MRI and histologic studies on early markers of Alzheimer's disease

Duijn, S. van

Chapter 1

General Introduction

This thesis addresses a variety of aspects of the early diagnosis of Alzheimer's disease (AD). AD is a complex clinical syndrome characterized by a cluster of symptoms and signs comprising difficulties in memory, changes in behaviour, disturbance in language and other cognitive functions causing impairments in activities of daily living. (Ferri et al., 2005; Qiu et al., 2009; Villemagne et al., 2013). In 2010, 5,4% of the European population at the age of 60+ had dementia, which was (at that time) 6.3 million people (Wittchen et al., 2011). AD accounts for 75% of all dementia cases, implying 4.7 million people in Europe had AD in 2010 and this number is further increasing. Brookmeyer et al., predicted an AD incidence of 1 in 85 persons worldwide in 2050 (Brookmeyer et al., 2007). As a result, AD represents an important socio-economic and public health concern. Results from human studies suggest that females are at higher risk for developing AD than men (Andersen et al., 1999; Corder et al., 2004; Grimm et al., 2012; Janicki and Schupf, 2010; Musicco, 2009)

Due to an incomplete understanding of the pathophysiology of the disease, an effective therapy is currently not available. Limitations in studying the early stages of the disease during life, have been partly responsible for lack of knowledge about the pathophysiology of AD. However, there are promising strategies, some of which are already effective in animal models and some are tested in clinical trials including immunotherapy (Lambracht-Washington and Rosenberg, 2015; Landlinger et al., 2015), inhibition of Aβ production (Howell et al., 2015; Wang et al., 2012) or tau aggregation (Harrington et al., 2015; Richter et al., 2014; Wischik et al., 2014; Wischik et al., 2015). The ability to detect the disease in an early stage would help increasing the knowledge on the pathophysiology of AD, would improve the chances for developing effective treatments, and would widen the therapeutic window for effective treatment.

However, at the moment AD is difficult to diagnose at an early stage and even at advanced stages of the disease a definitive diagnosis of AD still requires an autopsy. Therefore, diagnostic methods are needed allowing early in vivo detection of AD pathology (Jack, Jr. et al., 2010).

Pathology

The main pathological hallmarks of AD are atrophy, neurofibrillary degeneration and extracellular amyloid plaques (figure 1 and 2) (Doens and Fernandez, 2014; Dore et al., 2013; Ma et al., 2014; Price et al., 1991; Takahashi et al., 2010; Villemagne et al., 2013). All these can also be demonstrated in non-demented elderly subjects and therefore, are not

specific for AD but their quantity and distribution in relation to the clinical symptoms is specific (Thal et al., 2014). It has been demonstrated that plaques and tangles lead to synaptic dysfunction, mitochondrial damage, inflammation and neuronal death (Doens and Fernandez, 2014; Takahashi et al., 2010). It still remains unknown how these pathological hallmarks are related to each other. Another frequent finding in AD is cerebral amyloid angiopathy (CAA) that can also contribute to the cognitive decline (Weller et al., 2009). More recently, changes in iron distribution have been noted (Bartzokis, 2011; Crichton et al., 2002; Haacke et al., 2005; Meadowcroft et al., 2015a; Meadowcroft et al., 2015b).

Atrophy

Shrinkage of the brain associated with AD is regarded as a valid marker of disease state and progression. Brain atrophy is correlated to neurofibrillary tangles (NFT) and neuropsychological deficits. It starts, in the majority of AD patients, in the hippocampus and the enthorinal cortex, extending to the temporal, parietal and frontal neocortices during the disease progression (Frisoni et al., 2010).

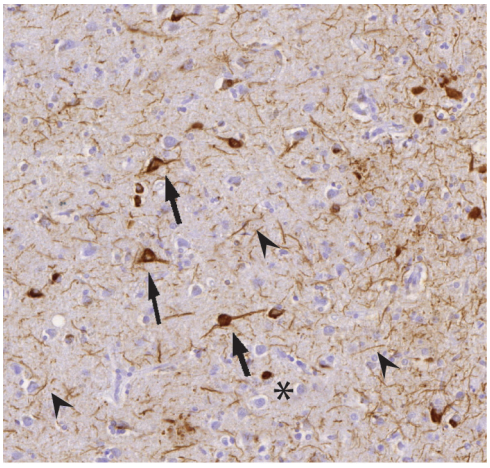

Figure 1: Examples of neurofibrillary tangles (arrows), neuropil threads (arrowheads) and dystrophic neurites (asterix).

Neurofibrillary degeneration

Neurofibrillary tangles (NFT) are one of the manifestations of neurofibrillary degeneration, the other being neuropil threads (NT) and dystrophic neurites (DN) (figure 1). In all these lesions there is intracellular accumulation of hyper-phosphorylated tau protein, forming soluble aggregates and paired helical filaments (PHF) (Kidd, 1963). NFT are neuronal cell bodies filled with PHF whereas in NT these PHF are present in the neuronal processes. DN are NT with irregular, dilated and distorted shapes. Normally tau proteins are involved in structural and reg-

ulatory function of the cytoskeleton where they promote the assembly of microtubule and their stability (Alonso et al., 2008; Grundke-Iqbal et al., 1986). However, when tau becomes hyper phosphorylated it exerts the exact opposite effect, leading to the dismantling of the same microtubule.

The resulting loss of neuronal structure impairs axonal transport, leading to disturbed proper synaptic, neuronal signalling and eventually leads to neuronal death (Ballatore et al., 2007). The degree of tau pathology correlates very well with dementia but neurofibrillary degeneration is not specific for AD: it is also seen in other neurodegenerative diseases, although with a different distribution in the brain.

Plaques

Plaques represent a wide array of lesions that contain extracellular deposits of amyloid β protein (Aβ) of which variable amounts are present as amyloid. Histologically, plaques are classified as diffuse, compact, classical and neuritic plaques. The type of plaque depends on the density and circumscription of Aβ (diffuse vs. compact plaques), the presence of an Aβ amyloid core (classical plaque) and the coexistence of dystrophic neurites (neuritic plaque) (figure 2) (Duyckaerts et al., 2009). Aβ plaques in an extensive amount is typical for AD but there is poor correlation between the amount of plaques and the degree of dementia.

Cerebral Aβ is generally cleaved by α-secretase and either degraded or cleared from the brain across the blood-brain barrier. Aβ peptide is generated by β- and γ-secretase induced cleavage of the amyloid precursor protein (APP), a transmembrane protein, forming predominantly Aβ1-40 or Aβ1-42. According to the amyloid cascade hypothesis, AD is initiated by an imbalance in Aβ production and clearance (Hardy, 2009; Hardy and Selkoe, 2002). This hypothesis is supported by the finding that APP gene mutations around the α, β- and γ-cleavage sites and gene mutations in proteins involved in cleavage at the APP γ-site lead to increased Aβ production and often early onset AD with an autosomal dominant pattern of inheritance. However, these mutations account for only < 5% of all AD cases. The other 95% of sporadic AD probably has a more complex multifactorial etiology (Minati et al., 2009).

Due to its fibrillogenic nature, high local concentrations of Aβ1-42 aggregate into soluble oligomers. These oligomers cluster into larger insoluble Aβ fibrils that allow the formation of β-sheet structures, which are characteristic for amyloid. This clustering of oligomers triggers the misfolding

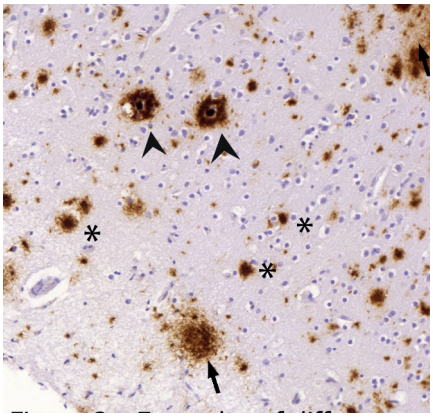

Figure 2: Examples of diffuse plaques (arrows), classical plaques (arrowheads) and compact plaques (asterix).

of other Aβ species, including the more soluble Aβ1-40, forming plaques (Duyckaerts et al., 2009).

The study of Corder et al. suggested an acceleration of amyloid deposition in women of late middle age associated with APOE4 (Corder et al., 2004). In some AD mouse models, a similar sex-related difference was found, showing more Aβ accumulation in female mice (Callahan et al., 2001; Wang et al., 2003).

CAA

In the majority of AD cases different amounts of CAA are found in the brain (Natte et al., 2001; van Rooden et al., 2009; Weller et al., 2009). CAA is caused by the same Aβ deposits as in plaques, mainly Aβ 1-40 and always forms amyloid which leads to stiffness and a loss of structure of the vesselwall. CAA can occur as a sporadic disease with little or no parenchymal Aβ deposits and is considered a major cause of cerebral microbleeds, haemorrhages and cognitive loss.

Inflammation

In AD, microglia may play an important role in disease progression by activating different inflammatory cytokines, causing neuronal damage and cell death (Doens and Fernandez, 2014; Hardy and Selkoe, 2002; Kettenmann et al., 2011). Microglia cluster especially around plaques and CAA.

Iron

Iron was recently identified as one of the pathological changes in the AD brain (Bartzokis, 2011; Meadowcroft et al., 2009; van Duijn et al., 2013). There are two hypotheses on the role of iron in AD. The first hypothesis claims that iron would directly contribute to the development of AD, due to its neurotoxic characteristics when not properly regulated (Bartzokis, 2011). The second is the idea of iron deposits being secondary to the formation of plaques and tangles (Peters et al., 2015); not playing a leading role in the development of AD, but following the formation of plaques. Iron also has relevance in AD research because it can be detected with

high sensitivity by magnetic resonance imaging (MRI) and may serve as an in vivo marker for AD.

Magnetic resonance imaging (MRI) and spectroscopy (MRS) in AD

The in vivo diagnosis of AD is now based on clinical and neuropsychological criteria, with additional techniques such as neuroimaging and cerebrospinal fluid biomarkers playing a supportive role, resulting in "probable" AD at best. This diagnosis is not always accurate and needs post mortem histological confirmation (Fox et al., 1996; Hyman et al., 2012; Jack, Jr. et al., 2010). An MR-based hallmark for AD is hippocampal atrophy. This measurement, however, is neither conclusive nor specific for AD and consequently of limited use in clinical setting (Nasrallah and Wolk, 2014). Furthermore, cerebral atrophy in AD is found in a late stage of the disease (Jack, Jr. et al., 2010) and therefore intrinsically a poor candidate for early diagnosis. Iron is a potential interesting target for the detection of early changes in AD. MRI is particularly sensitive to iron deposition in tissues, due to the changes it induces in the magnetic field.

Earlier studies demonstrated that increased iron accumulation in amyloid plaques induces a magnetic susceptibility effect. This is visible as hypointens foci on T2*-weighted or susceptibility-weighted (SW) MRI in the cerebral cortex of transgenic AD mouse models and in human post-mortem brain slices (Chamberlain et al., 2011; Meadowcroft et al., 2009; van Rooden et al., 2009). The high magnetic field strengths needed to obtain these results only recently became available for in vivo human use. These high field human MRI systems (> 7 Tesla) may offer new possibilities to specifically detect the neuropathological hallmarks of AD, with iron as main field of focus. Perhaps changes in iron distribution can be detected even at an earlier stage than the traditional hippocampal atrophy.

MRS is a non-invasive tool which can be used to measure the concentration of various brain metabolites in vivo (Marjanska et al., 2005; Oberg et al., 2008; Rupsingh et al., 2011). MRS uses MR to study the quantity of metabolites by measuring the interaction of a radiofrequency electromagnetic field with molecular nuclei inside an external high magnetic field (Azevedo et al., 2008). Measuring these metabolic changes in vivo, could help identify AD at an early stage since metabolic levels are believed to precede structural changes (Jack, Jr. et al., 2010).

Numerous MRS studies have been performed in transgenic (tg) mouse models of AD. Several tg mouse models are available that develop sim-

ilar, but not identical, pathology as compared to human AD. The results of previous MRS studies have shown AD-related abnormalities for several metabolites (Braakman et al., 2008; Chen et al., 2009; Choi et al., 2010; Dedeoglu et al., 2004; Marjanska et al., 2005; Oberg et al., 2008; von Kienlin M. et al., 2005; Westman et al., 2009; Xu et al., 2010). N-acetylaspartate (NAA) in the brain is predominantly present in neuronal cell bodies. Decreased NAA levels, indicating neuronal damage, have been found in tg mice in comparison to wild type (wt) mice (Chen et al., 2009; Choi et al., 2010; Dedeoglu et al., 2004; Marjanska et al., 2005; Oberg et al., 2008; von Kienlin M. et al., 2005). Myo inositol (mIns) and taurine play a role in osmoregulation and are mainly found in astrocytes of brain tissue. These metabolites were found to be higher in tg mice than in wt mice (Chen et al., 2009; Choi et al., 2010; Dedeoglu et al., 2004; Marjanska et al., 2005; Westman et al., 2009). Glutamate (glu) is an excitatory neurotransmitter, involved in learning, memory formation, and cognition, which is found to be decreased in mice with AD (Braakman et al., 2008; Choi et al., 2010; Dedeoglu et al., 2004; Marjanska et al., 2005; Oberg et al., 2008; von Kienlin M. et al., 2005).

Tg mouse models allow monitoring of the pathological and metabolic changes from the onset of AD in a longitudinal study, which is an effective way to investigate the early changes in the AD brain. However, no longitudinal study has been performed on AD mouse models using the non-invasive technique of MRS. Following mice from birth and investigating metabolic changes using MRS, the early start of AD might be detected making treatment more effective and giving us more insight in the pathogenesis of this disease.

Scope of this thesis

The overall aim of this thesis was to investigate MRI-based early markers of AD. We focused on correlation of radiological findings in AD with histology and we used MRS to study metabolic changes in brains of transgenic mice with AD.

Chapter 2 describes the effects of prolonged formalin fixation on MRI signal of brain tissue. This is important because such long fixed material is more readily available than brain tissue which is fixed for less than a year. In chapter 3 we compare different histological techniques to visualize iron in human brain tissue. Selection of the best techniques is crucial for reliable histological-radiological studies to assess the value of brain iron as an

MRI-based biomarker for AD.
Chapter 4 illustrates the relation between AD pathology and iron distribution in brain tissue of AD patients compared with normal aging subjects in different age groups. The difference of iron distribution between AD patients and aging was investigated in the frontal cortex.
Chapter 5 demonstrates a disturbed iron accumulation and myelin architecture in AD using MRI with histological correlation on ex vivo brain tissue.
In Chapter 6 we describe the first systematic longitudinal MRS study to investigate the differences in metabolic changes during development of AD in a transgenic mouse model.
Results of this thesis and recommendations for future studies are discussed in chapter 7.

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