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Author: Nabuurs, Rob Johannes Antonius

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Chapter 5

MR Microscopy of human A β deposits -
characterization of parenchymal amyloid,
diffuse plaques and vascular amyloid

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Rob J.A. Nabuurs¹

Remco Natté²

Fenna M. de Ronde²

Ingrid Hegeman-Kleinn³

Jouke Dijkstra⁴

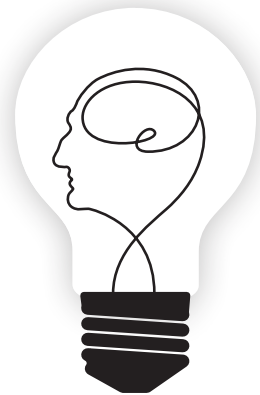
Sjoerd G. van Duinen²

Andrew G. Webb¹

Annemieke J. Rozemuller⁵

Mark A. van Buchem¹

Louise van der Weerd^{1,6,7}



Abstract

Cerebral deposits of amyloid-beta peptides ($A\beta$) form the neuropathological hallmarks of Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). In the brain $A\beta$ can aggregate as insoluble fibrils present in amyloid plaques and vascular amyloid, or as diffuse plaques consisting of mainly non-fibrillar $A\beta$. Previously, magnetic resonance imaging (MRI) has been shown to be capable of detecting individual amyloid plaques, not only via the associated iron, but also $A\beta$ itself has been suggested to be responsible for a decrease in the image intensity. In this current study we aim to investigate the MRI properties of the different cerebral $A\beta$ deposits including diffuse plaques and vascular amyloid. Post-mortem 60 μm thick brain sections of AD, CAA and Down's syndrome patients, known to contain $A\beta$, were studied. High resolution T_2^* - and T_2 -weighted MRI scans and quantitative relaxation maps were acquired using a microcoil on a Bruker 9.4T MRI system. Specific MRI characteristics of each type of $A\beta$ deposit were examined by co-registration of the MRI with Congo Red and $A\beta$ -immunostainings of the same sections. Our results show that only fibrillar $A\beta$, present in both vascular and parenchymal amyloid, induced a significant change in T_2^* and T_2 values. However, signal changes were not as consistent for all of the vessels affected by CAA, irrespective of possible dyshoric changes. In contrast, the non-fibrillar diffuse plaques did not create any detectable MRI signal changes. These findings are relevant for the interpretation and further development of (quantitative) MRI methods for the detection and follow-up of AD and CAA.

Introduction

Diagnosis of Alzheimer's disease (AD) *in vivo* remains a problematic issue. Despite recent efforts to sharpen clinical criteria, a definitive diagnosis still requires histopathological evidence showing the cerebral presence of amyloid- β peptides (A β) aggregated into amyloid plaques and neurofibrillary tangles (NFTs).^{1,2} Although the precise role of amyloid in AD pathology is still not completely understood, accumulation of amyloid plaques is thought to precede the onset of the first clinical symptoms by up to two decades.^{3,4} Clinical imaging techniques capable of visualizing and quantifying these early changes might enable early diagnosis.

Thus far the clinical role of magnetic resonance imaging (MRI) has been confined to depicting brain atrophy from mid-stage AD onwards.³ However, clinically MRI has not been able to detect earlier pathophysiological alterations, despite preclinical evidence that showed detection of individual amyloid plaques by MRI was feasible in post mortem human brain tissue⁵⁻⁸ as well as *ex vivo* and *in vivo* in several AD mouse models.^{6,9-16} The high magnetic field strength, high resolution and long acquisition time used in these studies prohibit a direct translation of appropriate imaging protocols to the clinic. Though detection of individual plaques is not realistic in a clinical setting, quantitative T₂ and T₂* measurements could be used to detect relaxation changes associated with amyloid plaque accumulation, or associated iron deposits, even at an image resolution that is far lower than the dimensions of amyloid deposits.^{9,17} Promising as it may be, this requires a full understanding of the MRI characteristics of all forms of A β deposits that may be present in AD.

The most renowned type of A β deposit in the cerebral cortex is the fibrillar plaque, which is one of the neuropathological criteria used post-mortem to confirm the diagnosis of AD. However, several other types of A β deposits can occur in the brain. Neuropathologically, the terminology used to describe the different A β deposits is diverse. In line with Duyckaerts *et al.* human parenchymal A β deposits can be divided into two main subtypes based on their A β content: diffuse plaques and focal plaques.¹⁸ Although the nomenclature of the different types of focal plaques is rather heterogeneous (primitive, classic, neuritic, senile and burn-out plaques), they generally contain A β peptides aggregated into a typical fibrillar β -sheet pleated conformation known as amyloid. For the remainder of this paper this type of fibrillar A β deposits are referred to as amyloid plaques. The presence of amyloid is presumed to lead to neuronal dysfunction and even the complete loss of neurons, and correlations between amyloid plaques and clinical symptoms have been reported.¹⁸

In contrast, the so-called diffuse plaques are not specific for AD and although abundant in AD and healthy controls, they correlate poorly or not at all with dementia.¹⁹⁻²³ They are histopathologically defined as large, ill-defined patches of parenchymal deposits of A β peptides but with hardly to no fibrillar amyloid or dystrophic neurites present.¹⁸

The formation of amyloid may also stretch along the vascular wall of leptomeningeal and parenchymal arteries and arterioles, and to a lesser extent the brain capillaries. This vascular amyloid is commonly referred to as cerebral amyloid angiopathy (CAA).^{18,24} Although CAA is often found to co-exist with AD, it can also appear as an entity on its own leading to microbleeds and severe cerebral hemorrhages.²⁵

Thus far, MRI studies have only examined the A β -related MR contrast changes with respect to parenchymal amyloid plaques.^{6,9-16} Observed contrast changes were primarily attributed to the

accumulation of iron within the plaques.^{9,10,12,15} However, similar MRI contrast was also observed in amyloid plaques without iron, and therefore it has been hypothesized that A β by itself contributes to the relaxation changes, presumably due to its hydrophobic nature.^{6,16}

The aim of this study, therefore, was to study the MRI characteristics of these different types of human cerebral A β deposits. In addition to the known induced MRI contrast changes due to the presence of amyloid plaques, we investigated the MRI characteristics of diffuse plaques and CAA in post-mortem human brain material. Ultimately, a better understanding of the MRI correlates of A β deposits may help to interpret the observed relaxation changes in quantitative MRI of AD patients.

Materials and Methods

Brain samples

Brain tissue was obtained from the tissue bank of our institution and from the Netherlands Brain Bank (NBB). Several hours post-mortem, the brains were resected, serially cut in 1 cm coronal sections and stored in 4 % paraformaldehyde. Routine autopsy of the brains included histological examination for CAA- and AD-related pathology respectively according to Attems *et al.*²⁶ and Braak *et al.*²⁷ Based on autopsy-confirmed diagnosis we selected tissue from subjects suffering from diseases that are known to be associated with cerebral A β . We selected patients with AD ($N = 5$), Down's syndrome (DS) ($N = 1$), CAA ($N = 6$) and clinically non-demented controls ($N = 3$). **(Table 5.1)** Patient anonymity was strictly maintained. All tissue samples were handled in a coded fashion, according to Dutch national ethical guidelines (Code for Proper Secondary Use of Human Tissue, Dutch Federation of Medical Scientific Societies). Previously described formalin-induced tissue artifacts that might affect MRI signal were avoided accordingly by careful visual and microscopic inspection and including material fixed for maximal 32 months.^{28,29} **(Table 5.1)**

Sample preparation

MR samples were prepared according to methods that have been described previously.⁷ In short, from each subject a cortical tissue block of approximately 12 x 12 x 10 mm³ was resected based on known predilection sides for the different types of A β . To study parenchymal deposits samples were obtained from a coronal section that contained a section of the hippocampus to enable imaging of the neocortex in the medial temporal lobe adjacent to the entorhinal cortex. To investigate the vascular A β deposits, tissue blocks were obtained from occipital lobe cranial within the sulcus, since the occipital lobe is the site of predilection of CAA. Remnants of the dura were removed from the pial surface, and 60- μ m-thick tissue sections were cut with a vibratome (VT1000S, Leica, Germany). Prior to imaging, any residual formalin was washed out by immersion in phosphate buffered saline (PBS) for at least one day to partially restore transverse relaxation times.³⁰ Sections were mounted on a standard microscope slide covered with a drop of PBS to prevent dehydration.⁷ Extreme care was taken to avoid the inclusion of any air bubbles by slowly lowering the coverslip, after which the section was sealed with nail polish.

Table 5.1 Subject characteristics and presence of A β deposits

Subject Characteristics				Sample scoring							
No.	Age / Sex	Diagnose	Braak	Cause of death	Fixation period (months)	Normal GM	Diffuse plaques	Amyloid plaques	CAA	dysCAA	capCAA
1	23 / F	Control	0	Myocarditis	26	scored	-	-	-	-	-
2	83 / F	Control	2	Arrhythmia	26	scored	-	-	-	-	-
3	89 / F	Control	3	Myocardial infarct / Pneumonia	20	-	scored	scored	-	-	-
4	60 / F	AD	5	Cachexia / dehydration	25	-	scored	scored	-	-	-
5	77 / M	AD	6	Cachexia / dehydration	26	-	scored	scored	-	-	-
6	85 / F	AD	4	Aspiration pneumonia	26	-	scored	scored	-	-	-
7	87 / M	AD	5	Cachexia / dehydration	25	-	scored	scored	-	-	-
8	88 / F	AD	4	Unknown	17	-	scored	scored	-	-	-
9	31 / M	DS	0	Myocardial infarct	23	scored	scored	-	-	-	-
10	72 / M	sCAA	3	Intracerebral haemorrhage	32	-	-	-	scored	-	-
11	57 / M	AD / sCAA	6	Intracerebral haemorrhage	30	-	-	-	scored	scored	-
12	84 / M	sCAA	1	Pneumonia	22	-	-	-	scored	-	scored
13	61 / F	sCAA	0	Intracerebral haemorrhage	4	-	-	-	scored	-	-
14	80 / M	sCAA	2	Pneumonia	32	-	-	-	scored	scored	-
15	81 / M	sCAA	3	Haemorrhagic infarcts	5	-	-	-	scored	scored	-

Characteristics of all subjects used for this study are shown based upon clinical information and standard neuropathologic examination according to Braak criteria for thin sections.²⁷ Per subject the type of A β deposits that were used for MRI analysis are stated as scored. A negative score does not necessarily imply they were not present but only that they were not included for that particular subject. AD = Alzheimer's disease; CAA = cerebral amyloid angiopathy; DS = Down's syndrome; sCAA = sporadic CAA; dysCAA = dyschoric CAA; capCAA = capillary CAA; GM = gray matter.

MRI acquisition and post-processing

All MRI experiments were performed on a vertical bore 9.4 T Bruker Avance 400 WB spectrometer, equipped with a 1 Tm⁻¹ actively shielded gradient insert. As previously described, a self-resonant microcoil was placed directly on top of the sample to obtain MR images of the 60- μ m-thick tissue sections.⁷ Multi-gradient-echo (MGE) images were acquired to assess T₂* with a repetition time (TR) = 750 ms, echo time (TE) = 4-70 ms (12 echoes with 6 ms spacing), flip angle (FA) = 30°, field of view (FOV) = 16 x 16 mm², data matrix [400 x 400] resulting in a resolution of (40 μ m)² with a total data acquisition time of 6 hours and 40 minutes. T₂ effects were investigated by using a multiple spin echo sequence acquired in 4 hours and 26 minutes with TR = 2000 ms, TE = 10-100 ms (10 echoes with 10 ms spacing), FOV = 16 x 16 mm², data matrix [200 x 200] giving a resolution of (80 μ m)². Post-processing was performed using a voxel-wise linear regression MatLab routine (MathWorks, Natick, MA, USA) to calculate quantitative T₂* and T₂ maps. T₂- and T₂*-weighted images were created as the sum of the third to the tenth echo image.

Despite extreme care in sample preparation, MRI hypo-intensities related to small inhomogeneities caused either by external dust particles, or tiny air bubbles were unavoidable. These artifacts were present throughout all samples, and were excluded from the analysis based on visual microscopic inspection of the sample.

Congo Red staining for amyloid

After MRI acquisition, Congo Red staining was performed for detection of amyloid on the same control, DS and AD sections that were analyzed by MRI. After removing the coverslip, the free floating tissue section was rinsed in distilled water three times for 10 minutes each. The section was counterstained with Harris' haematoxylin and rinsed in tap water for 10 minutes. After pretreatment of 20 minutes with 3% NaCl and 0.01% NaOH in 80% EtOH, the free floating section was again immersed in the same solution for 20 minutes with 0.5% Congo Red. The section was rinsed briefly in, in turn, 96 – 80 – 70 – 0 % EtOH in distilled water and air-dried prior to mounting (Micromount). This additional rehydration step with pure distilled water reversed most non-uniform shrinkage caused by the ethanol, thereby simplifying subsequent coregistration with the MRI data. Directly thereafter the section was digitized using a bright field microscope scanner (Pannoramic MIDI, 3DHistech, Hungary). Congo Red stained amyloid was confirmed using depolarized light, under which the red stained areas gave a characteristic green birefringence.

A β -immunostaining

To detect all isoforms of A β , including the diffuse plaques, a standard immunostaining procedure using a commercial monoclonal antibody (6F/3D, DakoCytomation, Denmark) was applied.³¹ The sections that had previously undergone Congo Red staining had the coverslip removed by overnight immersion in xylene, and were rehydrated using 96 – 80 – 70 – 0 % EtOH in distilled water. During this process both Congo Red and haematoxylin were completely removed. Endogenous peroxidase activity was blocked by applying 0.3% H₂O₂ in methanol for 20 minutes. For antigen retrieval the samples underwent 1 hour immersion in 85% formic acid, rinsing with distilled water and PBS, and 30 minutes immersion at 37 °C in 0.1% trypsin (Type II-S, Sigma) with 0.1 % CaCl₂ at pH 7.4. Next, the floating sections were incubated overnight with a 1:10 dilution of A β -antibody in 1% BSA in PBS at room temperature, and rinsed three times for 10

minutes each with PBS before biotinylated RAM secondary antibodies (1:200, DakoCytomation, Denmark) in 1% BSA in PBS were added for one hour. Next, the sections were rinsed three times with PBS and incubated for 30 minutes with freshly prepared HRP-labeled avidin-biotin complex (ABC, Vector Labs, CA, USA). Enhancement was completed by 5 – 10 minutes immersion in 100 ml distilled water containing 0.075% 3,3'-diaminobenzidine (DAB) (Sigma) and 15 μ l of 30% H₂O₂ after which the reaction was stopped in distilled water. The section was then mounted and digitized.

Coregistration and ROI analysis

For each section, the processed images were stacked prior to co-registration with their corresponding histology using the Cyttron visualization platform (CVP, Leiden, the Netherlands) and Adobe Photoshop CS3 (Adobe). Based on evaluation by a neuropathologist (R.N.), the A β positive areas on immunohistochemistry that were also Congo Red positive were classified as amyloid plaques. The poorly-defined larger patches of parenchymal A β were classified as diffuse plaques based on both their morphology and the lack of Congo Red staining according to the previous nomenclature.¹⁸ Further analysis included only those areas that clearly contained only a single subtype of A β deposition. CAA-loaded vessels were selected based on their distinct vascular morphology present in the immunostaining. Areas completely devoid of any staining were classified as normal appearing gray matter (GM). Based on these classifications, T₂*- and T₂-weighted images were analyzed to detect any corresponding changes in the MRI signal related to the specific A β -deposits. Quantitative T₂* and T₂ relaxometry of the different parenchymal A β deposits was assessed by region-of-interest (ROI) analyses based on the aforementioned areas using the Image J software package (National Institutes of Health, Bethesda, MA, USA). For amyloid plaques, an ROI of a single voxel was used since this corresponds best to the size of the plaques. In contrast, for the areas containing diffuse plaques, the ROI consisted of multiple voxels corresponding to the plaque's outline on histology. To assess normal-appearing GM, several ROIs of 100 voxels were selected in anatomically-similar cortical areas. Measurements across all samples were combined to calculate averages for each type of A β deposit. Possible differences in relaxation times for each subtype were tested using a Student t-test, with p-values < 0.05 considered statistically significant.

Results

Parenchymal A β deposits

All samples were taken from similar neo-cortical regions of the middle temporal lobe, and formalin fixation periods were between 3-24 months. Both diffuse and amyloid plaques were present in all samples except for two control subjects 1 and 2, and subject 9, whom only contained diffuse plaques. The distribution of both types of parenchymal A β , however, often co-localized with each other which hampered the selection for further analysis. As we aimed to select only those areas containing a single type of deposition, diffuse and amyloid plaques could not be scored in all samples. The scored amyloid types and other characteristics of each subject are presented in **Table 5.1**.

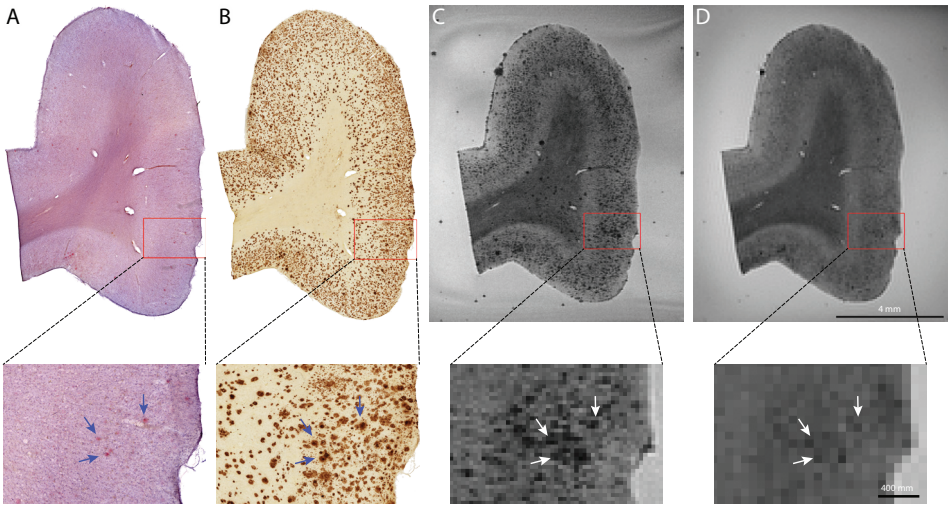


Figure 5.1 Qualitative MRI-histology correlation

Co-registration of (A) Congo Red and (B) Aβ stained section with corresponding (C) T2*- and (D) T2-weighted MR image from subject 4 diagnosed with Alzheimer's disease. The inhomogeneous signal in the cortex appeared to be caused by the deposition of Aβ into amyloid plaques: their distribution corresponded to the hypo-intensities as detected on both types of MR images. As an example, the more detailed comparison showed several larger amyloid plaques to be co-localized with these hypo-intensities (*arrows*). Those smaller in size, or corresponding to a lesser amount of CR staining, were more difficult to discriminate. The structural layering of the GM, visible in both MR images, appeared to be independent of the Aβ distribution.

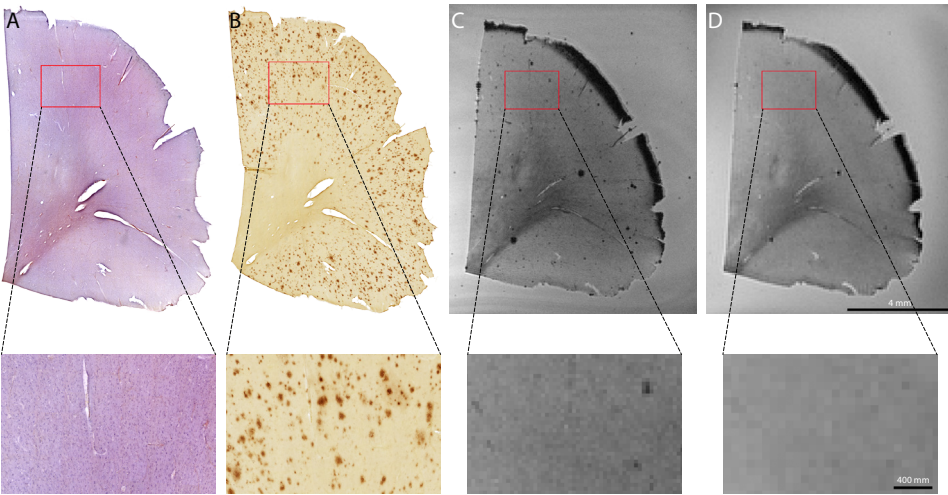


Figure 5.2 MRI characteristics of diffuse plaques

Co-registration of (A) Congo Red and (B) Aβ stains with the corresponding (C) T2*- and (D) T2-weighted MR images of subject 9 diagnosed with Down's syndrome. Since all Aβ deposits lacked Congo Red staining, they were classified as diffuse plaques, which resulted in a relatively homogenous MR image. No Aβ specific changes in MR signal intensity could be discriminated.

T₂*- and T₂-weighted MRI

On visual inspection of the T₂*- and T₂-weighted scans by trained observers, only the congophilic amyloid plaques were visible as hypointense foci. (**Figure 5.1**) Congophilic amyloid plaques with smaller diameters than the MRI voxel size were less conspicuous on MRI due to the partial volume effect. In general, the signal attenuation was more pronounced on the T₂*-weighted images; however, the resolution of the T₂-weighted images was four times lower than the T₂*-weighted images due to time restrictions. Despite the large amount of A β present in the diffuse plaques, their presence did not result in any visible change in MRI contrast as compared to the surrounding GM in either sequence. This was most strikingly seen on close examination of cortical sections of subject 9, who was diagnosed with Down's syndrome, showing abundant parenchymal A β deposits but completely lacking fibrillar amyloid. (**Figure 5.2**) MR images of normal appearing GM showed no cortical hypo-intensities apart from tissue preparation-induced artifacts. (**Figure 5.3**)

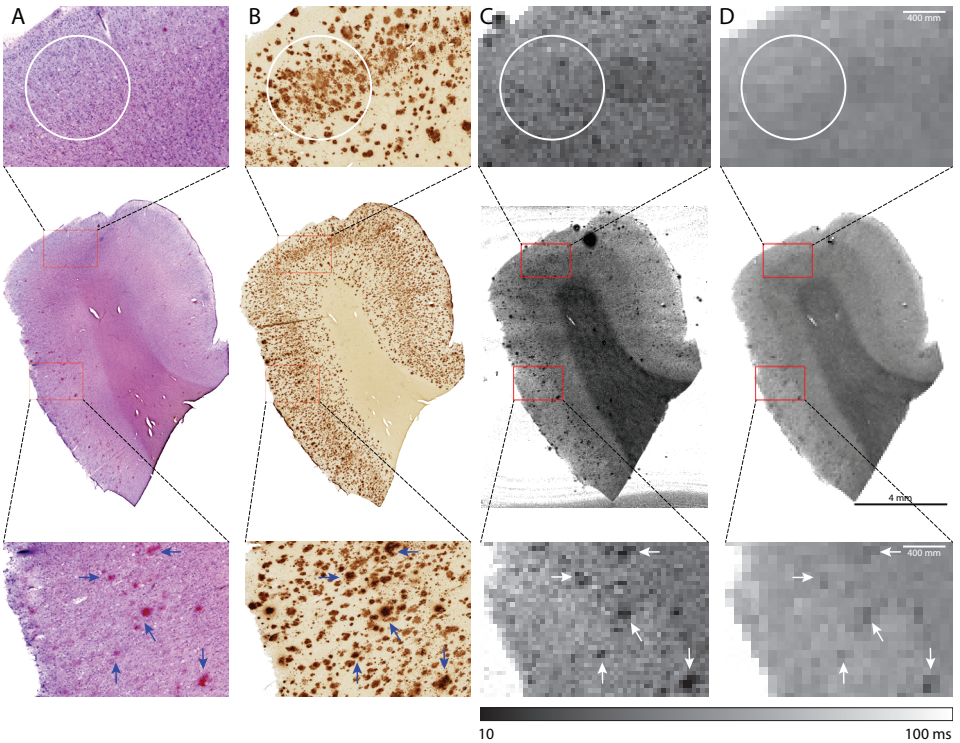


Figure 5.3 MRI characteristics of healthy age-matched brain tissue

Co-registration of (A) Congo Red and (B) A β stains with the corresponding (C) T₂*- weighted image and (E) T₂* map, and (E) T₂-weighted image and (F) T₂ map of control subject 2. No inhomogeneous hypo-intensities were found within the GM since no A β was present. Small circular hypo-intensities were observed both on and next to the section due to the inclusion of small air pockets underneath the coverslip.

Quantitative T_2^* and T_2 relaxation

Similar to the visual assessment, only the amyloid bearing deposits were associated with a significant decrease in T_2^* and T_2 relaxation times. (**Figure 5.4**) Further quantification of these findings by ROI analysis demonstrated that cortical areas containing diffuse plaques ($T_2 = 63.4 \pm 3.1$ ms (*mean $\pm$ SD*); $p = 0.051$; $T_2^* = 47.3 \pm 6.2$ ms; $p = 0.28$) were not associated with a significant difference in either T_2 or T_2^* relaxation when compared to normal appearing GM ($T_2 = 55.1 \pm 2.5$ ms; $T_2^* = 47.5 \pm 4.7$ ms). (**Figure 5.5**) In contrast, focal amyloid-bearing deposits showed a significant reduction in T_2 and particularly in T_2^* relaxation times ($T_2 = 49.9 \pm 10.0$ ms; $T_2^* = 17.7 \pm 6.4$ ms) when compared to normal appearing GM.

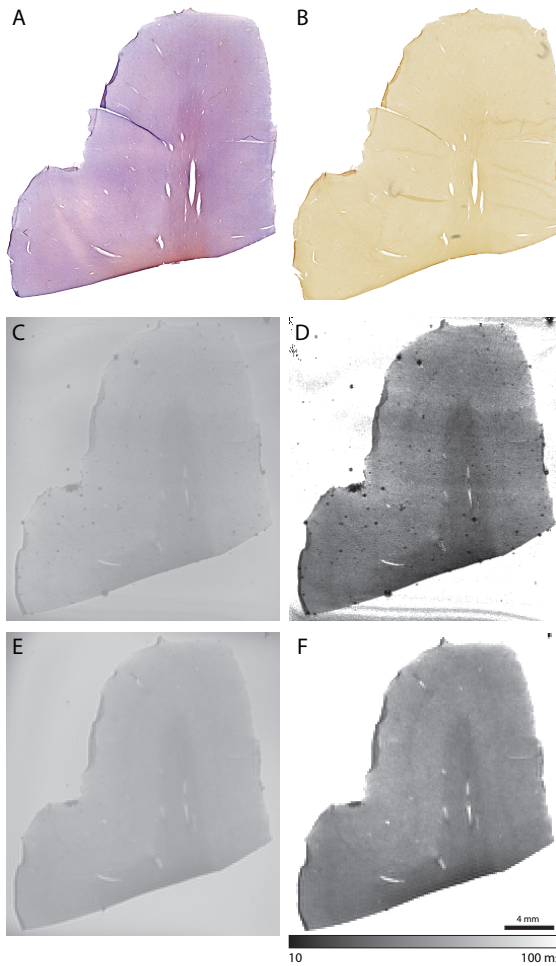


Figure 5.4 Quantitative MRI of parenchymal Aβ deposits

Co-registration of (A) Congo Red and (B) Aβ stains with corresponding quantitative (C) T_2^* and (D) T_2 maps of subject 5. No signal decrease was observed corresponding to the outline of areas with Aβ lacking CR positivity (*white circle*). In contrast, as an example several but not all amyloid plaques highlighted by the presence of both CR and Aβ clearly resulted in hypointensities seen on the corresponding MR images (*arrows*).

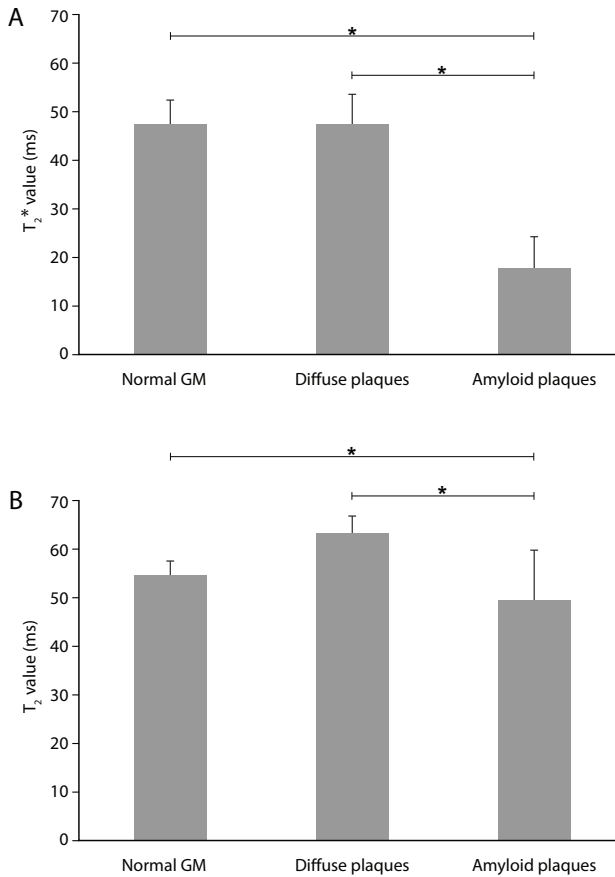


Figure 5.5 T₂* and T₂ values of parenchymal A β deposits

Bar graphs of average (A) T₂* and (B) T₂ values in milliseconds (ms) resulting for ROI analyses of all samples for normal appearing GM (N = 21), diffuse plaques (N = 26) and amyloid plaques (N = 40). *Significant difference of $p < 0.05$ after an unpaired two-tailed Student t-test. Error bars represent the standard deviation.

Vascular amyloid

Several subtypes of CAA were present within the subjects which, based upon the A β immunostaining, could be classified as: a) "large vessel" CAA without, or b) "large vessel" CAA with dyshoric changes (i.e. amyloid extending outside the vascular wall into the parenchyma), or c) capillary CAA. **(Table 5.1)** In four subjects more than one subtype of CAA was present, while capillary CAA, which is relatively rare, was only detected in one subject. None of the selected occipital sections showed evidence of microbleeds or aneurysms that are known to often accompany CAA.

Capillary CAA did not result in any detectable MR contrast change on either the T₂*- or T₂-weighted images. However, the large CAA-bearing vessels themselves were observed as hypointense structures in both scans, irrespective of accompanying dyshoric changes. **(Figure 5.6)** These findings were, however, not consistent for all large CAA vessels, as we also observed CAA vessels with a similar histological appearance, but without any signal attenuation on MRI. These large variations in appearance on MRI combined with the low number of subjects restricted further quantitative ROI analyses for CAA.

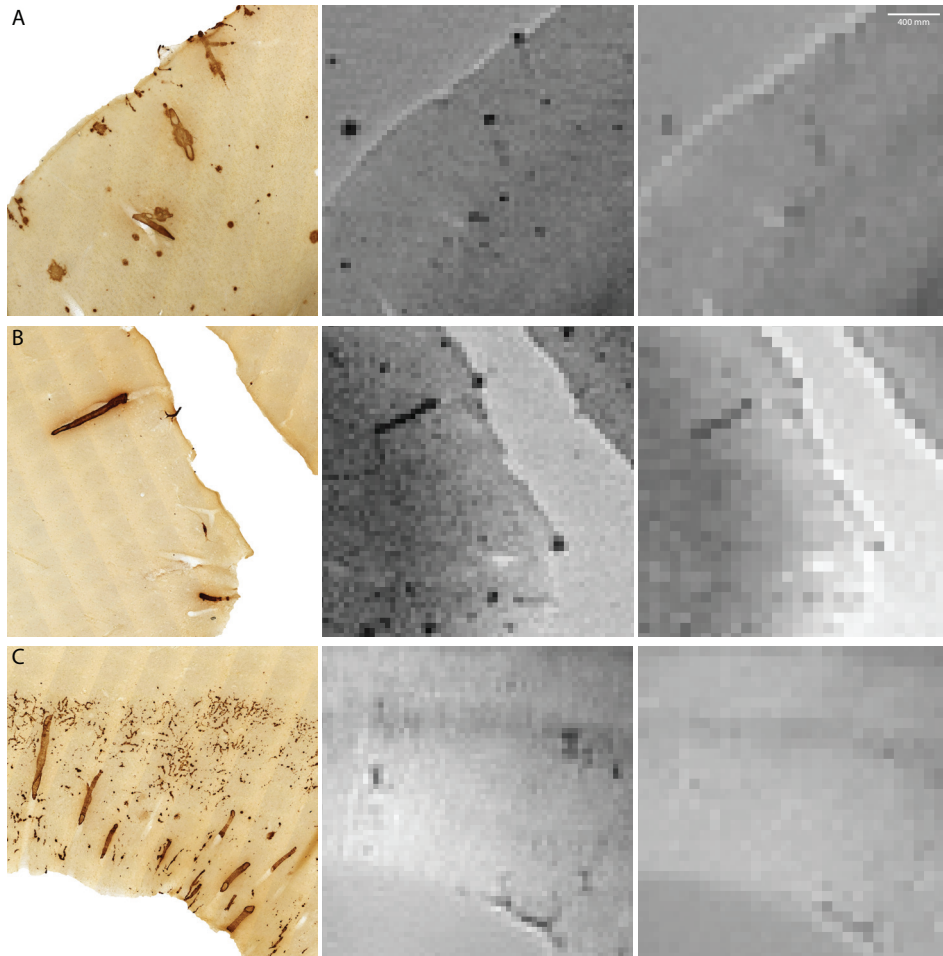


Figure 5.6 MRI presentation of different types of CAA

Detailed A β immunohistochemistry with corresponding T $_2^*$ - and T $_2$ -weighted images of several types of CAA: **(A)** dyschoric CAA (subject 14); **(B)** "large vessel" CAA (subject 12); and **(C)** both capillary CAA and "large vessel" CAA (subject 13). For (A) and (B) the majority of the CAA could be correlated to similarly-shaped hypo-intense structures. In (C) neither "large vessel" nor capillary CAA were associated with a change in MRI signal intensity.

Discussion

Previous studies have suggested that A β , irrespective of associated iron, might induce detectable MRI contrast. Our data, however, demonstrate that the mere presence of large amounts of A β peptides alone is not sufficient to change T $_2$ or T $_2^*$ relaxation significantly, since diffuse plaques are not associated with intensity changes on the MR images. Only when A β is deposited in its fibrillar amyloid conformation are T $_2^*$ and T $_2$ relaxation times reduced, which can be observed as the punctuate hypo-intensities described in previous studies.⁵⁻¹⁶ In addition, this study also showed that direct visualization of vascular amyloid is possible, although signal attenuation was

not observed in all CAA-affected vessels. With regard to the cerebral presence of A β , this would imply that quantitative MRI would depict the cerebral fibrillar amyloid load rather than the amount of cerebral A β peptides.

The current study used a magnetic field strength and spatial resolutions that are not clinically applicable; however, this study of the neocortical A β deposits may provide background information that can support the development of future clinical (quantitative) MRI methods. Currently clinical scans do not allow the detection of individual plaques limited by the maximum resolution. We therefore did not aim to colocalize individual amyloid plaques, but tried to characterize which types of A β deposits produce changes in MRI signal.

Diffuse plaques are hypothesized to be pathologic and are suggested to function as a seeding point for amyloid plaques. However, they are not specific for AD, they do not correlate with severity of dementia, and clinical evidence regarding their role in the pathogenesis of AD has not yet been reported.¹⁹⁻²³ In contrast, amyloid plaques are considered to be one of the definitive hallmarks of AD, and thus their presence forms one of the diagnostic criteria for AD. Any imaging technique aiming at the detection of amyloid should be sure to preclude the assignation of the A β deposited as diffuse plaques, especially since they are often found to be present in large quantities. **(Figure 5.4)**

The current clinical standard for *in vivo* amyloid plaque detection uses [¹¹C]-Pittsburgh-B compound (PiB) as an imaging tracer for positron emission tomography (PET). Numerous studies have demonstrated its specificity and sensitivity for plaque detection, showing that PiB binding is highly selective for fibrillar A β deposits in AD.³² However, its ability to delineate diffuse plaques and even NFTs has also been reported in a post-mortem study.³³ Furthermore, in cases of patients with other types of dementias, such as Parkinson's Disease Dementia, and Lewis Body Dementia, correlation of *in vivo* ¹¹C-PiB with post-mortem neuropathology showed that the high ¹¹C-PiB retention in these patients was primarily correlated to the presence of diffuse plaques.^{34,35} Thus, in the setting of these dementias, ¹¹C-PiB PET has insufficient specificity for *in vivo* diagnosis of comorbid AD due to its inability to distinguish between diffuse and neuritic amyloid plaques.³⁴ In these cases in particular, MRI could provide additional value to confirm the presence of amyloid plaque.

In addition to investigating parenchymal A β , this study is the first to present findings regarding MRI detection of vascular amyloid, another major site of cerebral A β deposition. Besides being present in the vast majority of AD cases, CAA is an entity in itself and a common age-related finding at autopsy.²⁵ CAA leads to local inflammatory responses and loss of vessel wall integrity that eventually result in lobar hemorrhages, as well as complete and partial infarcts.²⁴ Thus far, only these secondary CAA-related pathologies have been reported to be detectable by MRI, but not the vascular amyloid accumulations themselves.^{36,37} Our results showed that several CAA-affected vessels are associated with linear hypo-intensities. As these closely followed the outline of the CAA, this suggests that CAA itself in principle should be detectable with MRI.

However, not all CAA-affected vessels resulted in a similar loss of MR signal. Besides having vascular amyloid as a common denominator, various differences can be found amongst CAA.²⁴ These include not only the amount of A β deposition but also its position within the vascular wall, vasculature fragmentation and loss of structure, formation of fibrinoid necrosis, iron accumulation and signs of hemorrhages or microaneurysms. The current study was limited as

its current setup did not allow to take these differences into account, though these might explain the variation in MRI results found within the present CAA data.

In contrast to amyloid plaques, the usual site for the development of CAA is the occipital lobe rather than the medial temporal lobe. Besides differences in clinical appearance, the spatial pattern of areas of signal loss might be used for differentiation between parenchymal and

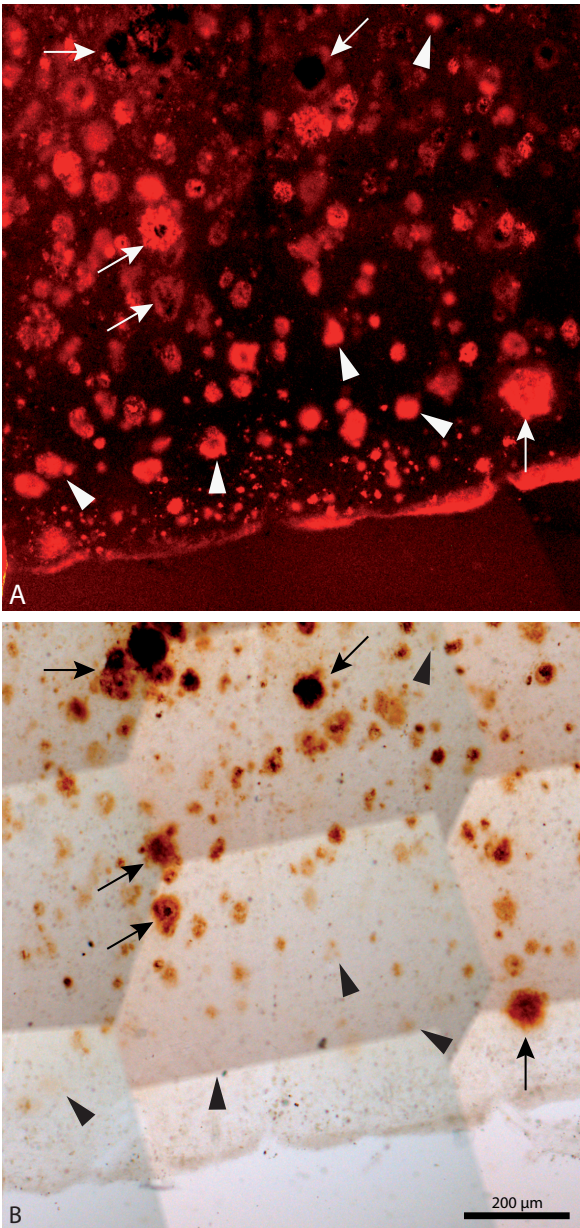


Figure 5.7 The presence of iron within Aβ deposits

Detailed immunofluorescence against Aβ (A) with corresponding 3'3-DAB enhanced Perls' iron stain (B) (*adapted from ref. 7*). In this small cortical areas a wide variety of Aβ deposits both rich (arrows) and low (arrow heads) in iron content is observed. Unable to define the diffuse plaques as the fibrillar status of each Aβ deposit is unknown, therefore it remains unclear whether this might be reflected in the iron colocalization.

vascular amyloid, analogous to the different distribution seen on PiB-PET images in CAA and AD patients.³⁸ Additionally, depending on spatial resolution, the typical tubular structure of amyloid-laden vessels might be a distinctive feature of CAA.

An effort was made to assess the contribution of capillary CAA to MR signal intensity. Compared to the other types, capillary CAA is a rather rare finding but its presence correlates well with clinical symptoms such as dementia.³⁹ It was present in only one sample of all our subjects. In this sample, direct detection of amyloid on the T_2^* -weighted MRI was hampered mainly by the small vessel size and the location of the capillary CAA within or near the stria of Gennari, a cortical layer of the occipital cortex known to result in a loss of signal using these sequences.⁴⁰ Further MRI studies including more capillary CAA samples will be essential to state with confidence whether capillary CAA leads to signal changes on MRI.

The possible effects of chemical fixation of tissue when acquiring post-mortem MRI data should always be taken into consideration and form an important limitation in the direct clinical translation of these types of studies.²⁸ Known effects such as altered water diffusion properties, reduced relaxation parameters and tissue changes due to prolonged fixation can be minimized by including only samples subjected to a well-defined, limited, fixation period and by thoroughly washing the sample with PBS to partially restore transverse relaxation times.^{28,30} This cannot, however, compensate for possible effects on T_2^* caused by protein cross-linking. In addition, tissue iron concentration are known to decrease due to prolonged formalin fixation, which influences MRI parameters.^{26,41} Whether this iron wash-out differs between the distinct types of A β deposits remains unknown. With regard to this study most samples were fixated for a similar period of just over two years, including the controls (**Table 5.1**), which makes it unlikely that the reported differences in MRI signal were induced by differences in fixation.

Future studies may include the use of fresh unfixed tissue as to circumvent these possible fixation effects.⁴² The 60 μ m thick sections required for the direct one-to-one correlation as performed within this study, however, hampers the use of fresh non-frozen tissue due to technical difficulties with sectioning and mounting of the tissue.

Iron has been indicated to play an important role in the MR contrast of amyloid plaques. However, the presence of iron within the different A β deposits remains to be further elucidated; a significant fraction of amyloid plaques have been reported not to contain any iron⁴³, whereas several analytical microscopy studies showed a high spatial correlation between amyloid and an increase iron concentration with respect to the surrounding tissue^{26,44,45}; only one study reported the presence of iron within vascular amyloid other than that associated with microbleeds⁴⁶; and as yet no relation of iron with human diffuse plaques has been reported. The main focus of this study, however, was to investigate the MR characteristics of the different types of A β pathology rather than to precisely unravel which constituent is responsible for the observed contrast. The potential role of iron within the non-fibrillar diffuse plaques remains an interesting one, both as a source of MRI contrast and as a neurotoxic species. In a previous study using similar sections, we showed that even within a small cortical area, the iron content was highly variable in areas with a relatively similar A β load. (**Figure 5.7**) Whether this difference can be explained by the presence of amyloid fibrils is yet unclear, because despite several attempts using different histological stains against fibrillar amyloid, we have not been able to perform triple stainings on these thick sections.

In conclusion, direct MR microscopy of histological sections offers a valuable tool to characterize MR changes in relation to different A β substrates. The results in this study show that both “large vessel” CAA and parenchymal amyloid cause hypo-intensities visible on T₂*- and T₂-weighted MRI. In contrast, diffuse plaques do not cause any MRI signal changes. These findings provide relevant background information for the interpretation and further development of specific MRI methods for the detection and follow-up of AD and CAA.

References

1. Dubois, B, Feldman, HH, Jacova, C, *et al.* Revising the definition of Alzheimer's disease: a new lexicon. *Lancet Neurol.* 2010; 9:1118-1127.
2. McKhann, GM, Knopman, DS, Chertkow, H, *et al.* The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011; 7:263-269.
3. Frisoni, GB, Fox, NC, Jack, CR, Jr., *et al.* The clinical use of structural MRI in Alzheimer disease. *Nat Rev Neurol.* 2010; 6:67-77.
4. Jack, CR, Jr., Knopman, DS, Jagust, WJ, *et al.* Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 2010; 9:119-128.
5. Benveniste, H, Einstein, G, Kim, KR, *et al.* Detection of neuritic plaques in Alzheimer's disease by magnetic resonance microscopy. *Proc Natl Acad Sci U S A.* 1999; 96:14079-14084.
6. Meadowcroft, MD, Connor, JR, Smith, MB, *et al.* MRI and histological analysis of beta-amyloid plaques in both human Alzheimer's disease and APP/PS1 transgenic mice. *J Magn Reson Imaging.* 2009; 29:997-1007.
7. Nabuurs, RJ, Hegeman, I, Natte, R, *et al.* High-field MRI of single histological slices using an inductively coupled, self-resonant microcoil: application to *ex vivo* samples of patients with Alzheimer's disease. *NMR Biomed.* 2010.
8. van Rooden, S, Aat-Schieman, ML, Nabuurs, RJ, *et al.* Cerebral amyloidosis: post-mortem detection with human 7.0-T MR imaging system. *Radiology.* 2009; 253:788-796.
9. Braakman, N, Matysik, J, van Duinen, SG, *et al.* Longitudinal assessment of Alzheimer's beta-amyloid plaque development in transgenic mice monitored by *in vivo* magnetic resonance microimaging. *J Magn Reson Imaging.* 2006; 24:530-536.
10. Chamberlain, R, Reyes, D, Curran, GL, *et al.* Comparison of amyloid plaque contrast generated by T₂-weighted, T₂*-weighted, and susceptibility-weighted imaging methods in transgenic mouse models of Alzheimer's disease. *Magn Reson Med.* 2009; 61:1158-1164.
11. Helpert, JA, Lee, SP, Falangola, MF, *et al.* MRI assessment of neuropathology in a transgenic mouse model of Alzheimer's disease. *Magn Reson Med.* 2004; 51:794-798.
12. Jack, CR, Jr., Garwood, M, Wengenack, TM, *et al.* *In vivo* visualization of Alzheimer's amyloid plaques by magnetic resonance imaging in transgenic mice without a contrast agent. *Magn Reson Med.* 2004; 52:1263-1271.
13. Jack, CR, Jr., Wengenack, TM, Reyes, DA, *et al.* *In vivo* magnetic resonance microimaging of individual amyloid plaques in Alzheimer's transgenic mice. *J Neurosci.* 2005; 25:10041-10048.
14. Lee, SP, Falangola, MF, Nixon, RA, *et al.* Visualization of beta-amyloid plaques in a transgenic mouse model of Alzheimer's disease using MR microscopy without contrast reagents. *Magn Reson Med.* 2004; 52:538-544.
15. Vanhoutte, G, Dewachter, I, Borghgraef, P, *et al.* Noninvasive *in vivo* MRI detection of neuritic plaques associated with iron in APP[V717I] transgenic mice, a model for Alzheimer's disease. *Magn Reson Med.* 2005; 53:607-613.
16. Wengenack, TM, Reyes, DA, Curran, GL, *et al.* Regional differences in MRI detection of amyloid plaques in AD transgenic mouse brain. *Neuroimage.* 2011; 54:113-122.
17. Ramani, A, Jensen, JH, and Helpert, JA. Quantitative MR imaging in Alzheimer disease. *Radiology.* 2006; 241:26-44.
18. Duyckaerts, C, Delatour, B, and Potier, MC. Classification and basic pathology of Alzheimer disease. *Acta Neuropathol.* 2009; 118:5-36.
19. Braak, H and Braak, E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991; 82:239-259.
20. Price, JL, Davis, PB, Morris, JC, *et al.* The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol Aging.* 1991; 12:295-312.
21. Thal, DR, Rub, U, Schultz, C, *et al.* Sequence of Abeta-protein deposition in the human medial temporal lobe. *J Neuropathol Exp Neurol.* 2000; 59:733-748.
22. Thal, DR, Rub, U, Orantes, M, *et al.* Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology.* 2002; 58:1791-1800.
23. Thal, DR, Griffin, WS, and Braak, H. Parenchymal and vascular Abeta-deposition and its effects on the degeneration of neurons and cognition in Alzheimer's disease. *J Cell Mol Med.* 2008; 12:1848-1862.
24. Thal, DR, Griffin, WS, de Vos, RA, *et al.* Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. *Acta Neuropathol.* 2008; 115:599-609.
25. Attems, J. Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms. *Acta Neuropathol.* 2005; 110:345-359.

26. Attems, J, Jellinger, K, Thal, DR, *et al.* Review: sporadic cerebral amyloid angiopathy. *Neuropathol Appl Neurobiol.* 2011; 37:75-93.
27. Braak, H, Alafuzoff, I, Arzberger, T, *et al.* Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 2006; 112:389-404.
28. van Duijn, S, Nabuurs, RJ, van, RS, *et al.* MRI artifacts in human brain tissue after prolonged formalin storage. *Magn Reson Med.* 2011.
29. Pfefferbaum, A, Sullivan, EV, Adalsteinsson, E, *et al.* Post-mortem MR imaging of formalin-fixed human brain. *Neuroimage.* 2004; 21:1585-1595.
30. Shepherd, TM, Thelwall, PE, Stanis, GJ, *et al.* Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. *Magn Reson Med.* 2009; 62:26-34.
31. Natta, R, Maat-Schieman, ML, Haan, J, *et al.* Dementia in hereditary cerebral hemorrhage with amyloidosis-Dutch type is associated with cerebral amyloid angiopathy but is independent of plaques and neurofibrillary tangles. *Ann Neurol.* 2001; 50:765-772.
32. Ikonomovic, MD, Klunk, WE, Abrahamson, EE, *et al.* Post-mortem correlates of *in vivo* PiB-PET amyloid imaging in a typical case of Alzheimer's disease. *Brain.* 2008; 131:1630-1645.
33. Lockhart, A, Lamb, JR, Osredkar, T, *et al.* PIB is a non-specific imaging marker of amyloid-beta (Abeta) peptide-related cerebral amyloidosis. *Brain.* 2007; 130:2607-2615.
34. Burack, MA, Hartlein, J, Flores, HP, *et al.* *In vivo* amyloid imaging in autopsy-confirmed Parkinson disease with dementia. *Neurology.* 2010; 74:77-84.
35. Kantarci, K, Yang, C, Schneider, JA, *et al.* Ante mortem amyloid imaging and beta-amyloid pathology in a case with dementia with Lewy bodies. *Neurobiol Aging.* 2012; 33:878-885.
36. Greenberg, SM, Vernooij, MW, Cordonnier, C, *et al.* Cerebral microbleeds: a guide to detection and interpretation. *Lancet Neurol.* 2009; 8:165-174.
37. Schrag, M, McAuley, G, Pomakian, J, *et al.* Correlation of hypo-intensities in susceptibility-weighted images to tissue histology in dementia patients with cerebral amyloid angiopathy: a post-mortem MRI study. *Acta Neuropathol.* 2009.
38. Johnson, KA, Gregas, M, Becker, JA, *et al.* Imaging of amyloid burden and distribution in cerebral amyloid angiopathy. *Ann Neurol.* 2007; 62:229-234.
39. Thal, DR, Ghebremedhin, E, Rub, U, *et al.* Two types of sporadic cerebral amyloid angiopathy. *J Neuropathol Exp Neurol.* 2002; 61:282-293.
40. Duyn, JH, van, GP, Li, TQ, *et al.* High-field MRI of brain cortical substructure based on signal phase. *Proc Natl Acad Sci U S A.* 2007; 104:11796-11801.
41. Schrag, M, Dickson, A, Jiffry, A, *et al.* The effect of formalin fixation on the levels of brain transition metals in archived samples. *Biometals.* 2010; 23:1123-1127.
42. Antharam, V, Collingwood, JF, Bullivant, JP, *et al.* High field magnetic resonance microscopy of the human hippocampus in Alzheimer's disease: quantitative imaging and correlation with iron. *Neuroimage.* 2012; 59:1249-1260.
43. Collingwood, JF, Chong, RK, Kasama, T, *et al.* Three-dimensional tomographic imaging and characterization of iron compounds within Alzheimer's plaque core material. *J Alzheimers Dis.* 2008; 14:235-245.
44. Miller, LM, Wang, Q, Telivala, TP, *et al.* Synchrotron-based infrared and X-ray imaging shows focalized accumulation of Cu and Zn co-localized with beta-amyloid deposits in Alzheimer's disease. *J Struct Biol.* 2006; 155:30-37.
45. Quintana, C, Bellefqih, S, Laval, JY, *et al.* Study of the localization of iron, ferritin, and hemosiderin in Alzheimer's disease hippocampus by analytical microscopy at the subcellular level. *J Struct Biol.* 2006; 153:42-54.
46. Schrag, M, Crofton, A, Zabel, M, *et al.* Effect of cerebral amyloid angiopathy on brain iron, copper, and zinc in Alzheimer's disease. *J Alzheimers Dis.* 2011; 24:137-149.

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Authors have contributed to this work equally

1 Department of Radiology, Leiden University Medical Center, Leiden, Netherlands

2 Department of Pathology, Leiden University Medical Center, Leiden, Netherlands

3 Division of Image Processing (LKEB), Department of Radiology, Leiden University Medical Center,
Leiden, Netherlands

4 C.S. Kubik Laboratory for Neuropathology, Massachusetts General Hospital and Harvard Medical School,
Boston, Massachusetts, USA

5 Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands
