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**Title:** Monitoring Alzheimer's disease in transgenic mice with ultra high field magnetic resonance imaging  
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1 General introduction

1.1 Alzheimer’s disease

Alzheimer's disease is one of the most common causes of dementia, accounting for approximately 60-80% of all cases, with no effective treatment or definitive ante-mortem diagnostic test (1,2). Increasing life expectancy along with the exponential rise of incidence of AD, position the disease as one of the main challenges to society and the health care system at present (1-3). The disease is characterised by abnormalities including a disruption of neuronal function, progressive memory loss, and a gradual cognitive, functional and behavioural deterioration (4). Despite noteworthy progress in unravelling the biological foundations of AD, the etiological mechanisms underlying the neuropathological changes in AD remain unclear. They probably involve environmental, epigenetic as well as genetic factors (5).

AD was named almost 100 years ago after a German physician, Alois Alzheimer (1864-1915) (6). According to Alzheimer’s original case report, published in 1907, the disease was characterised by the presence of miliary foci and neurofibrils in the post-mortem cerebral cortex region of a patient called “Auguste D”, who had shown progressive cognitive impairment, focal symptoms, hallucinations, delusions, and psychosocial incompetence (6). In this landmark paper, he remarkably identified the two hallmarks of AD that are known to be common to all forms of AD (7). These hallmarks are extracellular senile plaque deposition, which is mainly composed of Aβ peptide, and intracellular neurofibrillary tangle formation, which is composed of hyperphosphorylated forms of the microtubule associated protein tau (8). Presently, the disease is characterized by presence of these two hallmarks as well as vascular pathology (e.g. cerebral amyloid angiopathy) and brain atrophy (Fig. 1.1). These pathologies are often accompanied by gliosis, loss of white matter and synapses. Other features of AD pathologies include Lewy bodies, actin-immunoreactive Hirano bodies and inflammatory processes, e.g. oxidative stress and mitochondrial dysfunction (9). Furthermore, failure of neurotransmitter pathways, particularly those of the basal forebrain cholinergic system
Fig. 1.1. The pathological hallmarks of AD. (A) Photomicrograph shows an Aβ plaque (upper arrow) and a neurofibrillary tangle (lower arrow) in an 85-year-old individual with autopsy-verified AD. (B) Cerebral amyloid angiopathy is commonly observed in mouse models of AD and AD patients. Aβ40 is deposited around cerebral vessels. (C) Atrophy and clinical stage of AD: mild cognitive impairment (MCI) and AD patients showed atrophy compared to normal patients. Figs 1.1 A and C are reproduced, with permission, from Jack, 2012 (10). ©rsna.org.

have also been reported (8,11). Since learning and memory are thought to be associated with synaptic efficacy, such as long-term potentiation and long-term depression, synaptic alterations and loss are considered by some investigators to be the major neurobiological cause of cognitive dysfunction in AD (12-14).

While the etiology of the disease has been the subject of considerable debate, the amyloid cascade hypothesis suggests that altered cleavage of amyloid precursor protein, which leads to accumulation of different forms of Aβ peptide in the brain, is central to the pathological cascade that leads AD development (15). This hypothesis was initiated after
isolation of Aβ from cerebrovascular fibrils (16-18). Aβ40 and Aβ42 peptides, which are the major constituents of the Aβ plaques, are derived from an integral type 1 transmembrane integral protein through sequential cleavage by β-secretase and γ-secretase/presenilin complexes (19,20). These peptides can be either diffuse or compact in nature (17). Aβ peptides have the ability to self-aggregate and form large amyloid fibrils (21). Although both soluble oligomer forms of Aβ and insoluble forms, i.e. amyloid fibrils, have been associated with toxicity (22-24), recent studies have suggested that the soluble oligomer forms of Aβ are the most neurotoxic species (25), and have been associated with AD pathogenesis (26,27). How insoluble Aβ causes neurotoxicity is still not fully understood. Interestingly, the presence of insoluble Aβ does not correlate well with cognitive decline in AD (28,29), although some degree of correlation has been shown between Aβ load and cognitive decline (30). The main evidence supporting the amyloid cascade hypothesis has come from identification of mutations in the APP gene (31), which are linked to the early onset of autosomal-dominant familial type of AD (mean age at onset <65 years old). Additional support for this hypothesis comes from the identification of the APP gene's location on chromosome 21. Adults with Down's syndrome (trisomy 21), who have an extra chromosome 21, frequently (60%) develop AD in the sixth decade of life. This is thought to be due to excess production of the APP as a consequence of the trisomy, which leads to formation of amyloid plaques, neuronal cell dead and clinical AD (32).

Under normal physiological conditions, cleavage of APP by α-secretase precludes Aβ formation, whereas all FAD mutations near the β- or γ-secretase cleavage site of APP lead to an increase in the forms of Aβ that can aggregate. The amyloid cascade hypothesis was further supported by identification of mutations in PS1 (33) and PS2 (34), which are proteins that form the catalytic active site of the γ-secretase complex. The mutated form of these proteins can lead to production of more Aβ42, which leads to an increase in Aβ42/Aβ40 ratio (35,36).

Even though mutated genes in FAD are well characterized, the mutations in genes contributing to the disease formation in the LOAD are still under investigation. A few loci, such as the apolipoprotein E, have been derived from genome-wide association studies and have been linked with late onset AD (37,38). These AD gene candidates have been categorized into four groups, namely Aβ metabolism, lipid metabolism, innate immunity, and cell signalling (39) suggesting that multiple factors might be involved in the progression of AD. Clearly, there is an urgent need to have complete appreciation of
the full scope of AD pathology. To this end, it is essential to understand neuropathological factors that contribute to AD pathology. For example, cerebrovascular abnormalities leading to blood flow disturbances and white matter abnormalities, such as demyelination, are considered to be important risk factors for AD. However, how these abnormalities contribute to the disease progression remains unclear.

1.1.1 Cerebrovascular abnormalities and blood flow disturbances

Accumulating evidence suggests that AD is associated with cerebrovascular abnormalities that result in cerebral blood flow disturbances and impaired perfusion (40). The predominant source of cerebrovascular abnormalities of small blood vessels in AD are cerebral amyloid angiopathy and arteriosclerosis (41). Although CAA of the Aβ type is commonly observed in patients with AD and mouse models of the disease (42,43), the *in vivo* relationship between CAA related blood flow alterations in the brain of AD patients and Aβ deposits has not been systematically studied. Understanding the contribution of cerebrovascular abnormalities to the progression of AD is an important step for the development of new treatment strategies to slow and/or prevent the progression of the disease.

CAA is defined as the deposition of Aβ in the cerebral vessel walls (Fig 1.1b). The evidence for CAA in AD brain mainly comes from post-mortem studies (44,45). For example, Yamada has reported that among AD patients, the frequency of CAA was 87% (46). The CAA was associated with blood vessel wall disruption, loss of vascular smooth muscle cell and aneurismal vasodilation that lead to an increased incidence of CAA-related cerebral haemorrhage (47).

CAA usually affects cortical capillaries, small to middle-size arteries of the cerebral cortex and the leptomeninges (48). CAA may cause a failure in perivascular lymphatic drainage pathways, which drains interstitial fluid and solutes drain from the brain. Eventually, failure in drainage pathways could result in decreased clearance of Aβ from the brain (49). Finally, some evidence suggests that CAA could be an important cause of cerebral blood flow disturbances (50). There is a need for direct non-invasive *in vivo* methods to probe blood flow disturbances during AD development. Non-invasive visualization of these disturbances and correlation of them with *in vivo* plaque load could be an important step in the development of new therapies for the prevention of AD but also contribute toward better understanding of AD mechanism.
1.1.2 White matter alterations in AD

Along with grey matter pathologies, white matter microstructural alterations are also implicated in the pathogenesis of AD (51-55). The evidence for white matter alterations came mainly from neuropathological examinations of AD brain (52,54-57). Around 60% of the confirmed cases of AD showed white matter abnormalities, such as myelin breakdown (52). Although there is evidence that white matter abnormalities are associated with cognitive impairment, contribution of these abnormalities to the onset and progression of AD, however, remains unclear (58).

There is converging evidence that different patterns of white matter abnormalities in corpus callosum, which is the largest white matter structure in the brain, are associated with AD progression (59). Since the corpus callosum plays an important role in functional connectivity and cognitive processing, abnormalities in this region may lead to impairment in connections between brain regions, and disturb cognitive process (60). The disconnection between brain regions can play a critical role in progression of AD pathophysiology (59). Even though multiple AD studies have reported microstructural changes in the corpus callosum (61,62), little is known about age-dependent changes and tissue characteristics of these abnormalities. Biochemical analyses of AD white matter revealed that increased Aβ load is accompanied by significant decreases in the amount of myelin basic protein, myelin proteolipid protein, cholesterol, and leads to increased total fatty acid content (57). In addition, neuropathological examination depicted abnormalities, such as gliosis, reduction of oligodendrocytes, partial loss of axons and myelin breakdown in AD white matter (51,52,54,55). To approach these changes in vivo, however, is challenging. There is a need for in vivo non-invasive techniques to monitor age-dependent microstructural changes in corpus callosum. Altered white matter integrity in corpus callosum might be an early key event in the development of AD pathology. Non-invasive measures may have the potential to follow deteriorating white matter integrity during normal aging and the development of AD and, thus, may be a useful marker to evaluate treatment success.

The lack of histological correlation forms a limitation of in vivo studies. Therefore, animal models of AD can serve as valuable biological tools to characterize vascular and white matter abnormalities in vivo, and correlate these alterations with histology.
1.2 Mouse models of AD

More than 200 early onset-FAD mutations have been reported in genes producing APP, PS1 and PS2 proteins (39). Based on mutated FAD genes, and mutations on the gene of microtubular associated protein tau, in vivo mouse models of AD have been developed. Several groups have combined APP, PS1, PS2 and/or MAPT mutations to generate double or triple transgenic mice (63). Transgenic mouse models of AD play a very fundamental role in the study of pathogenic mechanisms in AD and allow development of novel therapeutic approaches that ameliorate or prevent AD within the next decade (64). These models replicate various features of human AD including senile plaques, cognitive deficits and neurofibrillary tangles pathology (11). A brief summary of the most commonly used transgenic mouse models of AD is presented in Table 1.1 and an updated overview of these models is available on the following website, http://www.alzforum.org, which also shows mouse models based on late-onset AD genetic risk factors, such as apolipoprotein.

Tg2576 is one of the most commonly used and well-characterized transgenic mouse models of the disease (Table 1.1) (65,66). This transgenic mouse line overexpresses a mutant human APP cDNA transgene with the K670M/N671L double mutation (also called Swedish mutation) under the control of the hamster prion protein promoter (65). These mutations lead to formation of soluble Aβ42 and Aβ40 peptides, resulting in Aβ plaque development. Aβ deposits start to appear at approximately 9 months of age and are similar to those found in AD. The plaques become visible in cortical and limbic brain regions of Tg2576 mice together with indications of cellular inflammation and behavioural defects (67-69). Neuronal loss, including loss of cholinergic neurons is not a feature of Tg2576 mice whereas CAA and white matter abnormalities are well developed in this model (70,71). Furthermore, development of age-dependent memory defects as assessed by Morris water maze testing, was also demonstrated in this model (65). Many of these findings fit well with biochemical and epidemiological findings in AD. Even though mice carrying FAD mutations represent a very small percentage of AD cases (1%<), the development and progression of AD pathology in these mouse models is of great interest to researchers since mouse models of AD present many pathologic features, similar to those found in patients with LOAD (in which symptoms develop at age 65 or later). Future treatment strategies that are effective in transgenic mouse models carrying FAD genes may provide clues to effective treatments for people with late-onset disease.
Over the past decade, in vivo non-invasive magnetic resonance based techniques, such as magnetic resonance imaging and spectroscopy, have emerged as a powerful means to monitor different biological processes longitudinally over time in transgenic mouse models of AD. Current efforts to extend the use of MRI and MRS to evaluate brain changes in these models of AD present a major opportunity to enhance the

<table>
<thead>
<tr>
<th>Species</th>
<th>Promoter</th>
<th>Aβ deposition onset</th>
<th>Transgene characteristics</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg2576</td>
<td>hamster PrP</td>
<td>9-11 months</td>
<td>human(h) APP695 with Swedish mutation (K670/M671L)</td>
<td>Deterioration in white matter integrity with age (71); CAA formation; memory deficits; brain lipid peroxidation; gliosis.</td>
<td>(65)</td>
</tr>
<tr>
<td>APP23</td>
<td>Thy-1.2</td>
<td>6 months</td>
<td>hAPP751 with Swedish mutation</td>
<td>Learning impairment; CAA; gliosis; tau hyperphosphorylation.</td>
<td>(66)</td>
</tr>
<tr>
<td>CNRD8</td>
<td>hamster PrP</td>
<td>3 months</td>
<td>hAPP695 with Swedish and Indiana (V717F) mutations</td>
<td>At 8 month of age cognitive deficits; CAA</td>
<td>(72)</td>
</tr>
<tr>
<td>APP/PS1</td>
<td>Thy-1</td>
<td>1.5 months</td>
<td>hAPP751 with Swedish mutation and PS1 with L166 in a single locus</td>
<td>At 4 months of age cognitive deficits; At 6 months of age deficits in long-term synaptic plasticity which correlate with the accumulation of intraneuronal Aβ; Tau pathology develops at 12 months of age.</td>
<td>(73)</td>
</tr>
<tr>
<td>3xTgAD</td>
<td>Thy-1.2</td>
<td>6 months</td>
<td>hAPP695 with Swedish, PS1M146V, and tauP301L</td>
<td>At 1.5 months Aβ42 accumulation, Aβ deposition and gliosis (2 months), synapse degeneration (4 months), neuron loss, and memory deficits (4-5 months).</td>
<td>(74, 75)</td>
</tr>
<tr>
<td>5x FAD</td>
<td>Thy-1.2</td>
<td>1.5-2 months</td>
<td>hAPP695 with Swedish, Florida (I716V), London (V717I) and PS1 double mutation (M146L, L286V)</td>
<td></td>
<td>(76)</td>
</tr>
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development of treatments and provide a potentially efficient approach for investigating promising biomarkers and evaluating candidate therapies. In the following paragraph, a brief introduction of theory of MR-based techniques that were employed in this thesis, and their relevance to mouse imaging is presented.

1.3 Magnetic resonance imaging

Magnetic resonance imaging is a highly advanced non-invasive medical imaging technique, with a very high spatial resolution. It is commonly used in preclinical and clinical studies as a noninvasive tool to investigate the structural, biochemical and functional changes in the brain (77).

The physical principles of MRI are based upon the nuclear magnetic resonance phenomenon, which was first experimentally demonstrated in 1946 independently by Purcell et al (78) and Bloch (79). NMR is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. The nuclei with an odd number of neutrons or protons exhibit spin. One of the most abundant nuclei with spin in the human body is the $^1$H atom. The $^1$H nuclear magnetic response to an external magnetic field is one of the strongest found in nature. For this reason protons are the most frequently studied nuclei. Several other nuclei including $^{31}$P, $^{13}$C, $^{23}$Na, and $^{19}$F have nuclear magnetic moments corresponding with spin ½ and are also suitable for detection by magnetic resonance (77). However their sensitivity is not as high as for the proton.

When nuclei possessing a spin are placed in an external magnetic field ($B_0$), the nuclear spins behave like a compass needle or small magnet (Fig. 1.2). The different nuclear states produce a net magnetization vector $M_z$ along the direction of $B_0$ (Fig. 1.2b). When the magnetization is tilted away from the $B_0$ direction by a radiofrequency pulse irradiation, the spins will precess around the magnetic field axis (z-axis) at a frequency proportional to the magnitude of the external field, according to the Larmor frequency in the MHz to GHz range, determined by $\gamma$, the gyromagnetic ratio. Protons have a higher $\gamma$ (42.575 MHz/T) compared other nuclei of biomedical interest. When an alternating magnetic field ($B_1$) is applied perpendicular to $B_0$ by using RF at the Lamor frequency (resonant frequency), the nucleus absorbs the portion of this energy. Subsequently it induces a transition between different spin states. The spins, which have different possible energy states, absorb the energy. This phenomenon can lead to a full or partial inversion of the nuclear spin population, with an increase in the fraction of the higher
energy state spins at the expense of those in the lower energy states decrease. When $B_1$ is applied in the presence of $B_0$, the magnetization vector $M_z$ will rotate away from the $z$-axis towards the $xy$-plane or $-z$-axis (Fig. 1.2c). Immediately after the radio frequency signal is switched off, the nucleus reemits the absorbed energy and the polarization relaxes back to the original alignment (Figs. 1.3a, b). This return to equilibrium is referred to as relaxation. The recovery process of magnetization along the longitudinal axis is called $T_1$ relaxation, spin-lattice relaxation or longitudinal relaxation (Fig. 1.3b). During this process an exchange of energy occurs between the spin system and the surrounding and is described as:

$$M_z(t) = M_{z \text{ max}} \left(1 - e^{-\frac{t}{T_1}}\right)$$  \hspace{1cm} (1.1)$$

The number $T_1$ represents the time it takes for 63% of the nuclear magnetization associated with the excited protons to realign with the $B_0$ (80). The transverse (or spin-spin) relaxation time $T_2$ reveals loss of the net magnetization in the $xy$-plane, due to dephasing (Fig. 1.3c). During $T_2$ relaxation, the energy is transferred between neighbouring nuclear spins. The number $T_2$ represents the time for $M_{xy}$ to drop to 37% of its original magnitude due to dephasing (80). During dephasing, the net magnetization in the $xy$-plane decays exponentially to zero over time. The rate at which this occurs is dependent on $T_2$ and the $T_2$ process is described as:

$$M_{xy}(t) = M_{xy \text{ max}} e^{-\frac{t}{T_2}}.$$  \hspace{1cm} (1.2)
When an image is acquired, $T_1$ or $T_2$ or a combination determines the amplitude of the MR signal and intensity of the image. Relaxation time parameters form the basis of tissue contrast in MRI, which allows identification of white and gray matter regions. During a relaxation period, the nuclei lose energy by emitting their own RF signal. This NMR signal is referred to as the free-induction decay. In MRI, the FID signal is measured by a surface or volume coil placed on or around the object being imaged. This measurement is processed or reconstructed to obtain grey-scale MR images. The resonance condition forms the basis of MRI, which can form an image by representing a display of spatially localized signal intensities. The image formation is achieved in two steps. First a slice of tissue is selected for imaging. Subsequently the location of the signal is determined through the use of a nonuniform magnetic field created by magnetic field gradients, which causes the spin location to be encoded in terms of its Larmor frequency (77). The gradient field is parallel to $B_0$ and its strength varies with position relative to $B_0$. The gradient has three components, $G_x$, $G_y$, and $G_z$, associated with the $x$, $y$, or $z$ spatial axis, respectively. When a magnetic field gradient along the $z$ axis formed, the magnitude of the applied magnetic field changes in proportion to the $z$ axis while remaining constant for any point in any $xy$ plane (77). Application of the gradient field allows the spins to experience different field strengths, which depend on the location of the spins within the gradient field. The gradient direction ($x$, $y$, $z$) determines the slice orientation, whereas the gradient amplitude together with the RF pulse characteristics determine both the slice thickness and slice position. Two most commonly employed pulse sequences for MRI are the spin echo sequence and the gradient echo sequence (Figs 1.4a,b) (81,82).

SE sequences use two RF pulses, an excitation pulse, which is a slice selective 90° pulse, and one or more 180° refocusing pulses that generate spin echoes. The two important parameters in a SE sequence are the repetition time and the echo time. TR is defined as the time between successive excitation pulses for a given slice. TE is the time from the

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**Fig. 1.3.** (a) Application of a 90° radiofrequency pulse produces magnetization in the $xy$ plane $M_{xy}$. When RF pulse is stopped, the nucleus reemits the absorbed energy and relaxes back to the original alignments at a rate determined by $T_1$ (b) and $T_2$ (c) relaxation times.
excitation pulse to the echo maximum. This sequence can be adjusted to give $T_1$-weighted, proton density and $T_2$-weighted images. The images acquired with the SE sequence are less sensitive to susceptibility artifacts than the data obtained with gradient echo sequences. In the SE sequence the 180° refocusing pulse can correct the susceptibility-induced dephasing of spins from $T_2^*$ effects (82). The GE sequence is used in a wide variety of imaging techniques, such as MR angiography. This sequence uses only a single selective pulse, which changes from 10° to 90°, in combination with readout gradient reversal. This sequence provides a fast imaging possibility with short TR (82).

1.3.1 Rapid acquisition with relaxation-enhancement imaging

Rapid acquisition with relaxation-enhancement imaging (83,84) is a fast magnetic resonance spin echo-imaging method. It is based on the Carr-Purcell-Meiboom-Gill echo train in which multiple spin echoes are generated by employing multiple 180° refocusing pulses. This imaging method delivers excellent $T_2$ contrast, which makes this method ideal for screening in most neurodegenerative disorders.
The primary contrast in RARE is $T_2$-based, however the contrast in the final image can be modified depending on the choice of TE, and TR. $T_2$-weighted images usually have a long TR >1500 ms and long TE, whereas a $T_1$-weighted image has a short TR <500msec and short TE. In a $T_2$-weighted image, the elements with long $T_2$ will appear bright, and elements with a short $T_2$ will appear dark. TE refers to the time between the excitation pulses and the echo. A practical implementation of RARE imaging in visualizing Alzheimer’s Aβ plaques is demonstrated in Chapter 2.

1.3.2 Magnetic resonance angiography

Magnetic resonance angiography offers a non-invasive approach to visualize cerebrovasculature and blood flow defects. One well-established MRA technique is the time of flight angiography (Fig. 1.5), which is commonly employed for the clinical evaluation of many vascular diseases (85). This technique is based on a nuclear magnetic resonance phenomenon in flowing liquids (86). This phenomenon, known as the time-of-flight effect, arises from the saturation of stationary tissue during RF pulse application, which leads to a net magnetization approaching zero and weak signal. The RF pulses, however, cannot fully saturate a flowing liquid, where the polarization is refreshed continuously with spins with full magnetization, and eventually this increases the signal (Fig. 1.4) (81,87). This phenomenon forms the basis of the contrast mechanism between a blood vessel and stationary tissue (85,87).

The time of flight MRA method is sensitive to fast flowing blood. In this method, vessel visibility depends on several object parameters, such as velocity of blood flow, relaxation times ($T_1$, $T_2$, $T_2^*$) of tissue and vessel diameter, and the imaging parameters, such as flip angle, slice profile, thickness and orientation, voxel size or field of view, resolution, TR, and TE (85,88,89). For example, increasing TR and decreasing the flip angle increases the visibility of vessels with lower lumen diameter where the blood flow is slow (88,90). In addition, moving to higher fields can significantly improve vessel visibility. Higher magnetic field strengths provide a double advantage for vessel/tissue contrast (91). First, an increase in stationary tissue $T_1$ value with increasing magnetic field strength provides better suppression of tissue signal. Second, higher magnetic field strength increases the magnetization of the flowing blood and eventually this leads to a stronger signal from the
flowing blood (91). The 3-D MRA acquired with 3D gradient echo sequences provides visualization of the large and small size arteries in a particular volume with a superior resolution (Fig. 1.4) (81,85,91). Additional post-processing of cross-sectional slices of these images with special techniques such as a maximum intensity projection algorithm allows 3D visual inspection of the vessel anatomy at an arbitrary view angle (92). Chapter 2 contains a practical implementation of this technique.

Figure 1.5. In-flow enhancement in GRE imaging results in bright signal of blood entering the imaging slice. In combination with saturation of static tissue this effect can be exploited to provide high blood-tissue contrast as in time of flight MR angiography. $V_{\text{blood}} = $ Velocity of blood; $TR =$ Repetition time; $TE =$ Echo time; $\alpha =$ flip angle. Reproduced, with permission, from Markly and Leupold, 2012 (81). © John Wiley & Sons, Inc
1.3.3 $T_2$ relaxation mapping

As described in the previous chapter, the relaxation times (e.g. $T_1$ and $T_2$) are intrinsic tissue parameters that are sensitive to changes in the biophysical water environment, and help determine the contrast in MRI (93). For example, myelin breakdown increases the $T_2$ relaxation time. Thus $T_2$ mapping has been proposed as a sensitive tool to investigate microstructural changes in white matter during AD progression (See Chapter 3, 4).

The $T_2$ relaxation time can be measured using multi-echo data sets acquired during a spin echo, enabling time efficient multi-slice imaging. $T_2$ can be calculated using different fit functions. In this thesis, to calculate $T_2$ maps of brain regions, regions of interest were drawn manually on the images by using an image sequence analysis tool package (Paravision 5, Bruker), which uses a fit function $[y = A + C*\exp(-t/T_2)]$, where $A$ is the absolute bias, $C$ represents the signal intensity, and $T_2$ is the transverse relaxation time. Estimating true (absolute) $T_2$ values of tissue is challenging in biological systems due to increased field inhomogenities and magnetic susceptibility differences, which lead to signal loss. The spin echo technique used in this thesis rephrases the signal before collection, which decreases the effect of susceptibility differences on the signal (81,82). Therefore the calculated $T_2$ is closer to the true $T_2$ values than for images acquired with traditional methods.

1.3.4 Magnetic resonance spectroscopy

Magnetic resonance spectroscopy is a specialized method that offers a non-invasive means for the characterization of metabolic composition of tissues from the particular region of interest in vivo. This method along with MRI has been commonly employed in preclinical studies to study a variety of neurodegenerative disorders such as Alzheimer’s disease. MRS and MRI are closely related techniques. The same scanner is used for both MRI and MRS. Both techniques use signals from the same nuclei, in particular $^1$H. One of the main differences between MRI and MRS is that while MRI provides information on the physical-chemical state of tissue and on motion, MRS provides only information about the chemical composition of tissue and is less sensitive than MRI.

The MR spectra are acquired by applying a RF pulse via a surface or volume coil at a particular resonant frequency to detect the signal of specific nuclei (e.g., $^1$H, $^{31}$P, etc.) in the region of interest. When the excitation RF pulse is turned off, the spins relax to their equilibrium state by emitting RF waves. These RF waves are collected as an FID in the
time domain by the RF coil (94). The sensitivity of the experiment can be increased by repeating the experiment and accumulating the FIDs. The FIDs contain (1) intensity, (2) frequency, (3) half-life, or $t_{1/2}$ and (4) phase information. The FID is converted to frequency-domain data \(i.e\). the spectrum) using a technique called Fourier transformation where the data are presented in the form of a one dimensional or two dimensional spectrum.

### 1.4 Monitoring AD using MR- based techniques

In recent years increasing effort has been directed towards the development of non-invasive high resolution \textit{in vivo} neuroimaging methods to monitor pathological changes in AD brain. Two most commonly employed advanced imaging technologies are PET and MR-based techniques, such as MRI. Unlike PET, MRI uses non-ionizing radiofrequency pulses and a strong magnetic field to acquire brain images with high spatial resolution. With continuing developments in the precision of MRI, it is increasingly feasible to use these techniques in the study of subtle brain changes during AD progression (10, 95).

Currently a variety of MR based techniques is commonly employed to characterize the anatomical, functional, neurochemical and blood flow changes observed during AD progression. For example, progressive cerebral atrophy, such as hippocampal atrophy, is a characteristic feature of AD, which can be detected with structural MRI (10, 96, 97). Non-invasive, \textit{in vivo} methods to image Aβ plaque-load would be very valuable for monitoring Aβ formation with age in the same patient, and to evaluate the success of treatments for plaque clearance (98). MRI can detect Aβ plaque-load with higher spatial resolution compared to PET without using ionization radiation. Theoretically, MRI has the potential to resolve individual Aβ plaques, which range from 2 to 200 μm in size (99). Until now two main strategies have been employed to detect Aβ plaques in AD brain. These strategies involve contrast-enhancing agents, which bind to Aβ plaques, and susceptibility artefacts. \textit{In vivo} MRI methods to image Aβ plaques without contrast agents in human AD patients are underway (100) but are currently not available in clinical magnetic fields. Recent \textit{ex vivo} and \textit{in vivo} higher field MRI studies using susceptibility artefacts have visualized Aβ plaques in the brain of AD mice as hypointense signals (98, 101, 102). These studies suggested that the signal hypointensities observed on MRI images represent Aβ plaques, containing metals, particularly iron. In addition, reduction of the water content in Aβ plaques can be the source of signal
hypointensities observed in MRI (103). Also, indirect MRI techniques, such as diffusion kurtosis imaging, magnetization transfer contrast and relaxation time maps have been employed to detect amyloid load in AD brains (10).

Microstructural changes, such as white matter degeneration, which accompany AD development, can be detected by intrinsic relaxation time parameters or DTI. DTI maps diffusion processes of water molecules in the brain, which serves as useful tools to detect changes in white matter integrity. DTI is being tested in an attempt to distinguish patients with AD from healthy elderly individuals (10). The neurobiological substrate of diffusion changes in AD remains unresolved. It remains unclear whether DTI has sufficient sensitivity to separate AD patients from controls, and is awaiting sufficient validation to be accorded into wider clinical use (10). Recent studies depicted that in vivo quantitative $T_2$ relaxation measurements are capable of characterizing white matter abnormalities in demyelinating disorders such as Multiple Sclerosis (104). However, few studies have employed $T_2$ measurement to assess white matter integrity in AD (105). Studies to characterize these changes longitudinally are missing. Functional MRI task free and task-based methods, which measure brain activity, are under evaluation to investigate their potential to discriminate different states of AD from healthy elderly individuals. In particular, a task free method, namely the resting state functional connectivity MRI method is increasingly employed to investigate subtle brain network abnormalities in AD (106). Loss of intra-network connectivity in large scale networks, including the default mode network, dorsal attention network, control network, salience network, and sensory motor network, has been shown in AD using the rs-fc MRI method, suggesting that soluble and/or insoluble Aβ causes disordered neural communication (106). The diagnostic accuracy of this method is under evaluation.

Biochemical changes in the brain can be measured by employing MR spectroscopy, which provides information about brain chemistry. Proton ($^{1}H$) MRS, among other spectroscopy methods, is one of the most commonly employed in vivo MR techniques for diagnosis of AD. This method has great potential to be used in clinical diagnosis of AD in the near future. For example, a decrease in NAA/mIns ratio provides very high sensitivity and specificity to distinguish AD from healthy individuals (95,107,108). Several cerebrovascular alterations, such as CAA, accompany AD neuropathology in AD (42,46,70). Cerebral blood flow abnormalities can be detected non-invasively in vivo in transgenic mouse models of AD (109) and human AD patients (110) by a variety of methods such as MRA and arterial spin labelling. A brief overview of MR based
techniques for visualizing blood flow and white matter abnormalities to assess AD pathology is given below.

1.4.1 MRA to detect blood flow abnormalities

The role of cerebrovascular abnormalities in the etiology of AD is increasingly becoming a topic of research (111). How CAA related abnormalities in cerebral blood flow contribute to the onset and progression of AD, and the interrelationship between these abnormalities and parenchymal amyloid load, however, remains unclear. *In vivo* non-invasive methods to visualize the blood flow abnormalities in brain are needed to address these questions.

Cerebrovascular blood flow abnormalities can be detected without contrast agent administration via MR-based techniques such as MRA and ASL. MRA of the mouse brain can be performed in spontaneously respiring animals. Determining the basal perfusion alterations in mice using ASL techniques is, however, challenging. Stable physiological conditions are warranted during *in vivo* scanning. This can be obtained by intubating and ventilating the animal, allowing a more controlled setting. However, these procedures are not routinely performed. MRA has been employed to investigate vascular alterations in a variety of mouse models of AD (109,112,113) and in clinics (114,115), the resolution of these studies, however, was hampered by the use of low magnetic field strengths. The MRA studies at ultra high magnetic field strengths, such as 17.6 T, which can provide better visualization of moderate to smaller vessels, are missing. The ability of the ultra-high field MRA to detect small flow disturbances makes it attractive as a diagnostic tool in AD (109). The application of this method in a mouse model of AD at 17.6 T is presented in Chapter 2.

1.4.2 $T_2$ measurements to assess white matter abnormalities

Tissue relaxation time parameters, such as $T_1$ and $T_2$, are sensitive to the biophysical environment, and can provide tissue specificity allowing enhanced characterization of pathological tissue (116). Thus a variety of aging and AD studies have employed relaxation time data in order to detect changes in tissue integrity (117-123). However, changes in relaxation values of white matter of AD brain with age and correlation of these values with AD pathological features, such as amyloid load and demyelination, is not yet well characterized.
A variety of transgenic mouse models of AD have been used to investigate the correlation between intrinsic relaxation values and AD pathology (e.g., demyelination, gliosis, αβ plaque deposition). An overview of these studies in AD mice is depicted in Table 1.2.

The changes in gray matter are well characterized by these studies using relaxation values, e.g., $T_1$, $T_2$, and $T_2^*$ and $T_1\text{rho}$. However, little is known about changes in the...
white matter. There is evidence that altered white matter integrity, such as demyelination, is an early event in the course of the AD, but the nature of these changes and their contribution to the onset and progression of the disease remains unclear (52,133-136). There is an emerging need for detection of these changes in vivo with MRI, which has potential to aid early diagnosis of AD. $T_2$ values are sensitive to myelination and demyelination processes (124).

Thus, $T_2$ measurements can serve as a sensitive non-invasive tool to longitudinally monitor subtle microstructural changes in corpus callosum and other white matter regions. However, a proper longitudinal study, which follows $T_2$ changes in white matter integrity of AD mice models, is missing.

### 1.4.3 Ultra-high field MRI in mouse models of AD

Transgenic mouse models of human diseases have been commonly employed in biomedical research for testing treatments and monitoring disease progression. Being a non-invasive method, MRI allows longitudinally monitoring of structural and functional changes in these murine models of human diseases. The small size of the mouse brain however, has considerable limitations such as low signal-to noise ratio for a given measurement time. To overcome these limitations, several strategies can be employed such as conventional signal averaging, using a cryogenic radio frequency coil, or moving to higher magnetic field strength. In particular, the use of ultra-high magnetic fields ($\geq 7$T) in MRI and MRS will be highly beneficial in order to obtain well-defined chemical shift spectra, larger data acquisition rates, higher-resolution and signal-to-noise ratio (137). Currently the best available horizontal MRI system operates at field strength of 14.1 T, whereas vertical systems are at much higher field strength ($\geq 17.6$ T). Use of ultra-high systems might cause a paradigm shift in preclinical AD studies through the in vivo observation of metabolism and subtle microscopic tissue changes. Many MR based techniques benefit from ultra-high fields. In particular, MRA clearly benefits from high magnetic field. Increased $T_1$ relaxation time and high magnetization at higher fields provides improved higher vessel-to-tissue contrast and the signal-to-noise ratio that may contribute to the improved quality of MRA (138). Thus abnormalities in smaller vessels can be monitored without the use of contrast enhancing agents. In addition to increased SNR, in vivo localized MRS, which provides the unique prospect of longitudinally monitoring brain metabolism in living animals or humans, benefits from high magnetic fields from the increased spectral resolution (137)
Relaxation time parameters \((e.g., T_1 \text{ and } T_2)\) are known to change substantially with increasing field strength in biological tissues, but the amount of change depends, among other factors, on the biophysical water environment and the presence of metal ions such as iron in such a way that it cannot be estimated accurately with a theoretical calculation \((139)\). Thus, an accurate determination of proton relaxation times with ultra high field strength MRI is an essential first step in exploring the capabilities of ultra high field MRI. While such values have been reported in lower field strength, to our knowledge normative \(T_2\) values of healthy mouse brain for 17.6 T are currently not available (See Chapter 4).

1.5 Thesis scope

An advanced characterization of AD pathology can be achieved by application of non-invasive \textit{in vivo} imaging methods, such as MRI, to monitor the disease progression longitudinally in transgenic mouse models of AD. In particular \textit{in vivo} imaging of these mice models at ultra high magnetic field strengths can permit a better understanding of the underlying cellular mechanism of AD. This knowledge can be used to develop potential biomarkers and promising treatment strategies to impede and/or prevent the disease progression.

There are important developments in AD translational research, especially with respect to the \textit{in vivo} imaging of vascular defects and white abnormalities using MRI in transgenic mouse models of AD. AD is linked to abnormalities in the vascular system such as CAA. Although CAA is very common in the advanced stages of AD, its contribution to the onset and progression of AD remains unclear. In addition, the \textit{in vivo} relationship between Aβ plaque deposition in brain tissue and its correlation with blood flow alterations is unknown. Longitudinal high-resolution MR studies to monitor blood flow abnormalities are required to study blood flow abnormalities and to investigate whether they are associated with Aβ plaque deposition. Furthermore there is a need for monitoring white matter changes in corpus callosum with age during AD development. The converging evidence suggests that different patterns of white matter abnormalities in corpus callosum are associated with AD progression \((59)\). These changes might be an early biomarker for AD. How and when the integrity of white matter decreases in corpus callosum remains unclear.
Since ultra-high field systems are increasingly used in AD research, there is a need for accurate determination of proton relaxation times at 17.6 T to explore the capabilities of ultra high field MRI. While such values have been reported at lower field strength, to our knowledge normative $T_2$ values of healthy mouse brain for ultra high magnetic field strengths (17.6 T) are currently not available. These values can serve as normative values against which disease related relaxation changes can be assessed in mice.

The aim of this thesis is to implement and optimize MR based techniques, namely MRA and $T_2$ relaxometry at high and ultra high magnetic field (i.e. 9.4 and 17.6 T) to longitudinally monitor blood flow and white matter abnormalities in a mouse model of AD to establish potential neuroimaging biomarkers.

In Chapter 2, high resolution $\mu$MRA at 17.6 T has been optimized and successfully implemented to longitudinally monitor morphological changes in cerebral arteries in a Tg2576 mouse model. These changes were correlated with Aβ plaque development. In Chapter 3 in vivo $T_2$ changes were longitudinally monitored in the corpus callosum of the Tg2576 mice. Since $T_2$ values have been shown to be sensitive to axonal injury, gliosis and demyelination, probing changes in $T_2$ values will provide a sensitive marker of dynamic microscopic changes in the corpus callosum, and will eventually yield new insights into the pathology underlying AD. In Chapter 4, regional $T_2$ values of the healthy mouse brain were established at 17.6 T, and regional brain $T_1$ and $T_2$ changes were followed with age. The estimates of in vivo $T_1$ and $T_2$ will be useful to optimize pulse sequences for optimal image contrast at 17.6 T and will serve as baseline values against which disease related relaxation changes could be assessed in mice. Chapter 5 provides a general discussion to the work presented in this thesis, and presents some future prospects.
References


