Chapter 3.4  Joint assessment of white matter integrity, cortical and subcortical atrophy to distinguish AD from behavioral variant FTD: a two-center study

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Highlights

- There are gray and clear white matter differences between AD and bvFTD.
- Gray matter atrophy contributed most to distinguish controls from AD and bvFTD patients.
- White matter integrity measurements contributed most to distinguish bvFTD from controls and AD.
- White matter integrity measures support the hypotheses of a network disorder in bvFTD.
- White matter integrity measures allow more precise differentiation between AD and bvFTD.
Abstract
We investigated the ability of cortical and subcortical gray matter (GM) atrophy in combination with white matter (WM) integrity to distinguish behavioral variant frontotemporal dementia (bvFTD) from Alzheimer’s disease (AD) and from controls using voxel-based morphometry, subcortical structure segmentation, and tract-based spatial statistics. To determine which combination of MR markers differentiated the three groups with the highest accuracy, we conducted discriminant function analyses. Adjusted for age, sex and center, both types of dementia had more GM atrophy, lower fractional anisotropy (FA) and higher mean (MD), axial (L1) and radial diffusivity (L23) values than controls. BvFTD patients had more GM atrophy in orbitofrontal and inferior frontal areas than AD patients. In addition, caudate nucleus and nucleus accumbens were smaller in bvFTD than in AD. FA values were lower, MD, L1 and L23 values were higher, especially in frontal areas of the brain for bvFTD compared to AD patients. The combination of cortical GM, hippocampal volume and WM integrity measurements, classified 97-100% of controls, 81-100% of AD and 67-75% of bvFTD patients correctly. Our results suggest that WM integrity measures add complementary information to measures of GM atrophy, thereby improving the classification between AD and bvFTD.

Key words: Alzheimer’s disease, frontotemporal dementia, gray matter atrophy, white matter integrity, discriminant analyses, diagnosis
Introduction
Alzheimer’s disease (AD) and behavioral variant Frontotemporal dementia (bvFTD) are the most common causes of young onset dementia [1]. Clinical diagnostic criteria have been proposed [2,3], but the frequent overlap of the clinical symptoms associated with AD and bvFTD pose serious problems in the differential diagnosis. Although the definite diagnosis of both types of dementia is only possible at autopsy, magnetic resonance imaging (MRI), providing measurements of gray matter (GM) atrophy and white matter (WM) integrity, have been shown to detect brain changes in an early disease stage. Studies on GM atrophy have shown precuneus, lateral parietal and occipital cortices to be more atrophic in AD than in bvFTD, whereas atrophy of anterior cingulate, anterior insula, subcallosal gyrus, and caudate nucleus was more severe in bvFTD compared to AD [4-6]. GM loss in dorsolateral prefrontal cortex, medial temporal lobes, hippocampus and amygdala is found in both AD and bvFTD and does not help to discriminate between the two disorders [4,7-9]. In addition to local GM damage, a decrease of FA in WM, suggesting WM tract damage has been shown, especially in bvFTD. Previous studies showed that compared to AD, WM integrity was lost in bvFTD especially in the frontal and bilateral temporal regions [10,11].

Most former studies focused on either GM or WM damage, while only a few investigated the extent to which the loss of WM integrity and GM atrophy are related and how they jointly contribute to the clinical classification of patients [12-14]. Generalizability of these findings is limited as in one study patients from the whole FTLD spectrum were compare to AD patients [12] and in other studies the different imaging modalities were only linked to each other but not used for diagnostic discrimination [13,14].

In this multi-center study we compared patterns of cortical and subcortical GM atrophy and of WM integrity between patients with bvFTD, AD and controls with the ultimate goal to facilitate clinical diagnosis. In addition, we investigated the joint discriminative ability of GM atrophy and WM integrity measurement to distinguish both patient groups from controls and from each other.

Materials and Methods
Patients
In this two center study, we included 39 patients with probable AD and 30 patients with bvFTD, who visited either the Alzheimer Center of the VU University Medical center (VUmc) (probable AD: n=23; probable bvFTD: n=16; possible bvFTD: n=4) or the Alzheimer Center of the Erasmus University Medical Center Rotterdam (probable AD: n=16 ; probable bvFTD: n=9; possible bvFTD: n=1). All patients underwent a standardized one-day assessment including medical history, informant-based history, physical and neurological examination, blood tests, neuropsychological assessment, and MRI of the brain. 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 [3,15] and according to the clinical diagnostic criteria of FTD for bvFTD [2]. To minimize center effects, all diagnoses were re-evaluated in a panel including clinicians from both centers. In
addition, we included 41 cognitively normal controls (VUMc: n=23; Rotterdam: n=18), who were recruited by advertisement in local newspapers. Before inclusion in the present study, controls were screened for memory complaints, family history of dementia, drugs- or alcohol abuse, major psychiatric disorder, and neurological or cerebrovascular diseases. They underwent an assessment including medical history, physical examination, neuropsychological assessment, and MRI of the brain comparable to the work-up of patients.

Inclusion criteria for both cohorts were: (1) availability of a T1-weighted 3-dimensional MRI (3DT1) scan and a diffusion tensor imaging (DTI) image at 3T, and (2) age between 50 and 80 years. Exclusion criteria were: (1) large image artifacts (n=12); (2) failure of imaging analyzing software to process MR scans (n=6) (details see sections below); and (3) gross brain pathology other than atrophy, including severe white matter hyperintensities and/or lacunar infarction in deep gray matter structures. Level of education was rated on a seven-point scale [16]. The study was conducted in accordance with regional research regulations and conformed to the Declaration of Helsinki. The local medical ethics committee of both centers approved the study. All patients gave written informed consent for their clinical and biological data to be used for research purposes.

MR image acquisition and review
Imaging at the VUMc was carried out on a 3T scanner (Signa HDxt, GE Healthcare, Milwaukee, WI, USA), using an 8-channel head coil with foam padding to restrict head motion. Patients and controls from the Erasmus University Medical Center Rotterdam were all scanned at the Leiden University Medical Center (LUMC). Imaging at LUMC was performed on a 3T scanner (Achieva, Philips Medical Systems, Best, the Netherlands) using an 8-channel SENSE head coil.

The scan protocol included a whole-brain near-isotropic 3DT1-weighted sequence for cortical and subcortical segmentation. At VUMc this was a fast spoiled gradient echo sequence (FSPGR; repetition time TR 7.8 ms, echo time TE 3 ms, inversion time TI 450 ms, flip angle 12°, 180 sagittal slices, voxel size 0.98x0.98x1 mm, total scan time 4.57 minutes). At LUMC this was a turbo field echo sequence (T1TFE; TR 9.8 ms, TE 4.6 ms, flip angle 8°, 140 transversal slices, voxel size 0.88x0.88x1.2 mm, total scan time 4.57 minutes). In addition DTI was performed using EPI based sequences. At the VUMc, DTI consisted of five volumes without directional weighting (i.e. b=0 s/mm^2) and 30 volumes with noncollinear diffusion gradients (i.e. 30 directions, b=1000 s/mm^2) and TR 13000 ms, TE 87.8 ms, 45 contiguous axial slices of 2.4 mm, voxel size=2x2x2.4 mm, parallel imaging with factor 2, total scan time 7.8 minutes. At the LUMC DTI consisted of one volume without directional weighting (i.e. b=0 s/mm^2) and 60 volumes with noncollinear diffusion gradients (i.e. 60 directions, b=1000 s/mm^2) and TR 8250 ms, TE 80 ms, 70 axial slices, voxel size=2x2x2 mm, parallel imaging with factor 2, total scan time 9 minutes).

In addition, the MRI protocol included a 3D Fluid Attenuated Inversion Recovery (FLAIR) sequence, dual-echo T2-weighted sequence, and susceptibility weighted imaging (SWI) which were reviewed for brain pathology other than atrophy by an experienced radiologist.
Gray matter volume
DICOM images of the 3DT1-weighted sequence were corrected for gradient nonlinearity distortions and converted to Nifti format. The linear transformation matrix to MNI space was calculated using FSL-FLIRT [17] and used to place the image coordinate origin (0,0,0) on the anterior commissure by using the Nifti s-form. The structural 3DT1 images were then analyzed using the voxel-based morphometry toolbox (VBM8; version 435; University of Jena, Department of Psychiatry) in Statistical Parametric Mapping (SPM8, Functional Imaging Laboratory, University College London, London, UK) implemented in MATLAB 7.12 (MathWorks, Natick, MA). The first module of the VBM8 Toolbox ("Estimate and Write") segments the 3DT1 volumes into GM, WM and cerebrospinal fluid (CSF), apply a registration to MNI space (affine) and subsequently a non-linear deformation. The non-linear deformation parameters are calculated via the high dimensional DARTEL algorithm and the MNI 152 template. Remaining non-brain tissue was removed by the 'light clean-up' option. Tissue classes are normalized in alignment with the template with the 'non-linear only' option which allows comparing the absolute amount of tissue corrected for individual brain size. The correction is applied directly to the data, which makes a head-size correction to the statistical model redundant. In the second module, images were smoothed using a 8 mm full width at half maximum (FWHM) isotropic Gaussian kernel. Images were visually inspected after every processing step.

Voxelwise statistical comparisons between groups were made to localize GM differences by means of a full factorial design with diagnosis (AD, bvFTD, controls) as factor with independent levels with unequal variance, using absolute threshold masking with a threshold of 0.1 and implicit masking. Age, sex and center were entered as covariates. Post hoc, we compared AD with controls, bvFTD with controls, and AD with bvFTD. The threshold for significance in all VBM analyses was set to p<0.05 with family wise error correction (FWE) at the voxel level and an extent threshold of 0 voxels.

Volumes of deep gray matter (DGM) structures
The algorithm FIRST (FMRIB’s integrated registration and segmentation tool) [18] was applied to estimate left and right volumes of seven structures: hippocampus, amygdala, thalamus, caudate nucleus, putamen, globus pallidus, and nucleus accumbens. Left and right volumes were summed to obtain total volume for each structure. FIRST is integrated in FMRIB’s software library (FSL 4.15) [19] and performed both registration and segmentation of the above mentioned anatomical structures. A two-stage linear registration was performed to achieve a more robust and accurate pre-alignment of the seven structures. During the first-stage registration, the 3DT1 images were registered linearly to a common space based on the Montreal Neurological Institute (MNI) 152 template with 1x1x1 mm resolution using 12 degrees of freedom. After registration, a second stage registration using a subcortical mask or weighting image, defined in MNI space, was performed to improve registration for the seven structures. Both stages used 12 degrees of freedom. This 2-stage registration was followed by segmentation based on shape models and voxel intensities. Volumes of the seven structures were extracted in native space, taking into account the transformations matrices during registration. The final
step was a boundary correction based on local signal intensities. All registrations and segmentations were visually checked for errors.

To correct the volumes of the DGM structures for head size we used a volumetric scaling factor (VSF) derived from the normalization transform matrix from SIENAX (Structural Image Evaluation using Normalization of Atrophy Cross-sectional) [20], also part of FSL. In short, SIENAX extracted skull and brain from the 3DT1 input whole-head image. In our study, brain extraction was performed using optimized parameters [21]. These were then used to register the subject’s brain and skull image to standard space brain and skull (derived from MNI152 template) to estimate the scaling factor (VSF) between the subject’s image and standard space. Normalization for head size differences was done by multiplying the raw volumes of the DGM structures by the VSF. Next to the VSF, we also obtained brain tissue volumes of GM and WM [22]. Total volumes of the seven DGM structures and volumes of GM and WM, and VSF were transferred to SPSS for further statistical analyses.

**White matter integrity**

All preprocessing steps were performed using FSL [19,23], including motion- and eddy-current correction on images and gradient-vectors, followed by diffusion tensor fitting. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (L1), and radial diffusivity (average of L2 and L3, L23) were derived for each voxel. Each subject’s FA image was used to calculate nonlinear registration parameters to the FMRIB58_FA brain, which were then applied to all four parameter images. The registered FA images were averaged into a mean FA image, which was skeletonized for tract-based spatial statistics (TBSS) [24]. The skeleton was thresholded at 0.2 to include only WM and used for TBSS statistics in all diffusion parameters. Each subject’s aligned FA data was then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics. The projection parameters for each voxel were then also applied to the MD, L1 and L23 data to create skeletonized data in standard space for each subject. Differences in FA, MD, L1 and L23 between controls, AD and bvFTD patients were analyzed in a voxelwise fashion using FSL’s randomise with age, sex and center as covariates. A family wise error (FWE) corrected Threshold-Free Cluster Enhancement (TFCE) significance level of p<0.05 was used to correct for multiple comparisons.

**Extraction of regions of interest (ROI)**

As a next step, we extracted ROIs from the VBM and TBSS group analyses, to be able to combine the most promising MR markers in one statistical model.

Gray matter: From the resulting T-map from the comparisons AD<controls, bvFTD<controls, and bvFTD<AD from the VBM analyses all significant voxels were extracted as one ROI per groups comparison (in total 3 ROIs: GM ROI AD<Controls, GM ROI bvFTD<Controls, GM ROI bvFTD<AD). This was done by merging the normalized modulated GM segments of all subjects into a 4D file. The T-maps of all contrasts were thresholded at p<0.05 (FWE corrected) and binarised. We then calculated the mean GM fraction of every voxel in the ROI and the size of the ROI (voxels and mm^3). By multiplying the GM fraction with the size of the ROI we
maintained the GM volume of the whole ROI which was transferred to SPSS for further analysis.

White matter integrity: We calculated the mean FA value under the total skeleton using the fslstats function from FSL. Significant voxels (TFCE, FWE corrected p<0.05) from the contrast (statistical) images from the two-group comparisons AD<controls, bvFTD<controls, AD<bvFTD, and bvFTD<AD were extracted in a ROI (in total 3 ROIs: FA ROI AD<Controls, FA ROI bvFTD<Controls, FA ROI bvFTD<AD) using fslmaths and fslstats. We then calculated the mean FA and region size (voxel count and mm³) in the ROI. The same was done for MD, L1 and L23. For these measurements we used the comparisons AD<controls, bvFTD<controls, bvFTD>AD to create the ROIs (in total 3 per diffusivity measurement: MD ROI AD<controls, MD ROI bvFTD<controls, MD ROI bvFTD>AD; L1 ROI AD<controls, L1 ROI bvFTD<controls, L1 ROI bvFTD>AD; L23 ROI AD<controls, L23 ROI bvFTD<controls, L23 ROI bvFTD>AD). Values of the 12 ROIs were transferred to SPSS for further analysis.

Statistical analysis
SPSS version 20.0 for Windows was used for statistical analysis. Differences between groups were assessed using univariate analysis of variance (ANOVA), Kruskal-Wallis tests and χ² tests, where appropriate. Multivariate analysis of variance (MANOVA) was used to compare raw total volumes of the medial temporal lobe structures hippocampus and amygdala, and of the DGM structures thalamus, caudate nucleus, putamen, globus pallidus, and nucleus accumbens (dependent variables) between the different diagnostic groups (between-subjects factor). Post hoc, ANOVAs and Bonferroni adjusted t-tests were performed with age, sex and center as covariates.

To determine which combination of MR markers based on VBM, DGM structures and TBSS measurements differentiated the three patient groups with the highest accuracy, we conducted a discriminant function analysis with leave-one-out cross validation. As predictors we entered the following variables: three GM ROIs from the comparison AD<controls, bvFTD<controls, bvFTD<AD; DGM volumes of hippocampus, thalamus, caudate nucleus, putamen and nucleus accumbens (as these structures significantly differed between the groups); three FA ROIs from the comparison AD<controls, bvFTD<controls, bvFTD<AD; as well as sex, age, and center. Because of collinearity we performed another discriminant function analyses with the other diffusion parameters L1 and L23 instead of FA. In this discriminant function we used the following variables as predictors: three GM ROIs from the comparison AD<controls, bvFTD<controls, bvFTD<AD; DGM volumes of hippocampus, thalamus, caudate nucleus, putamen and nucleus accumbens; six L1 and L23 ROIs from the comparisons AD<controls, bvFTD<controls, bvFTD<AD; as well as sex, age, and center. In general, a discriminant analysis creates k-1 linear combinations (discriminant functions) of the entered predictor variables which provides the best discrimination between the groups (k). To identify the most optimal combination of variables for best discrimination, stepwise forward analysis was used with a decision scheme based on the F-value of Wilks’ lambda (entry: 3.84; removal: 2.71). Statistical significance for all analyses was set at p<0.05.
Results

Demographics
Demographic data for all patients (AD: n=32; bvFTD: n=24) and controls (n=37) fulfilling inclusion criteria are summarized in Table 1. AD patients were older than controls (p<0.001); there were no differences in gender distribution or education. Both dementia groups had smaller normalized brain volumes than controls (p<0.001). AD patients had lower MMSE scores than both other groups (p<0.05). CDR and GDS scores were lowest in controls (p<0.002) but did not differ between the two dementia groups.

Gray matter volume
The full factorial design showed main effects of diagnosis (Figure 1). Post hoc comparisons showed that compared to controls, AD patients showed a reduction of GM in superior and middle temporal gyrus, parahippocampal gyrus, hippocampus, posterior cingulate, cuneus, precuneus, superior parietal lobule and inferior frontal gyrus (p<0.05, FWE corrected). BvFTD patients had less GM compared to controls in superior, middle, and inferior frontal gyrus, orbito-frontal gyrus, insular, temporal gyrus, parahippocampal gyrus and hippocampus. Controls did not show any regions with less GM than AD or bvFTD (p<0.05, FWE corrected). Compared to AD patients, bvFTD patients had less GM matter in left inferior and medial frontal gyrus, in right inferior frontal gyrus, and in orbito-frontal gyrus (p<0.05, FWE corrected). AD patients did not show any regions of significantly reduced GM compared to bvFTD patients.

Volumes of deep gray matter structures
Normalized volumes of MTL and DGM structures are summarized in Table 2. MANOVA adjusted for age, sex and center revealed group differences in hippocampus, thalamus, caudate nucleus, putamen and nucleus accumbens (Figure 2). Post hoc tests showed that nucleus accumbens and caudate nucleus volume discriminated all groups, with bvFTD having most severe atrophy. Hippocampus and thalamus discriminated dementia patients from controls. bvFTD patients had smaller putaminal volumes than controls.

White matter integrity
Figure 3 shows the mean skeleton with significant regions in FA, MD, L1 and L23 for different group comparisons. Compared with controls, AD patients showed widespread patterns of lower FA values, incorporating 44% of the WM skeleton voxels, in areas including the fornix, corpus callosum, forceps minor, thalamus, posterior thalamic radiation, superior and inferior longitudinal fasciculus. Furthermore, they had higher MD values in 36% of the WM skeleton voxels including the fornix, corpus callosum, forceps minor and forceps major, higher L1 values in 23% of the WM skeleton voxels including the corpus callosum, the corticospinal tract and inferior longitudinal fasciculus, and higher L23 values in 42% of the WM skeleton voxels including the forceps major, inferior fronto-occipital fasciculus, inferior and superior longitudinal fasciculus and the corpus callosum compared with controls. Compared to controls, bvFTD patients showed widespread patterns of lower FA values in 58% of the investigated WM voxels throughout the whole brain, in areas
including the fornix, corpus callosum, forceps minor, thalamus, anterior thalamic radiation, superior and inferior longitudinal fasciculus and inferior fronto-occipital fasciculus. Furthermore, they had higher MD values in 55% of the investigated WM voxels including the inferior fronto-occipital fasciculus, uncinate fasciculus and the forceps minor, higher L1 values in 39% of the WM skeleton voxels including the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, corticospinal tract and corpus callosum, and higher L23 values in 62% of the investigated WM voxels in the inferior and superior longitudinal fasciculus, corticospinal tract, corpus callosum, fornix, inferior fronto-occipital fasciculus and the anterior thalamic radiation compared to controls.

In direct comparison between the two dementia groups, bvFTD patients had lower FA values in 17% of the investigated voxels, solely located in the frontal parts of the brain, like the rostrum and the genu of the corpus callosum, forceps minor, anterior part of the internal and external capsule, anterior parts of the fronto-occipital fasciculus and superior longitudinal fasciculus. Furthermore, bvFTD patients had higher MD values in 21% and higher radial diffusivity values in 23% of the investigated WM voxels including forceps minor, uncinate fasciculus, inferior fronto-occipital fasciculus and anterior thalamic radiation, higher axial diffusivity values in 14% of the investigated WM voxels including inferior fronto-occipital fasciculus, uncinate fasciculus and forceps minor compared to AD patients. AD patients had no areas of reduced diffusivity of fractional anisotropy compared to bvFTD.

**Extraction of regions of interest (ROI)**

Figure 4 illustrates which areas composite the ROIs per contrast. GM ROI AD<Controls was composed of significant voxels in temporal gyrus, posterior cingulate, cuneus, precuneus, parietal lobule and inferior frontal gyrus. GM ROI bvFTD<Controls was composed of significant voxels in frontal gyrus, orbito-frontal gyrus, insular, and temporal gyrus. GM ROI bvFTD<AD was composed of significant voxels in left inferior and medial frontal gyrus, right inferior frontal gyrus, and orbito-frontal gyrus.

FA ROI AD<Controls consisted of significant voxels in fornix, corpus callosum, forceps minor, thalamus, posterior thalamic radiation, superior and inferior longitudinal fasciculus. FA ROI bvFTD<Controls consisted of significant voxels in fornix, corpus callosum, forceps minor, thalamus, anterior thalamic radiation, superior and inferior longitudinal fasciculus and inferior fronto-occipital fasciculus. FA ROI bvFTD<AD consisted of significant voxels in rostrum and the genu of the corpus callosum, forceps minor, anterior part of the internal and external capsule, anterior parts of the fronto-occipital fasciculus and superior longitudinal fasciculus.

MD ROI AD>controls consisted of significant voxels in fornix, corpus callosum, forceps minor and forceps major. MD ROI bvFTD>controls consisted of significant voxels in inferior fronto-occipital fasciculus, uncinate fasciculus and the forceps minor. MD ROI bvFTD>AD consisted of significant voxels in forceps minor, uncinate fasciculus, inferior fronto-occipital fasciculus and anterior thalamic radiation.

L1 ROI AD>controls consisted of significant voxels in corpus callosum, the corticospinal tract and inferior longitudinal fasciculus. L1 ROI bvFTD>controls consisted of significant voxels in inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, corticospinal tract and corpus callosum. L1 ROI bvFTD>AD
consisted of significant voxels in inferior fronto-occipital fasciculus, uncinate fasciculus and forceps minor.

L23 ROI AD>controls consisted of significant voxels in forceps major, inferior fronto-occipital fasciculus, inferior and superior longitudinal fasciculus and the corpus callosum. L23 ROI bvFTD>controls consisted of significant voxels in inferior and superior longitudinal fasciculus, corticospinal tract, corpus callosum, fornix, inferior fronto-occipital fasciculus and the anterior thalamic radiation. L23 ROI bvFTD>AD consisted of forceps minor, uncinate fasciculus, inferior fronto-occipital fasciculus and anterior thalamic radiation.

Predictive value of GM volume, volumes of DGM structures, and white matter integrity

Subsequently, we used discriminant analysis to identify the combination of MR-markers providing optimal classification. Using stepwise forward method, the first discriminant analysis selected the following predictors: (1) GM ROI AD<Controls; (2) hippocampal volume; (3) volume of putamen (4) FA ROI AD<Controls; (5) FA ROI bvFTD<Controls; (6) center; (7) age; and (8) sex. The two resulting discriminant functions had a Wilk's lambda of 0.082 (p≤0.001) and 0.388 (p≤0.001). Figure 5A shows the projection plot of the two canonical discriminant functions for discrimination of the three groups. Discriminant function 1 discriminated AD from bvFTD and controls. Discriminant function 2 discriminated bvFTD from AD and controls. The loadings of the individual predictors for each function are shown in Table 3A. GM ROI AD<Controls had the highest loading on discriminant function 1. Discriminant function 2 was primarily composed of the variables FA ROI bvFTD<Controls, hippocampal volume, FA ROI AD<Controls, and GM ROI AD<Controls. Cross-validation successfully classified 91.4 % of all cases correctly, with correct classification of 100% of controls, 100% of AD patients, and 66.7% of bvFTD patients.

The second discriminant analysis selected the following predictors: (1) GM ROI AD<Controls; (2) GM bvFTD<AD; (3) L1 ROI AD>Controls; (4) L1 ROI bvFTD<Controls; and (5) L1 ROI bvFTD>AD. The two resulting discriminant functions had a Wilk’s lambda of 0.134 (p≤0.001) and 0.437 (p≤0.001). Figure 5B shows the projection plot of the two canonical discriminant functions for discrimination of the three groups. Discriminant function 1 discriminated AD from bvFTD and controls. Discriminant function 2 discriminated bvFTD from AD and controls. The loadings of the individual predictors for each function are shown in Table 3B. GM ROI AD<Controls and L1 ROI AD<Controls had the highest loadings on discriminant function 1. Discriminant function 2 was primarily composed of GM ROI bvFTD<AD, L1 ROI bvFTD<AD, L1 ROI bvFTD>Controls, GM ROI AD<Controls, and L1 ROI AD>Controls. Cross-validation successfully classified 86% of all cases correctly, with correct classification of 97.3% of controls, 81.3% of AD patients, and 75% of bvFTD patients.

Discussion

The main finding of this study is that there are GM and clear WM differences between AD and bvFTD which both independently contributed to the classification of both types of dementia. Despite a comparable disease stage, bvFTD patients had more atrophy in orbitofrontal, medial frontal and inferior frontal areas, caudate nucleus and
nucleus accumbens than AD patients. Furthermore, they had more severe loss of FA, higher MD, L1 and L23 values, especially in the frontal areas. Combination of modalities led to 86-91.4% correct classification of patients. GM contributed most to distinguishing AD patients from controls and bvFTD patients, while WM integrity measurements, especially L1, contributed to distinguish bvFTD from controls and AD.

A large number of studies investigated the differences between controls and AD or bvFTD patients with regard to either GM or WM pathology. Their results are in line with the current study showing GM atrophy of medial temporal lobe structures and temporoparietal lobes in AD [25-27] and atrophy of orbitofrontal, anterior cingulate, lateral temporal cortices, and caudate nucleus in bvFTD [28-31]. DTI studies on AD reported a rather consistent pattern of FA reductions in widely distributed WM tracts exceeding MTL regions [32-34]. In patients with bvFTD significant FA reductions in the superior and inferior longitudinal fasciculus, as well as additional FA decreases in the uncinate fasciculus and the genu of the corpus callosum have been reported [35,36].

To determine whether GM atrophy or WM integrity have potential diagnostic use, a direct comparison between AD and bvFTD is more important than the comparison with a control group. With respect to GM atrophy, precuneus, lateral parietal and occipital cortices have been shown to be more atrophic in AD than in bvFTD, whereas atrophy of anterior cingulate, anterior insula, subcallosal gyrus, and caudate nucleus are more atrophic in bvFTD compared to AD [4-6]. In our study, we did not find any areas which are more atrophic in AD compared to bvFTD. This could be explained by the strict FWE-correct VBM approach in our study, as in one study the decrease GM areas found in AD compared to FTD did not survive multiple comparisons correction [4]. Another explanation that we did not find any GM reductions in AD could be that our patients are included in an early disease stage, with relatively higher MMSE scores compared to another study [5]. Nevertheless, patterns of GM atrophy often overlap, as there are numerous regions of GM loss which are found in both AD and bvFTD [4,7-9]. The few existing DTI studies demonstrated WM alterations in FTD compared to AD, including more widespread FA reductions in the frontal, anterior temporal, anterior corpus callosum, inferior fronto-occipital fasciculus and bilateral anterior cingulum [10,12,14,29,37]. One of these studies also investigated the MD, L1 and L23 differences between FTD and AD and found increased L1 and L23 values in FTD compared to AD [10]. Our study is in line with these previous studies, failing to observe reduced FA and increased MD, L1 and/or L23 in AD relative to bvFTD. The same is seen in the DGM structures, where bvFTD patients have more subcortical brain damage compared to AD patients but not the other way around [6,38,39]. This is suggestive that bvFTD is more a network disease, with involvement of the whole frontal-striatal circuits, including the connecting white matter tracts and DGM structures, while AD is seemingly more a cortical disease.

We attempted to combine GM and WM measures to increase the discrimination of patient groups and showed that next to GM atrophy, WM integrity measures helped in distinguishing AD from bvFTD. A few earlier studies have combined WM and GM information with the objective to better discriminate between AD and bvFTD. They
found that FTD patients exhibited more WM damage than AD patients in an early stage of the disease suggesting that measuring of WM damage add up in the discrimination between these two dementias [13, 14]. Another study only linked the two imaging modalities and support the idea of a network disease in FTD but did not examine diagnostic value of GM and WM [37]. Only two studies actually used a multimodal combination of WM and GM. In one study they achieved a classification with 87% sensitivity and 83% specificity between AD and bvFTD [12]. In another study they developed a new metric which gives a measure of the amount of WM connectivity disruption for a GM region and showed classification rates were 8-13% higher when adding WM measurements to GM measurements [40].

The novelty of the study lies in the combination of three measures to separate AD from bvFTD. We combined VBM based measures of cortical atrophy, FIRST based measures of atrophy of DGM structures and DTI based measures of WM integrity to yield an optimal classifier.

Both discriminant analyses revealed that cortical GM matter contributed to the separation of AD from the other two groups and WM integrity measurements contributed to the discrimination of bvFTD from the other groups. Especially axial diffusivity increased the discriminatory power for bvFTD. This could be explained by the notion that, despite some involvement of DGM and WM, AD is assumed to be a cortical dementia with specific GM regions being affected whereas bvFTD predominantly affect areas (frontal-insula-anterior cingulate) which are part of structurally and functionally connected neural networks. These networks are connected by specific WM tracts located within damaged GM areas as the frontal lobes and are preferentially affected, contributing to network failure in bvFTD. The finding of more severe damage of DGM structures add up to the hypotheses of bvFTD being a network disorder as DGM structures can be seen as relay stations in the fronto-striatal brain networks. These findings are further supported by the fact that bvFTD had the same disease stage (comparable MMSE, CDR, duration of symptoms) as AD patients but have more WM and DGM structure damage.

A possible limitation of this study is that we did not have post-mortem data available, so the possibility of misdiagnosis cannot be excluded. Nevertheless, we used an extensive standardized work-up and all AD patients fulfilled clinical criteria of probable AD, 19 patients fulfilled the criteria for probable bvFTD and 5 patients for possible bvFTD. All diagnoses were re-evaluated in a panel including clinicians from both centers to minimize sample effects. Because this is a multicenter study, the differences in data acquisition parameters between the two centers might introduce some noise in the DTI analysis. However, we adjusted for center in all models and moreover, a recent study showed that when considering scanner effects in the statistical model, no relevant differences between scanners were found [41]. Among the strengths of this study is the sample size and its multi-center nature. Most of the studies comparing AD with bvFTD use smaller sample sizes. We had enough power to detect differences using FWE and FWE-TFCE correction to adjust for multiple comparisons. Another strength is the unique combination of three imaging parameters in this study to achieve optimal discrimination between AD and bvFTD.
Conclusion
Accurate diagnosis of patients in life is increasingly important, both on clinical and scientific grounds. It is a guide to prognosis and prerequisite for optimal clinical care and management. AD and bvFTD are difficult to discriminate due to overlapping clinical and imaging features. Therefore, there is an urgent need to improve diagnostic accuracy in a quantitative manner. This study has shown that DTI measures not only support the hypotheses of a network disorder in bvFTD but also add complementary information to measures of cortical and subcortical atrophy, thereby allowing a more precise diagnosis between AD and bvFTD.

Acknowledgements / Disclosures
The VUmc Alzheimer Center is supported by Alzheimer Nederland and Stichting VUMC Fonds. The study was supported by the Netherlands Organisation for Scientific Research (NWO). Research of the VUmc Alzheimer Center is part of the neurodegeneration research program of the Neuroscience Campus Amsterdam. The clinical database structure was developed with funding from Stichting Dioraphte. This project is funded by the Netherlands Initiative Brain and Cognition (NIHC), a part of the Netherlands Organization for Scientific Research (NWO) under grant numbers 056-13-014 and 056-13-010. The gradient non-linearity correction was kindly provided by GE medical systems, Milwaukee. Prof. Dr. Serge Rombouts is supported by The Netherlands Organisation for Scientific Research (NWO), Vici project nr. 016.130.677. Dr. Wiesje van der Flier is recipient of the Alzheimer Nederland grant (Influence of age on the endophenotype of AD on MRI, project number 2010-002).
### Tables and Figures

**Table 1. Demographics**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AD</th>
<th>bvFTD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>37</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>60.4 ± 6.2</td>
<td>66.7 ± 7.7</td>
<td>63.2 ± 7.5</td>
</tr>
<tr>
<td><strong>Sex, females</strong></td>
<td>16 (43%)</td>
<td>12 (38%)</td>
<td>6 (25%)</td>
</tr>
<tr>
<td><strong>Center, Vumc</strong></td>
<td>22 (60%)</td>
<td>22 (69%)</td>
<td>18 (75%)</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td>5.6 ± 1.0</td>
<td>5.0 ± 1.4</td>
<td>4.8 ± 1.6</td>
</tr>
<tr>
<td><strong>Duration of symptoms (months)</strong></td>
<td>-</td>
<td>40.2 ± 4.6</td>
<td>50.0 ± 8.9</td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td>28.9 ± 1.4</td>
<td>23.2 ± 3.1</td>
<td>25.1 ± 3.1</td>
</tr>
<tr>
<td><strong>CDR</strong></td>
<td>0 ± 0</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td><strong>GDS</strong></td>
<td>1.1 ± 1.3</td>
<td>3.0 ± 3.1</td>
<td>3.8 ± 2.9</td>
</tr>
<tr>
<td><strong>NBV (cm³)</strong></td>
<td>1493.7 ± 64.1</td>
<td>1395.2 ± 76.2</td>
<td>1394.81 ± 87.6</td>
</tr>
<tr>
<td><strong>VSF</strong></td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

Values presented as mean ± standard deviation or n (%). Level of education is determined according to the Verhage-system. Differences between groups for demographics were assessed using ANOVA. Kruskall-Wallis tests and χ² tests, where appropriate.

Key: MMSE: Mini-Mental State Examination; CDR: Dementia Rating Scale; GDS: Geriatric Depression Scale; NBV: normalized brain volume; VSF: volumetric scaling factor

- different from controls (p<0.05),  
- different from AD (p<0.05)
<table>
<thead>
<tr>
<th>Structure</th>
<th>Controls</th>
<th>AD</th>
<th>bvFTD</th>
<th>p</th>
<th>Ctrl &gt; AD Mean difference</th>
<th>p</th>
<th>Ctrl &gt; bvFTD Mean difference</th>
<th>p</th>
<th>AD &gt; bvFTD Mean difference</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>10.3 ± 1.0</td>
<td>8.4 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.3 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>1.707</td>
<td>&lt;0.001</td>
<td>1.847</td>
<td>&lt;0.001</td>
<td>0.140</td>
<td>1.000</td>
</tr>
<tr>
<td>Amygdala</td>
<td>3.6 ± 0.6</td>
<td>3.4 ± 1.5</td>
<td>3.3 ± 0.6</td>
<td>0.082</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>19.8 ± 1.7</td>
<td>18.1 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.1 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>1.227</td>
<td>0.006</td>
<td>1.689</td>
<td>&lt;0.001</td>
<td>0.461</td>
<td>0.793</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>9.0 ± 0.8</td>
<td>8.7 ± 0.9</td>
<td>8.0 ± 1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>0.128</td>
<td>1.000</td>
<td>0.978</td>
<td>&lt;0.001</td>
<td>0.850</td>
<td>0.002</td>
</tr>
<tr>
<td>Putamen</td>
<td>12.3 ± 1.1</td>
<td>11.2 ± 1.4</td>
<td>11.2 ± 1.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.231</td>
<td>0.631</td>
<td>1.118</td>
<td>0.001</td>
<td>0.485</td>
<td>0.364</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>4.8 ± 0.7</td>
<td>4.6 ± 0.8</td>
<td>4.3 ± 0.8</td>
<td>0.071</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>1.1 ± 0.2</td>
<td>0.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>0.143</td>
<td>0.031</td>
<td>0.331</td>
<td>&lt;0.001</td>
<td>0.187</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are presented as mean cm$^3$ ± standard deviation. Comparisons are Bonferroni corrected with age, sex and center as covariates.
<sup>a</sup> different from controls, <sup>b</sup> different from AD (p<0.05)
Table 3. Structure Matrix showing the discriminant loadings for each predictor. The structure matrix correlation coefficient represents the relative contribution of each predictor to group separation. (A) Discriminant analysis with GM ROIs, DGM structures and FA ROIs. (B) Discriminant analysis with GM ROIs, DGM structures and L1 and L23 ROIs.

(A) Table showing discriminant loadings for GM ROIs, DGM structures and FA ROIs.

<table>
<thead>
<tr>
<th>Function</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM ROI AD&lt;Controls</td>
<td>0.469</td>
<td>0.449</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.222</td>
<td>0.475</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.111</td>
<td>0.282</td>
</tr>
<tr>
<td>FA ROI AD&lt;Controls</td>
<td>0.232</td>
<td>0.451</td>
</tr>
<tr>
<td>FA ROI bvFTD&lt;Controls</td>
<td>0.131</td>
<td>0.476</td>
</tr>
<tr>
<td>Center</td>
<td>-0.021</td>
<td>-0.103</td>
</tr>
<tr>
<td>Age</td>
<td>0.187</td>
<td>0.111</td>
</tr>
<tr>
<td>Sex</td>
<td>0.003</td>
<td>-0.121</td>
</tr>
</tbody>
</table>

(B) Table showing discriminant loadings for GM ROIs, DGM structures and L1 ROI.

<table>
<thead>
<tr>
<th>Function</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM ROI AD&lt;Controls</td>
<td>0.642</td>
<td>0.400</td>
</tr>
<tr>
<td>GM ROI bvFTD&lt;AD</td>
<td>0.039</td>
<td>0.706</td>
</tr>
<tr>
<td>L1 ROI bvFTD&gt;AD</td>
<td>0.026</td>
<td>0.478</td>
</tr>
<tr>
<td>L1 ROI bvFTD&gt;Controls</td>
<td>0.183</td>
<td>0.433</td>
</tr>
<tr>
<td>L1 ROI AD&gt;Controls</td>
<td>0.303</td>
<td>0.329</td>
</tr>
</tbody>
</table>
Figure 1. VBM voxel-wise statistical analysis of GM reductions between groups. Figures are displayed with a threshold of p<0.05, FWE corrected. Brighter colors indicate higher t values.
Figure 2. Boxplot of raw volumes (cm$^3$) of MTL and DGM structures.

**p≤0.001, *p<0.05
Figure 3. TBSS voxelwise statistics displaying areas of white matter skeleton (green) with lower FA (red-yellow) and higher MD, L1, L23 (blue-lightblue) values, overlaid on the MNI-standard brain. Significance level of $p<0.05$ with correction for multiple comparisons was used. Skeletonized results are thickened to enhance figure clarity. These thickened results are based on the original p-maps. Percentages remained the same.
Figure 4. Composition of ROIs per contrast. (A) GM ROI AD<Controls: All significant voxels (p<0.05, FWE corrected) from the VBM group comparison where AD patients had less GM compared to controls are indicated in yellow. FA ROI AD<Controls: All significant areas (p<0.05, FWE TFCE corrected) from the TBSS group comparisons where AD patients had lower FA values compared to controls are indicated in red. (B) GM ROI bvFTD<Controls: All significant voxels (p<0.05, FWE corrected) from the VBM group comparison where bvFTD patients had less GM compared to controls are indicated in yellow. FA ROI bvFTD<Controls: All significant areas (p<0.05, FWE TFCE corrected) from the TBSS group comparisons where bvFTD patients had lower FA values compared to controls are indicated in red. (C) GM ROI bvFTD<AD: All significant voxels (p<0.05, FWE corrected) from the VBM group comparison where bvFTD patients had less GM compared to AD patients are indicated in yellow. FA ROI bvFTD<AD: All significant areas (p<0.05, FWE TFCE corrected) from the TBSS group comparisons where bvFTD patients had lower FA values compared to AD patients are indicated in red. (D) MD, L1, L23 ROI AD<Controls: All significant areas (p<0.05, FWE TFCE corrected) from the TBSS group comparisons where AD patients had higher MD (pink), higher L1 (blue) and higher L23 (green) values compared to controls. (E) MD, L1, L23 ROI bvFTD<Controls: All significant areas (p<0.05, FWE TFCE corrected) from the TBSS group comparisons where bvFTD patients had higher MD (pink), higher L1 (blue) and higher L23 (green) values compared to controls. (F) MD, L1, L23 ROI bvFTD<AD: All significant areas (p<0.05, FWE TFCE corrected) from the TBSS group comparisons where bvFTD patients had higher MD (pink), higher L1 (blue) and higher L23 (green) values compared to AD patients.
Figure 5. Projection plot of canonical discriminant functions for discrimination of healthy controls, AD and bvFTD patients. (A) Discriminant function consisted of GM ROI AD<Controls; hippocampal volume; volume of putamen; FA ROI AD<Controls; FA ROI bvFTD<Controls; center; age; and sex. (B) Discriminant function consisted of GM ROI AD<Controls; GM bvFTD<AD; L1 ROI AD>Controls; L1 ROI bvFTD>Controls; and L1 ROI bvFTD>AD. Blue squares indicate individual data of healthy controls, green dots indicate data of individual AD patients, red triangles indicate individual data of bvFTD patients. The black squares represent the group centroids.
Reference List


fluent aphasia and behavioral variant of frontotemporal dementia. *Front Hum Neurosci* 7, 467.


