/671NL (Swedish)	AD	52 (44-59)	Mullan, et al. 1992
H677R	AD	55 (55-56)	Janssen, et al. 2003
D678N (Tottori)	FAD	60	Wakutani, et al. 2004
E693Δ	AD		Tomiyama et al., 2008
D694N (Iowa)	AD or Cerebral Hemorrhage	69	Grabowski, et al. 2001
A713T	AD, but may not be pathogenic	59	Carter, et al., 1992
T714A (Iranian)	AD	52 (40-60)	Pasaler, et al., 2002
T714I (Austrian)	Affects γ-secretase cleavage directly, 11X increase in Aβ(42)/Aβ(40) ratio in vitro.		Kumar-Singh, et al.
V715A (German)	AD	47	De Jonghe, et al., 2001; Cruts, et al., 2003
V715M (French)	AD	52 (40-60)	Ancolio, et al., 1999
I716T	AD	55	Terrini, et al., 2002
I716V (Florida)	AD	55	Eckman, et al., 1997
V717F (Indiana)	AD	47 (42-52)	Murrell, et al. 1991
V717G	AD	55 (45-62)	Chartier-Harlin, et al. 1991
V717I (London)	AD	55 (50-60)	Goate, et al. 1991
T719P	AD	46	Ghidoni et al., 2009
L723P (Australian)	AD	56 (45-60)	Kwok JB, 2000

Table 1. Amyloid Precursor Protein Mutations

assembly and maturation of the complex, presenilin undergoes endoproteolitic processes generating stable N-and C-terminal fragments (NTF and CTF, respectively). Both fragments (NTF and CTF) contribute with one of the two catalytic aspartates that are, the active site which is responsible for the intramembranal proteolysis of AβPP and some other proteins as well [60, 80-85]. Both presenilins (PS1 and PS2) possess these conserved aspartate residues required for γ-secretase activity [85]. In addition, presenilins directly or indirectly regulate the trafficking and metabolism of select membrane proteins in neurons [86], as well as having a role in

synaptic function [87-88], learning and memory [89], neuronal survival in the adult brain, regulation of calcium homeostasis [90-91] and presynaptic neurotransmitter release [92].

PS1 is an integral membrane protein with eight transmembranal domains and a hydrophilic domain between the 6 and 7 domains. The PSEN1 gene is located on chromosome 14q24.2, comprises 12 exons and generates a protein with a length of 467 amino acids. To date, more than 180 mutations in PSEN1 have been described in 405 families (http://www.molgen.ua.ac.be/ADmutations), all of which are related to the appearance of the disease at younger ages (Figure 6A) [93-94]. The PSEN2 gene is located on chromosome 1q42.13 and comprises 12 exons, of which only 10 are translated to generate a protein with a length of 448 amino acid residues. This protein exhibits 9 transmembrane domains and displays tissue-specific alternative splicing [95]. Only 13 mutations in PS2 have been described among 22 families (Figure 6B). (http://www.molgen.ua.ac.be/ADmutations)

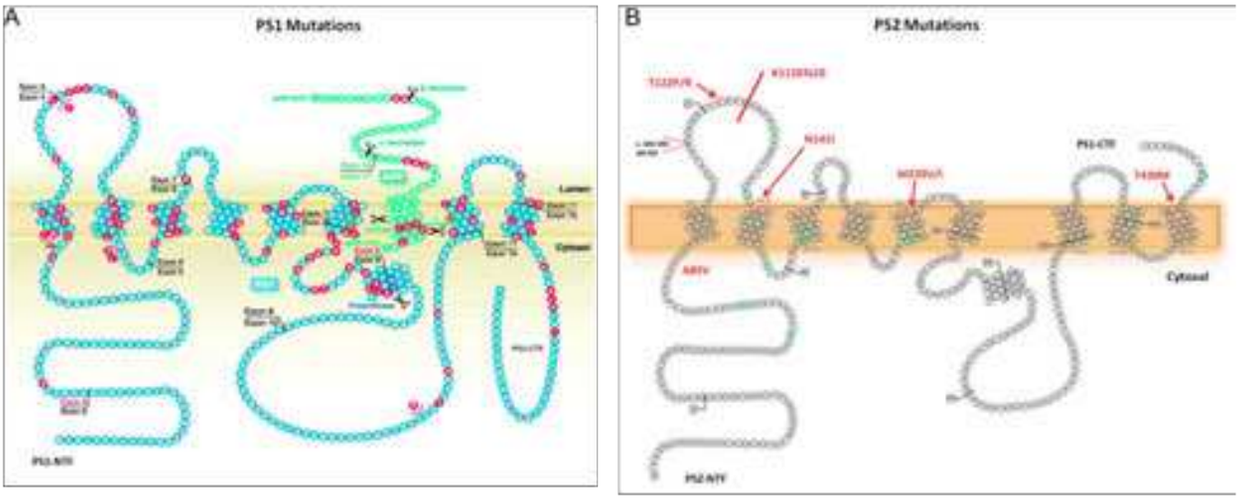


Figure 6. Schematic representation of Presenilis. (A) Presenilins are membrane proteins that form the catalytic core of the γ-secretase complex. The PSEN1 gene is located on chromosome 14q24.2 and comprises 12 exons. PS1 is an integral membrane protein with eight transmembrane domains and a hydrophilic domain between domains 6 and 7. Two aspartate residues in transmembrane domains (TMs) 6 and 7 constituting the catalytic site. To date, more than 185 mutations in PSEN1 have been described in 405 families all of which are related to the appearance of the disease at younger ages. Although mutations are found throughout the protein, most are located in the transmembrane region. **(B)** The PSEN2 gene is located on chromosome 1q42.13 and comprises 12 exons, of which only 10 are translated to generate a protein with a length of 448 amino acid residues. This protein exhibits 9 transmembrane domains and displays tissue-specific alternative splicing, major mutations found in the protein are identified.

Most familial cases of Alzheimer´s disease are associated with mutations in presenilins [50, 53, 96], the majority of PSEN mutations are single-nucleotide substitutions, but small deletions and insertions have been described as well. It has been suggested that these mutations induce overproduction of β-amyloid, apparently by increasing γ-secretase activity [51, 53, 97-102]. Although transgenic mice expressing presenilin mutations do not develop the formation of neuritic plaques, these animals showed changes similar to those observed in AD patients, such as neuronal damage, synaptic loss and vascular disease. The most severe mutation in *PSEN1* is a donor-acceptor splice mutation that causes a two-aminoacid substitution and an in-frame

deletion of exon 9. However, the biochemical consequences of these mutations for γ-secretase assembly seem to be limited [103-104]. PS1 also appears to modulate GSK3β activity and the release of kinesin-I from membrane-bound organelles at sites of vesicle delivery and membrane insertion. These findings suggest that mutations in PS1 may compromise neuronal function, affecting GSK-3 activity and kinesin-I-based motility and, thus, leading to neurodegeneration [105]. Although PS2 shows close homology to PS1, PS2 is less efficient with respect to amyloid peptide production [106]. *In vitro* expression of PSEN2 V393M cDNA did not result in a detectable increase in the secreted Aβ42/40 peptide ratio. However, patients heterozygous for this missense mutation are characterized by profound language impairment [107]. Although mutations are found throughout the protein, most are located in the transmembrane region.

6. Late onset alzheimer´s disease

6.1. Apoe risk gene

Allele 4 of apolipoprotein E (ApoE4) has been reported to represent the main identified risk factor for sporadic AD [44, 49, 108]. This gene has been associated with EOAD and LOAD in multiple ethnic groups, and carriers of APOE4 exhibit an earlier age of onset for AD [44, 109-110]. The frequency of the APOE4 allele varies among ethnic groups and it has been shown that ApoE4 is determinant for AD risk in white's individuals; however, in Hispanic and African populations, there is no correlation between the presence of the pathology and this allele. These results suggest that other genes or risk factors may contribute to the increased risk of AD in African and Hispanics [111-114].

The ApoE4 gene is located at chromosome 19q13.2 [115] and consists of 4 exons encoding a protein of 299 amino acid residues with a molecular weight of 34 kDa. APOE is a glycoprotein exhibiting variable levels of posttranslational sialylation due to O-linked glycosylation at threonine 194 [116]. The gene contains several single-nucleotide polymorphisms (SNPs) [117] leading to changes in the amino acid sequence of the protein, resulting in the production of three isoforms: ApoE2, ApoE3 and ApoE4, which are associated with different alleles (ε2, ε3, ε4). The three isoforms differ only by one or two amino acids, with the changes occurring at amino acid residues 112 and 158: ApoE2 (cys112, cys158), ApoE3 (cys112, arg158) and ApoE4 (arg112, arg158) [118-120]. The allelic distribution varies among ethnic groups, although it has generally been observed that allele 3 is the most frequent (77%), followed by allele 4 (15%), while allele 2 is less frequently observed (8%).

ApoE is a plasma protein that plays an important role in lipid metabolism and cholesterol transport in various tissues [108, 121-122]. The amino acid changes observed in the different isoforms of ApoE alter the 3-dimensional structure of the protein, affecting its lipid-binding properties, indicating that each isoform is metabolically different and varies in its affinity to bind to lipoprotein particles [123-124]. Apolipoproteins are synthesized primarily in the liver but can be processed and secreted in the brain by astrocytes and microglia. They are involved in neuronal regeneration [125], an increase in the synthesis of these proteins has been observed

in the central and peripheral nervous system during neuronal damage. The distinct human ApoE isoforms differ significantly in their long-term effects on neuronal integrity as well as in their ability to protect against exocitotoxicity [126-128]. When ApoE isoforms are expressed in brain cells of ApoE-knockout (APOE-/-) mice, it can be observed that ApoE3 has a protective effect against age-related Aβ toxicity and neurodegeneration [129-130]. These differences in the neuroprotective capacities of apoE3 and apoE4 could contribute to the increased susceptibility of human ApoE4 carriers to AD [131]. Cholesterol homeostasis in hippocampal neurons is affected by the presence of apoE4, while the presence of ApoE2 and ApoE3 is not associated with any alterations in homeostasis [132]. Other roles of APOE isoforms include proliferation, synaptogenesis, myelination and amyloid elimination and tau phosphorylation.

6.2. Apoe and amyloid

Overexpression of a mutated form of human APP has shown that the levels of amyloid and ApoE increase in the brain with age, which is associated with decreasing Aβ levels in plasma [133]. It is possible that ApoE increases Aβ sequestration, deregulating the clearance of amyloid and leading to cognitive impairment in transgenic mice expressing a mutant form of human APP [134-135]. Recent studies have shown that apolipoprotein E (ApoE) receptor 2 and other members of the low-density lipoprotein receptor family (LRP, LRP1B, SorLA/LR11) interact with AβPP and regulates its endocytic trafficking [136-137]. Stable expression of human APP in B103 rat neuroblastoma cells (B103-APP); demonstrated that the isoform-specific effects of ApoE on Aβ production result from an alteration of AβPP recycling due to more pronounced stimulation of AβPP recycling by apoE4 than ApoE3 [138]. However, other authors have noted that there is no clear evidence upon which to base conclusions regarding the isoform-specific effects on AβPP processing [127, 139].

Although clearance of Aβ by ApoE has not been extensively studied, ApoE may modulate the removal of Aβ from the brain (Figure 7). Nevertheless, it has been suggested that clearance of Aβ is regulated by low-density lipoprotein receptor related protein-1 (LRP) and the receptor for advanced glycation end products (RAGE); this function is compromised in AD, which may contribute to elevation of the levels of amyloid in the brain [135, 140-141].

6.3. Apoe and tau

Neither the mechanisms by which the tau and ApoE4 proteins confer pathogenicity nor the nature of the interaction between these proteins has yet been established. Some authors have suggested that there is a relationship between the dosage of the ApoE4 allele and the density of NTFs [142-143]. It is known that ApoE3 has the ability to form a stable complex with Tau protein, and this association is believed to decrease Tau phosphorylation, preventing abnormal phosphorylation of Tau protein and their aggregation into paired helical filaments (PHF) [144]. When tau is phosphorylated, it loses its ability to interact with ApoE3. In contrast, ApoE4 does not interact with Tau.

It has recently been shown that the expression of a carboxy-terminal truncated fragment of the ApoE4 protein (Δ272-299 carboxyl terminal) is sufficient to elicit AD-like neurodegeneration

and behavioral deficits *in vivo* [145]. Transgenic mice expressing apoE4Δ272–299 displayed AD-like neurodegenerative alterations in the cortex and hippocampus, including abnormally phosphorylated tau (p-tau) and Gallyas silver-positive neurons that contained cytosolic straight filaments with diameters of 15–20 nm, resembling pre-neurofibrillary tangles [145]. Similarly, overexpression of human ApoE4 in neurons results in hyperphosphorylation of the tau protein, which increases with age [146-147].

Finally, although the presence of allele 4 of ApoE is not a deterministic factor for AD, it has been observed that this allele may favor the development of the disease at younger ages [148].

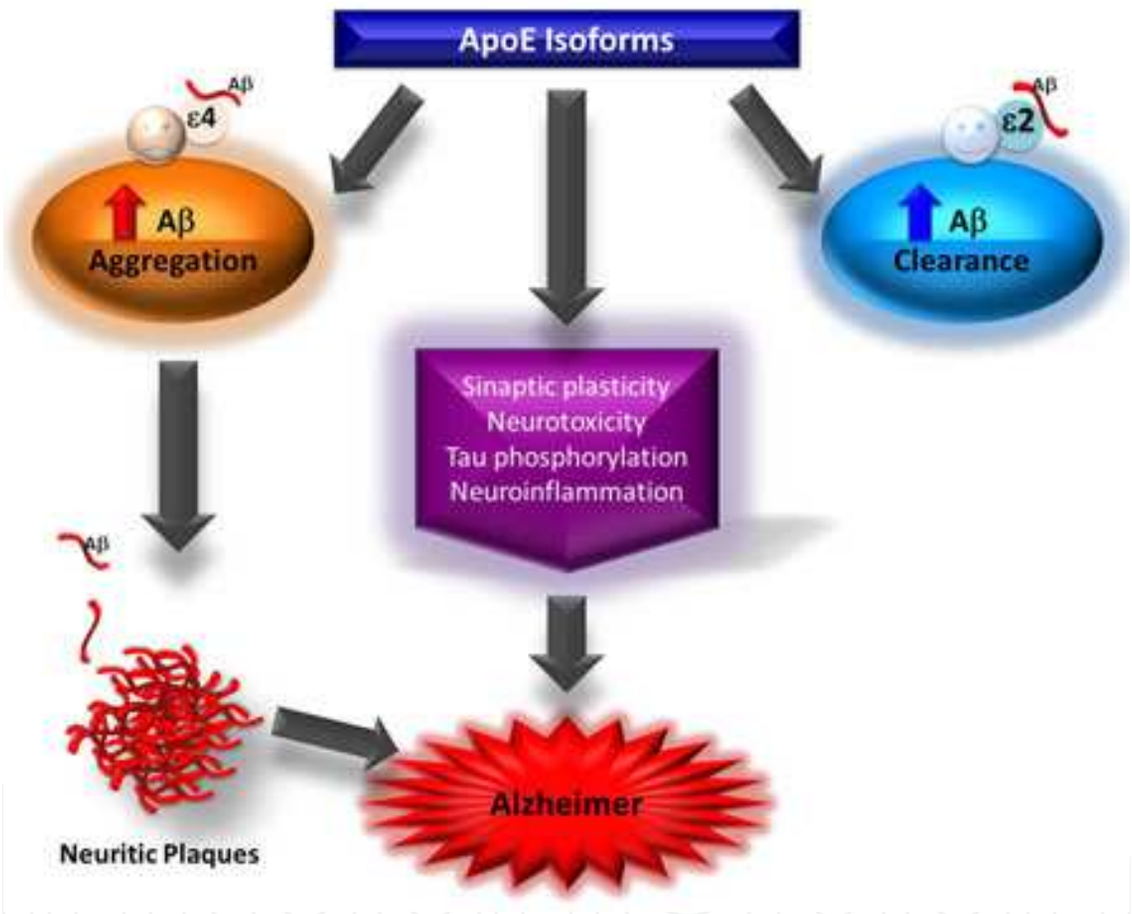


Figure 7. Interaction of Amyloid and ApoE. The ApoE4 gene is located on chromosome 19q13.2. It has been suggested that ApoE, could be involved in the Aβ aggregation and clearance. This process can be regulated of ApoE isoform and thereby promote the onset of Aβ aggregation. In this way other pathologic mechanisms could be favored

7. Genome-wide association studies (gwas)

The genetic causes of AD can be highly variable, even for familial forms. While EOAD is characterized by the presence of mutations with high penetrance in specific genes, the genetics of sporadic cases (LOAD) are more complex. LOAD susceptibility is determined by an

uncertain number of genetic risk factors exhibiting low penetrance that are present at a high frequency. This is particularly important because although patients who develop this subtype of the disease have been considered to represent *sporadic cases*, the genetic component of these cases is a feature that has not been established. A possible explanation for the difficulty involved in the identification of genetic risk factors is that LOAD is a multifactorial complex disorder that involves both genetic and environmental components.

In the last thirty years, a considerable number of studies have been developed aimed at identifying risk factors that confer susceptibility for developing AD. In this regard, genome-wide association studies (GWAS) represent a powerful approach for identifying putative candidate genes for common complex diseases, such as LOAD. These studies simultaneously analyze a large number of genetic markers, typically consisting of single-nucleotide polymorphisms (SNPs). Although they have also involved arrays for assessing copy-number variants (deletions or multiplications of chromosomal segments), other GWAS arrays only contain SNPs located in predicted or known coding regions (cSNPs). The Affymetrix GeneChip 500K platform exhibits 60% coverage of the phase II HapMAp (Affymetrix, Santa Clara, CA, USA), whereas the Illumina Hap300 platform presents 77% coverage (Illumina, Inc., San Diego, CA, USA). At least 12 GWAS addressing Alzheimer´s disease have been published to date, which have identified more than 40 genetic variants that might confer risk for developing this pathology. However, much remains to be learned regarding the pathology and the genetic risk factors associated with late onset Alzheimer´s disease. The main studies investigating the associations between cases and controls with LOAD using such platforms are described below (Table 2).

Genome-Wide Association Studies (GWAS)										
					AD Cases			**Normal Controls**		
Study	**Design**	**Type**	**Population**	**# of SNPs**	**DX**	**# Subjects GWAS**	**# Subjects (follow-up)**	**# Subjects GWAS**	**# Subjects (follow-up)**	**Featured Genes**
CAUSASIAN										
Abraham, 2008	CC	GWAS pooled	Overlaps with Harold, 2009	561494	C	1082	-	1239	1400	APOE, LRAT
Beecham, 2009	CC	GWAS	USA (CAP)	532000	M	492	238	496	220	APOE, FAM113B
Bertram, 2008	FBAT	GWAS	USA (NIMH)	484522	M	941	1767	404	838	APOE ATXN1 CD33 GWA 14q31.2
Carrasquillo, 2009	CC	GWAS	USA (Mayo)	313504	M	844	1547	1255	1209	APOE PCDH11X
Coon, 2007	CC	GWAS	USA, Netherlands (TGEN1)	502627	N	664	-	422	-	APOE
Grupe, 2007	CC	GWAS	USA & UK	17343	M	380	1428	396	1666	ACAN, APOE, BCR, CTSS, EBF3, FAM63A, GALP, GWA_14q32.13,

Genome-Wide Association Studies (GWAS)										
					AD Cases			Normal Controls		
Study	Design	Type	Population	# of SNPs	DX	# Subjects GWAS	# Subjects (follow-up)	# Subjects GWAS	# Subjects (follow-up)	Featured Genes
										GWA_7p15.2, LMNA, LOC651924, MY13, PCK1, PGBD1, TNK1, TRAK2, UBD
Harold, 2009	CC	GWAS	Europe & USA (GERAD1)	529205	M	3941	2023	7848	2340	APOE, CLU, PICALM
Heinzen, 2009	CC	GWAS +CNV	USA (CAP, DUKE)	n.g.	U	331	-	368	-	
Hollingworth, 2011	CC	GWAS	Europe & USA (GERAD1+2, EADI1+2, ADNI, TGEN1, MAYO2, CHARGE)	496763	M	6688	13182	13685	26261	ABCA7, BIN1, CD2AP, CD33, CR1, EPHA1, MS4A4E, MS4A6A
Hu, 2011	CC	GWAS	USA (Pfizer, ADNI), Canada (GenADA, Genizon)	509376	C	1831	751	1764	751	APOE, BIN1
Lambert, 2009	CC	GWAS	Europe (EADI1)	537029	C	2032	3978	5328	3297	APOE, CLU, CR1
Li, 2008	CC	GWAS	Canada (GenADA)	469438	C	753	418	736	249	APOE, GOLM1, GWA_15q21.2, GWA_9p24.3
Naj, 2011	CC	GWAS	USA (ADGC)	2,324,889 (imputed)	M	8309	3531	7366	3565	APOE, BIN1, CD2AP, CD33, CLU, CR1, EPHA1, MS4A4A, PICALM
Poduslo, 2009	CC, FBAT	GWAS	USA	489218	C	9	199	10	225	TRCP4AP
Potkin, 2009	CC, QT	GWAS	USA (ADNI)	516645	C	172	-	209	-	APOE, ARSB, CAND1, EFNA5, MAG12, PRUNE2 TOMM40
Reiman, 2007	CC	GWAS	USA, Netherlands (TGEN1)	312316	M	446	415	290	260	GAB2
Seshadri, 2010	CC	GWAS + meta-analysis	Europe & USA (CHARGE, EADI1, GERAD1)	2,540,000 (imputed)	M	3006	6505	22604	13532	APOE, BIN1, CLU, EXOC3L2, PICALM
Sherva, 2011	CC	GWAS	Israel (Wadi Ara)	2,540,000 (imputed)	C	124	-	142	-	AGPAT1, ATPVOA4, GLOD4, RGS6, TMEM132C
Wijsman, 2011	CC, FBAT	GWAS	USA (NIA, NCRAD)	565336	M	1848	617	1991	573	APOE, CELF2

Table 2. Genome-Wide Association Studies in Alzheimer's Disease.

7.1. Grupe 2007

The first GWAS addressing Alzheimer´s disease was reported in 2007 by Grupe *et al*. A total of 17, 343 SNPs, located in 11 221 unique genes were tested for an association with LOAD in a case–control discovery sample from the UK (1808 LOAD cases and 2062 controls) [149]. These researchers reported the identification of several candidate SNPs showing a significant association with LOAD. Three of these SNPs (**rs157581, rs405509** and **rs1132899**) are located on chromosome 19, close to the APOE gene, and exhibit genome-wide significance (P value=6.94E-81 to 0.0001) and linkage disequilibrium (LD) with the APOE4 and 2/3 variants ($0.09 < D0 < 1$). Furthermore, sixteen additional SNPs showed evidence of an association with LOAD [P=0.0010-0.00006; odds ratio (OR)=1.07–1.45].

Of these SNPs, one was a missense mutation (**rs3745833**) located in the galanin-like peptide precursor (**GALP**) gene. The associated SNP encodes a non-synonymous substitution (Ili72Met) in exon 4. In the Caucasian population, the common minor C-allele increases the risk for AD in 10% of individuals. The galanin gene has been implicated in neuronal survival, regeneration and neuroprotection as well as the inhibition of cholinergic neurotransmission and suppression of long-term potentiation [150-151]. In limbic brain regions of AD patients, galanin expression is upregulated and could conceivably worsen the symptoms of the disease. Transgenic mice overexpressing galanin display cognitive and neurochemical deficits similar to those observed in AD patients [152].

Another important SNP was found to be located in PGBD1 (piggyBack transposable element derived 1). The associated SNP (**rs3800324**) encodes a non-synonymous substitution (Gly244Glu) in exon 5, and the presence of the minor A (Glu) allele significantly increases the risk of AD by 20%. The function of this protein is not known, but it is specifically expressed in the brain. Finally, in this study, the authors showed that four additional SNPs showed evidence of association with LOAD. These variants include SNPs located in TNK1 and PCK1 as well as an intergenic SNP near SERPINA13.

TNK1 is a non-receptor tyrosine kinase that mediates phospholipid signal transduction. In addition, together with TRAK2, TNK1 may be involved in protein trafficking and signal transduction [153] and participate in the processing of amyloid precursor protein and amyloid β-production [154-155]. Aberrant TNK1 activity may increase the risk of LOAD [156].

7.2. Coon 2007, Reiman 2007

In the same year, Coon *et al.* employed an ultra-high-density whole-genome association analysis, demonstrating the ability to identify the APOE locus as a major susceptibility gene for late onset AD [157]. This study used the Affymetrix 500K platform, including 502,627 SNPs, and was performed in a population of 1086 histopathologically verified AD cases and controls. The results obtained showed that the APOE locus is the major susceptibility gene for late onset AD in the human genome, with an OR significantly greater than any other locus in the human genome (Bonferroni corrected OR=4.01). The polymorphism identified in this study (**rs4420638**) is located on chromosome 19 and is 14 kilobase pairs distal to the APOE epsilon variant.

In a subsequent study, the same group of researchers divided each cohort of LOAD cases and controls into two subgroups: allelic APOE ε4 carriers and APOE ε4 noncarriers. The results showed an association with six SNPs of the GRB-associated binding protein 2 (GAB2) gene and a common haplotype encompassing the entire **GAB2** gene [158]. SNP **rs2373115** was associated with an odds ratio of 4.06 (confidence interval 2.81–14.69) and interacts with APOE ε4 to further modify risk.

The GAB2 protein is involved in a number of different pathways, and thus, it is possible that GAB2 could affect mechanisms involved in cell survival, Tau phosphorylation and NFT formation. Additionally, GAB2 may be involved in the production of Aβ [158], contributing to the development of AD pathology. Finally, GAB2 has been found to be coexpressed with other putative AD-related genes [159].

7.3. Abraham 2008

The GWAS conducted by Abraham in 2008 differs from all other currently published GWAS addressing AD in that, in the initial screening in this study, DNA pools were utilized for genotyping rather than individual DNA samples [160]. DNA samples were collected from 1,082 individuals with LOAD and 1,239 control subjects. The age at onset ranged from 60 to 95 years, and controls were matched for age (mean=76.53 years, SD=33), gender and ethnicity. The construction of the pools was validated using the SNaPshot method. The pools were genotyped using Illumina HumanHap300 and Illumina Sentrix HumanHap240S arrays, testing 561,494 SNPs. The results showed an association of several SNPs close to the *APOE* locus with LOAD, including 7 SNPs within 71 kb, with allele frequency differences of between 6% – 14%. Five of the seven SNPs were individually genotyped and were confirmed to present highly significant associations with LOAD. Although these studies using pooled DNA samples considerably reduce costs, their results may not accurately represent real allele frequency distributions.

7.4. Bertram 2008.

Another GWAS addressing AD was performed by Bertram *et al.* in 2008. This study represented the first to employ family-based methods for the initial screening. This case, a genome-wide association (GWA) analysis was performed using 484,522 single-nucleotide polymorphisms (SNPs) on a large (1,376 samples from 410 families) sample of AD families of self-reported European descent. All 10,388 X chromosome markers were eliminated, as also were 5,758 SNPs that did not pass genotype quality assessment or showed a minor allele frequency (MAF). A total of 404,604 (80.8%) SNPs were finally used for screening [161].

In this study, five SNPs were identified as showing either a significant or marginally significant genome-wide association with a multivariate phenotype combining affection status and onset age. Four of these markers were not related to APOE4. The first marker, **rs4420638**, is located 340 bp 3′ of APOC1 on chromosome 19q13 and very likely reflects the effects of the APOE4 allele (**rs429358**). The other markers are **rs11159647** (located in predicted gene NT_026437.1360 on chromosome 14q31.2), **rs179943** (located in **ATXN1** [MIM 601556] on chromosome 6p22.3,

rs3826656 (located in predicted gene NT_011109.848 on 19q13.33), and **rs2049161** (located in cDNA BC040718 on 18p11.31). These four SNPs were tested in three additional independent AD family samples composed of nearly 2700 individuals from almost 900 families. SNP **rs11159647** on chromosome 14q31 was primarily associated with age of onset (p=0.006), with a median reduction in onset age of 1.1 years being observed. Evidence of an association with this allele was also found in GWA data generated in an independent sample of ~1,400 AD cases and controls (p=0.04). None of these markers were previously described as modifiers of AD risk or onset age (Bertram 2008). The SNP **rs179943** on chromosome 6p22.3 is located within an intron of the ataxin 1 (ATXN1) gene. Although the function of ataxin1 is not known, it has been proposed to be associated with spinocerebellar ataxia type 1 (**SCA1**), a progressive neurodegenerative disease. The SNP **rs3826656** on 19q33 is located less than 2 kb proximal of the transcription initiation site of **CD33**. This protein is a cell-surface receptor on cells of monocytic or myeloid lineages. Additionally, it is a member of the SIGLEC family of lectins that bind sialic acid and regulate the innate immune system via the activation of caspase-dependent and caspase-independent cell death pathways.

7.5. Beecham 2009

Another GWAS was carried out by Beecham in 2009. This GWAS included 998 individuals of European descent, including 492 LOAD cases and 496 cognitive controls, using Illumina's HumanHap550 BeadChip. An additional 238 cases and 220 controls were also used in this study as a validation dataset for single-nucleotide polymorphisms (SNPs) that met the genome-wide significance criteria. The results showed associations of 38 SNPs with LOAD with uncorrected p values < 0.00005, six of which were in or near the APOE gene [162].

The most significant non-APOE SNP was rs11610206 on chromosome 12q13 (45.92 Mb), which presented an uncorrected $p=1.93X10^{-6}$. This SNP was genotyped in an independent replication dataset of 238 cases and 220 controls, resulting in a p value of $3.452X10^{-7}$, which was more significant than in the initial dataset. This SNP is not located in a known gene but is less than 10 kb from the FAM113B gene. Additionally, there are a number of nearby candidate genes, such as the *vitamin D* (1,25-dihydroxyvitamin D3) *receptor* (*VDR* [MIM 601769]) and *adhesion molecule with Ig-like domain 2* (*AMIGO2*) genes.

These authors also compared their results with those obtained by Reiman, and four polymorphisms were found that showed an association in both studies. Two of these SNPs, 1q42 and 19q13, are located within genes; the two other signals replicated in both datasets are not in known genes. The 1q42 SNP (rs12044355) resides in the DISC1 gene, which has been associated with schizophrenia and is linked to bipolar disorder, depression, and cognitive function. The 19q13 signal is located in and near exon 6 of *zinc finger protein 224* (*ZNF224* [MIM 194555]); two of the associated markers (rs4508518 and rs3746319) are within the exon. The first SNP (rs4508518) is a coding but synonymous polymorphism, whereas the second (rs3746319) leads to a missense mutation.

Finally, nine candidate genes from the over 500 genes in the AlzGene candidate gene list present SNPs with a nominal association in both GWASs. These genes (**ADAM12, CSF1, GBP2,**

KCNMA1, NOS2A, SORCS2, SORCS3, SORL1, and **WWC1)** exhibited p values ranging from 0.003 to 0.05 in the individual GWAS and from 0.0001 to 0.01 in the joint analysis.

Of these genes, the main candidate associated with the development of LOAD in several populations is the *sortlin-related receptor* (**SORL1**) gene. The mechanism by which SORL1 affects the development of Alzheimer´s disease is unknown, but it has been established to have the ability to interact with APP and APOE, possibly affecting the formation and accumulation of amyloid beta peptides.

7.6. Carrasquillo 2009

This genome-wide association study was performed in two stages using the Illumina Human-Hap300 array. In stage I, 313,504 SNPs were analyzed in 844 cases and 1,255 controls (2100 subjects from the Mayo clinic), and only six *APOE*-linked SNPs showed genome-wide significance in this stage of study. Of these polymorphisms, only rs2075650 (located on chromosome 19) showed genome-wide significance, and this SNP shows strong linkage disequilibrium (LD) with APOE (P value $4.8x10^{-46}$). In stage II, the 25 SNPs showing the most significant associations in stage I were genotyped in an additional 845 cases and 1,000 controls. These 25 SNPs included 10 SNPs in the APOE region on chromosome 19, all of which presented *P* values ranging from $9.5X10^{-79}$ to 0.05. The other 15 SNPs are located on other chromosomes. One of two SNPs on the X chromosome, rs5984894 (P value 0.0006), is located within the gene encoding protocadherin 11, X-linked (**PCDH11X**) in the Xq21.3/Yp11.2 region. To extend the analysis of *PCDH11X*, three *PCDH11X* SNPs (rs5941047, rs4568761 and rs2573905) residing in the same haplotype block as rs5984894 were genotyped in all stages. Highly significant associations were observed for all three SNPs, with *P* values of 1.6×10^{-7} (rs2573905), 8.0×10^{-5} (rs5941047) and 0.001 (rs4568761) being obtained. rs2573905 is located 8,483 bp 3′ of rs5984894 and is in strong linkage disequilibrium with rs5984894 (r2=0.98, D'=0.99). Analysis of rs5984894 by multivariable logistic regression adjusted by sex showed that the association was stronger in female homozygotes (OR=1.75, $P=2.0x^{10-7}$) and heterozygotes (OR=1.26, P=0.01). For hemizygous males, a similar trend was observed (OR=1.18), although this did not reach statistical significance (P-value 0.07) [163].

The ***PCDH11X*** gene contains at least 17 exons spanning over 700 kb. Alternative splicing of *PCDH11X* produces several isoforms that are mainly expressed in the brain, with particularly strong expression being detected in the cortex and hippocampus and weaker expression being observed in the cerebellum. The PCDH11X protein plays a fundamental role in cell-cell recognition and it is essential for the segmental development and function of the central nervous system. However, among all published and reported AD GWASs, this is the only one that reports involvement of an X chromosome locus, which, if confirmed, could at least partially explain the well-established increased disease prevalence in women versus men.

7.7. Harold 2009

In the first stage of this study, an association with the APOE locus (rs2075650, $p=1.8\times10^{-157}$) was established in 3,941 patients and 7,848 controls. Additionally, this GWA analysis identified

strong associations of SNPs in two new loci: rs11136000, which is located in the ***CLU,*** or ***APOJ***, gene ($p=1.4\times10^{-9}$), and rs3851179, a SNP 5′ to the *PICALM* gene ($p=1.9\times10^{-8}$). rs11136000 is located within an intron of the clusterin (*CLU*, also known as *APOJ*) gene on chromosome 8, and rs3851179 is found 88.5 kb 5′ of ***PICALM*** on chromosome 11. In stage 2, these new SNPs were genotyped in 2,023 AD cases and 2,340 age-matched controls from an independent sample. Associations were found for both polymorphisms, with p=0.017 and OR=0.905 for rs11136000 and p=0.014 and OR=0.897 for rs3851179. A meta-analysis of stages 1 and 2 was also conducted in this study, and the results showed highly significant evidence of associations for the *CLU* and *PICALM* loci (rs11136000 $p=8.5\times10^{-10}$ and rs3851179 $p=1.3\times10^{-9}$, respectively). Finally, no significant interactions of novel SNPs associated with *APOE* status were observed to influence AD risk (rs11136000x*APOE*4 interaction p=0.674; rs3851179x*APOE*4 interaction p=0.735) [164].

CLU is a secreted chaperone that can also be found in the cytosol under some stress conditions. It has been suggested that CLU is involved in several basic biological events, including cell death, tumor progression, and neurodegenerative disorders. The genetic risk allele (C) of CLU gene variant rs11136000 is carried by ~88% of Caucasians; the C allele confers 1.16 greater odds of developing late onset AD than the T allele [165].

PICALM is a phosphatidylinositol-binding clathrin assembly protein. This protein plays a role in altering synaptic vesicle cycling or APP endocytosis. Although the presence of polymorphism rs3851179 was associated with high significance related to the development of AD in the Caucasian population, these results could not be replicated in Chinese or Italian populations [166-167]. The results obtained in recent studies by Piaceri showed that the segregation of the PICALM rs3851179 variant did not show a statistically significant difference between LOAD cases and controls, suggesting a reduced risk of developing late onset Alzheimer´s disease (LOAD).

7.8. Han 2010

Unlike the studies described above, this study additionally established a relationship between the allelic variants found by GWAS and cerebrospinal fluid (CSF) levels of amyloid Ab1-42, T-tau, and P-tau181P [168]. The data used in this study was obtained from the Alzheimer´s Disease Neuroimaging Initiative (ADNI). This database consists of approximately 800 adults with ages between 55-90 years, 243 of whom were normal subjects, while 235 presented mild cognitive impairment, and 340 had been diagnosed with Alzheimer´s disease. These participants were genotyped using Illumina Human Genome 610 Quad BeadChips, and the CSF levels of amyloid Ab1-42, T-tau, and P-tau181P were determined in 410 subjects (119 normal, 115 MCI and 247 AD), of which 247 were men and 163 were women. An association analysis using age and APOE4 genotype as covariates was also performed, but did not incorporate principal component analysis.

The results showed that T-Tau levels are higher in AD patients than in control subjects. When the results were adjusted using APOE and the age of individuals as covariates, it was not possible to observe an association between SNPs and CSF levels among patients. This study also identified polymorphisms associated with the development of AD that had been already

reported in previous studies: CYP19A1 (rs2899472, p=1.90 × 10-7) and NCAM2 (rs1022442, p=2.75 × 10-7).

8. Population genetics and genetic association studies: crucial issues to enhance the transparency of results.

Although efforts to obtain genetic biomarkers that help in anticipate diagnostic of Alzheimer disease, present-day the clinical research not have the results that expected. The publications that relate genes with Alzheimer disease has increased exponentially however, numerous lines of evidence have demostrated discrepant results among populations. These findings suggest that it is neccesary diminish the confounder factors and focus on identify the cause [169]. Once the causes are established, could integrated in practice of medicine helping with anticipate diagnostic.

In order to avoid spurious associations Little J. et al published an initiative that pretends increase the quality of reporting genetic association studies dissemining this information in different journals (epidemiology, clinical investigation, internal medicine and basic research) [170-176]. The publication refered as STREGA report (STrenghthening the REporting of Geenetic Association studies) provides additional comments to 22 items reported previously by STROBE (STrengthening the Reporting of OBservational Studies in Epidemiology) [177]. These comments include different items, however population genetics topics are crucial issues whose depreciation, increase statistical mistakes type I and II [173].

One of the most important topics in genetic association studies (GAS) is Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium (HWE) is represented by the equation $(p+q)^2=1$, whose perfect square binomial equation it is represented by $p^2+2pq+q^2=1$, where p^2 and q^2 represented homozygous state, whereas 2pq represented heterozygous state. Under random maiting and non-overlapping, homozygous and heterozygous states are in equal proportions (0.5 each one) maintaining the HWE. This equilibrium are also maintained when evolutionary forces are absents (mutation, random drift, genetic flow, natural selection), the population size it is nearby to the infinitum, and when frequencies of alleles in both sexes are equal [178]. However, some conditions could modify these proportions provoking a Hardy-Weinberg departure (HWD). HWD is related with an excess of homozygous individuals (with subsequent heterozygous deficit) or heterozygous individuals (with subsequent homozygous deficit). Therefore, Hardy-Weinberg model is an essential element used to analyze genetic data, and is the initial step for check the quality of genotyping, because genotyping errors due to poor quality provoke HWD as a consequence of distort in genotype distribution [179]. Nevertheless, HWD are not only related with genotyping mistakes because some factors as demographic events, young population, founder effect, inbreeding, and population stratification may provoke HWD.

Population stratification is the consequence of populations with a recent miscegenation. Admixture populations show different allele frequencies among different subpopulations that conform the whole population, which consequently is not a homogeneous population [180].

Indeed, admixture population is an heterogeneous population with dissimilar ancestry proportions. Consequently, the population stratification may lead to spurious associations because each subpopulation are not equally represented [169, 179]. Applying these premises to GAS, the differences of frequencies between cases and controls populations could be related with dissimilar frequencies among different population strata rather than association of genes with disease. Therefore, population stratification is the most common problem and one of the most important confounder factors in GAS [180-181].

At first glance could appear that HWD are only related with false associations nevertheless, HWD could also be a singnature of disease association, principally in case-control studies, because if some allele is associated with a disease this association break the random maiting provoking HWD [182-183]. This HWD is the result of differences between allele frequencies, where one allele are overrepresented in cases group (excess of homozygous), whereas the same allele are underrepresented in control cases (excess of heterozygous). In order to suport these findings it is neccesary to know the frequency of distribution of this allele in the general population. If the allele show a high frequency in the general population, this finding could be not related with the disease [182, 184-185]. Conversely, if the prevalence of the allele is low in the general population, these data may support a relationship between the allele and the disease suggesting the allele could be a risk allele. As a consequence, the HWD is particularly relevant in GAS.

In light of these evidence, several methods have been developed to detect HWD. The most used method is chi square, however this statistic only must be used in homogeneous population [186]. Other approach is detect the intrapoblational variance (F_{is}), where $F_{is} > 0$ means a homozygous excess, whereas $F_{is} < 0$ means heterozygous deficit [187]. Recently, Li M and Li C have developed a likelihood test that allows assessment of HWD taking into account potential association with the disease [182]. This method can differentitate HWD caused by disease association, diminishing the over estimation of type I error and avoiding the false exclusion of associated markers. Hence, is necessary diminish the effect of genetic structure in order to detect susceptibility loci for complex disease. Studies to date suggest different methods, among which are:

- Genomic control. This method diminish the population heterogeneity due to cryptic relatedmess or correlation across individuals, correcting the variance inflation, which is previously detected with unlinked null markers [188].
- Infer the number of populations. This Bayesian analysis inferrers the number of subpopulations (k) and correct them, decreasing the effect of admixture over GAS [189].
- Summarize the genetic background using hierarchical clustering through principal component analysis (PCA) and its variants enable the detecction of differences between samples, detect clinal distributions and suggest other demographic events as isolation-by-distance [190-191].

All of these bioinformatic models have been an excelent help to clarify the genetic associations in population-based genetic associations increasing the statistical power. These methods have detected limitations or errors in assessments genotypes (20-70%) [192], as well as spurious

association rates (40%) [193]. However, these methods help to identify rare variants that could have a role in common disease etiology [194]. Hence, all of these variants have an implications in desing, analysis and interpretation of GAS, and are a good strategy for developing markers to elucidate the origins of many human genetic diseases. This alternative approach of anticipated diagnosis can significantly reduce treatment costs by providing preventive medicine

9. Conclusions

Alzheimer´s disease is one of the main causes of dementia. This disease is clinically characterized by the irreversible and progressive loss of memory and it is histopathologically characterized by the presence of neuritic plaques (NP) and neurofibrillary tangles (NFT). Both types of lesions are formed due to the accumulation of insoluble protein aggregates, consisting of beta amyloid peptide and the microtubule binding protein Tau, respectively. Studies performed in the last thirty years have provided important advances in understanding the molecular mechanisms involved in the pathology of AD. Through genetic studies, it has been possible to identify the presence of mutations in the APP, PS1 and PS2 genes as causal factors for early onset Alzheimer´s disease (EOAD). These mutations are associated with beta amyloid peptide accumulation, which generates a series of molecular events that lead to a neurodegenerative process. With respect to late onset Alzheimer´s disease (LOAD), the results obtained to date do not support amyloid overproduction as a cause; in this case, it has been proposed that alterations in the mechanisms responsible for peptide clearance indirectly favor the amyloid accumulation. Amyloids have the ability to interact with several different receptor types, including the Frizzled, insulin, NMDA and NGF receptors, triggering events leading to neuronal death. Additionally, it is known that molecules such as APOE play an important role in the clearance and aggregation of amyloid beta and other risk factors that may eventually determine the conformational changes that allow amyloids to aggregate and form neuritic plaques. For LOAD, APOE is the single most important risk factor. However, a recent GWAS identified several susceptibility loci associated with disease development in different populations, although these studies provide a better understanding of the pathophysiology of the disease, these new genetic markers seem to have a weak genetic effect. Therefore, it is necessary to consider using other tools to detect genotyping errors that can be caused among other reasons, by population stratification.

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