

Current knowledge on the relationship between ApoE and Alzheimer's disease

BACHELOR ESSAY FOR THE PREMASTER MOLECULAR BIOLOGY AND
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Abstract

Alzheimer's disease, the leading cause of dementia worldwide, is a progressive age-dependent neurological disease that mostly impacts seniors (>65). Currently an estimated 50 million people are living with dementia, this number will double every 20 years due to rising life expectancies. There is currently no cure to treat or slow down disease progression in AD. However, a lot of effort is put into research to expand the current knowledge of the pathogenesis of- and risk-factors involved in the origin and development of AD. AD is mostly a sporadic disease, with only ~5% of patients suffering from familial AD (FAD). Amyloid beta (A β) plaques and neurofibrillary tau-tangles are hallmark features seen in AD patients brain. Moreover, chronic neuroinflammation, neuronal dystrophy and loss of neuronal connections are also signs of AD. Apolipoprotein E (ApoE) is a lipid transporter protein in the brain, humans can carry three ApoE genotypes, ApoE2, ApoE3 and ApoE4. ApoE4 is a well known risk factor for AD, whereas ApoE2 is believed to be protective and ApoE3 neutral. Research suggests that ApoE influences AD by affecting A β aggregation and plaque formation. However, there is no clear idea how ApoE is exactly involved in AD. Recent research provides novel insights in the molecular workings of the ApoE isoforms on Alzheimer's pathogenesis, novel pathways have been discovered that show involvement of ApoE in A β production and neuroinflammation. In this essay these recent findings concerning the relationship between AD and ApoE will be presented.

Abbreviations

ASO	Antisense oligonucleotide
A β	Amyloid Beta
AD	Alzheimer's disease
DLK	dual leucine-zipper kinase
ERK1/2	Extracellular signal-related kinase 1 and 2
MAPK	Mitogen activated protein kinase
MAPT	Microtubule associated protein tau
MEFs	Mouse embryonic fibroblasts
MGNd	Microglial neurodegenerative phenotype
MO	Microglial homeostatic molecular and functional signature
NFTs	Neurofibrillary tangles
PA	Physical activity
SP	Senile plaques
TGFb	Transforming growth factor b

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1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is marked by the decrease of connections between neurons and the loss of neurons. AD was first described by Dr. A. Alzheimer in 1907, he described a patient who was disoriented, confused, had impaired memory and was unable to understand certain questions. Post-mortem examination of the patient's brain showed accumulation of unusual neuro-fibrils and proteins and loss of neuronal cells¹. The risk of developing AD increases with age, with 95% of AD patients suffering from late-onset AD (LOAD) aged >65, only 5% develop symptoms earlier in life, suffering from early-onset AD (EOAD) or familial AD (FAD). Nowadays approximately 50 million people worldwide suffer from AD or any related disease, due to rising life-expectancy and the aging populations this number is estimated to double by 2050, generating major health-care costs and heavy strains on care-givers. Because of the higher occurrence the interest in AD has grown in the last decades. Even though AD has been studied for years, still not much is known about the exact pathogenesis, onset, development and molecular biology behind the disease. As previously mentioned 95% of AD patients are >65, however the disease starts developing years to decades before its onset and is possibly due to numerous risk- or protective factors².

1.1 Pathology of Alzheimer's disease

1.1.1 Disease progression

AD starts with a pre-clinical phase referred to as mild cognitive impairment (MCI), in this stage the patient has mild memory loss. MCI can progress to Alzheimer's disease. In AD patients, neurons and synapses are affected badly and patients are progressing through the disease. Patients will lose more memories along the way and become more disorientated. After a while of disease progression, AD patients reach severe cognitive impairment and are inevitably completely dependent on help and health-care.

1.1.2 Pathological features

AD is characterized by two hallmark features, extracellular senile plaques (SP) consisting mostly of amyloid beta protein ($A\beta$) and intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau protein. Post-mortem evaluation of AD brain also reveal several other abnormalities like: neuritic dystrophy, microglial activation, neuro-inflammation and loss of synapses. These changes occur foremost in regions that regulate memory and acquired skills.

1.1.2.1 Neurofibrillary tangles

The neurofibrillary tangles are composed of microtubule associated protein tau (MAPT). MAPT is mainly responsible for stabilizing microtubules. In AD tau is hyperphosphorylated, which causes the protein to form aggregates or tangles. These neurofibrillary tangles can eventually cause the neuronal cell undergo apoptosis in later stages of AD.

1.1.2.2 Amyloid β peptide and senile plaques

$A\beta$ peptides derive from the ~110 kDa amyloid precursor protein (APP), a transmembrane protein widely expressed in neurons. The most abundant forms of $A\beta$ peptides end respectively at amino-acids 40 (~90 %) and 42 (~10 %) and are products of amyloidogenic proteolytic cleavage of APP. APP is first cleaved by β -secretase (β -site APP cleaving enzyme 1; BACE1), this is also the rate-determining step in APP processing and secondly by γ -secretase. Proteolytic cleavage of APP with γ -secretase results in C-terminal heterogeneity, acquiring $A\beta$ peptides with different lengths. Figure 1 illustrates APP processing in both the amyloidogenic and non-amyloidogenic pathway³.

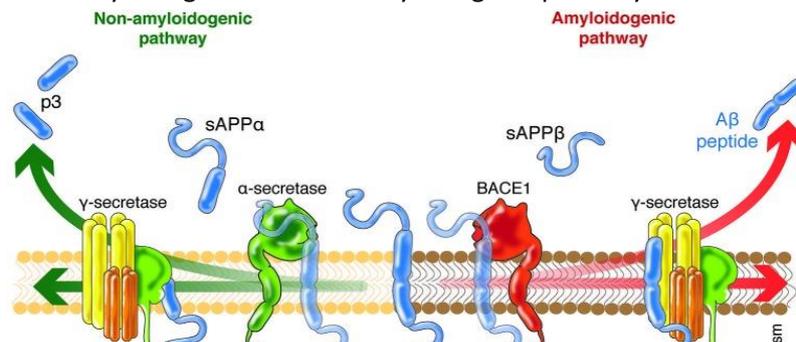


Figure 1: APP processing, the non-amyloidogenic pathway and the amyloidogenic pathway, where APP is cleaved by B- and Y-secretase

Although, $A\beta_{1-42}$ is less abundant, it is considered a more amyloidogenic peptide than $A\beta_{1-40}$, meaning that it is more prone to aggregation and is able to act as a seed to stimulate aggregation. Tendency to form aggregates is mostly determined by the C-terminus of $A\beta$, causing the peptide to become more hydrophobic. Here for, insoluble amyloid plaques in AD brain consist mainly of $A\beta_{1-42}$. Figure 2 shows the different aggregation species $A\beta$ can form.

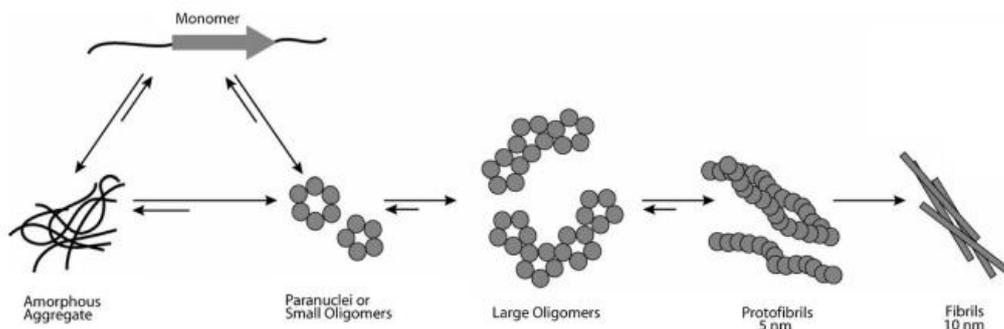


Figure 2: aggregation species of amyloid beta

Soluble oligomeric species are suggested to be a highly toxic form, causing a high neurotoxic response to neuronal cells and decreasing their viability⁴. According to the amyloid hypothesis it is the formation and seeding of amyloid plaques that trigger a toxic cascade which leads to the development of AD. However, amyloid deposition alone does not necessarily point towards AD, since it is also a common observation in post mortem non-AD brain. Studies have revealed that $A\beta$ deposition stretches for decades prior to the onset of AD⁵. The exact function of $A\beta$ is not entirely clear. However suspected is

that A β is involved in several processes under which: protection against oxidative stress and regulation of cholesterol metabolism.

1.2 Neuroinflammation and Alzheimer's disease

The presence of A β -plaques and neurofibrillary tangles activate neuro-inflammatory cells, such as astrocytes and microglia, and promote the release of pro-inflammatory cytokines, including TNF- α , reactive oxygen species (ROS) and chemokines. Microglial cells are permanently activated in AD, causing chronic neuroinflammation which promotes neuronal death.

1.3 Apolipoprotein E and Alzheimer's disease

Apolipoprotein E (ApoE) is a protein mainly produced by liver tissue (75%), followed by astrocytes and microglia in the central nervous system and brain. The primary neurological function of Apolipoprotein E is the transportation of lipids like cholesterol to neurons via cell surface ApoE receptors for the maintenance of synapses. The human ApoE gene contains several polymorphisms that result in three common allele isoforms in the gene namely: ApoE2, ApoE3 and ApoE4. The ApoE4 allele is known as the highest genetic risk factor for late-onset AD, ApoE4 carriers have a higher risk of developing AD with earlier and greater disease progression. While ApoE2 is associated with a lower risk of AD compared to the most prevalent and neutral form, ApoE3. Studies indicate that ApoE4 enhances the accumulation and deposition of A β and enhances A β -oligomerization⁶. However it is unclear what the exact influence of ApoE in the development, onset and progression of AD is and how ApoE has an effect on A β pathology. Increasing the biological knowledge of the influence of ApoE on AD could give new insights into ApoE targeting therapies to prevent, delay or even cure AD.

1.3.1 Apolipoprotein E and A β aggregation

Apolipoprotein E (ApoE) is a glycoprotein consisting of 299 amino acids. The N-terminal (residues 1-167) contains a receptor-binding region (residues 136-150) and the C-terminal domain (residues 206-299) contains a lipid-binding region (residues 244-272). ApoE also contains heparin binding sites within the N-terminal and C-terminal domain. Sequencing of the complete ApoE amino acid showed differences between the ApoE isoforms. ApoE2 has Cys residues at positions 112 and 158, ApoE3 has a Cys residue at 112 and an Arg residue at 158 and ApoE4 has Arg residues at both positions. ApoE3 and ApoE2 prefer high-density lipoproteins (HDL), whereas ApoE4 binds more to very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL). These differences likely induce changes in the protein which influence the biological function of ApoE. Figure 3 shows ApoE with binding-sites and the locations of polymorphisms in the isotypes⁷. Neurons contain immunoreactive ApoE but do not contain ApoE mRNA, so a metabolic pathway must exist where (lipidated) ApoE is taken up by neuronal cells. Major neurological ApoE receptors are those of the low-density lipoprotein receptor (LDLR) family, these include LDLR, LDLR-related protein 1 (LRP1), ApoE can bind directly to these receptors.

Also, A β can bind directly to LDLR, in vitro experiments showed that overexpression of LDLR increases cellular uptake of A β in astrocytes⁸. Furthermore, LDLR importance in ApoE metabolism is clear since LDLR deficiency in mice leads to higher ApoE levels in the brain⁹. ApoE2 catabolism through LDLR uptake is probably slower compared to ApoE3 and ApoE4, since ApoE2 has lower affinity to LDLR. Because of this, ApoE2 carrying individuals might be protected from A β accumulation in the brain. In addition, ApoE also binds to heparan sulfate proteoglycans (HSPG), where the ApoE C-terminal shows highest affinity for binding to heparin. Binding of ApoE to HSPG is most likely a two-step pathway, where first the C-terminal of ApoE binds to HSPG, where after its transferred to LRP1 and ApoE is taken up. Interestingly, ApoE isoforms show different bindings affinity's to HSPG, where ApoE2 has the lowest binding affinity and ApoE4 the highest¹⁰. A β also has been shown to bind to HSPG through its heparin binding domain (amino-acids 13 through 17)¹¹, indicating that A β and ApoE are taken up into cells by a common pathway, competing with each other through their heparin binding domains¹⁰.

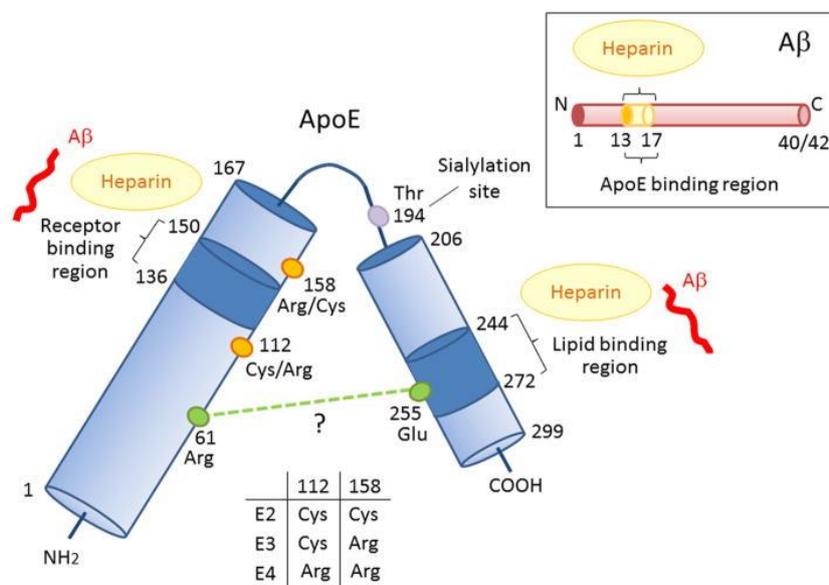


Figure 3: ApoE, AB and heparin. Illustrated image showing functional sites in ApoE as well as the binding sites of ApoE and AB with heparin, also showing the differences between the isoforms of ApoE.

2 Novel insights in the relationship between Apolipoprotein E and Alzheimer's disease

It is clear that ApoE influences AD pathology and progression, however it is not clear why ApoE4 is such a high risk-factor for AD and why ApoE2 seems to be protective. Plenty of research has been done in the past few years and many of these provide novel insights into the mechanisms behind the ApoE-AD relationship. In the next chapter some of the new findings in this field will be discussed.

2.1 ApoE affects amyloid pathology during early A β seeding stage

2.1.1 ApoE4 accelerates early seeding of amyloid pathology:

As previously mentioned, A β pathology is affected by ApoE, where ApoE4 is the highest genetic risk factor for late-onset AD, enhancing A β aggregation and deposition. As amyloid pathogenesis has been shown to develop decades before the onset of AD, an important question to ask is if there is a critical window of time where ApoE4 especially impacts A β aggregation and amyloid seeding. A recent study done by *Liu et al* revealed that ApoE4 accelerates and promotes amyloid deposition in mice during the early amyloid seeding stage¹². In this study ApoE3 or ApoE4 expression was induced in mice either during the seeding stage (0-6 months) the rapid growth period (6-9 months) or for the entire periods (9 months). During the seeding stage small aggregates are formed, in the rapid growth period these aggregates extent rapidly and eventually form A β -plaques. What was interesting is that the expression of ApoE4 during the seeding period (0-6 months) significantly increased soluble and insoluble A β ₁₋₄₀ and A β ₁₋₄₂ and promoted fibrillar plaque load and amyloid pathology, whilst ApoE3 expression did not have this effect. Expression of ApoE4 during the rapid growth period had no significant effect on A β ₁₋₄₀ and A β ₁₋₄₂ levels, suggesting that ApoE4 affects amyloid pathology during the early seeding stages of A β . Furthermore, ApoE4 increased levels of neurotoxic A β -oligomers when expressed during the seeding stage, but not during the rapid growth period. Finally, neuronal dystrophy, a pathological feature of AD which is induced by fibrillar A β ¹³, was shown to be increased near amyloid plaques in the ApoE4 0-6 and 0-9 months mice but not in the ApoE3 expressing mice. These results suggest that ApoE4 may serve as a A β seeding factor in the early stage of AD and that ApoE isoforms have differential effects on A β pathology, such as neuritic dystrophy.

2.1.2 Age-dependent effect of ApoE reduction using antisense oligonucleotides in a model of β -amyloidosis

Another recent study performed by *Huynh et al* investigated if reduction of ApoE levels before and after the onset of A β pathology could affect amyloid deposition¹⁴. In this study, a specific antisense oligonucleotide (ASO) that reduces ApoE expression by 50% was used in APP/PS1-21 mice homozygous for either ApoE4 or ApoE3. ASO treatment was either started at the postnatal day 0 (PO, before the onset of A β deposition) or after 6 weeks of age (at the onset of A β deposition). They found that A β -pathology was significantly reduced when ASO treatment started at PO, but not when ASO treatment was given at 6-weeks, regardless of ApoE isoform. Interestingly, a significant decline in dystrophic

neurites compared to control mice was found in the PO and 6 week ASO-treated ApoE4 mice, independently of A β -plaque size. Implying a role for ApoE4 in neurotoxic response to A β -plaques. Dystrophic neurites are commonly found in AD-brains, but there is not yet a good understanding to what causes the development of neurotoxic dystrophy. Finally, they established that the decline of A β pathology found in ASO PO-treated mice was not due to changes in the APP metabolism pathway. These results suggest that ApoE possibly has a role in the early nucleation/seeding stage of A β -pathology, but does not likely influence the plaque formation during the growth period or APP metabolism, this is also in line with the results of *Liu et al* that were described before.

2.2 ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy

A β pathology is probably a key initiator in AD. However, A β accumulation and aggregation poorly correlate with disease symptoms or tissue loss in AD patients¹⁵. Moreover, tau-pathology, the second major pathology found in AD brains, strongly correlates with clinical signs and neurodegeneration in AD and other tauopathies¹⁶. So far it was not clear if ApoE influences tau-pathology and if this happens independently from A β pathology. A recent study by *Shi et al* investigated if and how the presence of ApoE affected tau pathology, they found that ApoE affects neurodegeneration in context of tau pathology independently from A β pathology and that ApoE4 aggravates neurodegeneration whilst the absence of ApoE was strongly neuroprotective¹⁷. In this study a mouse model was generated overexpressing 1N4R human tau with the P301S tauopathy mutation on either a ApoE knock-in (KI) or knock-out (KO) background. Significantly more brain atrophy was found in 9-month old P301S/ApoE4 mice compared to P301S/ApoE2 and P301S/ApoE3 mice. Interestingly, neuronal loss and brain atrophy was considerably declined in P301S/ApoE KO (TEKO) mice. Revealing a role of ApoE in regulating tau-mediated neurodegeneration by possibly affecting tau conformation and progression. Chronic neuroinflammation is found in several tauopathies including AD and can lead to neuronal death and thus brain atrophy. Interestingly, this study found microglial upregulation of proinflammatory genes in P301S/ApoE4 mice, microglia in TEKO mice remained mostly homeostatic. The enhanced ApoE4 related neuroinflammation might increase neurodegeneration. Moreover, severe neuronal death and high levels of TNF- α were observed whilst co-culturing P301S tau expressing neurons with ApoE expressing glia cells, with the ApoE4 glia/P301S neuron co-culture displaying the highest neuronal death. Finally, post-mortem evaluation of individuals with tauopathies showed that possession of the ApoE4 allele is associated with more severe neurodegeneration, with or without A β pathology.

2.3 ApoE2, ApoE3 and ApoE4 differentially stimulate APP transcription and A β secretion

It is established that ApoE effect AD pathogenesis, possibly in multiple ways. Several studies have found that ApoE activates MAP-kinase kinase kinase DLK (dual leucine-zipper kinase, DLK) in rodent neurons, which are prominently expressed in brain¹⁸. However, the exact relationship of ApoE and the activation of MAP-kinase and the consequences had not been investigated. *Huang et al* investigated the effect of ApoE2, ApoE3 and ApoE4 on human neurons and showed that ApoE isoforms function as signalling molecules and differentially activate a MAP-kinase pathway that eventually stimulates APP transcription¹⁹. Neurons co-cultured with glia cells excreted 2-3 fold more A β 40 and A β 42 than neurons co-cultured with mouse embryonic fibroblasts (MEFs), they found that ApoE, IGF2 and IGFbp2 molecules secreted by glia cells increased A β 40 and A β 42 production. Further analysis revealed that A β secretion in human neurons was stimulated by ApoE in different potencies of ApoE4>ApoE3>ApoE2. It was confirmed that ApoE activated a MAP kinase (MAPK) pathway in neurons through binding with ApoE receptors which increased phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) 2 to 5 fold. Furthermore, they also found that ApoE induced DLK protein levels human neurons 2 to 4 times. It was later established that ApoE activates a non-canonical MAPK signal transduction pathway where ApoE increases DLK levels, after which DLK phosphorylates MKK7 which in turn phosphorylates ERK1/2. The ApoE isoforms differentially influenced DLK levels, MKK7 and ERK1/2 phosphorylation in a potency of ApoE4>ApoE3>ApoE2, this pathway is shown in Figure 4.

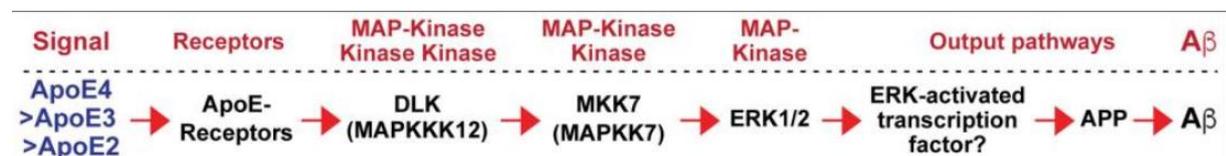


Figure 4: ApoE induced non-canonical MAPK pathway, *Huang et al 2017*, ¹⁹

Following this discovery, the effect of ApoE on APP levels were measured to establish how ERK1/2 induced or stimulated A β production. ApoE increased APP mRNA and protein levels 3-5 times in human neurons co-cultured with MEFs, again in a ApoE4>ApoE3>ApoE2 potency. This research demonstrates a novel pathway in which ApoE binding to receptors activates DLK, which in turn activates MKK7 and ERK1/2. Eventually phosphorylated ERK1/2 induces cFos phosphorylation, which stimulates transcription factor AP-1, AP-1 enhances APP transcription and finally the increase of APP causes an increase of A β 40 and A β 42. Thus, ApoE can possibly also influence AD pathology by stimulating APP transcription. The full pathway is shown in Figure 5.

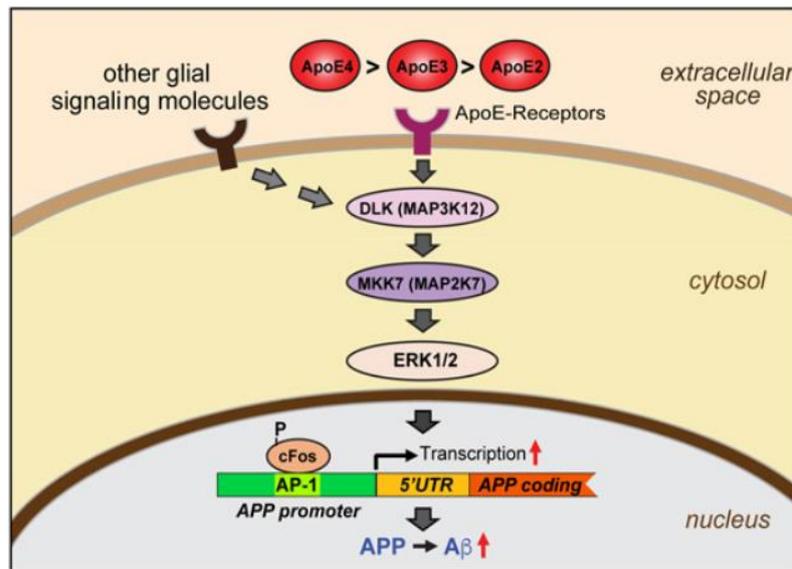


Figure 5: ApoE activated MAP kinase pathway which ultimately increase APP and Aβ levels (Huangh et al, 2017)

The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases

Among other features, neuronal cell death and neuroinflammation are also common aspects of AD and other neurodegenerative diseases. Microglia in a healthy brain have a unique homeostatic signature (M0) which is regulated by transforming growth factor B (TGFb) signalling. During development of AD microglia become chronically inflamed and display a disease-associated microglia signature. So far, the mechanisms that regulate the microglial phenotypes in neurodegenerative diseases has not been identified. A recent study done by *Krasemann et al*²⁰ identified a ApoE-TREM2 pathway that influences the neurodegenerative phenotypic switch, the exact findings of this research will now be laid out. By performing gene expression analysis on aging and neurodegenerative microglia, two gene clusters were identified, cluster 1 was associated with loss of 68 homeostatic genes and cluster 2 was associated with the upregulation of 28 inflammatory molecules, this distinct phenotype will be referred to as microglial neurodegenerative phenotype (MGnD). Ingenuity pathway analysis (IPA) revealed that ApoE upregulation and TGFb signalling suppression are major regulators of MGnD microglia. Furthermore, demonstrated was that the microglial phenotypic switch from M0 to MGnD in APP-PS1 mice and AD patients brains was associated with neuritic plaques, another feature of AD. Microglia associated to neuritic plaques were identified by expression of the Clec7a (Clec7a+), an inflammatory molecule, and suppression of P2ry12 (P2ry12-), a homeostatic gene. The transition of M0 microglia to Clec7a+P2ry12- MGnD microglia was also associated with upregulation of ApoE signalling. Further data showed that MGnD microglia and ApoE signalling were induced by phagocytosis of apoptotic neurons (dNs). Moreover, ApoE signalling and the latter microglial phenotypic switch was mediated by TREM2. TREM2 is a receptor of the innate immune system, which also has been suggested to affect neuroinflammation. *Krasemann et al* demonstrated a TREM2-ApoE

pathway that induces and regulates a phenotypic switch in microglia from M0 to MGnD. Figure 6 summarises the findings of this research.

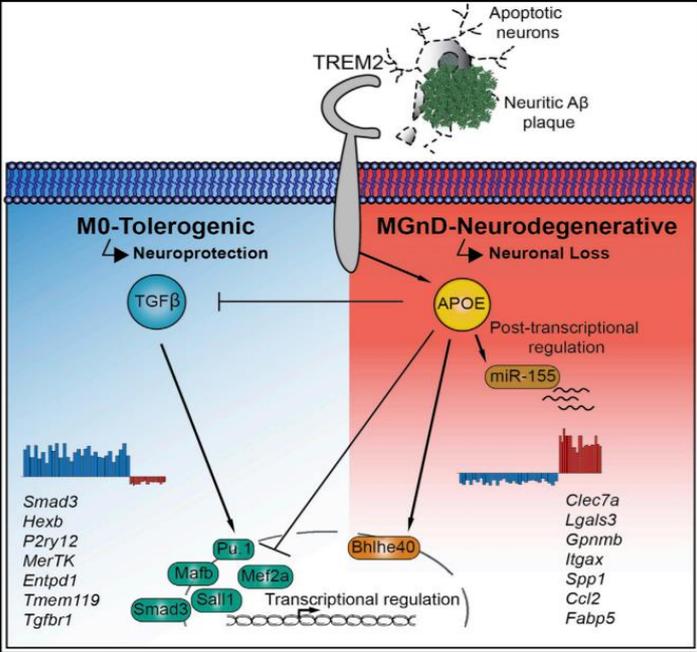


Figure 6: M0 microglia express homeostatic genes such as *Smad3*, *P2ry12*, *MerTK* and *Tmem119*, MGnD microglia express inflammatory genes such as *Clec7a*, *Gpnmb*, *Spp1* *Fabp5*. ApoE signalling 1) suppresses microglial homeostatic transcriptional factors such as *Mef2a*, *Mafk*, *Smad3* and 2) induces inflammatory gene transcription factors such as *Bhlhe40* and transcriptional and translational regulator *miR-155*²⁰

3 Discussion

It can be established that Alzheimer's disease is a very complicated disease. The likelihood of developing sporadic AD presumably depends on predisposed genetic risk-factors and lifestyle choices. In this essay novel insights from recent studies have been presented.

It has been established that ApoE isoforms influence A β aggregation and clearance distinctively²¹, however novel insights presented by *Liu et al.* and *Huynh et al.* both indicate that ApoE4 especially influences A β deposition and aggregation in the early seeding stage. Both papers suggest that ApoE4 might serve as a 'seed' to promote A β and aggregation. As regards to neuritic dystrophy, *Lui et al* found an increase in neuronal dystrophy near amyloid plaques in mice expressing ApoE4 but not in ApoE3 (0-6 m, 0-9, 6-9 months) and ApoE4 (6-9 months) expressing mice. *Huynh et al* found a significant decline in dystrophic neurites in mice where ApoE4 expression was reduced with ASO-treatment. These results imply that ApoE is a possibly participates or influences the toxic response of neurons towards A β -plaques. If ApoE4 is indeed most involved in A β -pathology in the early seeding stages, therapy could be developed in the future that down-regulates ApoE4 expression. Thinking of ASO-treatment, gene therapy of mRNA intervention to reduce amyloid deposition and neuritic dystrophy.

Moreover, it was mentioned that even though A β is a pathological feature of AD it poorly correlates with AD progression and disease. In contrary, the presence of phosphorylated tau does strongly correlate with AD symptoms and disease progression. A study of *Shi et al* showed that ApoE2, ApoE3 and ApoE4 influence tau-pathology independently of A β pathology. Furthermore, neuroinflammation and brain atrophy was elevated in ApoE4 expressing mice. ApoE4 also had the greatest effect on neuroinflammation, neurodegeneration and neuronal viability. ApoE isoforms each influenced tau and AD pathology distinctively, with ApoE4 displaying to be the most toxic isoform in regards to AD pathology. This research is relevant because it displays a possible novel pathway in which ApoE is involved in AD pathology.

In addition, *Huang et al* uncovered a novel pathway where glial ApoE stimulates neuronal A β secretion by activating a MAP-kinase cascade that increases APP transcription. This pathway is differentially stimulated by the three ApoE isoforms in a ApoE4>ApoE3>ApoE2 potency order. *Huynh et al* described that ApoE silencing by ASO-treatment had no effect on APP processing and thus A β production and levels, which is contradictory to what *Huang et al* showed. However, *Huynh et al* tested APP processing in PS1/APP transgenic mice, whereas *Huang et al* conducted research in vitro on cultured human neurons. These different research settings possibly gave the contrasting results.

Finally, *Krasemann et al* found that phagocytosis of apoptotic neurons stimulate ApoE signalling in an ApoE-TREM2 signalling pathway which triggered the switch from homeostatic microglia to

neurodegenerative microglia, which are chronically inflamed. Thus, ApoE is possibly also involved in initiating neuroinflammation in AD. AD brains are chronically inflamed, this causes disease progression and neurodegeneration. Targeting the ApoE-TREM2 pathway induced the switch from neurodegenerative microglia to homeostatic microglia. Hence, targeting this pathway in AD could possibly restore homeostatis and slow down disease progression.

All findings considered, it can be concluded that the effect that ApoE has on AD pathogenesis is very intricate. The recent findings give rise to many future possibilities in which AD can be targeted. Of course, more research is needed to investigate more fully these novel pathways that influence the pathogenesis of Alzheimer's disease.

4 References

1. Stelzmann, R. A., Norman Schnitzlein, H. & Reed Murtagh, F. An english translation of alzheimer's 1907 paper, 'Über eine eigenartige erkankung der hirnrinde'. *Clin. Anat.* **8**, 429–431 (1995).
2. Bateman, R. J. *et al.* Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *N. Engl. J. Med.* **367**, 795–804 (2012).
3. Zolezzi, J. M., Bastías-Candia, S., Santos, M. J. & Inestrosa, N. C. Alzheimer's disease: Relevant molecular and physiopathological events affecting amyloid- β brain balance and the putative role of PPARs. *Frontiers in Aging Neuroscience* **6**, (2014).
4. Chromy, B. A. *et al.* Self-assembly of Abeta(1-42) into globular neurotoxins. *Biochemistry* **42**, 12749–12760 (2003).
5. Villemagne, V. L. *et al.* Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: A prospective cohort study. *Lancet Neurol.* **12**, 357–367 (2013).
6. Hashimoto, T. *et al.* Apolipoprotein E, Especially Apolipoprotein E4, Increases the Oligomerization of Amyloid Peptide. *J. Neurosci.* **32**, 15181–15192 (2012).
7. Kanekiyo, T. *et al.* ApoE and A β in Alzheimer's disease: accidental encounters or partners? *Neuron* **81**, 740–54 (2014).
8. Basak, J. M., Verghese, P. B., Yoon, H., Kim, J. & Holtzman, D. M. Low-density lipoprotein receptor represents an apolipoprotein E-independent pathway of A β uptake and degradation by astrocytes. *J. Biol. Chem.* **287**, 13959–13971 (2012).
9. Fryer, J. D. *et al.* The low density lipoprotein receptor regulates the level of central nervous system human and murine apolipoprotein E but does not modify amyloid plaque pathology in PDAPP mice. *J. Biol. Chem.* **280**, 25754–25759 (2005).
10. Winkler, K. *et al.* Competition of A β amyloid peptide and apolipoprotein E for receptor-mediated endocytosis. *J. Lipid Res.* **40**, 447–455 (1999).
11. Kanekiyo, T. *et al.* Heparan Sulphate Proteoglycan and the Low-Density Lipoprotein Receptor-Related Protein 1 Constitute Major Pathways for Neuronal Amyloid- Uptake. *J. Neurosci.* **31**, 1644–1651 (2011).
12. Liu, C. C. *et al.* ApoE4 Accelerates Early Seeding of Amyloid Pathology. *Neuron* **96**, 1024–1032.e3 (2017).
13. Grace, E. A., Rabiner, C. A. & Busciglio, J. Characterization of neuronal dystrophy induced by fibrillar amyloid β : Implications for Alzheimer's disease. *Neuroscience* **114**, 265–273 (2002).
14. Huynh, T. P. V. *et al.* Age-Dependent Effects of apoE Reduction Using Antisense Oligonucleotides in a Model of β -amyloidosis. *Neuron* **96**, 1013–1023.e4 (2017).
15. Josephs, K. a. *et al.* Beta-Amyloid Burden Is Not Associated With Rates of Brain Atrophy. *Ann. Neurol.* **63**, 204–212 (2008).
16. Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T. & Hyman, B. T. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–631 (1992).
17. Shi, Y. *et al.* ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* **549**, 523–527 (2017).
18. Qiu, Z., Hyman, B. T. & Rebeck, G. W. Apolipoprotein E receptors mediate neurite outgrowth through activation of p44/42 mitogen-activated protein kinase in primary neurons. *J. Biol. Chem.* **279**, 34948–34956 (2004).
19. Huang, Y. W. A., Zhou, B., Wernig, M. & Südhof, T. C. ApoE2, ApoE3, and ApoE4 Differentially

- Stimulate APP Transcription and A β Secretion. *Cell* **168**, 427–441.e21 (2017).
20. Krasemann, S. *et al.* The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity* **47**, 566–581.e9 (2017).
 21. Kim, J. *et al.* Anti-apoE immunotherapy inhibits amyloid accumulation in a transgenic mouse model of A β amyloidosis. *J. Exp. Med.* **209**, 2149–2156 (2012).