Chapter 4 – Discussion
The main findings of this thesis were that patterns of gray matter (GM) atrophy differ between different manifestations of AD as well as between AD and bvFTD. This is applicable for some cortical areas as well as for certain subcortical structures. In the discrimination of AD and bvFTD, the measurement of white matter (WM) integrity increases the diagnostic accuracy and gives more information about the underlying pathological processes. GM, WM and deep gray matter (DGM) structures have the potential of serving as a diagnostic tool as they allow reliable discrimination of groups. However, at this point, these results are applicable only for groups of patients and not for individual subjects. Image analysis techniques have to become more reliable and suitable for single-subject diagnosis. In the present chapter, the main findings of this thesis are summarized, followed by a discussion of the diagnostic value of MRI derived measures and of the disease pathology underlying AD and bvFTD. The chapter closes with suggestions for further research.

Summary
Chapter 2. Patterns of gray matter loss in different manifestations of AD
In chapter 2.1 we assessed patterns of GM atrophy according to age-at-onset in a large sample of AD patients and controls with VBM. By comparing AD patients with controls and early-onset with late-onset AD patients, we found that age and diagnosis independently affected hippocampus; moreover, the interaction between age and diagnosis showed that precuneus atrophy was most prominent in early-onset AD. This suggests that patterns of atrophy may vary in the spectrum of AD and that it is important to compare patients with a reference of their own age category. Moreover, in younger patients, the posterior part of the brain – especially the precuneus – may provide the most valuable information when evaluating their MRI scans.

In chapter 2.2 we determined if the use of the 4-point visual rating scale for posterior cortical atrophy (PCA) in clinical practice is justified. For this we used quantitative GM volumetry and VBM. The visual PCA rating scale turned out to reliably reflect GM atrophy in posterior cortical regions. There was a clear separation between brains rated as having PCA and those rated as having no atrophy. Moreover, the different severity scores in the rating scale corresponded to different quantitative degrees of atrophy. Finally, especially the volume of the inferior parietal gyrus affected the visual PCA scoring. These results suggest that the visual PCA rating scale is a valuable tool for the daily radiological assessment of dementia.

In chapter 2.3 we investigated whether DGM atrophy predicts progression from mild cognitive impairment (MCI) to AD, and compared subcortical volumes between AD, MCI and controls. Furthermore we analysed associations with severity of cognitive impairment. We found that in addition to baseline hippocampal volume, also baseline nucleus accumbens volume predicted progression from MCI to AD during two years of clinical follow-up. Baseline volumes of other subcortical structures were not predictive of progression to AD, despite the observation of decreasing volumes from controls to MCI to AD, and significant associations with cognition for all structures except globus pallidus.
Chapter 3. Patterns of gray matter loss in AD and bvFTD

Because the involvement of frontostriatal circuits in bvFTD suggests that DGM structures may be affected in this disease, we investigated whether volumes of DGM structures differed between patients with bvFTD, AD and controls with subjective complaints (SC) and explored the relationships between DGM structures, cognition and neuropsychiatric signs and symptoms in Chapter 3.1. The results suggest that nucleus accumbens, caudate nucleus, and globus pallidus are more severely affected in bvFTD than in AD and SC. The associations between cognition and DGM structures varied between the diagnostic groups. The observed difference in volume of these DGM structures supports the idea that in addition to frontal cortical atrophy, DGM structures, as parts of the frontal circuits, are damaged in bvFTD rather than in AD. In Chapter 3.2 we explored the diagnostic accuracy of an automated classifier for individual patients based on a generally available 3D T1-weighted from which the GM content of each voxel was quantified using a widely used, standardized procedures. To increase generalizability we used MRI scans from different scanners and centers and two independent data sets in a cross-sectional design. The results showed that with this automated classifier it was possible to discriminate between scans of patients with different forms of dementia and controls with high accuracy, based solely on their GM patterns. We also demonstrated that automated classifiers can be used in single-subject diagnosis, as the diagnostic accuracy of our classifier in an independent dataset of patients and controls was good to excellent.

In Chapter 3.3 we examined the loss of cortical thickness and cognitive functioning over time in AD, bvFTD and controls in a longitudinal study. We found that both, AD and bvFTD showed more cortical thinning per year compared to controls, with AD showing decline in memory and language. Compared to controls, AD patients lost cortical thickness over the whole brain with a clear posterior gradient, whereas bvFTD patients only showed cortical thinning in the frontal cortex and in the anterior parts of the temporal lobes. Compared to each other, AD patients showed cortical thinning in the insula, temporal and parietal regions, bvFTD patients only progressed faster in a small frontal region. These results suggest that decrease of thickness is more generalized in AD, whereas bvFTD have a more selective loss of thickness.

In Chapter 3.4 we investigated the ability of cortical and subcortical GM atrophy in combination with WM integrity to distinguish bvFTD from AD and from controls using VBM, subcortical GM structure segmentation, and WM integrity as assessed using TBSS. Furthermore, we were interested in the question which combination of imaging markers differentiated the three groups with the highest accuracy. We showed that there were clear GM and WM differences between AD and bvFTD which independently contributed to the classification of both types of dementia. Despite a comparable disease stage, bvFTD patients had more GM and DGM atrophy, and had more severe loss of fractional anisotropy, and higher mean, axial and radial diffusivity values, especially in the frontal areas. Combining modalities led to 86-92% correct classification of patients. GM contributed most to distinguishing AD patients from controls and bvFTD patients, while WM integrity measurements, especially axial diffusivity, contributed to distinguishing bvFTD from controls and AD. These results suggest that WM integrity measures add complementary information to measures of GM atrophy, thereby improving the classification between AD and bvFTD.
General discussion
Using the consensus clinical diagnostic criteria for AD and bvFTD, diagnostic certainty is variable, but suboptimal. The criteria for probable AD have a diagnostic sensitivity and specificity compared to the pathological diagnosis, ranging between 50% and 90%, mainly depending on the clinical expertise, the age and other characteristics of the patients studied [1,2]. Diagnostic uncertainty remains in other clinical criteria as well, as the ultimate ‘gold standard’ for diagnosis does not exist [3]. When imaging is included in the criteria a higher degree of specificity (>90%) can be reached. In general, the use of imaging has shifted from an exclusionary to an inclusionary approach over the past decades. Using MRI to identify specific abnormalities that may aid the clinician to diagnose underlying disease became increasingly more relevant and adds positive predictive value to the diagnosis of dementia. Therefore, current international guidelines recommend the use of brain imaging techniques in diagnosing dementia syndromes [4-6].

Diagnostic value of MRI derived measures of brain tissues
Gray matter
In most memory clinics, patients are usually scanned once during dementia screening, with a standardized protocol generally including a structural T1-weighted 3-dimensional (3DT1) MRI sequence. The main features of this sequence include both high spatial resolution and high contrast between GM and WM. Many dementias are characterized by GM atrophy as it reflects a loss of neurons irrespective of the underlying protein defect. GM atrophy may be focal or spread over the whole brain, and the patterns of GM atrophy may be diagnostic in itself as they are linked to specific diagnoses. A 3DT1 image is useful for the detection of these disease-specific patterns of GM atrophy and therefore supports a clinical diagnosis. For the interpretation of structural images, standardized assessment by the use of visual rating scales can improve the diagnostic accuracy [7]. In chapter 2.2 we support this notion by showing that a recently developed rating scale for posterior cortical atrophy is a reliable tool for the daily radiological assessment of dementia. AD is typically associated with medial temporal lobe atrophy (MTA) [8-11]. The presence of MTA on MR imaging improves the discrimination of AD from healthy controls and predicts progression to dementia in patients with mild cognitive impairment (MCI) [11,12]. Visual MTA rating based on established rating scales, has proven to be useful for a good and reproducible assessment in clinical practice and correlates well with volumetric assessments [13,14]. However, MTA is also present in other dementias and may be seen in normal aging [15-17]. While MTA is the hallmark finding in senile-onset AD, especially in APOE4 positive patients with an amnestic presentation, it can be relatively mild in subjects with presenile-onset, or without APOE4 and non-amnestic presentations [18,19]. Occasionally, AD patients present with a striking posterior atrophy pattern. In such patients, the pattern of atrophy will be dominated by posterior/parietal atrophy. In chapter 2.1 we affirm these findings by means of voxel-based comparisons of GM. We demonstrated that, when compared to their age-matched controls, AD patients with an early disease onset also showed more widespread atrophy throughout the brain (medial temporal lobe, precuneus,
cingulate gyrus, frontal lobe), whereas late-onset AD patients showed a more specific pattern of GM atrophy, predominantly restricted to the medial temporal lobe and cerebellum. Direct comparisons revealed more pronounced GM atrophy in the precuneus of early-onset AD patients despite their younger age. Posterior cortical atrophy appears to be characteristic of AD in patients with typical and atypical clinical presentations and may assist in the clinical distinction of AD from bvFTD. Moreover, combined with relative sparing of the medial temporal lobe, posterior cortical atrophy has found to be characteristic for patients with atypical clinical presentations [20-24]. As posterior cortical atrophy should be assessed in a standardized manner, a visual rating scale has been developed [25]. To establish the validity of this visual posterior cortical atrophy rating scale and thereby determine whether its use in the clinical practice is justified, it should be compared against quantitative brain volumetry. In chapter 2.2 we addressed this issue to stimulate the clinical applicability of the visual rating scale. We demonstrated that the visual rating scale for PCA reliably reflects GM atrophy in posterior regions and that its simplicity has great advantage for clinical practice, making it a useful tool in the daily radiological assessment of dementia.

In the differentiation between AD and bvFTD, GM atrophy only plays a restricted role, as patterns of atrophy are often not exclusive for one diagnosis. The typical AD atrophy of medial temporal lobe, including hippocampus and amygdala [26-28] is also common in bvFTD [16,29-40]. The other way around, bvFTD is typically characterized by atrophy of frontal and anterior temporal lobes [29,41-43], but atrophy in these regions does not exclude a diagnosis of AD [44-46]. This was confirmed by our results in chapter 3.4 where AD patients did not show any regions of significantly reduced GM compared to bvFTD patients. The other way around bvFTD patients show only small areas of reduced GM in left inferior and medial frontal gyrus, in right inferior frontal gyrus, and in orbito-frontal gyrus.

On the other hand, studies have also shown areas as the precuneus, lateral parietal and occipital cortices being exclusive for AD, and atrophy of anterior cingulate, anterior insula, subcallosal gyrus and caudate nucleus being more severe in bvFTD compared to AD [20,29,47,48]. This is confirmed by our findings that the insular cortex was more severely atrophied in bvFTD, in the beginning of the disease, and still discriminated bvFTD from AD at follow-up, even though AD patients showed a steeper rate of atrophy compared to bvFTD patients (chapter 3.3).

But even if there are small regions found with quantitative image analysis methods, these regions are hard to detect by eyeballing (insula, caudate nucleus) and still refer to results of groups analyses.

To be of diagnostic value, patterns of GM atrophy should have the potential to be able to predict a diagnosis for a single subject, taking into account information from the entire brain. As we showed in chapter 3.2, it was possible to discriminate between AD and bvFTD solely based on a T1-weighted GM image with 78.7% accuracy. We used a support vector machine to apply learned GM patterns of groups of AD and bvFTD patients to new individual patients and achieved 82.1% accuracy in deciding with patient has AD or bvFTD.

To summarize, GM atrophy has proven to be useful for differentiation of dementia from controls. For the differentiation between forms of dementia however, patterns
of GM atrophy often overlap. Furthermore, in the beginning of the disease, when GM atrophy is hardly visible by eyeballing even the discrimination from healthy controls can be challenging. Another point of concern are the atypical cases, as early-onset AD patients or patients with genetic mutations, which display unusual patterns of GM atrophy, will not be recognized based on solely GM atrophy. Quantitative image analysis methods add a lot to the diagnostic certainty, as it allows a closer look at vanishing GM. However, these methods only allow comparisons on a group level, while for application in diagnostic routine, results need to applicable at the single subject level. Automatic classifiers for single-subject diagnosis are available but further research is needed to be able to use them reliably in the daily practice.

White matter
Dementia has been associated with both macro- as well as microscopic WM pathology [49-51]. Neuroimaging, especially MRI, has played a major role in the identification of macroscopic WM pathology thereby providing a significant contribution to both diagnosis and therapeutics [52,53]. Conventional MRI in neurodegenerative disorders lost in credibility as studies quantifying WM changes resulted in differing outcomes, WM lesions seen on MRI did not correlate well with cognitive disabilities, and differentiating between types of dementia proved problematic [54,55]. A likely explanation for this could be that pathological WM changes occurring at the microscopic level - particularly in the early disease stages - cannot be detected by these relatively insensitive conventional MRI techniques, rendering WM to appear normal [56]. However, the development of novel MRI techniques, especially diffusion tensor imaging (DTI), now provides us with the ability to quantify even the most subtle microstructural alterations in WM in ways that were impossible before [56-58]. By applying a strong gradient magnetic field in a single direction, the so-called diffusion-sensitizing gradient, the signal becomes sensitized for diffusion in that direction. Fiber tracts parallel to this gradient field will show maximal signal loss, whereas the effect is minimal if the gradient field is perpendicular to the fiber tracts. By applying gradients in three or more different directions, one can display the anisotropy of tissue, especially in the WM. By using more than six non-collinear diffusion gradients, it is possible to determine the full diffusion tensor, which is the starting point for techniques like fiber tracking and quantitative analyses of the principal eigenvectors of diffusion. Studies of DTI in dementia have consistently shown altered diffusion (tract) properties in accordance with the pattern of neurodegenerative pathology [59-61]. For example, widespread abnormalities in the temporal lobe (but also elsewhere in the brain) were found in AD [60]. Microstructural WM changes can even already be detected in cognitively normal individuals in the pre-MCI stage, and may serve as a potential imaging marker of early AD-related brain changes [62,63]. WM tract damage has also been shown in bvFTD. Previous studies showed that compared to AD, WM integrity was lost in bvFTD especially in the frontal and bilateral temporal regions [64,65]. By using DTI, tract-specific pathology can be demonstrated, which may be specifically linked to the clinical syndrome at hand and therefore helps in distinguishing AD from bvFTD.
In chapter 3.4 we found that measuring WM damage can improve the discrimination between AD and bvFTD, which is in line with other studies showing increased classification rates between AD and bvFTD in an early disease stage when combining GM with WM measurements [66,67]. In a study on patients with corticobasal degeneration and progressive supranuclear palsy, the authors even showed the potential of DTI as a diagnostic marker at the single-subject level [68]. DTI, therefore, constitutes a promising tool for differentiation of various types of dementia, even in the earliest disease stages, quantifying damage in normal appearing WM. Furthermore, more knowledge regarding the exact timing and anatomical location of pathological WM changes will contribute to more insight into the molecular substrates of the different dementias, thereby expanding treatment options and increasing chances of a good clinical response and, eventually, aid in designing and timing of future interventions for disease prevention. However, DTI sequences are not yet implemented in standard scanning protocols and are not yet suitable for the daily clinical practice as they still lack reliability and stability of measures across multiple sites employing different scanners with different field strengths and scan parameters [69]. Furthermore, post-processing methods are not technically mature enough to use DTI images in the radiological assessment.

**Imaging brain tissue for a better understanding of disease pathology**

The characteristic lesions of AD at the microscopic level are extracellular neuritic plaques, consisting of a core composed of beta amyloid (Aβ), and intracellular neurofibrillary tangles (NFT) consisting of hyperphosphorylated tau-protein (tau). Neuritic plaques are amyloid plaques surrounded by degenerating neuritis filled with tau pathology and are the best histological markers of AD. Amyloid plaques are found in both non-demented and demented patients, while neuritic plaques are only found in demented patients. The staging system developed by Braak and Braak describes the extent, location and the presumed sequence of accumulating neurofibrillary tangle pathology, which in AD is thought to start in the transentorhinal and entorhinal areas, before spreading to the hippocampus, the association cortices, and the rest of the cortex [37]. Imaging GM, could help us to draw conclusions about the distribution of microscopic pathology as plaques and tangles are responsible for GM atrophy. Besides this prototypic distribution of Aβ and tau, which is often found in AD patients with the typical clinical presentation and MRI profile, atypical variants, e.g. early-onset patients with posterior cortical atrophy, suggest that the origin and spread of tau pathology originating from the transentorhinal and hippocampal area might not be the only pattern of pathological progression in AD. As we showed in chapter 2.1, spreading of AD pathology might differ between individuals and certain subtypes of AD may have proportionally greater involvement of the cortex than of the hippocampus. Age of onset could be a driving factor in this regional vulnerability as other studies showed that AD patients with a hippocampal sparing subtype were younger at age-at-onset, and had more widespread cortical involvement than the typical and limbic-predominant AD subtypes [70,71]. This might imply that the Braak stages as described in the early nineties do not hold for all AD patients but need to be adapted for specific subgroups [37,72].
Imaging of GM atrophy can also give information about the chronology of pathological processes, as we showed in chapter 2.3. Baseline volumes of DGM structures in MCI were not predictive of progression to AD, despite the observation of decreasing volumes from controls to MCI to AD. These results could indicate that subcortical involvement is a late phenomenon of AD and that AD could be regarded as a primarily cortical disease. This hypothesis is supported by clinical evidence that symptoms related to frontostriatal circuit disruption in AD generally appear in the later disease stages [73-75].

FTD is genetically and pathologically heterogeneous without a clear relationship between the clinical phenotypes and the underlying pathogenetics. Up to a third of patients with FTD will have an autosomal dominant family history of the disease. Mutations in six genes have been associated with genetic FTD although only two of these, progranulin (GRN) and microtubule-associated protein tau (MAPT) are common causes. FTD clinical syndromes are usually associated with one of the frontotemporal lobar degeneration (FTLD) pathologies. Two major pathological pathological types of FTLD were described, those with tau-positive (FTLD-tau) and those with tau-negative, ubiquitin-positive pathology (FTLD-U). However, it has been shown that FTLD-U actually consists of three separate groups: TDP-43-positive pathology (FTLD-TDP), fused-in-sarcoma protein positive pathology (FTLD-FUS) and cases which are both TDP-43 and FUS-negative. Each of these major pathological types also has a number of subtypes [76,77]. Because of this wide heterogeneity of patients with FTD, it is extremely challenging to study this group. Neuroimaging may help in explaining the underlying pathology.

In contrast to AD, FTD is associated with prominent volume loss of the DGM structures, even in earlier disease stages [29,48,78,79]. This is in line with our findings in chapter 3.1 that volumes of caudate nucleus, globus pallidus and nucleus accumbens are smaller in FTD compared to both AD patients and patients with subjective complaints. These findings help to explain some of the symptomatology of this multifaceted disorder, as the involvement of basal nuclei as part of the frontostriatal circuits in FTD fits with the signs and symptoms of this disease, that include behavioral abnormalities and extrapyramidal symptoms [48,80]. Structural changes in components of these circuits could lead to Wallerian degeneration of the connecting fibers and eventually to failure of the whole circuit. Furthermore, these imaging findings can also give more information about the different underlying neuropathology of FTD. Whereas amyloid deposits are mainly found in the cerebral cortex, tau inclusions are found in subcortical regions as well [81,82]. Next to tau, FUS is also associated with atrophy of caudate nucleus [83], Pick bodies (PiD) have been found in the putamen [84] and MAPT mutations are associated with basal ganglia atrophy [85]. These findings point to an important crux of determining whether neuroanatomical patterns can be useful to predict pathology in groups of patients who present with the same clinical diagnosis, especially in syndromes that have heterogeneous pathology as FTD. Correlations between imaging and pathology have been observed in patients with bvFTD: FTLD-TDP type 1 pathology was associated with a different pattern of atrophy from those seen in CBD and PiD pathologies [86-166]
These findings suggest that imaging in patients with FTD syndromes could be used to predict pathology in these individuals, regardless of their clinical presentation. It is also important to recognize that each of the FTD clinical syndromes can also be associated with AD pathology i.e. a prominent and primary behavioral syndrome indistinguishable from bvFTD may occur [89,90]. Also, the term 'frontal AD' is used by some people to indicate a clinical syndrome in which patients have features of episodic memory impairment characteristic of AD and also early behavioral symptoms characteristic of bvFTD [91]. Some patients that present with the clinical picture of bvFTD may turn out to have AD. An imaging signature of AD, showing temporoparietal or parietal atrophy with relative sparing of the medial temporal lobes, can be seen on MRI scans of patients who have a clinical presentation suggestive of an FTD syndrome [23,24]. In these cases, neuroimaging showed that a combination of reduced temporoparietal volumes and large hippocampal volumes enabled discrimination of patients with FTLD-like clinical symptoms and AD pathology from individuals with identical clinical diagnoses but with FTLD pathology [24]. In addition, in individuals with CBS, reduced temporoparietal volume could be used to predict which patients had AD pathology, as opposed to CBD pathology [92].

Imaging the WM could also provide more information about the underlying pathology and how this pathology spreads through the brain. Neurotoxic processes may target the neuron first, leading to neuronal death, followed by degeneration of dendrites and axons. In this case, axonal degeneration in the WM thought to result from decreased axonal transport subsequent to dysfunction or degeneration of cell bodies in the GM. This process of WM degeneration secondary to cortical neurodegeneration is comparable to Wallerian degenerative processes. Wallerian degeneration assumes preferential WM loss or disconnection along the posterior-anterior axis of the brain, secondary to GM pathology in neighboring cortical areas [93,94]. On the other hand, WM could also be damaged more directly [95,96]. This type of WM pathology, known as demyelination, could interfere with transmission velocity by conduction delay and increased refractory period of the axon [97]. Delaying this transmission mechanism may influence synchronization of impulses which are essential for integration of information across the distributed neuronal networks underlying higher cognitive functions [98]. Elaborating on this, GM could be secondary to disconnected WM pathways.

This may be the case in bvFTD, where DTI measurements show widespread FA reductions in the frontal, anterior temporal, anterior corpus callosum, inferior fronto-occipital fasciculus and bilateral anterior cingulum [64,66,99-101] and increased L1 and L23 values in FTD compared to AD [64]. We showed in chapter 3.4 that this WM integrity loss was more widespread than the GM atrophy found in bvFTD. This could be indicative for a direct targeting of the WM instead of a secondary consequence of GM damage. Together with the results on DGM structures from chapter 3.1, where bvFTD patients have more subcortical brain damage compared to AD patients [48,78,79] these results are suggestive that bvFTD is more a network disease, with involvement of the whole frontal-striatal circuits, including the connecting WM tracts and DGM structures. These network failures may also explain the clinical symptoms of bvFTD, as impaired self-monitoring, theory of mind capabilities, perception of
emotions, and changes in personality, which are all coordinated by frontal networks [80]. DTI measurements of WM could shed more light on the anatomical networks and at which point of the pathological cascade these networks start to fail. Finally, a combination of DTI and resting-state functional MRI (rs-fMRI) data would offer a unique opportunity to improve the understanding of the structural basis underlying brain functional changes.

Even though DTI has not been used so far to study the underlying pathology, there is some evidence that changes in the individual eigenvalues of the tensor can provide information about the specifics of WM damage. Increased diffusivity found in the WM is indicative for tissue degeneration and findings of reduced degree of anisotropy are considered to reflect cytoarchitecture changes i.e. axonal degeneration and/or demyelination. Decreased axial diffusion has been associated with axonal damage in mouse models [102], perhaps reflecting increased barriers to organized diffusion in the axial plane. Increased radial diffusion has been associated with damage to myelin [103], perhaps reflecting increased diffusion in the plane orthogonal to the axial plane. However it is still unclear what pathological mechanism causes such microstructural WM damage. The question of whether such tissue loss and cytoarchitecture changes are secondary to or independent of GM pathology remains.

To summarize, discovering different patterns of GM and WM atrophy with MRI sheds light on the microscopic histological changes in the neurodegenerative diseases as they are inevitably associated with each other. Especially in patients where the clinicopathological correlations are weak, a strong imaging signature consisting of cortical, subcortical and WM tract information could be vital to help target future treatments and provide an accurate prognosis.

Methodological considerations
Patient selection
All patients who were included in the studies presented in this thesis were included from the Amsterdam Dementia cohort and collected within the framework of the National Program Brain and Cognition (both VU university medical center and Leiden University medical center/Erasmus medical center Rotterdam). They underwent a one-day thorough examination for clinical evaluation. All diagnoses were made in a multidisciplinary consensus meeting according to the core clinical criteria of the National Institute on Aging and the Alzheimer’s Association workgroup for probable AD and according to the clinical diagnostic criteria of FTD [1,2,4,5]. Even though, patients were carefully screened, the possibility of misdiagnosis cannot be ruled out. The absence of pathological and genetic data also contributes to possible misdiagnoses. Due to the focus of the Alzheimer Center VUmc on early-onset dementia, AD patients that participated in the studies were relatively young, which may hamper generalizability of our results to an average AD patient, who on average is ten years older. Some of the studies in this thesis are multicenter studies as patients were included in the VUmc and LUMC. A potential strength of multicenter studies is
that the generalizability of the results is strong. However it also has limitations, as patient groups will be less homogenous than in one-center studies. When studying atrophy patterns of AD or bvFTD it is important to be aware of the composition of the groups one intends to examine, as different pathological and genetic subtypes can present with different forms of atrophy. In the case of bvFTD patients can also have a ‘benign’ or ‘nonprogressive’ form [104]. These so called phenocopy syndromes should not be included when studying patterns of atrophy in FTD. In the current project, we took care only to include patients with probable bvFTD.

Data acquisition and analysis
In many clinical situations, a qualitative visual assessment of images is sufficient for diagnostic purposes. With trained eyes, it is reproducible and differentiates reasonably well a normal from a diseased state [11, 25, 105-107]. However, many scans differ from the predicted patterns of atrophy, which combined with large between–rater variability results in low sensitivity of these scales. More complex analytical tools such as volume measures of specific structures are more sensitive but are very time-consuming, and also do not provide enough accuracy for differential diagnosis. Because of their high sensitivity and detailed information on a more microscopic level, quantitative image post-processing techniques, may overcome the shortcomings of visual rating scales and volumetric measurements.

Besides a lot of advantages, these image analysis techniques should be used only with a sufficient amount of expertise and results should always be interpreted with caution. In general, one should bear in mind that segmentation of brain images (e.g. VBM, FreeSurfer) critically depends on the quality of the input image, as well as on availability of adequate computing power, and that the image analysis is suited for the type of image available. Furthermore, quantitative image post-processing techniques require sophisticated post-imaging analysis and even though they are mostly automatic, they still require a certain amount of human interaction.

Gray matter analysis – VBM, FreeSurfer and FIRST
Voxel-based morphometry involves a voxel-wise comparison of the local concentration of GM between groups of subjects. It calculates brain volume by segmenting tissue into GM and WM using prior probability maps, whereas FreeSurfer calculates cortical thickness using an estimate of the width of the cortical GM. Although these techniques assess two different metrics - volume and thickness - they are both essentially markers of neurodegeneration and of atrophy of the cortex. The advantages of these techniques are that they assess atrophy throughout the whole brain and do not require prespecifying and prelocalising regions or features of interest. Therefore a priori hypotheses are not required for these data-driven methods. In addition, it reduces the likelihood of a Type II error that can occur when ROIs are not placed in the brain region that differs most between groups or that correlates most strongly with some dependant variable (e.g. cognitive performance). However, evaluation on a voxel-by-voxel basis is complicated by Type I error inflation due to multiple comparisons. Other disadvantages arise from the fact that the exact image-processing is not uniform across studies, and that the spatial extent of the
results will depend on how user-specified options are changed. Varying the type and level of statistical correction, e.g. using uncorrected data versus family-wise error corrected data, largely influences the results of a study. In all studies in this thesis the results are corrected for multiple-comparisons minimizing the probability of Type 1 error.

Another problem is the need for spatial smoothing, and arbitrariness of choosing the spatial smoothing extent. It has been shown that the extent of the significant findings depends on the size of the smoothing kernel [108]. Especially when studying neurodegeneration, greater smoothing tends to increase sensitivity at the expense of specificity and makes it harder to localize an effect anatomically [109].

Next to user-specific changes, another disadvantage of voxel-based morphometry is that it only assesses mean differences between groups, and does not allow for variability within groups or provide data at the individual level, which makes precise prognostication in a given individual difficult. However, as we have shown, the techniques provide valuable data on imaging signatures across types of dementia and provide the necessary first step to the development of useful biomarkers.

The algorithm FIRST (FMRIB’s integrated registration and segmentation tool) [110] is a model-based segmentation/registration tool for 15 different subcortical structures. For the segmentation, it uses shape/appearance models, which are constructed from 336 manually segmented images. FIRST is easy to use and no deviation from the standard instructions are necessary to obtain good results. However, visual checks after every step of the image analysis pipeline, which has been done in all of our studies, is advisable. FIRST has been shown to give accurate and robust results for the segmentation of subcortical structures and that it performs comparable or better to other automatic methods [110-112].

**White matter analysis – TBSS**

TBSS bring together the strengths of voxel-based and tractography-based approaches. It is fully automated, and investigates WM in the whole brain, not requiring prespecification of tracts of interest. It overcomes alignment problems by working in the space of individual subjects’ tractography results and do not necessarily require presmoothing [113].

Next to these advantages, careful interpretation is needed if there are confounding effects, such as within-scan head motion. The most obvious effects of increased head motion are increased image blurring and biased FA. This could lead to misinterpretation of apparent subject group differences, if for example a patient group had greater head motion than a control group. This problem is not in general resolved through the use of the TBSS approach.

Another area where careful interpretation is needed is in regions of crossing tracts or tract junctions. Voxelwise statistics are difficult to estimate and interpret at tract junctions or crossings. An apparent reduction in FA at junctions can in fact be due to an increase in one of the tracts feeding into the junction [113,114]. Furthermore, there is the possibility that pathology could reduce FA so strongly that potential areas of interest may be wrongly excluded from analysis. Pathologies like gross stroke or large tumors, which are likely to seriously disrupt tracts (and FA) are unlikely to be suitable for TBSS analysis.
However, considering these shortcomings, TBSS is a reliable method for estimating localized change in fractional anisotropy, a useful marker for anatomical brain connectivity across different subjects. As the above discussed problems are not in general be resolved through the use of the TBSS approach, we excluded subjects with severe WM damage and movement artifacts. Furthermore, all images were carefully inspected after every analysis step.

**Diagnostic Systems for Single-Subject Diagnosis - PRONTO**

The holy grail of computational techniques may be the one of diagnostic assistants. Multivariate pattern recognition approaches can be extremely useful in distinguishing between groups of subjects (e.g. healthy controls versus patients), and their predictive power can potentially be used as a diagnostic tool for single subjects in a clinical setting [115]. PRoNTo automatically discovers regularities in data through the use of computer algorithms, and with the use of these regularities takes actions such as classifying the data into different categories [116]. Not only diagnosis but also prognosis can be performed [117].

One common mistake, when using linear models, relates to the temptation of interpreting the model weights images as statistical parametric maps (SPMs). Contrary to SPMs, it is the combination of all weights that defines the model and therefore the weights at each voxel are dependent on one another. No voxel-wise statistical tests assuming independence can be performed on them. This leads to interpretability issues, since most neuroscientists look to find not only how information is encoded in the brain but also where in the brain this information resides.

Even though PRONTO has the potential to be used as a multiclass classifier, one should bear in mind that it can only classify cases according to already learned patterns. New cases which do not belong to one of the learned groups will be incorrectly assigned to one of these. In chapter 3.2 we showed that PRoNTo provides a comprehensive and user-friendly software framework for multivariate analysis based on machine learning models for neuroimaging data. It constitutes a promising diagnostic assistant for the daily practice and showed good to excellent accuracy in classifying new subjects to a diagnostic group.

**Advanced MR techniques and second line imaging**

When structural imaging is equivocal or does not lead to the diagnosis, functional imaging may add diagnostic value. Newer imaging techniques such as ASL and resting state fMRI are likely to be increasingly used as studies already showed their additional value in the workup for dementia. In an earlier study we showed that region-of-interest-based cerebral blood flow comparisons showed different perfusion patterns in AD and FTD patients. Partial volume corrected cortical cerebral blood flow values were lowest in the frontal lobes in FTD patients, and in the temporal lobes in AD patients [118]. Within the framework of the Brain & Cognition project, resting-state functional MRI (rs-fMRI) was used to study functional connectivity between AD and bvFTD. We showed that whole-brain functional connectivity differed between patients with bvFTD and patients with AD. Compared to AD bvFTD patients had
decreased functional connectivity between the lateral visual cortical network and the lateral occipital cortex and cuneal cortex, and between the auditory system network and the angular gyrus. Patients with AD showed decreased functional connectivity between the dorsal visual stream network and lateral occipital cortex and opercular cortex [119]. These findings support the idea that imaging of resting state functional connectivity is sensitive to detect disease-related functional connectivity network changes in neurodegenerative diseases and could be useful for differentiation at the group level. Two other studies have shown that changes in functional connectivity precede the onset of clinical symptoms and atrophy in individuals with MAPT (microtubule-associated protein tau) or GRN (progranulin) mutations, suggesting that functional MRI could provide useful biomarkers for FTLD at the preclinical stage [120,121]. Nevertheless, more research is needed to validate these sequences on group level. For the implication in the daily clinical practice as well as for single-subject diagnosis, acquisition of rs-fMRI has to be optimized and analysis techniques have to be validated.

Imaging techniques other than volumetric MRI could also provide promising biomarker options. Second-line neuroimaging investigation includes metabolic information obtained by using single-photon emission computed tomography (SPECT) or positron emission tomography (PET). As in the early stages of FTD there may not exist any discernible atrophy, Fludeoxyglucose(18F)-PET or SPECT might demonstrate decreased metabolism or hypoperfusion preceding tissue loss on structural imaging. Abnormalities on molecular imaging could also be useful in predicting the presence of AD pathology. In a study employing SPECT, a characteristic AD-like pattern of parietal hypoperfusion was identified in patients with CBS, and could distinguish these individuals from those with CBD pathology [122]. However, one should note that temporoparietal abnormalities on functional imaging are not restricted to patients with AD, and have also been observed in patients with FTLD pathology [123]. The predictive value of patterns observed using PET and SPECT imaging may, therefore, be limited. Although detection of amyloid by means of PET provides an excellent biomarker for AD pathology in patients with FTD syndromes, for FTLD pathology such markers are insufficiently investigated so far. However, newer PET imaging methods have recently been investigated (e.g. cholinergic imaging in FTD, PSP and CBS [124] and newer 18F amyloid labelling compounds) and in the future the implementation of PET ligands that would bind to tau, TDP-43 or FUS may offer a huge advance in the ability to make a molecular diagnosis for FTLD [125-128].

Conclusions and future perspectives
The standard MRI sequences generally used in dementia screening are very useful in discriminating between dementia and healthy aging. In the differentiation between AD and bvFTD these sequences are sufficient when the radiological diagnosis fits well with the clinical criteria and patients present with the typical atrophy patterns. However, there can be a gray area where clinical and imaging data do not fit, patients are showing with atypical presentations or overlapping imaging signatures. Nevertheless it is important to use these sequences in the radiological assessment in a
standardized manner, e.g. by the use of visual rating scales to quantify objectively what can be seen by eyeballing.

DTI has been shown to be promising for an early and differential diagnosis between AD and bvFTD but is not yet eligible for the daily radiological practice for single-subject diagnosis as this sequence needs post-processing and image analysis before conclusions can be drawn. As DTI measures have been shown to be sensitive for early WM damage not visible on conventional MRI sequences, DTI could elucidate the earliest point at which structural changes occur by focusing on patients with normal structural GM scans.

The combination of patterns of GM atrophy and WM integrity sheds light on the microscopic histological changes in AD and bvFTD. Especially in patients where the clinicopathological correlations are weak, a strong imaging signature consisting of cortical, subcortical and WM tract information could be vital to help target future treatments and provide an accurate prognosis. The combination of DTI and rs-fMRI data would offer a unique opportunity to improve the understanding of the structural basis underlying brain functional changes. Furthermore, a multimodal approach that combines different imaging sequences and techniques, or a combination of neuropsychological testing and MRI, can improve non-invasive, in vivo distinction between AD and bvFTD.

For clinical implications it is therefore important to develop and evaluate image analysis methods which can be used in the daily practice and for single subject diagnosis to eventually support the clinical diagnosis on a daily basis. To do this it is necessary to a) find MRI signatures for all pathological subtypes of AD and FTD based on GM and WM - these imaging signatures have the potential to help in the diagnosis and prognosis of dementia as it can provide information on which abnormal protein is causing the disease; b) to use pattern recognition approaches more to eventually serve as diagnostic assistants; and c) to conduct larger longitudinal studies to find regional distribution of tissue loss that would provide measurements for the assessment of disease modifying treatments.
Reference List


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