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Genetické rizikové faktory Alzheimerovy choroby
Genetic risk factors of Alzheimer's disease

Bakalářská práce

Školitel: Mgr. Petr Telenský, Ph.D.

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Prohlášení:

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Alice Foltýnová

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Abstrakt

Alzheimerova choroba je neurodegenerativní onemocnění, které postihuje především starší populaci a vzhledem k faktu, že se dožíváme stále vyššího věku, tak se Alzheimerova choroba považuje za jedno z nejvážnějších onemocnění 21. století. Existují dva typy Alzheimerovy choroby, familiární a sporadická. Liší se ve věku, kdy se onemocnění projeví a začne vykazovat symptomy a také různým typem dědičnost – familiární forma je geneticky podmíněná a u sporadické formy se jedná spíše o rizikové faktory. Jejich klinický obraz je však srovnatelný – v prvních stádiích se objevuje charakteristická zapomnětlivost a do dalších fází se pacient dostává postupně a obvykle nedochází k výrazným zhoršením ze dne na den. Stále je hodně nesrovnalostí ohledně Alzheimerovy choroby a existuje vice směrů, kterými se lze ve výzkumu vydat. Jeden z možných přístupů je genetický – cílem je najít další genetické lokusy zapojené do patologie Alzheimerovy choroby a poznat nové mechanismy skrz studium úlohy nově identifikovaných genů.

Klíčová slova: sporadická forma Alzheimerovy choroby, familiární forma Alzheimerovy choroby, beta amyloid, apolipoprotein E, amyloidový prekurzorový protein, presenilin, celogenomová asociační studie

Abstract

Alzheimer´s disease is a neurodegenerative disorder that mostly affects the elderly population and since our lifespan increases, Alzheimer´s disease is one of the most serious diseases of the 21st century. There are two types of Alzheimer´s disease, namely familial (FAD) and sporadic Alzheimer´s disease (SAD) that differ in the age of onset and contribution of the genetic factors – the familial form is genetically predisposed whereas the genes involved in the sporadic form are perceived as risk factors. However, their clinical manifestation is similar. Alzheimer´s disease causes dementia that is characterized by memory loss and a steady decline in the early stages. Unfortunately, there are still many discrepancies regarding Alzheimer´s disease and there are multiple approaches in research concerning Alzheimer´s disease. One of the possible approaches in finding new mechanisms involved in Alzheimer´s disease is in genetics – we can findnew genetic loci involved in this disease and investigate new mechanisms via studying newly identified genes.

Key words: sporadic form of AD, familiar form of AD, beta amyloid, apolipoprotein E, amyloid precursor protein, presenilin, genome wide association study

Contents:

List of abbreviations

AD – **A**lzheimer´s **d**isease

GWAS – **g**enome **w**ide **a**ssociation **s**tudy

FAD - **f**amilial **A**lzheimer´s **d**isease

SAD - **s**poradic **A**lzheimer´s **d**isease

ApoE – **Apo**lipoprotein **E**

AD – **a**utosomal **d**ominant

SPs - **s**enile **p**laques

NFTs - **n**euro**f**ibrillary **t**angles

Aβ - **b**eta **a**myloid

APP - **a**myloid **p**recursor **p**rotein

AICD - **A**PP **i**ntracellular **c**ytoplasmatic **d**omain

CTFα - **c**arboxy-**t**erminus **f**ragment **α**

sAPPα - APP fragment α

sAPPβ - APP fragment β

PSEN-2 - **Pre**s**en**ilin-**2**

PSEN-1 - **Pre**s**en**ilin-**1**

FTO - **fat** mass and **o**besity associated (gene)

T1D and T2D -**d**iabetes mellitus **t**ype I and II

APLP1 and APLP2 - **a**myloid **p**recursor-**l**ike **p**roteins

APPL - **a**myloid **p**recursor **p**rotein-like

KPI - **K**unitz type serin-**p**rotease **i**nhibitor

sAPP - **s**ecreted **APP**

KO - **k**nock**o**uts

VLDLs - **v**ery **l**ow **d**ensity **l**ipoproteins

HDLs - **h**igh **d**ensity **l**ipoproteins

1. Introduction

Alzheimer´s disease (AD) is becoming a greater threat nowadays than ever before because age is the biggest risk factor for the familial form of the disease (FAD) and our lifespan increases. For easier demonstration, every 66 seconds a person developed AD in 2017 and the mentioned number is predicted to be half its value in 2050 (Alzheimer's Association, 2011). At the age of 65, one in ten people suffer from this disease and the percentage increases up to 32 % once the person is 85 years old or older. These numbers describe the situation in the United States even though other western countries have similar statistics (Alzheimer's Association, 2011).

The incidence of the disease is raising and the medicaments available for the patients have beneficial effects on mental focus and energy and they are able to slow the progress of the disease but none of them can reverse and cure AD.

The main objective of my bachelor thesis is to summarize the prominent genetic risk factors involved in AD and describe their contribution to developing the dementia. However, the importance of their role differs between the sporadic form (SAD) and the familial form (FAD) of AD which is another essential part of my thesis. Furthermore, I will expound the principles of GWAS (genome wide association study) in order to elucidate the method used in finding the genetic risk factors for AD.

2. Alzheimer´s disease

AD is the most common cause of dementia affecting primarily the elderly population. Dementia represents a set of symptoms which lead to a significantly impaired intellectual functioning with two or more brain functions, such as memory or learning skills, severely damaged. As a result, the patient is not able to engage in everyday life activities and requires around-the-clock care (Dening & Sandilyan, 2015).

2.1. Clinical manifestation

AD can be hard to recognize because everyone has their own individual manifestation and pace of progression. The first dominant clinical symptom of AD is usually progressive memory loss with impairment of other cognitive functions over the years. Unfortunatelly, in less typical presentations of AD, other cognitive dysfunction can be most prominent which could lead to an incorrect diagnosis. Another less common symptoms include aphasia, spastic paraparesis and visuospatial skills. In later stages, patients with AD usually develop some extrapyramidal dysfunctions (parkinsonism, dystonia, tremor etc.) (Geldmacher & Whitehouse, 1997).

2.2. Forms of AD

The clinical classification of AD distinguishes two forms – the first one is FAD and is caused by genetic abnormalities. It affects people younger than 65 years old and the symptoms can start when the person is around 40 years old (Joshi et al., 2012).

99% of AD cases are described as SAD. The symptoms are identical with these two forms. The genetic risk factors of SAD convey an increased probability in developing the disease but unlike in FAD they are neither sufficient nor necessary to trigger the disease. Therefore, we cannot explain the origin by a single cause and a multifactorial approach thus seems to be a more appropriate perspective (Van der Linden & Van der Linden, 2016). The factors with the highest risk involve older age, family history of AD (Fratiglioni et al., 2016) and having the *ApoE-e4* (Apolipoprotein E*)* allele (Chartier-Harlin et al., 1994).

To date, it is widely accepted that AD as a clinical unit is in fact a heterogenous group of diseases with various causes, including mutations in different genes, and while the clinical classification of AD is a well known and useful tool for clinical practice and clinical research, it does not say much about the underlying pathology of the disease.

From the genetic point of view, we recognize 4 well-accepted types of AD (AD1-4), but the evidence nowadays suggest that another 15 genetic forms of disease may exist (AD 5-19), but they are relatively rare(some of them were described only in the form of case reports).The related genes are summarized in a table in Figure 1.

The genetic classification is more precise but the clinical approach that differentiates only FAD and SAD is sufficient for my thesis. Before I explore the genetic backround of the two types and the methods used in finding the genetic loci, I will summarize the main pathological events of AD and especially how does beta amyloid (Aβ) become toxic.

Table 1– Table of genetic classification – AD is divided into 19 forms and for each form there is a name, loci and heredity type – AD means autosomal dominant. (retrieved from: OMIM, [Internet] : (Victor A. McKusick, 1960).

NAME	GENE NAME (S)	LOCUS	HEREDITY TYPE	REFERENCES
AD 1	*APP, AAA, ABETA, ABPP, ADI, CTFgamma, CVAP, PN-II, PN2, preA4, APP*	21q21.3	AD	Murrell et al., 1991
AD 2	*APOE, AD2, APO-E, ApoE4, LDLCQ5, LPG*	19q13.32	AD	Reiman et al., 1996
AD 3	*PSEN1, ACNINV3, AD3, FAD, PS-1, PS1, S182*	14q24.2	AD	George-Hyslop et al., 2012
AD 4	*PSEN2, AD3L, AD4, CMD1V, PS2, STM2*	1q42.13	AD	Levy-Lahad et al., 1995
AD 5	*AD5*	12p11.23-q13.12	AD	Pericak-Vance et al., 1997
AD 6	*AD6*	*10q24*	unknown	Bertra, et al, 2000
AD 7	*AD7*	*10p13*	unknown	Zubenko et al., 1998
AD 8	*AD8*	*20p*	unknown	Crawfor et al., 2000
AD 9	*ABCA7,ABCA-SSN, ABCX, AD9*	19p13.3	AD	Wijsman et al., 2004
AD 10	*AD10*	7q36	AD	Rademakers et al, 2005
AD 11	*AD11*	9p22.1	unknown	Pericak-Vance et al., 2000
AD 12	*AD12*	8p12-q22	unknown	Giedraitis et al., 2006
AD 13	*AD13*	1q21	unknown	Sanchez-Chuan et al.,2007
AD 14	*AD14*	1q25	unknown	Sanchez-Chuan et al.,2007
AD 15	*AD15*	3q22-q24	unknown	Sanchez-Chuan et al.,2007
AD 16	*AD16*	Xq21.3	unknown	Carrasquillo, et al., 2009
AD 17	*AD17*	6p21.2	unknown	Jonsson et al., 2013
AD 18	*AD18*	15q21.3	unknown	Kim et al., 2009
AD 19	*PLD3,AD19,HU-K4, HUK4, SCA46*	19q13.2	AD	Cruchaga et al., 2014

2.3. **Pathophysiology**

There are several pathophysiological events occuring in AD, some of which begin to appear even decades before the dementia starts to show clinical symptoms. I will abbreviate the whole problematic to the main hallmarks that are crucial for my thesis.

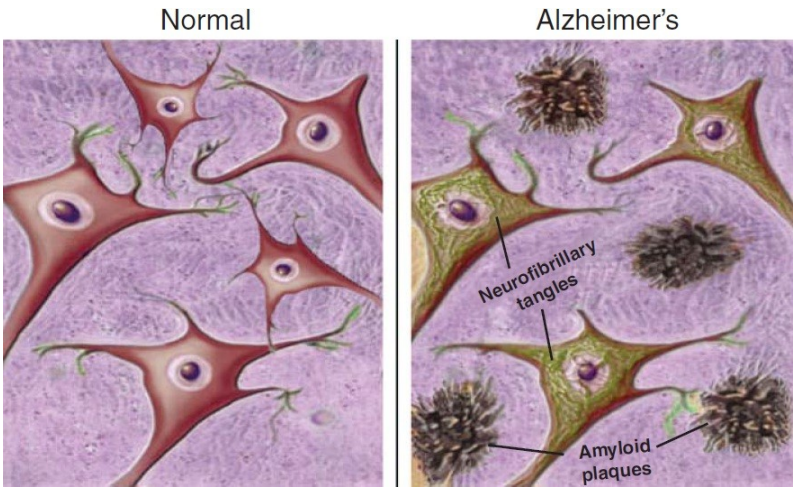

Figure 1 – Illustration of pathophysiological changes in the brain represented by senile plaques and neurofibrillary tangles (Chopra, 2011).

The main pathophysiological features of AD are senile plaques (SPs) and neurofibrillary tangles (NFTs) (as shown in Figure 2). Even though these hallmarks can be found in the brains of individuals with no cognitive deficit, SPs and NFTs still play a major role in AD development. NFTs correlate with the severity of the stage of AD but that is not the case with SPs (Arriagada et al., 1992).

Deposits of beta amyloid (Aβ) create SPs in a process explained in the next chapter. SPs are insoluble fragments of proteins that are positioned extracellularly and they disturb cell communication. and In AD, entorhinal cortex and hippocampus are the first structures that they appear in which corresponds with the fact that memory is usually the first damaged structure (Bouras et al.,1994).

Tau protein is a microtubule associated protein which stabilizes microtubules. If the phosphorylation of tau is extensive and the hyperphosphorylation occurs, the microtubules can fall apart due to the instabilitity. In consequence, the tau proteins disconnect and form

NFTs. Their location is therefore mainly intracellular and they appear in the same structures as SPs. Their density is high in the temporal lobe. Before they tau pathology, the levels of Aβ are elevated which suggests involvement of Aβ in affecting the NFTs (Näslund et al., 2000). The pathophysiological processes can trigger the cell apoptosis.

3. Amyloid precursor protein processing

The amyloid precursor protein (APP) plays a pivotal role in AD pathogenesis by generating Aβ.

The cleavage of APP is enabled by the proteolytic activity of enzymes called secretases via pathways that either create an amyloidogenic product or are non-amyloidogenic (as shown in Figure 3).

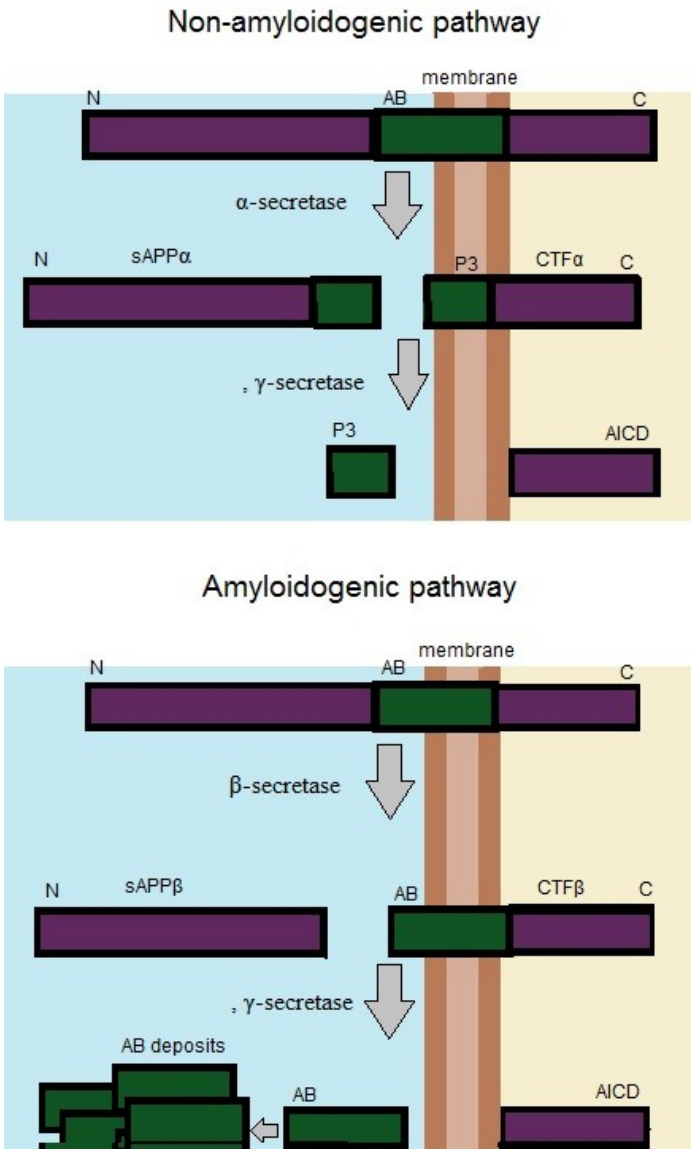

Figure 2 – Demonstration of non-amyloidogenic and amyloidogenic pathway. As a first step in the non-amyloidogenic pathway Aβ is cleaved by α-secretase and in the non-amyloidogenic pathway is cleaved by β-secretase. The second cleavage is enabled by γ-secretase. The non-amyloidogenic pathway creates soluable P3 whereas amyloidogenic toxic Aβ. (©Foltýnová)

3.1. The non-amyloidogenic pathway

The non-amyloidogenic pathway takes place in the cell membrane. The α-secretase cleaves within the Aβ region of APP so this process results in creating carboxy-terminus fragment α (CTFα) and soluble APP fragment α (sAPPα). Later on, γ-secretase takes action by cleaving the CTF-α fragment that is divided into the APP intracellular cytoplasmatic domain (AICD) and the soluble peptide P3. None of these products were proven to be toxic (Lammich et al., 1999).

3.2. The amyloidogenic pathway

The amyloidogenic pathway occurs in molecules of APP that failed to by cleaved by α-secretase. The process begins with internalization of APP and β-secretase into an endosome with lower pH than the surface of the cell which is necessary for the β-secretase to fuction properly. β-secretase cleaves APP into Carboxy-terminus fragment β (CTFβ) and soluble APP fragment β (sAPPβ) (Vasaar,1999). Subsequently, γ-secretase cleaves the CTFβ into AICD and the Aβ segment. These products are transported to the extracellular space by exocytosis. Aβ40 is secreted more commonly but the most toxic form of Aβ is Aβ42 and its aggregation forms SPs (Stroud, Liu, Teng, Eisenberg, 2012).

3.3. Amyloid and tau hypothesis

The amyloid hypothesis of AD expects the abnormal levels of Aβ to play a major role in triggering the disease. Since Aβ accumulation in the brain usually precedes hyperfosforylation of tau, Aβ is believed to be at the beggining of the cascade resulting in neurodegeneration. The amyloid hypothesis assumes that Aβ is responsible for the creation of NFTs. Also, some mutations in the APP gene cause FAD but on the other hand, other APP variants reduce formation of Aβ (Jonsson, 2012). Another reason to not overlook the importance of Aβ is the neurotoxicity of Aβ oligomers named Aβ-derived diffusible ligands involved in AD pathology (Lambert,1998). According to these observations, there was a hope that the clearance of APP and regulation of Aβ levels could provide a solid foundation for therapeutic approaches.

The effect of anti-amyloid drugs did not live up to the original expectations. A number of clinical trials failed (Egan et al., 2018) (Green et al, 2009) including the third clinical phase of solanezumab trial (Honig et al., 2018). Even though the results still did not show a significant amelioration in the probands medicated by the solanezumab drug, the clinical efficacy increases when applied in the mild stage of AD. This finding may suggest that the anti-amyloid treatment could work in the initial stages of AD or maybe even earlier since SPs accumulate decades before the symptoms appear.

From another point of view, the low efficiency of the antiamyloid agents could occur due to the discrepancies in the hypothesis. For example, the post-mortem analysis that compared brain tissue of elderly patients who suffered from AD and who did not have dementia and the amount of SPs in the brain does not correlate with the severity of the dementia.

Another perspective puts the hyperphosphorylated tau above Aβ accumulation. The treatment approach based on the tau hypothesis is not as explored as the amyloid one since we are not fully aware of why the hyperphosporylation occurs and also targeting drugs inside the cell is a lot harder. Nonetheless, the tau vaccine AADvac1 had good results regarding imunogenicity and safety in the first clinical trial so hopefully further trials could prove a significant clinical effect (Novak et al., 2016).

Currently, we have a wide range of hypotheses regarding the cause of AD, some of the explications vary from hormonal deficiencies to a viral reactivation. However, none of these hypotheses received general acceptance.

The role of SPs and NFTs in the pathogenesis of AD stays unclear – the debates on whether these hallmarks are essential in forming the pathology or rise as by-products of other events continue.

While none of the theories is capable of explaining the causes of AD in their own right, perhaps the solution is to combine all the hypotheses rather than leaving the amyloid hypothesis behind and balance the antitau and antiamyloid avenues with other factors that were proven to reduce the risk of developping AD such as diet (Refolo et al.,2000) or exercise (Teri et al., 2003).

4. Methods

In this chapter, I would like to summarize the main approaches and methods used to find the genes involved in AD pathology. The widely used method is GWAS. Different approaches lie in finding the candidate genes and searching for associated genes via comorbidities.

4.1. GWAS

According to Nature (retrieved from Nature [Internet]) „GWASs are unbiased genome screens of unrelated individuals and appropriately matched controls or parent-affected child trios to establish whether any genetic variant is associated with a trait. These studies typically focus on associations between single-nucleotide polymorphisms (SNPs) and major diseases.“ Majority of gene loci portraying a role in AD were found using GWAS, some of them are named in chapter 5. The benefit of this method is that it discovers loci that are also targets for other diseases which can be beneficial.

4.2. Linkage studies

According to Nature (retrieved from Nature [Internet]) „A genetic linkage study is a family-based method used to map a trait to a genomic location by demonstrating co-segregation of the disease with genetic markers of known chromosomal location; locations identified are more likely to contain a causal genetic variant.“ For example, Presenilin-2 (PSEN-2) was originally identifed being homologous to PSEN-1 (Levy-lahad et al., 1995).

4.3. Identifing new genetic risk factors through comorbidities

Another approach is to scan the diseases and conditions that are comorbid with or express similar pathology to AD and to find some genetic correlations. Both diabetes mellitus type 1 and 1 (T1D and T2D) share biochemical dysfunctions with AD. GWAS studies even found some shared genetic loci in the case of T2D and AD (Hao et al., 2015). It was found that dysregulations of insulin signaling contribute to the AD pathophysiology. The

significance of impaired insulin signaling led some scientists to propose that it is in fact the underlying cause of AD, creating the nickname „type III diabetes“ for the disease (Steen et al., 2005). An example of a shared genetic risk factor is the fat mass and obesity associated gene (FTO) which has been linked toobesity and diabetes but also significantly elevates the risk of AD especially in ApoE4 carriers (Reitz et al., 2012).

5. Familial AD

The origin of FAD remains unknown in some patients but the vast majority of cases have a genetic cause. The early-onset form of AD is predominated by the mutations in the genes for PSEN-1, PSEN-2 and amyloid precursor protein (APP). If a person inherits the PSEN-1 mutation or any other mutation on chromosome 21q where the locus of APP is, he or she develops AD with an absolute certainty whereas the risk represented by PSEN-2 accounts for 95 % penetrance (St. George-Hyslop et al.,1998).

In total, there are 32 *APP*, 179 *PSEN1* and 14 *PSEN2* gene mutations that have been linked to FAD. The APP mutations either increase the formation of Aβ42 directly or move the ratio of production so that Aβ42 is cleaved more frequently than Aβ40 or other safer peptides (Borchelt,1994).

5.1. APP

APP is a membrane protein that is known for its involvement in AD by generating the Aβ. The family of related proteins consists of the APP, the amyloid precursor-like proteins (APLP1 and APLP2) identified in mammals and one of the proteins present in the genome of Drosophila titled as the amyloid precursor protein-like (APPL). The processing is similar throughout all the family but only APP produces Aβ.

As hinted, APP is permanently positioned within the biological membrane thus it is classified as a single-pass integral membrane protein. As mentioned earlier, the gene lies on chromosome 21q, spans 240 kb and is composed of 17 exons. There are 2 exons encoding Aβ – particularly the 16^{th} and the 17^{th}. The alternative splicing produces 3 mRNA variants and two of them encode the Kunitz type serin-protease inhibitor (KPI). The KPI is a small ectodomain that is the active site of secreted APP (sAPP) which can improve some cognitive skills like memory retention by affecting the synapses in healthy animals (Roch et al., 1994). The sAPP is a product of the non-amyloidogenic pathway and is overall neuroprotective and positively affects the neuronal excitability and synaptic plasticity (Turner, 2003).

5.1.1. Structure

The various isoforms of APP contain between 365 to 700 amino-acid residues. The APP transcript offers 8 possible variants due to the alternative splicing with 3 isoforms being the most common - the 695 amino acid isoform is highly expressed in the central nervous system (CNS) and the other two variants with 751 and 770 amino acids have a wider range of expression.

APP consists of two domains and the extracellular domain is much larger than the intracellular one. The ectodomain of APP contains two heparin-binding domains (Mok et al. 1997) that seem to be the most active sites. One of these domains binds F-spondin which is a signaling molecule with protective and developmental cell function (Ho et al., 2004) but unfortunaly there are no findings suggesting the involment of APP in cellular development.

APP is being compared to a signaling molecule Notch because they share structural components as well as cleavage strategies. Also, along with other molecules like colagen or netrin-1, Notch is one of the possible binding partners to the N-terminus of APP (Chen, 2006).

Another important site of APP is the C-terminus bearing two functions. The first one is to regulate transcription and the second is intracellular sorting via the YENPTY domain that positively affects anterograde transport during which synthetises molecules that move from the cell body to synapses (Krishnan et al., 2006). The YENPTY motif is also used as a binding site for cytosol proteins. Although the evidence is unclear, YENPTY domain probably bears some of the physiological functions of APP as the knockout animal studies have suggested. The APP KO (knockouts) caused a decrease in total body weight and brain, impaired long-term potentiation and destruction of synapses and the locomotor functions were compromised as well. Some of these conditions were present in animals with a KO targeted to the YENPTY domain which is the reason for the assumed physiological role of YENPTY motif (Matrone et al., 2013).

5.1.2. Function

The physiological role of APP in the cell is not yet fully understood. However, APP affects binding with other cells while acting like a cell adhesion molecule (Soba, 2005). Furthermore, APP has positive effects on viability of the neurons and axogenesis as well as prolonging the branches of the dendrites (Perez et al., 1997).

An in vivo study revealed that holoAPP is necessary for a proper migration of precursor cells to the cortex in the early stages of embryogenesis (Pearse et al., 2007). Whether APP in the adult brain involves in similar biochemical pathways in order to have an organizational function is a subject of debate. It is possible that APP is needed to regulate the migration of progenitor cells to the injured tissue. On the other hand, APP, APLP1 and APLP2 triple-knockouts in mice (Herms et al., 2004) did not cause any significant morphological defects on the whole body even though they were lethal – 20 % of the brains show a usual brain development which does not support the fact that APP is crucial for cell migration suggesting that this matter requieres a further debate.

One of the appropriate models for explaining the APP function is the Swedish mutation because it elevates the Aβ production. Mice that carry this construct have high levels of Aβ and show amyloid pathology. A study revealed that an overexpression of APP with familial AD Swedish mutation causes hypertrophy of cortical neurons (Oh et al., 2009). This finding hints that APP may have a positive neurotrophic effect but whether this function is enabled by the full-lenght APP or one of the cleavage products remains unclear.

5.1.3. Role of AB peptide

Although Aβ represents a pivotal role in AD pathology, the peptide itself may provide a needed negative feedback in excitatory synapsis when the transmission is too high in normal condition (Kamenetz, 2003). Furthermore, endogenic Aβ has a regulatory function in releasing synaptic vesicles (Abramov et al., 2009).

As Aβ accumulates, the pathological dysfunctions begin to appear. Interestingly, the APP is not upregulated when the levels of Aβ elevate which is due to the SPs that interfere with the cell funtion (Barger et al., 2008). Since APP is downregulated and does not fulfill its

protective function entirely, this could contribute to the pathophysiology of the disease as well.

5.2. Presenilins

Presenilins are proteins which are components of a multi-subunit intramembrane protease complex called the γ -secretase. The locus of genes encoding presenilin differs, *PSEN-1* is positioned on chromosome 14q and the candidate gene *PSEN-2* is placed on chromosome 1q (Sherrington et al., 1995).

Since *PSEN-2* mutations are rare, I am summarizing most of the studies on *PSEN-1*. Also, the proteins PSEN-1 and PSEN-2 share a number of similarities in their structure, amino acid sequence and expression pattern in the brain which leads to a conclusion that their function could be connected.

5.2.1. Structure

The amino-acid sequence of PSEN-1 consists of 467 amino-acids and the PSEN-2 is 448 amino-acids long according to expressed-sequence-tag databases (Sherrington et al., 1995).

Presenilins are rich in hydrophobic regions which makes it harder to identify their exact structure. However, PSEN-1 is a polytopic protein located within the membrane, more specifically in endoplasmic reticulum and Golgi complex, and the FAD mutations generally do not interfere with their position (Kovacs et al., 1996). Along with nicastrin, PS-2, and APH-1, PS-1 forms γ- secretase (Spasic et al., 2006).

Presenilins are well conserved proteins just like APP with homologues throughout all animal species and probably even plants. Vertebrates have two – PSEN-1 and PSEN-2. The homology between primary sequence of PSEN-1 and PSEN-2 is estimated around 67%. In comparison with other animals, human presenilins can be even 90 % identical to *Xenopus laevis*. Some of the regions of the proteins are identical in most species– the hydrophobic parts such as the carboxy terminus are present in most of the species as opposite to the hydrophilic parts that for example in the case of *Arabidopsis thaliana* are not conserved. The

carboxy terminus plays an important role in apoptosis which may be the reason for its high conservation level (Tsujimura et al., 1997).

5.2.2. Function

Presenilins are also being compared to Notch because they bear similar function. KO studies in mice show similar phenotypes and both presenilins and Notch KO are lethal in the early embryogenic stages. According to these experiments, PSEN-1 is essential for neurogenesis, development of axial skeleton and neuronal survival (Shen et al., 1997).

Also, Notch receptors are cleaved by presenilins and since Notch proteins are pivotal in development, the lethality of KO mice is not surprising. The involment of presenilins in Notch pathways could lead to a conclusion that they take part in AD pathology but a recent study denies this theory – there is a *PSEN-1* mutation that does not affect Notch signaling (Zhang et al., 2018).

Presenilins represent a key role in neurotransmitter funtion by modulating the levels of Ca^{2+} within the cell which then causes decrease in glutamate (Zhang et al., 2009).

5.2.3. Contribution to AD

The studies revealed single nucleotid changes in codons for presenilins causing the protein to be translated with different amino acids. The missense codons playing role in FAD were found in both presenilins – there were 2 for *PSEN-2* and 50 for *PSEN-1*. In consequence, the mutations influence cleavage of Aβ in the same way as mutations in APP meaning the fragments were made of the less soluable Aβ42 (Citron et al., 1997).

To sum up the mechanisms explained above, in order for Aβ to be formed, APP must by cleaved by two enzymes – β- and γ- secretases and presenilins are the components of γ-secretase that take part in APP cleavage. The γ- secretase cleaves APP fragments of different sizes – they differ by two amino acid residues and are called Aβ42 and Aβ40. Aβ42 is the most toxic form and in subjects with the high-risk presenilin mutations, Aβ42 is more abundant although the overall production of Aβ remains the same (Citron et al., 1997).

6. Sporadic AD

In contrary to the FAD, we are not aware of a single cause that would explain the origin of SAD. The environmental factors affect the incidence and the genetic backround contributes to the development of the disease as well (Gatz et al., 2006). Therefore, we should portray the respective genes merely as risk factors – after all, a subset of SAD do not carry any of the identified risk alelles, while many people bearing the most significant genetic risk factor ApoE-ε4 genotype never expressed any symptoms of dementia. A recent study shed light on this matter from the perspective of mental health. Positive attitude towards aging in people who are 60 years old or older alter the effect of ApoE-ε4, the main genetic risk factor for SAD, in a positive way – individuals with the ε4 allele who thought positively about their age had a 49.8% lesser chance to go through the stages of dementia (Levy et al., 2018).

The ApoE-ε4 is not the only genetic risk factor that should be considered in SAD, the GWAS linked the increased risk of AD to several other gene loci but the strongest association still lies with the ApoE-ε4 (Corder et al., 1993). At least one ε4 allele is carried in 40 to 80% of AD patients and in the healthy population ε4 is also present in 25-30 % of the cases (Farrer et al., 1997) which means that even though ApoE-ε4 is the strongest genetic risk factor, its penetrance is still relatively low. The presence of the ε4 variant is increased in the FAD as well compared to a healthy population.

In 2013, a metanaanalysis of GWAS constisting of Europeans summarized all the genetic risk factors of SAD and in addition to the ApoE-ε4 locus, there are another 19 loci representing a risk, 11 of them being newly identified (Lambert et al., 2013). The genes are: *CASS4, CELF1, FERMT2, HLADRB5, INPP5D, MEF2C, NME8, PTK2B, SORL1, ZCWPW1, SlC24A4, CLU, PICALM, CR1, BIN1, MS4A, ABCA7, EPHA1,* and *CD2AP.* (Lambert et al.,, 2013) As hinted earlier, the ApoE-ε4 accounts for most of the genetic variability, therefore the contribution of the other candidate genes is believed to be minor. For example, the *ABCA7* mutation elevates the relative risk 1.7 times which is negligible compared to the effect of ApoE-ε4 that could modificate the relative risk up to 15 times as discussed further in the thesis.

6.1. ApoE

ApoE is a protein essential in metabolism of lipids. The gene for ApoE is located on chromosome 19 (Mahley et al., 1996).

Overall, three isoforms were identified namely E2, E3, and E4. Since there are 3 known alleles, 6 genotype combinations are possible: three homozygous (E2/2, E3/3, and E4/4) and three heterozygous ones (E3/2, E4/3, and E4/2). The frequency of the alleles varies among countries and ethnicities. Of the six possible, there are 3 most common genotypes - E3/2 (7-16.9%), E3/3 (39.8-72.1%) and E3/4 (11.3-35.9%) (Hallman et al., 1991).

According to a study, compared to non-carriers, probands who had at least one copy of the ε4 allele had an increased relative risk of developing AD 15 times higher. (Nalbantoglu et al., 1994)

6.1.1. Structure

The main structure of ApoE is alike in all the variants and the alleles vary in their residues – ApoE-ε-3 contains Cys-112 and Arg-158, ApoE-ε4 only has arginine residues at position 112 so its structure is very similar to ApoE- ε3 except it lacks cysteine. Apo-ε2 consists only of cysteines that do not bind properly which results in hyperlipoproteinemia and this condition leads to elevated cholesterol and triglycerides levels (Weisgraber et al., 1990).

The E4 isoform tends to form molten globules which could alter the physiological funtion of ApoE perhaps by exposing the hydrophobic core more (Morrow et al., 2002). ApoE is formed by two domains: an amino-domain and a carboxyl-terminal domain (Dong et al., 1996).

The low density lipoprotein receptor is equally effective in binding in ApoE-ε3 and ApoE-ε4 but their preferences for lipoproteins differ – ApoE-ε4 binds with very low density lipoproteins (VLDLs) because of the two domain interactions that do not occur in ApoE- ε2 and ApoE-ε3 and they prefer to bind with high density lipoproteins (HDLs) (Weisgraber et al., 1990).

6.1.2. Function

In relation to the molecular function, ApoE contributes to cleaning the deposits in brain parenchyma and decreases the deposition of AB.

As lipid-carrying molecules, apolipoproteins regulate metabolism throughout the whole body, including the CNS. In case oftissue injury, lipids are transported to the affected tissue in order to ameliorate healing (Ignatius et al., 1986). ApoE allows neurons to repair and grow with the ε3 allele being the most effective and ε4 allele malfunctioning (Mahley et al., 1996).

ApoE regulates the cholesterol levels in the brain and its importance is emphasized by the lack of some other major apolipoliproteins in the brain tissue such as ApoB and LDL (Roheim et al., 1979).

6.1.3. The pathophysiological role of ε4 allele

ApoE-ε4 does not only represent a risk factor for AD but for a number of other neurodegenerative and cardiovascular diseases – for example Parkinson´s disease (PD) (Harhangi et al., 2000), central nervous system (CNS) or stroke (Zhu et al., 2000).

ApoE has an important influence of on metabolism. As discussed above, ApoE transports cholesterol in the brain and the ε4 allele is less efficient than the other isoforms. Cholesterol and phospolipids are critical in re-innervation of the hippocampus when injured (Poirier et al., 1994).

Lipids are not the only substrates which metabolic pathways are altered by ApoE. Metabolism of glucose is impaired under the influence of the ε4 allele. The structures that are amongst the first to degenerate in AD such as cortex or hippocampus show lower cerebral glycolytic rate (CMRgl) if the E4 variant is present. According to the study, the CMRgl could be used as a biomarker in the future in favor of a better prevention (Reiman et al., 2001). Since we can only measure the levels of Aβ from the cerebrospinal fluid, it would be of benefit to find an associated blood biomarker.

The ε4 allele affects the age of onset (Meyer et al., 1998) with each copy accelerating the beginning of symptoms by almost 10 years. (Corder et al., 1993) The cognitive functions

are negatively affected even in the clinically healthy elderly ε4-carriers and this effect progresses with age. (Deary et al., 2002) The ε2 allele seems to have a protective effect against neurodegeneration whereas the ε3 variant is neither protective, nor riskant (Corder et al., 1994).

The involvement of ApoE-ε4 in AD is not yet comprehended in its full complexity but still we gathered enough data to assume its role in neuropathology of the disease from transgenic mice expressing human Apo-ε4 and Apo-ε3. In mice carrying the ε4 allele, the studies have shown a number of reduced presynaptic terminals which was not influenced by the SP deposition even though the overexpression of AB peptides contributed to the reduction as well (Buttini et al., 2002). The long-term potentiation is altered as well (Klein et al., 2010). The levels of ApoE are increased when Aβ produced more with even greater concentration in SPs (Barger et al., 2008). ApoE-ε4 does not only influence the Aβ but is also responsible for increased tau hyperphosporylation (Tesseur et al., 2000). The ε4 allele has an impact on cognitive decline – a study proves an impaired memory retention (Raber et al., 2000).

As for the mechanisms of neurotoxicity, the high reactivity of ε4 variant leads to increased proteolysis by ApoE cleaving enzyme which creates fragments resulting in increased neurodegeneration since the repair function of ApoE is less effective. This process results into neurofibrillary tangle-like intracellular inclusions in neurons (Huang et al., 2001).

6.1.4. Interaction with AB

Apo-ε4 induces formation of SPs by inhibiting AB clearance and enhancing AB production (LaDu et al., 1994). It is possible that ApoE acts as a detrimental chaperon which leads to AB deposition and transformation to a fibrillar form. An in vivo study proposes that role of ApoE-ε4 is essential in forming AD-like pathology (Holtzman et al., 2000).

6.1.5. Interactions with other genetic factors

One of the contributing genes is FTO. The genetic variability in the FTO has been shown to modulate the risk of developing AD in ApoE- ε4 carriers. The risk of developing AD is APOE4 carriers is increased 3-fold in people who are also bearing the rs9939609SNP variant of FTO, which is a known risk factor for obesity and diabetes. In addition, the FTO

AA-genotype interacts directly with Apo-ε4 resulting in impaired removal of lipids from the blood (Keller et al., 2011).

6.1.6. Apo-ε4 in general population

Whether the person is a homozygote or a heterozygote for the ε4 allele and other factors as the described genetic interactions is not enough to determine the risk of developing AD – there is evidence that some aspects of human individuality should be explored more.

The risk of developing AD connected to ApoE-ε4 is not the same for all of the races. Asians and Europeans report a stronger correlation (Duijn et al., 1994).

A study has shown differences in men and women regarding Apo-ε4 effect on AD. Women seem to be more susceptible in developing AD since they are more affected by the Apo-ε4 genotype (Altmann et al., 2014). The possible explanation could be in estrogen and Apo-ε4 binding but this interaction needs further explication. Interestingly, higher levels of estrogen have positive cognitive effects on women who are not carriers of the ε4 allele and who were estrogen users (Yaffe et al., 2000). ApoE transports lipids in CNS and ε4 causes artherosclerosis whereas estrogen is a vasodilatant and therefore can ameliorate artherosclerosis which could be one possible mechanism of how ApoE and estrogen interact. Accordimg to these studies, it is possible that estrogen has a positive effect.

7. Conclusion

Since AD represents a higher threat to our population each decade, the search for the cure for the disease is becoming urgent. To date, we have no treatment that could possibly reverse the progress of AD and the available options only slow down its course.

Understanding of the genetic backround and the impaired function of the mutated genes could shed more light on the disease etiology and thus offer new therapeutic avenues. Explanation of the β-secretase activity led to the discovery that β-secretase inhibitors are efficient in lowering the Aβ levels. (Kennedy et al., 2016) However, as promising as this treatment was, it did not have any positive significant effect on AD in mild stages of the disease (Egan et al., 2018) and the trial did not continue. Perhaps the clinical trials in the early stage of the disease will provide better results or maybe it it is time to move on to explore another mechanisms. Another important genetic finding is FTO gene. FTO connects the metabolic diseases and neurodegeneration which offers the possibility of discovering new targets for AD treatment. For the SAD form, the strongest risk factor is ApoE-ε4 and even though none of the attempts to treat AD was based on this genetic risk factor, the studies provided a better undestanding of AD pathology.

Nonetheless, we can still actively participate in prevention of the disease and lower our chances in developing dementia by a number of variables some of which we can actually change. This is another reason why pay attention to gene mutations and how they are influenced. A good example is physical activity. For the elderly, especially walks are recommended. Another important factor is to have a social network or a person to talk to – especially old people get isolated sometimes and being lonely is a proven risk factor in developing AD.

In conclusion, there is still a lot that we do not know about AD and a better understanding of genetic risk factors could help to shed light into this matter. Genes and their protein products are often first identified as heritable risk factors for a disease and subsequently their role is studied on the molecular level. In many instances, this approach led to discovering new mechanisms and molecular pathways in ethiology of diseases, or even to identification of prospective molecular targets for treatment. Even though only a small fraction of candidate drugs makes it to the clinical testing, we still need to focus on the

primary research to come up with new hypotheses to try to fill in the missing links in AD pathology.

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