Diploma thesis

Structural covariance networks in Alzheimer's disease

Submitted by

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List of Abbreviations

Aβ	Amyloid beta
ACh	Acetylcholine
AD	Alzheimer's disease
APOE	Apolipoprotein E
APP	Amyloid-Precursor-Protein
ASPFS	Austrian Stroke Prevention Family Study
BET	Brain extraction tool
BOLD	Blood oxygenation level dependent
CSF	Cerebral spinal fluid
CDR	Clinical Dementia Rating
DMN	Default mode network
DWI	Diffusion weighted imaging
EOAD	Early onset form of Alzheimer's disease
GDS	Geriatric depression scale
GM	Grey matter
IC	Independent component
ICA	Independent component analysis
LOAD	Late onset of Alzheimer's disease
MCI	Mild cognitive impairment
MCI-C	Mild cognitive impairment converters
MCI-S	Mild cognitive impairment stable
MMSE	Mini Mental State Examination
MTL	Medial temporal lobe
MRI	Magnetic resonance imaging
sMRI	Structural magnetic resonance imaging
NDC	Non-demented controls
NFTs	Neurofibrillary tau tangles
NP	Neuritic plaques
PRODEM	Prospective registry on dementia in Austria
SCN	Structural covariance network
SN	Salience network
WM	White matter

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Abstract

The cognitive symptoms of Alzheimer's disease (AD) might be the consequence of disrupted connectivity of various brain regions. Different network-based approaches have been established to examine inter-relationships between different brain regions. In comparison to functional magnetic resonance and diffusion weighted imaging, the analysis of structural covariance networks (SCNs) is a relatively new approach that provides complementary information about inter-regional structural brain connectivity in general and in neurodegenerative disorders such as AD in particular. The objective of this study was the identification of grey matter structural covariance networks and their integrity in AD. T1-weighted MRI images of 78 patients with probable AD and 78 age and sex matched healthy elderly, retrieved from the Prospective Registry On Dementia in Austria (PRODEM) and the Austrian Stroke Prevention Family Study (ASPFS), respectively, were analysed using independent component analysis. Results revealed 16 SCNs composed of cortical, subcortical and/or cerebellar structures. 10 SCNs demonstrated decreased structural covariance network integrity in AD when compared to the control group. These results highlight that AD causes a reduction in network integrity in specific SCNs, modelling AD as a disconnection syndrome and moreover corroborates the sensitivity of the method. Future studies will have to clarify the association with cognitive decline.

Contents

1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by grey matter atrophy including the cortex, but also sub- cortical brain structures such as the hippocampus. Also, AD is associated with progressive cognitive decline, primarily in episodic memory. This process begins with mild memory complaints and develops into an all-encompassing disabling disease.

1.1 Epidemiology

The global prevalence of dementia is estimated on 46,8 million affected individuals (1). As AD is with an estimated proportion of 60-80 per cent the most common form of all dementia types (2), approximately 28-37.4 million people suffer from it. In Austria 60.000-80.000 individuals suffer under this form of dementia (3). Following calculations (1), every year roughly 10 million additional dementia cases will arise worldwide. As the life expectancy increases and age is seen as the most important risk factor, according to estimates, there will be 131.5 million dementia patients (78,9-105.2 million with AD) in 2050 (1).

1.2 Clinical Stages and Symptoms

AD can be classified in three different merging stages (2):
1. A preclinical stage,
2. Mild Cognitive Impairment (MCI), and
3. A clinical stage, which is divided in probable and possible AD

Preclinical stage

AD can develop up to 20 years before the clinical onset (2). In 2011 the "National Institute on Aging and the Alzheimer's Association", defined four sub-stages of preclinical AD (4):
-Stage 0 is defined as the absence of all AD typical pathology.
-Stage 1 is characterized by the presence of amyloid deposits.
-Stage 2 shows next to the amyloid deposits neurodegenerative pathology.
As an early sign of the disease a reduced concentration of beta amyloid in the cerebral spinal fluid (CSF) is present.

-In addition to stage 2, stage 3 presents the occurrence of subtle cognitive dysfunction. If this cognitive decline progresses, individuals can further be classified in a stage termed *mild cognitive impairment (MCI)*. Difficulties in memorization and thinking are present. Nonetheless, daily life activities are not yet impaired. MCI individuals can remain in this stage (MCI stable, MCI-S), convert to AD (MCI converters MCI-C) or even regress to a normal cognitive status (5).

Clinical Alzheimer's disease

As the disease progresses, subjects show increasing impairments in memory, activities of daily living, spatial and temporal orientation, cognitive flexibility, information processing speed or language. Aggressiveness and depression complicate the clinical picture and may be of great concern for the caregivers. In final stages, subjects lose the capacity of meaningful communication. Problems in walking, speaking and swallowing can appear. Patients become bedridden, which makes them even more vulnerable to contract infections such as pneumonia (6). Pneumonia is a frequent contributing element to death of AD patients (2). According to the National Centre for Health Statistics, AD is the sixth-leading cause of death in the United States (7). In Austria, AD is the fourth-leading cause of dead after ischaemic heart disease, lung cancer and stroke (8).

1.3 Pathogenesis

The exact pathogenesis is still unknown. Three dominant theories have been established to explain the molecular basis of AD

The cholinergic hypothesis

The cholinergic hypothesis was founded in the late 1960s (9) and is based upon observations that in patients with AD a cholinergic deficit is present. The neurotransmitter acetylcholine (ACh) was identified to be altered in patients with AD. As reviewed by Francis et al. (9) in AD a reduction of the ACh-synthesizing enzyme acetyltransferase was observed. Further studies revealed reduced ACh uptake (10) and ACh release (11) in AD patients. Moreover a reduction of cholinergic neurons in the nucleus basalis of Meynert (12) was found.. The basal and rostral forebrain cholinergic pathways were identified to play an important role in cognitive functions

such as attention, working memory and awareness (13). The reduction of cholinergic neurons in the rostral forebrain pathways (14) might result in the cognitive degradation in AD patients (15).

The amyloid hypothesis

*Around two decades later the amyloid theory was established. It centres the amyloid beta (*Aβ*) protein, one of the two neuropathological hallmarks of AD.* Aβ is formed by the transmembrane protein amyloid precursor protein (APP). APP can be processed into smaller peptides by either the α-secretase or the β-secretase (BACE1). The resulting peptide of the BACE1 is further processed by the γ-secretase. This leads to the formation of different sized beta amyloid peptides. Aβ40 is composed of 40 and Aβ42 of respectively 42 aminoacids (16) (please compare figure 1).
In AD, a dysregulation of the APP processing might lead to an increased formation of the more pathogenic Aβ42. Also, there are genetic and environmental influences that are known to play a role in the increase of Aβ42. These aberrant extracellular Aβ protein deposits result in the formation of neuritic plaques (NP) (17). This process is assumed to trigger further neurotoxic processes such as the evolution of Neurofibrillary tau tangles (NFTs) (17). The inter-neuronal communication at synapses is disturbed and cell destruction might follow.
As a consequence of NPs and NFTs, a decrease in synaptic plasticity and axonal dysfunction occur. A massive loss of neurons, white matter and synapses is the consequence. As a reduction of neurons is observed in brain regions that are involved in cognitive functions, such as the memory (18), a cognitive decline is the consequence (19).

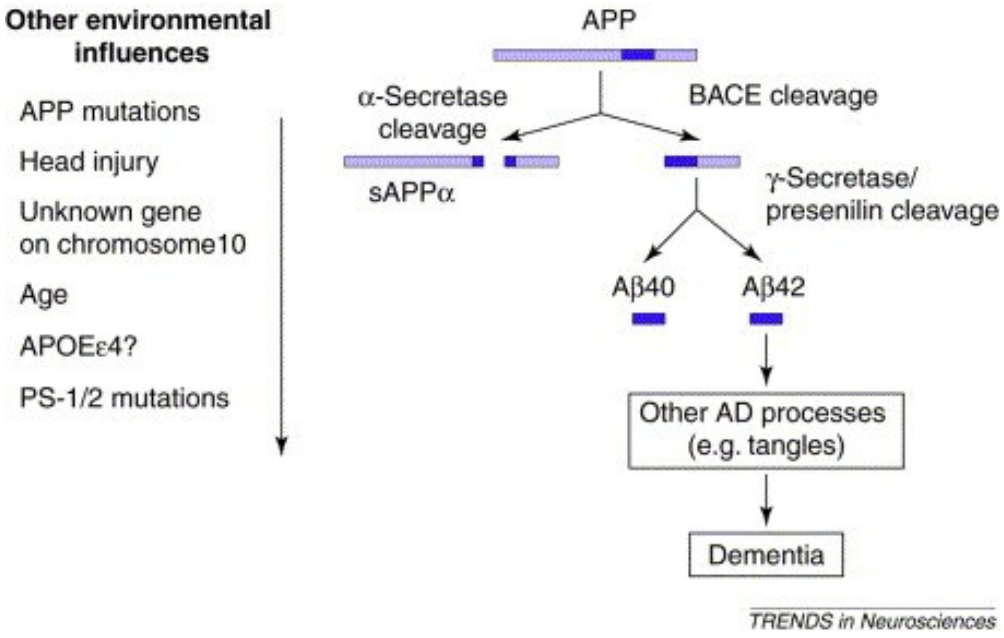


Figure 1: The amyloid hypothesis of AD: Some environmental factors are known to increase Aβ42 production. [Reprinted from Trends in Neuroscience, Volume 25, Issue number 1, Mudher A, Lovestone S., "Alzheimer's disease –do tauists and Baptist finally shake hands?" (17), Pages 22-26, Copyright (2016), with permission from Elsevier.]

The tau hypothesis

In contrast to the previous theory, the tau hypothesis (17) assumes changes in the intracellular tau protein temporally even before the evidence of amyloid beta plaques. The tau protein is linked with microtubules of the cytoskeleton. In AD a hyperphosphorylation of the microtubule associated tau protein is present. As a result of this hyperphosphorylation, the tau protein might dissolve from the microtubule, which leads to a reduction in its function (17). Furthermore, an aggregation of the deliberated tau proteins might result in the formation of abnormal neurofibrillary tangles. Patients with AD show a moderate replacement of the neuronal microtubules by tau tangles (20). The intracellular aggregation of tangles and the dysfunction of microtubules probably lead to neuronal loss. The aggregation of tau tangles is observed in regions, that are involved in memory (21). Hyperphosphorylated tau can be measured in the CSF and is correlated with cognitive decline. (22).

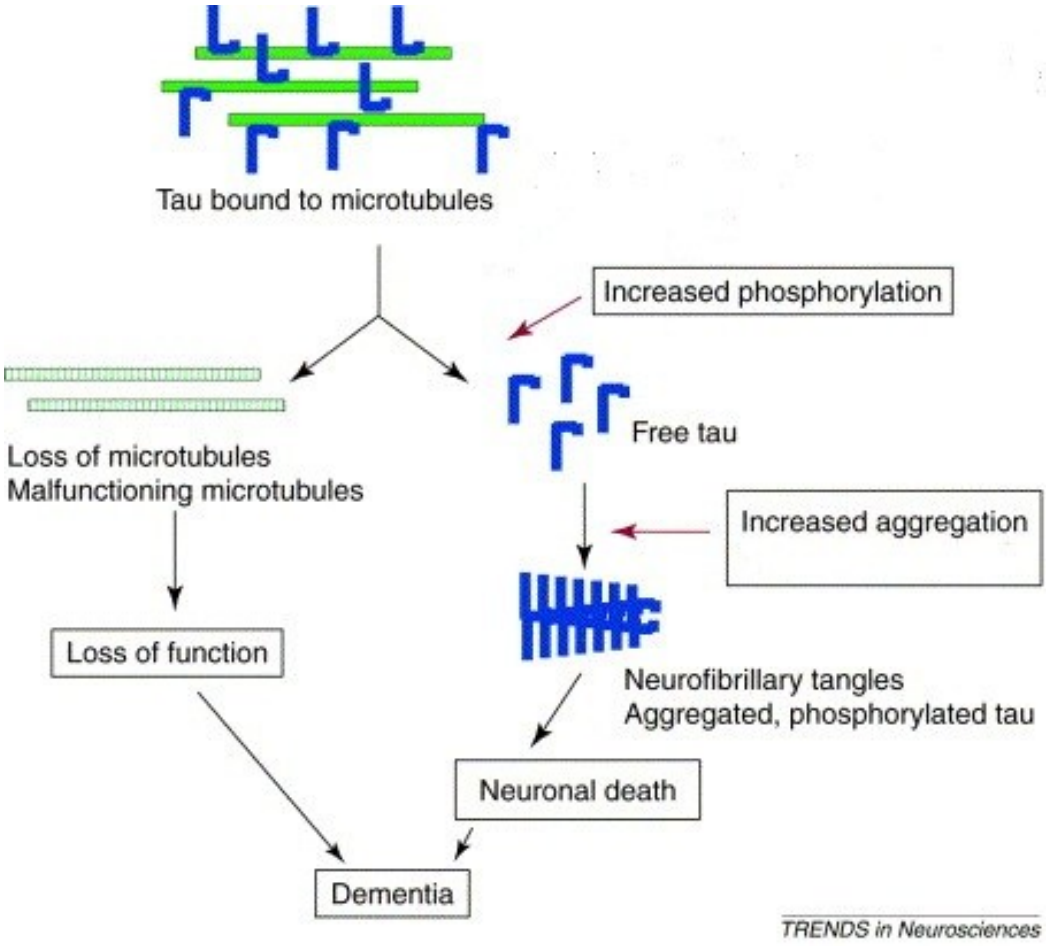


Figure 2: Tau-Hypothesis of AD. [Reprinted from Trends in Neuroscience, Volume 25, Issue number 1, Mudher A, Lovestone S., "Alzheimer's disease –do tauists and Baptist finally shake hands?" (17), Pages 22-26, Copyright (2016), with permission from Elsevier, slightly modified for simplification]

Cerebral distribution of neurofibrillary tangles and neuritic plaques

Whereas the amyloid plaque pathology is predominantly found in cortical regions, the accumulation of NFTs progresses from medial temporal lobe structures to enclose the whole cortex (23). Based on the distribution of NTFs Braak and Braak (23) introduced a six staged model of disease progression in 1995: While subjects are clinically symptom-free, the deposition of NFTs can already be observed in the transenthorinal cortex (Stage I and II). From there, the NFTs spreads further to the entorhinal cortex and via the tractus perforantes to the hippocampus, the parahippocampus, amygdala and uncus, regions that are all parts of the medial temporal lobe (MTL), (Braak III and IV). Moreover, those structures are part of the limbic system and play an important role in cognitive functions. As the distribution of NFTs goes hand in hand with atrophy (24), a mild cognitive decline is observed.

Amnestic AD typically starts with decreased episodic memory functions related to early neurofibrillary pathology in the medial temporal lobe (25).
In final AD the NFTs and the destruction advance to subcortical regions and the neocortex (Braak stages V and VI).

The amyloid deposition begins in basal areas of the isocortex. Relatively sparing the hippocampal formation, all isocortical association areas are subsequently affected. In the end stage, amyloid deposits are observed in all regions of the isocortex, including motor and sensory core fields (26)

1.4 Genetics

Two forms of AD have been established based on the age of manifestation (27).
- Early onset form of AD (EOAD): Individuals develop AD before the age of 65 years. This familial form represents only 1-5 per cent of all AD cases. Three genes were identified for the EOAD form. These genes code for amyloid precursor protein on chromosome 21, presenilin 1 on chromosome 14, and presenilin 2 on chromosome 1. Due to predominantly autosomal inherited mutations, an overproduction of amyloid beta occurs.
- Late onset form of AD (LOAD): This form is responsible for about 95 per cent of AD cases and manifests usually after age 65. Despite intensive research the genetic background of LOAD remains unclear. It is estimated that LOAD is genetically determined to a substantial degree (60-80 per cent) (27). A polymorphism on chromosome 19 is seen as a polygenic risk factor (28). This gene contains the transcription information for the APOE protein, which is involved in lipid metabolism. Three different alleles are known. A single exchange of an amino acid at position 112 or 158 results in three different proteins: APOEε-2/3/4. The APOEε-4 variation is associated with a higher production of Amyloid-β than in individuals without this variant (29). Therefore APOEε-4 is considered to be a risk factor for AD (28). A single APOEε-4 allele doubles or triples the risk of developing AD, two alleles might increase the risk by a factor of 11 (30–32). APOEε-4 is thought to be responsible for 20-30 per cent of the risk to develop LOAD (28). However, the mere presence of this variant is not enough for the establishment of AD; and moreover it is not required for the development of the disease (33).

1.5 Diagnosis

The current diagnostic classification of the “National Institute on Aging and the Alzheimer’s Association“ (34) comprises criteria for 1) probable AD, 2) possible AD and lastly, 3+4) probable or possible AD with the proof of typical pathophysiological formations. Detailed information is provided below.

Probable dementia due to Alzheimer’s disease

For the diagnosis of probable AD dementia, general core criteria of cognitive and executive decline must be met. The onset of disease must be subtle, over months to years. Next to changes in behaviour and personality, the ability to acquire and remember new information is lost. For a detailed list of required impairments to fulfil the probable AD dementia criteria please compare McKhann et al. (34).
Based on medical history, laboratory tests, physical examination, neuropsychological assessment and neuroimaging methods, the diagnosis of probable AD can presently be determined with a confidence of >90 per cent (35). A definite diagnosis of AD is possible exclusively post mortem.

Possible AD dementia

A possible diagnosis is present if the course appears to be atypical, or if the subject shows a mixed presentation of aetiology:
• In the atypical course, core criteria (34) of AD are met, but the onset of impairment is abrupt.
• An aetiologically mixed presentation matches all core clinical criteria for AD, but shows also the presence of accessory cerebrovascular or neurological diseases, non-neurological comorbidities and/or a medication that effects cognition.

Probable AD dementia with evidence of AD related pathophysiological processes

The core clinical criteria for probable AD dementia must be met. Biomarkers (compare chapter 1.7) can be used to demonstrate that the cause for the dementia is related to AD typical pathophysiological processes (34).
Testing of biomarker are mainly used for research purposes. The implementation of biomarkers in routine diagnostic is not yet recommended, as for most AD patients, the core clinical criteria give very good accuracy and utility for the diagnosis.

Possible AD dementia with evidence of the AD pathophysiological processes

This category is for subjects who present AD related biomarkers or pathophysiological formations, but do not meet AD dementia criteria (34).

1.6 Therapy

There is no cure for AD. Nevertheless, there are drugs to decelerate the progression and to alleviate the symptoms. In spite of an enormous effort to discover novel treatments, the outcome has been rather poor: Over the past 30 years only memantine and cholinesterase inhibitors have been approved for the treatment of AD (36).

The most effective drugs are the acetylcholine-esterase inhibitors (37), which augment the concentration of Ach in the synaptic space. Donepezil, galantamine, and rivastigmine are three approved drugs for the treatment of mild to moderate AD. Among them Donepezil is the most prescribed AD drug worldwide (38).

Memantine is a N-methyl-D-aspartate (NMDA) receptor antagonist and can inhibit the destruction of cholinergic neurons (39). It is used for patients with moderate to severe AD and can be prescribed in combination with acetyl cholinesterase inhibitors (37).

Selegiline and α-tocopherol have an antioxidant effect and are approved for clinical usage; however their effectiveness is viewed controversially. Neuroleptics are used for their antipsychotic and calming effects. A symptomatic depression can be treated with selective serotonin reuptake inhibitors (37).

Besides the pharmacological therapy it is very important to implement non-pharmacological treatments like behavioural therapy, physiotherapy, ergo therapy, cognitive training, reality orientation and music-therapy (40).

Ballard and colleagues (41) found that non-pharmacological measures, such as cognitive training, cognitive rehabilitation and cognitive stimulation therapy have a modest but significant influence on the treatment of cognitive symptoms in AD patients.

1.7 Biomarkers

In 1993 Morris introduced the Clinical Dementia Rating scale (CDR) to facilitate the comparability between affected patients and to classify stages (42). However, clinical measures lack the possibility of objectification as they show an immense variation between and even within patients. A major objective of the AD related research is to develop specific and sensitive biomarkers, which enable early identification, monitoring and an objective classification of AD. Biomarkers are indicators of biological normal or pathological processes. The APOEε-4 status represents one possible marker of AD (43). It was shown that the presence of at least one APOEε-4 allele increases the atrophy rate of grey matter (GM) in the hippocampus and in the entorhinal cortex (44).

For the early diagnosis of AD pre-stages, the most promising biomarkers are: Concentrations of amyloid-β42 (Aβ), total tau-protein and phosphorylated-tau-181 in the CSF (45). These biomarkers in combination increase the diagnostic validity for AD and reach a specificity of >85 per cent and a sensitivity of >95 per cent (35).

In AD, the concentration of amyloid-β 42 (Aβ) is decreased in the CSF, while the values of phospho-tau or total-tau are increased. The level of hyperphosphorylated tau in the CSF correlates with the reduction in cognitive functions (22).

However, a liquor puncture is an invasive method that entails risks such as infection and meningitis. Non-invasive techniques, such as volumetric magnetic resonance imaging or the *in vivo* visualization of Aβ and tau-protein using PET with Aβ-binding ligands (46) (47) might represent promising alternatives.

Magnetic resonance imaging as a biomarker

Structural magnetic resonance imaging (sMRI) is a non-invasive, safe and reliable technique. It can be used to measure global and regional brain volumes *in vivo*. Based on brain wide atrophy patterns, this method discriminates non-demented controls (NDC) from pathologically confirmed AD with a sensitivity of 97 per cent and a specificity of 94 per cent (48). Moreover it delimits other dementia forms such as Lewy-body disease or frontotemporal lobar degeneration from AD with a sensitivity of 91 per cent and a specificity 84 per cent (49).

Next to the volume of global (50) and local cortical grey matter (GM) as important markers, the volumetric measurement of the hippocampus is currently the best-

established local imaging biomarker (51).
De Leon and colleagues (52) first established the atrophy of the MTL as a significant biomarker for AD. AD patients reveal expanded grey matter loss in medial temporal lobe structures such as the entorhinal cortex, hippocampus, parahippocampus and in the amygdala (53, 54). Up to five years before the probable diagnosis, a deterioration can be observed in these regions (55). The left hemispherical amygdala-hippocampal-complex (part of the MTL) already shows a reduced volume even up to ten years prior to onset of clinical AD (55). In AD, the left hemispheric MTL shows a lower volume than the right MTL (53).

Additionally, MRI has the potential to improve the diagnostic staging of AD and can be useful in the prediction of the conversion of MCI to AD (56). Subjects with MCI that convert to AD show, in comparison with MCI subjects that do not convert to AD, a higher atrophy of medial temporal lobe structures, especially of the left hippocampus and parahippocampal gyrus (57). Thus, the most consistent anatomic biomarker for the prediction of evolution of amnestic MCI into AD is the atrophy of the left MTL (57). Neuronal connections between MTL structures and the neocortex are important for building up and maintaining long-term-memory (58). As a consequence of decreased GM volume of the left entorhinal cortex, a disconnection between the hippocampus and the neocortex occurs. This hampers the episodic consolidation of memory in Alzheimer patients (59).
Furthermore, the atrophy of the hippocampus itself is a significant marker for the diagnosis of AD (60). The hippocampus of AD patients and MCI-C subjects shows an accelerated atrophy rate when compared to NDC and MCI-S subjects (44). Barnes et al. (61), revealed a mean annual hippocampal atrophy rate of -4.66 per cent in patients with AD. MCI-C patients had an annual rate of -3 per cent, while NDC showed an annual atrophy rate of merely -1.41 per cent.

Next to medial lobe structures, precuneus (62), thalamus, parietal, temporal, frontal, insular, cingulate cortices (53), hypothalamus and putamen show reduced GM volumes in AD patients when compared to NDC. The volumes of the left hemispherical thalamus, hippocampus and putamen seem to be correlated with a decrease in cognitive functions (63).

To date, biomarkers merely contribute to an increased specificity for the diagnosis (27). However, no biomarker has shown the accuracy and reliability to diagnose AD

unequivocally (64). Additional studies are needed to identify their validity in practice and in research settings (34).

2 Background

Local anatomical atrophy markers neglect inter-relationships between different brain regions, as brain functions are arranged into vast communicating and complex functional networks with circumscribed topological organization (65).
Brain disorders are not restricted to limited brain regions, but appear to emerge in different networks of many brain regions (66) (67). For a better understanding of the pathophysiology of AD it is crucial to examine the changes in the underlying connectivity patterns (68).

A network model of AD pathophysiology

In 2003 Delbeuck et al. (69) proposed a “disconnection” model of AD. Brier et al. (70) further hypothesized that amyloid and/or tau depositions might cause a toxic environment, that leads to a disconnection between previously connected regions. As a result of this disconnection memory deficits might appear. As the accumulation of amyloid and tau progressively occurs over years in a network of regions (26), brain regions deteriorate gradually and might stay in a lowered functional state. Nevertheless, an interaction with other regions of the network might still be possible. Directly affected regions described by Braak and Braak (26), might maintain their in- and outputs with related regions in a disturbed way. Thus, it is conceivable that the damaging processes can be distributed among different network regions. This might cause decreased function between connected regions. Observations showed that a disruption in functional systems is at hand, even if they are not directly affected by pathological deposits (70).

2.1 Structural covariance

Some brain regions co-vary in their morphological characteristics. Cortical thickness in one region can impact the thickness of functionally and/or structurally related brain parts. Subjects who present a greater cortical thickness of Wernicke’s area, also present a greater thickness of Broca’s area (71). This example of structural covariance demonstrates that variations in local structures between subjects can develop in an organized manner (72).
Early observations demonstrated that the post-mortem volume of visual system

components such as primary visual cortex, lateral geniculate nucleus and optical tract co-vary across different persons (73). Also, a correlation between brain regions participating in cognitive functions and their size was reported (72). For example the hippocampal GM volume showed strong co-variation in GM volume with the amygdala, the perirhinal, entorhinal, parahippocampal and orbitofrontal cortices, brain regions associated with the memory system (74).
Underlying mechanisms of structural covariance might be based on common trophic influences and/or of mutually experience-related plasticity (75, 76). Nevertheless, it is nebulous to what degree covariance is caused as a result of aging and development, genetic effects or experience-linked plasticity (66). The study of networks of correlation between regional GM volumes as calculated across subjects has been consistently portrayed in several studies and was called structural covariance MRI. It allows the analysis of the extent to which inter-individual variations in regional structures are systematically organized within networks of GM volumes or cortical thickness (66, 77, 77, 78). These networks are called structural covariance networks (SCNs).
Methodologically, compared to Diffusion Weighted Imaging (DWI) and functional MRI, structural covariance mapping provides a deeper insight into inter-regional connectivity and the structural organization of the brain.
For the investigation of structural covariance, there are mainly three experimental approaches (72):

1) Seed based analysis studies the morphology of a pre-specified “seed” region in contrast to the remaining brain. The result is a whole-brain correlation map of structural covariance with the seed region.
2) Independent component analysis (ICA) (for further detail see chapter 3.2), and
3) Graph theoretical approaches. Here, a network can be described as a graph G, built up of nodes, that symbolize brain regions and edges between those nodes, which equal interregional relationships (79).

The definition of either a seed region or specific network nodes is confounded by a selection bias. We therefore used ICA, because this method does not require pre-defined seeds, and is thus preventing bias and allows hypotheses-free examination

of the data. Despite some limitations (see chapter 5), the investigation of SCNs is believed to reveal complementary information about the topological arrangement of the brain (19, 77).

Does covariance signify connectivity?

Several studies try to relate structural covariance to either structural or functional connectivity. The functional connectivity is based on the blood oxygenation level dependent (BOLD) signal and is examined by functional MRI imaging. In contrast, structural connectivity implies direct anatomical connections.
Based on DWI (80), it has been speculated that the pattern of structural covariance might be related to the pattern of structural connectivity. Hence, interregional structural covariance could be the result of direct white matter tracks. It has been shown that many brain areas that show direct axonal connections strongly covary in their morphology. Contralateral homologue regions of the cortex (81) or posterior and anterior language regions (71) are just two examples. In addition to structural imaging, functional MRI studies suggested a direct connection between the organization of intrinsic functional networks and the pattern of structural covariance (82, 83). Regions that show a covariance in morphological traits could belong to identical functional networks (84). Overlap is possible as some functionally correlated brain regions show not only a covariance in the thickness of the cortex (71, 84), but also axonal connections (68, 85). Nevertheless a generally valid coherence between SCNs and functional connectivity has not yet been proven, because the fundamental mechanisms of this relationship remain unknown.

2.1.1 Genetics of covariance structures

As it is known that global indices such as total GM/WM volume, total intra-cranial-volume and mean cortical thickness are highly heritable (86–88), it is supposed that the coordinated development of locally separated brain regions might also be partly genetically modified. Twin analysis of single brain regions can reveal the range about inter-individual structural differences that are caused by genetic factors. These studies demonstrated that some brain gyri show a higher heritability in cortical thickness than others (89). As reviewed by Alexander-Bloch et al. (72), different

genetic factors influence structural covariance patterns of cortical thickness most powerful in distinct networks of the brain such as the fronto-parietal network. This highlights a network specific association between genetic determinants and networks. Interestingly, carriers of the APOE ε4 allele show a reduced covariation of GM volume decades before the potential onset of AD in dorsolateral and medial prefrontal, parietal and lateral temporal cortices. In contrast, some regions demonstrate an increase of covariation in GM. These regions are bilateral cerebellar, occipital, bilateral thalamus, bilateral fusiform and right lingual gyri areas (90).

2.1.2 Gender differences in structural covariance

Grey matter correlation studies (81) revealed, that there is little gender divergence in structural covariance. In both genders, a specific seed region (with the exclusion of primary visual cortex) predicts the density of the homologue region of the contralateral hemisphere (91). Nevertheless, some gender differences have been discovered: Females present a stronger covariance between a) left middle occipical and lateral occipito-temporal gyri and b) right inferior and middle temporal gyri. These regions are involved in semantic memory processing and language. These differences of covariance might be associated with the advantages of females in verbal processing (92). Further, significant gender differences were found in the left amygdala which in females is positively associated with the right angular gyrus. In male subjects the left amygdale is associated with the left and right anterior inferior temporal cortex (81). Despite the general equivalence between genders, these exceptions emphasize that the gender modulates distinct brain areas. As reviewed by Mechelli et al. (81) only male subjects show an activation of the left amygdala for long-term incidental memory of arousing material. Additional, the identified structures (the right angular gyrus and anterior inferior temporal cortex) are reviewed to play a role in several memory functions, such as memory formation, maintenance and retrieval. On the base of these findings Mechelli et al. proposed that identified gender-related structural covariance patterns influence gender-related brain responses in the left amygdala.

2.1.3 SCNs in brain maturation and normal aging

During childhood and adolescence global and regional brain structures undergo great changes. In order to investigate structural covariance changes in the developing brain, Zielinski et al. (93) conducted a seed-based-GM density analysis. In the youngest age group, structural covariance was limited to the region of interest and the contralateral homologous region. Subsequently, in early childhood, networks, derived from primary sensorimotor seeds were well established. Interestingly, in early adolescence these networks enlarge, before they shrink into a smaller topological organization in late adolescence.
In comparison to these findings, socio-emotional, language and other cognitive networks, presented a lower level of organization at early age and an age related growth of organization (93).
In early childhood and adolescence, brain maturation is defined by nonsimultaneous changes of cortical thickness. Regions that show a synchronization in maturation might be subsumed in a “maturational network” (94). Studies showed that there is a considerable overlap between maturational structural and maturational functional networks in the developing brain (77). SCNs seem to display synchronized maturational changes in disseminated cortical regions (77).
In normal elderly not only a decrease in the integrity of structural networks (95, 96) is observed, but also a GM reduction in brain parts that reveal high inter-regional correlations in morphology. When comparing a group of 60-84 years with a younger group of individuals (18-35 years), a reduction of structural covariance is existent in language and cognitive networks (97).

Principal component analysis studies showed correlated atrophy patterns within subcortical and fronto-temporal networks (98–100). Age related alterations in structural covariance impact the functional level, as the stability of these brain-wide covariance networks is correlated with the individual neuropsychological performance including attention, cognitive and memory tests (98, 101). Structural covariance network changes occur not only in subcortical structures but rather also in lateral occipital, posterior and anterior cingulate cortices. Highest association with age was found in an SCN including the thalamus, nucleus caudatus, nucleus accumbens and the hippocampus (102).

2.1.4 Structural Covariance in Alzheimer's disease

The analysis of structural covariance represents an important tool to better understand the neuropathology of AD, as this method provides a deeper insight into the organization of neural activity that in turn influences clinical manifestation of the disease (19, 103, 104). Accordingly, the clinical symptomatology of AD might be due to alterations within distinct large-scale networks, instead of being caused by local independent loss of neurons.

On a cellular level this approach is supported by neuropathological evidence, that neurodegenerative diseases might spread along neural connections (105, 106). Misfolded tau and amyloid beta can be transported trans-synaptically in a "prion-like" manner (107). Oligomeric amyloid beta can likely act trans-synaptically (108) and hyper-phosphorylated tau can be directly transferred from pre- to postsynaptic cells (109).

Five types of dementia (behavioral variant frontotemporal dementia, semantic dementia, progressive nonfluent aphasia, corticobasal syndrome and AD), cause a well-defined disease-related atrophy pattern in five different networks of the brain (82). These networks show a powerful covariance in GM volume in healthy subjects. Moreover, these networks approximately overlap with intrinsic functional connectivity networks in healthy subjects, which are associated with cognitive and behavioural tasks. In neurodegenerative diseases, including AD, these networks are reduced in GM volume, thus causing a reduction in cognitive performance (82). This indicates that functional connectivity is correlated with structural connectivity in healthy subjects (66).

AD causes a reduction in GM structural covariance between distinct areas of the brain. These brain regions include inter-hemispheric (110) and long-distance correlations (72). Compared with healthy subjects, AD patients show a less integrated and more segregated whole-brain-network (72). Based on He et al. (103, 110), who used cortical thickness correlations for the study of network mediated changes, Evans (66) proposed that a reduction of long distant anatomical fibres and a subsequent reorganization in predominantly local arrangements define AD. This assumption models AD as a disconnection syndrome (19).

In AD, further reductions of structural associations were found between the precuneus and the entorhinal cortex (111). Due to changes of structural covariance

between the entorhinal cortex and association cortices, the detachment of the hippocampal formation might be promoted. This might cause progressive memory deterioration in AD (111). In contrast to NDC, AD subjects also showed decreased network integrity in the hippocampal SCN (112), which is composed of the hippocampus, temporal fusiform cortex, occipital and temporal lobe.

Moreover, in AD a decreased integrity of the precuneal SCN (112), including the posterior cingulate cortex, lateral occipital cortex, precuneal cortex and lingual gyrus was detected. Here, a positive association of general cognitive performance and the integrity of this SCN has been reported. Changes in structural covariance were further observed in the default mode system (DMN) in patients with early AD and abnormal Aβ42 levels (111). Finally, the medial and dorsolateral prefrontal cortices and the entorhinal cortex showed a less extended structural association when compared to NDC (111). The medial prefrontal cortex has considerable connections with the hippocampus and might play a role in learning and memory consolidation (113).

Although the underlying mechanisms of structural covariance are not yet understood, the investigation of SCNs might help to understand the pathology of AD. The milestone work of Seeley et al. (82) showed that AD causes a reduction of GM in a well-defined structural covariance network of healthy subjects, that overlaps closely with cognition associated intrinsic functional connectivity networks. In AD, an integrity reduction of the precuneal SCN has been shown to be correlated with cognitive capacities (112). Furthermore, in AD a reduction of integrity is observed in the DMN (111), the hippocampal and the precuneal SCNs (112). Hence, AD causes not only a decreased connectivity in functional networks, but further results in a reduction of integrity in specific SCNs.

2.2 Objectives of the work

Due to growing life expectancy, the prevalence of AD will increase significantly in the future. This provides great challenges not only for caring relatives, but also for the society, as health cost expenses for AD will explode. It is a major priority to develop methods for the early identification of AD, because therapies might be more effective when applied in early stages. Grey matter volume reduction is a major hallmark of the

disease and thus a potential valid morphological biomarker in AD staging. Structural magnetic resonance imaging is a non-invasive and safe method to analyse these reductions. Instead of focusing on single structures, the contemplation of SCNs can provide a more coherent and deeper insight of pathological processes in AD. This method provides complementary information about brain connectivity in general and in neurodegenerative disorders such as AD (19, 77). In comparison to other covariance approaches, the analysis of SCNs using ICA does not need prior defined regions and thus prevents selection bias.

The objective of this work is the identification of SCNs in NDC and AD patients, examining the group differences in grey matter network integrity and to get a first impression of the sensitivity of the method.

3 Methods and Material

3.1 Data samples

3.1.1 Participants

In total, 156 participants were included in our study. Seventy-eight structural T1-weighted MRI scans from patients with probable AD (according to the NINCDS-ADRDA guidelines) were provided by the prospective registry on dementia in Austria (PRODEM) (compare Seiler et al. (114)).
PRODEM Inclusion criteria are:
• Diagnosis of dementia according to DSM-IV guidelines.
• No institutionalisation, no dependence of nurture.
• Participation of a caregiver, who is willing to give information about the patient.
• Patients do not reveal other neurological diseases or symptoms.
Subjects included 32 men and 46 women, aged between 51 and 87 years with an average of 70.65 years (SD=7.8). All participants went through a broad medical screening. Cognitive functions were evaluated by an extensive neuropsychological assessment including the Mini Mental Status Examination, the CERAD-Plus test battery and a screening for depressive symptoms.
Another 78 MRI scans derived from the Austrian Stroke Prevention Family Study (ASPFS) were used to provide healthy, sex- and aged (+/-3 years) matched controls. Subjects were randomly selected and included 32 men and 46 women, aged between 53 and 87 years, with a mean of 70.05 years (SD=7.0).
Both groups were scanned over the same period, under equivalent conditions, with the same scan protocol and with the same MRI scanner.

3.1.2 Data acquisition

Image acquisition was performed on a Siemens Magnetom TrioTim 3 T.
For the realization of the anatomical three-dimensional T1-weighted images, following parameters were pre-determined: TR=1900 ms, TE=2.19 ms, flip angle= 9° and isotropic voxel size of 1mm. Participants were directed to stay motionless.

3.2 Identification of grey matter networks

First, all T1-weighted scans were visually checked for gross artefacts to ensure data quality. Data analysis was conducted with FSL (FSL 5.0, Oxford, England, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM) (115). Second, preprocessing involved non-brain tissue extraction from the T1-weighted scans using Brain Extraction Tool (BET, part of FSL) (116). Data were checked for extraction quality, and whenever necessary, additional adjustments were conducted manually. Next, tissue type segmentation into grey and white matter as well as cerebrospinal fluid was performed using voxel-based morphometric analysis by Ashburner and Friston (117). Then, data were again quality checked. The resulting individual grey matter volume images were aligned to the grey matter MNI 152 standard space image and non-linearly registered (118). A study specific grey matter template was created using all subjects' grey matter image, to which the native grey matter images were again non-linearly re-registered. A further modulation step, to correct for enlargements and contractions of single voxels, was conducted. These modulated images were then spatially smoothed with a Gaussian kernel of sigma=3mm. A more detailed explanation of brain extraction, segmentation, modulation and smoothing are provided below.

1) Brain Extraction and Segmentation

The FSL semi-automated brain extraction tool (BET) (119) was employed to erase scalp and other non-brain tissues. Remaining non-brain tissue was removed manually. Segmentation includes the voxel-based morphometric (117) segmentation of the brain tissue in white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) *(120)*.

2) Non-linear registration

Due to different forms of the skull and variations in the brain development, the brain anatomy differs slightly from a person to another person. In order to conduct a voxel-wise analysis, every GM segment must fulfil the same spatial layout. The objective is to compensate for differences in the head position in the scanner and for further macro-anatomical differences.
To overcome this issue non-linear registration (118) of every grey matter image on the template image is applied (121). FSL uses the MNI152 template (Montreal

Neurological Institute, Montreal, QC, Canada) It is composed of 152 MRI images from healthy young adults which are aligned on a common coordinate system ("MNI space") to provide standardized research conditions.

3) Modulation

The step of 'spatial normalisation' induces a change in volume. By the adjustment of the MRI images to the MNI 152 template, some regions shrink, while others expand. The objective of the modulation step is to compensate for these modifications (117, 122). Voxel intensities of the GM must be multiplied by implementing the Jacobian determinant. Hence, changes in volume will be amended (117). Through the step of modulation the initial regional volumes will be conserved, even if they are now converted to the new stereotactical space (123).

5) Smoothing (120)

The grey matter segments are smoothed with a Gaussian kernel of 3mm. In consequence each voxel receives the average grey scale value of the voxels around itself. Smoothing leads in fact to a reduction in image resolution, but transforms the data in a normal distribution. Smoothing also corrects for impreciseness of spatial normalization (123). The modulated images were concatenated into a four-dimensional data set.

After ensuring sufficient pre-processing quality, ICA was performed. The "multivariate exploratory linear optimized decomposition into independent components" (MELODIC) (124) is a statistical analysing method to fragment the signals with a maximum of statistical independence into spatial component maps (125).
ICA is administered on the concatenated four-dimensional GM images. Based on inter-individual covariance of GM volumes, spatial components are defined automatically. For this, no prior selection of regions is needed. Each component represents a SCN (102, 126).

We conducted a single session ICA with n=156 subjects by using the auto-estimation function yielding 19 components of which three were identified as artefact.
For the investigation of cross-sectional group differences, a nonparametric permutation test (127) was performed. Based on general linear model approaches FSL calculates the subject-specific individual SCN integrity. This process results in a

beta value for all subjects and networks and ranges from negative to positive values. The beta value can be seen as a SCN integrity score (112). The higher the value, the higher is the integrity of the network.

To identify the anatomical localization of the clusters, the Harvard-Oxford Cortical- and Subcortical Structural Atlas, and the Cerebellar Atlas in MNI152 space, all integrated in FSL, were applied. We considered clusters exclusively above k=100 voxels.

3.3 Statistical Analysis

IBM SPSS Statistics version 23 by IBM Corp., Somers, NY, USA, was used for analyses. We performed the Shapiro-Wilk test to check our data for normal distribution. Six out of 16 networks presented a normal distribution (SCNs 5,6,8,16,18,19). A logarithmic transformation was applied to the remaining data. This transformation resulted in a normal distribution for additional six networks (SCNs 2,3,4,7,9,12).
The remaining four networks (SCNs 1,10,15,17) did not present normal distribution. Group differences between AD and NDC were calculated using two sample independent t-test, if data was normally distributed. For the non-normal distributed data, the Mann-Whitney-U test was applied. The Bonferroni correction was applied to correct for multiple comparisons. The corrected statistical threshold was set at $p = 0.0015$ (2x16 = 32 comparisons, two-tailed and 16 networks).

4 Results

4.1 Demographic characteristics

Our analysis included 78 patients with AD and 78 age and sex-matched non-demented controls. Demographic data is presented in table 1.

	Alzheimer's patients			Non-demented controls		
Characteristics	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Males/females participants (n)	32/46			32/46		
Age (years)	70.65	7.80	51-87	70.05	7.04	53-87
Disease duration (months)	24.61	23.06	3-156	n.a.		
MMSE (max score: 30)	21.91	4.08	10-29	28.09	1.51	23-30
CDR (max score: 3)	0.80	0.37	0.5-2.0	n.a.		
GDS (max score: 15)	2.97	3.0	0-12	2.51	2.38	0-11
Positive ApOEε-4 status (n)	18			7		
Education (n)						
-Primary education	24			16		
-Lower secondary education	30			39		
-Upper secondary education	13			15		
-University	11			8		

Table 1: Demographics of Alzheimer's patients (AD) and non-demented controls.
Abbreviations: SD = standard deviation; n.a. = not applicable, MMSE = Mini Mental State Examination; CDR = Clinical Dementia Rating Scale; GDS = Geriatric Depression Scale;

4.2 Identification of structural covariance networks and group differences

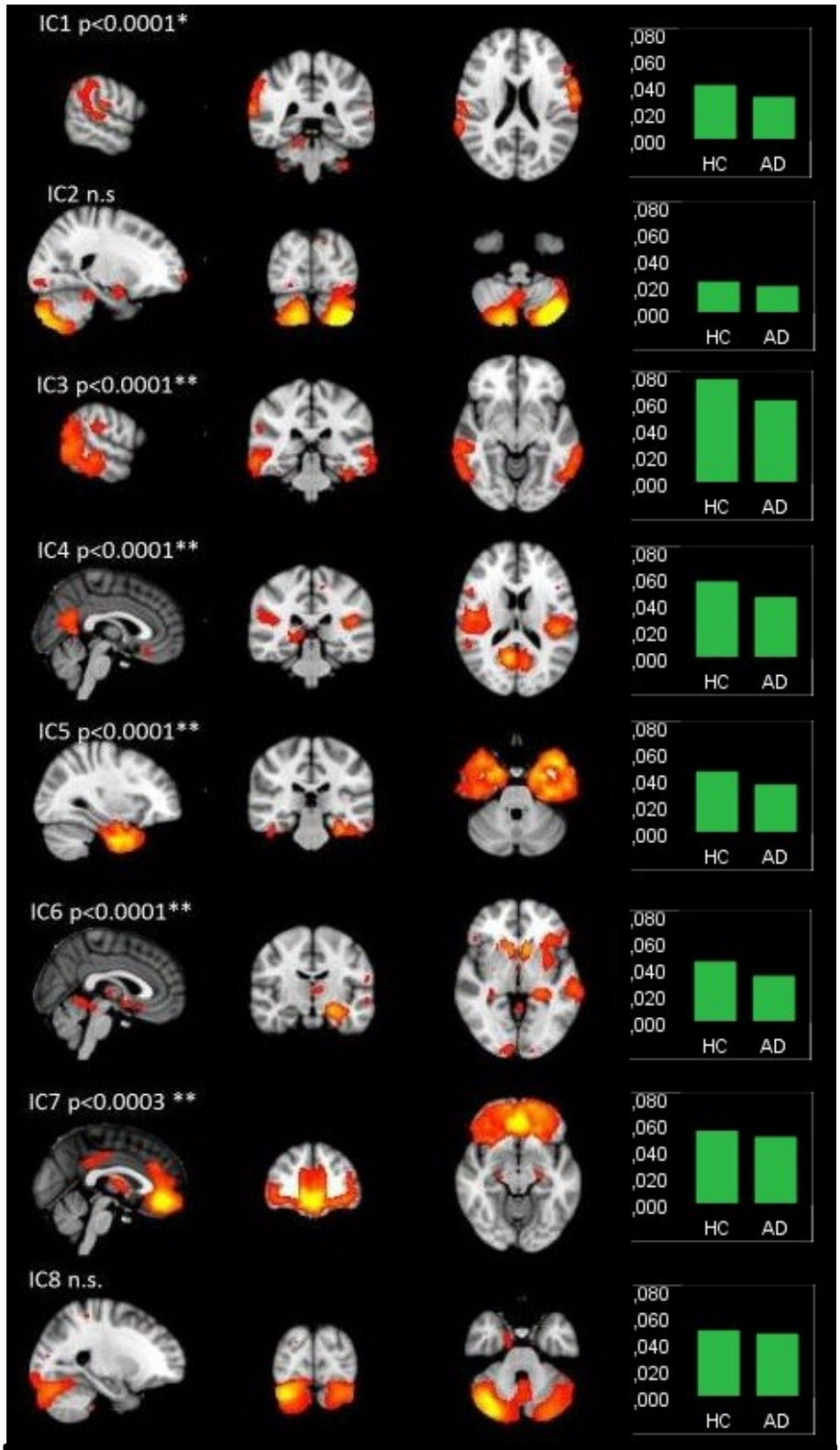
Based on grey matter structural covariance analysis, 19 independent components (ICs) were identified. Each grey matter IC represents an SCN and is composed of distinct anatomical structures. Ten networks are predominantly composed of cortical structures. Two networks include subcortical structures, four networks consist of cerebellar structures and three networks were identified as artefacts (Fig. 1 and 2; Table 1; please compare Table A-1 in the annex for more details on the composition of the SCNs and the hemispherical localization of their components).

Based on an α-level of 0.0015, significant cross-sectional group differences in network integrity were found in ten SCNs, which are composed essentially of the pre-

and postcentral gyrus (SCN1), lateral occipital cortex (SCN3), precuneus (SCN4, SCN10, SCN12), inferior and middle temporal gyrus (SCN5), left amygdala (SCN6), frontal cortex (SCN7), fronto-parietal (SCN 17), and the cerebellum (SCN9).

Independent component	Main anatomical structures
Cortical networks	
SCN1, SCN18	Pre-and postcentral gyrus
SCN3	Lateral occipital cortex and middle temporal gyrus
SCN4	Precuneus, lingual gyrus, central and parietal opercular cortices
SCN5	Inferior and middle temporal gyrus, left parahippocampal gyrus and right temporal fusiform cortex,
SCN7	Paracingulate gyrus, anterior and posterior cingulate cortices, frontal medial cortex and frontal pole
SCN10, SCN12	Inferior and Superior part of the precuneus cortex
SCN17	Frontal pole and middle frontal, angular and lateral occipital gyri
SCN19	Cuneus, supracalcarine cortex and frontal pole
Cortical-subcortical networks	
SCN6	Left amygdala, caudate, insular cortex, occipital cortex
SCN16	Putamen, insular cortex and thalamus
Cerebellar networks	
SCN2, SCN8, SCN9, SCN15	Cerebellar structures
Artefacts	
SCN11, SCN13, SCN14	

Table 2: Anatomical structures of the identified structural covariance networks



* Significant difference based on Mann-Whitney U-Test
** Significant difference based on two independent sample t-test

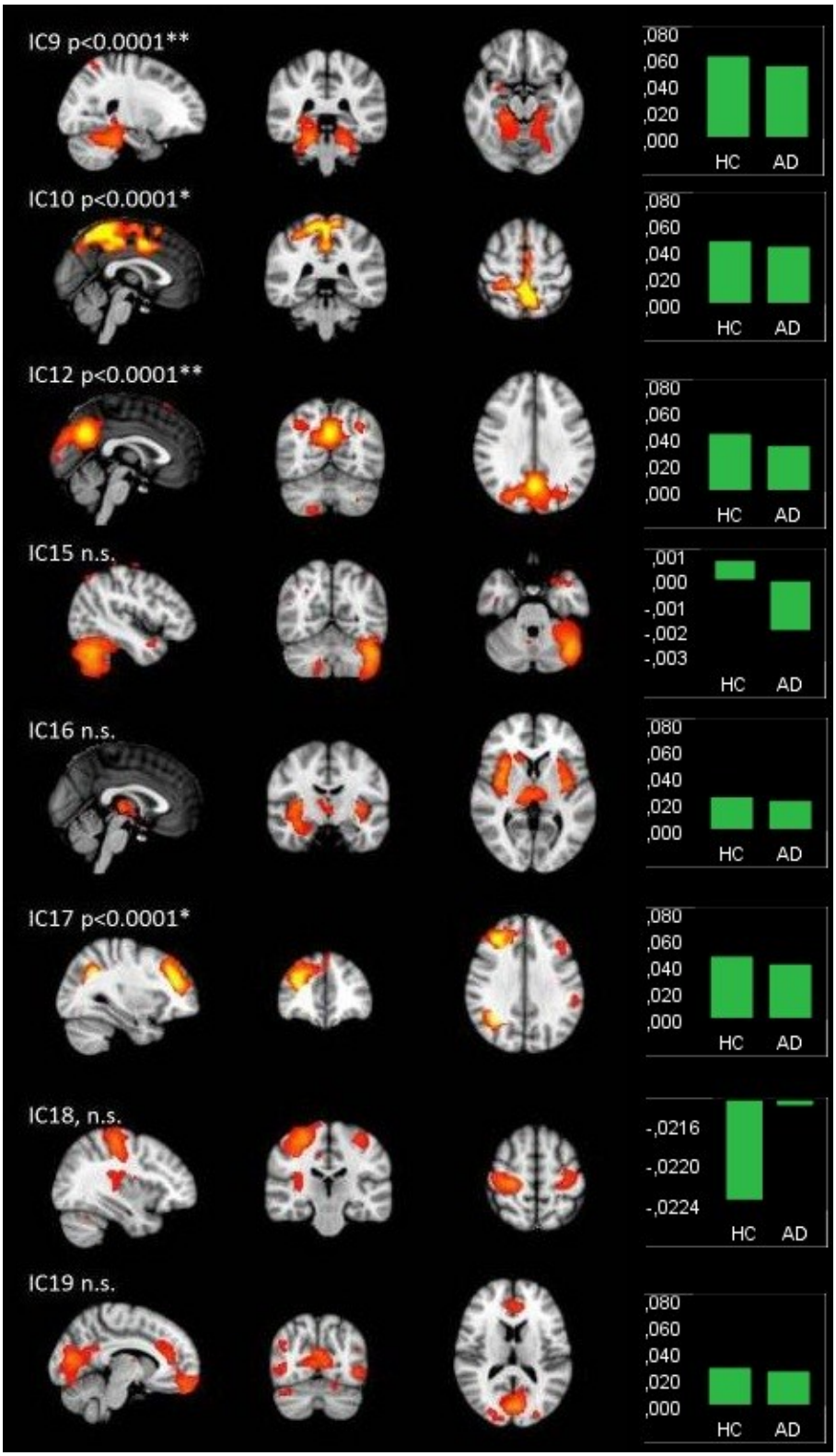


Figure 3: 16 structural covariance networks identified in 78 non-demented controls and 78 AD patients. The most informative sagittal, coronal and transversal slices are shown. Based on α-level of 0.0015, significant group differences were found in IC 1,3,4,5,6,7,9,10,12,17. The corresponding bar charts compare the mean beta-value of the two groups (AD patients & non-demented controls (NDC)). Non-demented controls show higher mean beta-values when compared to patients in all networks. This suggests disrupted network integrity in patients with Alzheimer's disease. * Significance assessed with Mann-Whitney U-Test, ** Significance assessed with two-tailed independent sample t-test, n.s. = non-significant. Please mind the divergent scale labelling in IC15 and IC18, as values are negative. For a detailed anatomical description of the networks please see table A-1 in the annex.

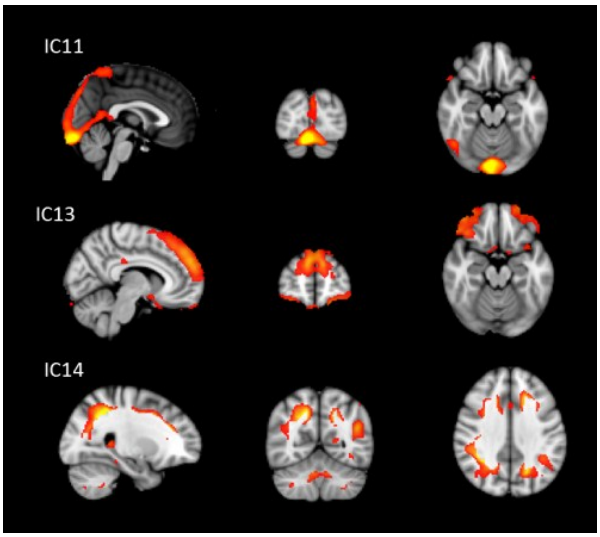


Figure 4: Three components of the ICA were identified as artefacts.

5 Discussion

Grey matter atrophy is a major hallmark of AD. Recent research highlighted that the brain functions in networks rather than on independent single areas. In this context, AD has been identified as a disconnection syndrome. Prior research has shown that areas that mature together also die together, i.e. in specific GM networks. The aim of this study therefore was to evaluate differences between AD patients and controls in GM network integrity. Using independent component analysis on T1-weighted preprocessed GM images revealed 16 distinct GM covariance networks, of which 10 showed less GM covariance integrity in AD patients than in controls. These involved a visual network (SCN3), a parieto-temporal auditory network (SCN4), sensory motor networks (SCN1, 10), a cerebellar network (SCN9), and networks associated with higher cognitive functions (SCN5, 6, 7, 12, 17). These results give a first reference on the sensitivity of the method to examine integrity differences between patients and controls. Moreover, our results highlight a disease specific network vulnerability, sparing motor (SCN18), cerebellar (SCN2,8,15) and a basic visual networks (SCN19). Surprisingly, one network of interest, the thalamic-insulate network (SCN16), did not show reduced integrity in AD patients when compared to controls, although based on prior literature one could have expected otherwise (128).

The most pronounced reduction of network integrity was found in a subcortical network, comprising the left amygdala, the caudate nuclei and the occipital cortex (SCN6). Interestingly, already ten years prior to clinical onset, the left amygdala has been reported to show a reduced volume (55), which highlights the sensitivity of this network as a possible classification network for disease staging.
Additionally to the amygdala network, other cognition-associated networks, such as the precuneal, frontal, fronto-parietal, and the inferior and middle frontal networks, showed decreased integrity in AD patients. The precuneus has been reported to constitute the major hub (129)(130) of the default mode network (DMN), a functional network that has most commonly been associated with wakeful rest or deactivation during tasks (task negative network) (129). Some studies reported on progressive functional disconnection within the DMN during the course of AD (131–133). Also, an association between the DMN and episodic memory has repeatedly been reported (134, 135). Most interestingly, the DMN has also been identified as a network

densely affected by amyloid, because high amyloid-β loads are most pronounced in the posterior cingulate cortex and precuneus. Taken together, functional and structural deterioration together with presence of amyloid-β pathology and the correlation with memory emphasize the specificity of this network for AD.
In contrast, the frontal (136) as well as the fronto-parietal networks (137) have been associated with executive functions, and the inferior and middle temporal cortices with memory and language (138).
Interestingly, many of the GM networks identified here have already been detected in functional resting state data and were reported in the groundbreaking resting state imaging work of Smith et al. (139). These investigators compared in a meta-analysis the functional networks of task-related fMRI studies of nearly 30.000 subjects (dataset 1) with a resting state fMRI sequence in 36 subjects (dataset 2). Using decomposition, they reduced the functional networks of both datasets into ten major explicit activation patterns. Activation networks during task execution (dataset 1) were found to mirror resting state fMRI (dataset 2) while the brain is not adhered to a specific task. Accordingly the brain does not adhere to a specific task during resting state, equivalent networks can be identified. Based on visual inspection between the Smith-10 and our networks, equivalence was found for two visual networks (SCN3, 19), the default mode network (SCN12), the cerebellar (SCN2,8,15), the sensorimotor (SCN10,18), the auditory (SCN4), the executive control (SCN7) and the fronto-parietal (SCN17) networks. These functional networks are found consistently across different individuals. The majority of these networks demonstrate a mirror-symmetric organization, which suggests a transcallosal interhemispherical connection of homologue areas.
The SCNs, identified in the current study, may partly be a morphologically correspond to functional networks. Prior studies reveal that several specific functional networks disconnect both during healthy aging (140) and during the course of AD (141). The reduction of structural covariance integrity seen in our AD patient sample is a further indication that the pathology of the disease targets the brain at the network level, rather than at specific isolated areas. Using an equivalent approach, similar results for grey matter network deterioration during healthy aging have been reported by Hafkemeijer et al. (112), although network integrity reduction due to aging was less pronounced.

Although the major aim of this study was to determine the difference in grey matter integrity between AD patients and controls, the approach may also prove to be beneficial in the diagnosis of different types of dementia, because GM pathology differs between different types. Further studies are needed to clarify the value of SCNs as classification markers.

5.1 Advantages of structural covariance network analysis

Compared to other neuroimaging methods, structural covariance analysis provides complementary information on inter-regional connectivity in general and on neurodegenerative disorders including AD in particular (19, 77).
The analysis of SCNs is based on magnetic resonance imaging. MRI scanners are readily available in most clinical centres focussing on neurodegenerative disorders and represent a non-invasive, safe and reliable method to detect atrophy. High resolution volumetric images can be obtained within a few minutes. Based on a selection of different MRI sequences, one single MRI session can deliver both clinical and research data, thus entailing cost optimisation.

5.2 Limitations of the study

The fundamental mechanisms of structural covariance and its interrelationship with functional connectivity are complicated and not yet explained. The growth of anatomical structures and the inter-regional covariance is influenced by genetic, developmental and environmental factors (77). The process of learning and the development of cognitive and motor skills are based on activity-dependent plasticity. We must understand the microstructural mechanisms, which underlie morphologic changes in order to clinically interpret SCNs in a meaningful manner.

In contrast to diffusion MRI and fMRI networks, the method of SCNs is based on inter-regional correlations that are calculated on the basis of a group of individual images (72). Therefore it cannot be applied in single images. For clinical application, a method to extract valid information about one single object needs to be developed.

A further limitation is based on the low number of subjects. We conducted our study with 78 AD subjects and 78 controls. For obtaining more robust results, a bigger sample size is required. Another limitation of the study is the lack of longitudinal data. Further studies are needed to investigate individual grey matter network integrity decline and associate these with cognitive functions.

5.3 Future directions

In order to increase the clinical value of structural covariance analysis in AD, it is of great importance to understand its underlying mechanisms. Due to homotopic cortical and subcortical SCNs in the mouse brain, this species is appropriate to further investigate the neuroanatomical and biological basis for SCNs and their alterations in disease (78). Animal studies enable monitored investigations highly controlled for genetic background that might explain underlying mechanisms. Therefore, animal models will contribute to understand the influence of genotype and developmental stage on structural covariance and how microstructural changes influence network properties.

Having identified ten SCNs that show significantly reduced integrities in AD, further investigations are necessary to evaluate the scientific value of our findings.
There are a considerable number of studies that have tried to relate grey matter density or cortical thickness of specific brain areas to neuropsychological achievement. Next to regional structures – for example, the size of the amygdala alters with achievement in memory functions (147) – networks can also be related to specific functions. Age related changes in the subcortical and fronto-parietal networks correlate with individual neuropsychological achievement, including attention, cognition and memory (98, 101). In AD the integrity of the precuneal SCN is correlated with MMSE results (112). Accordingly, the analysis of SCNs provides the possibility to relate structural covariance data to inter-individual differences.

Cognitive decline is the most common symptom in AD. For the assessment of the cognitive status, different neuropsychological tests are available. Among them, the Mini-Mental State Examination (MMSE) was published in 1975 as a test to assess cognitive impairment in adults (148). It has found international acceptance and is

used for the screening of cognitive impairment. Additionally it can be used to monitor cognitive changes in persons with dementia over a certain time. The test consists of 30 questions and examines orientation, short-term memory, calculation, attention, praxis and language. The cognitive status of Alzheimer's patients deteriorates about 2.8 MMSE points every year. A faster decline is observed in severe stages, while a slower decline is present in milder stages of AD (149). As some of our identified SCNs are related to cognitive functions(112), it might be possible to correlate certain SCNs with cognitive decline (MMSE) and use it as a marker for cognitive deterioration. Maybe a combination of different SCNs raises the validity. Because the parameters sex, age and education have a substantial influence on cognitive decline, studies must also be adjusted for these factors.

Longitudinal studies are required to investigate the changes of network integrity in the course of AD. As it is known that carriers of the APOE ε4 allele show a reduced/increased covariation in GM volume in some regions (90) decades before the potential onset of AD it might be interesting to investigate carriers of this allele separately.

The possible value of network integrity regarding early diagnosis of AD or classification of various dementia types is worthy of further studies. Is it possible to distinguish between different stages of AD on the basis of the network integrity of an SCN? Could it be possible to establish a network integrity score that enables a classification of patients in NDC, MCI-S, MCI-C and AD? If a single SCN has only little informative value, there might be a combination of certain SCNs to increase this significance. Additionally, studies could investigate if SCNs can be applied for the prediction of the different stages of AD. For the prediction of the progression of amnestic MCI into AD, MTL atrophy could be added to this model, as this marker is the most consistent currently known biomarker (57). Longitudinal studies will provide answers to these questions.

Nevertheless, the value of a marker that predicts cognitive decline must be discussed critically in terms of specificity and sensitivity. Obviously, both should be high prior to broad scaled clinical use; alternatively a set of markers may achieve this goal. Furthermore, ethical issues are important: On the positive side, an individual, who gets diagnosed with a positive SCN marker for cognitive decline, could initiate appropriate precautions in order to organize future health care and family affairs.

Decisions could be made in a yet normal cognitive status. In later disease stages, patients might be unable to decide about their own health care. On the negative side, individuals who are diagnosed with a rapid cognitive decline might become highly anxious as to their future. Psychological distress and even suicide could be triggered. Currently, neither clinical suggestion nor guidance would be possible. Finally no therapy options are available as of today (51).

The analysis of SCNs is a promising new approach to better understand interregional connectivity in AD. We found decreased integrity in 10 identified SCNs. This finding substantiate that AD targets specific SCNs, rather than local anatomical structures. However, before it can be used as a clinical tool, more research is required to understand its basic principles. Accordingly, further studies are required to understand the underlying mechanisms of structural covariance and to better judge the value of SCNs regarding early diagnosis and prediction of clinical stages or cognitive status.

6 Annex

		MNI coordinates			Voxel count	Inten-sity	Side
ICs	**Anatomical structures in the Cluster**	**X**	**Y**	**Z**	**n**	**Max**	**L/R**
IC1	Pre- and Postcentral Gyrus	-62	-4	12	5321	7.34	L
	Right Crus I (Cerebellum)	44	-60	-42	3780	11.7	R
	post. Supramarginal Gyrus	68	-38	14	2318	6.09	R
	Left VIIb (Cerebellum)	-36	-48	-48	1952	9.49	L
	Frontal Medial Cortex, Paracingulate Gyrus	-12	44	-8	339	4.91	L
	Frontal Pole	20	56	-2	310	4.99	R
	Frontal Medial Cortex	-2	42	-30	194	3.69	L
	Frontal Pole	-16	52	-26	102	5.36	L
IC 2	Left Crus II (Cerebellum)	-36	-72	-52	4443	21.8	L
	Right Crus II (Cerebellum)	20	-84	-44	3195	9.74	R
	Inferior Frontal Gyrus	60	22	22	143	3.41	R
IC3	inf. Lateral Occipital Cortex,	56	-64	-2	6870	6.31	R
	Middle Temporal Gyrus temporooccipital part	-62	-54	0	5144	6.14	L
	ant. Supramarginal Gyrus	-62	-24	28	429	3.99	L
IC4	Precuneous Cortex. Lingual Gyrus	14	-58	6	4988	10.4	R
	Central Opercular Cortex. Heschl's Gyrus (includes H1 and H2)	54	-16	10	3830	6.5	R
	Parietal Operculum Cortex	-46	-28	16	1535	6.81	L
	Frontal Orbital Cortex	24	18	-10	718	5.56	R
	ant. Parahippocampal Gyrus, right Hippocampus	34	-10	-24	653	4.31	R
	Frontal Orbital Cortex	-28	24	-10	616	4.72	L
	inf. Lateral Occipital Cortex	-32	-90	-12	427	3.92	L
	Subcallosal Cortex,	0	26	-16	176	3.64	R
	Inferior Temporal Gyrus temporooccipital part	58	-46	-12	117	3.82	R
	Precentral Gyrus	6	-22	54	114	3.93	R

IC 5	Inferior and middle temporal gyrus, ant. Parahippocampal Gyrus, temporal pole	-22	0	-38	9946	10.6	L
	ant. Temporal Fusiform Cortex ant. Parahippocampal Gyrus	30	-6	-38	7383	9.52	R
	Frontal Medial Cortex	-2	38	-12	547	4.1	L
	Occipital Pole, Cuneal cortex	-12	-90	24	328	3.87	L
	Precentral Gyrus	4	-16	68	154	3.63	R

IC6	Left Amygdala	-22	-8	-12	12503	9.86	L
	Occipital Pole	18	-96	-8	825	4.91	R
	Occipital Pole	-16	-96	-4	421	3.46	L
	Vermis VIIa (Cerebellum)	0	-60	-32	131	3.4	R

IC7	Paracingulate Gyrus, anterior cingulate cortex, frontal medial cortex, frontal pole	0	44	-10	17101	13	R
	Left Crus I (cerebellum)	-26	-46	-40	602	5.09	L
	post. Cingulate Gyrus	0	-38	34	503	4.1	R
	Left Thalamus	-2	-6	8	360	4.48	L
	Right VIIIa (cerebellum)	26	-46	-42	245	4.79	R
	Right Crus II (Cerebellum)	38	-78	-56	143	3.49	R
	Left Hippocampus	-24	-20	-14	141	4.14	L
	Left VII a (Cerebellum)	-12	-74	-56	129	3.32	L

IC8	Right Crus I (Cerebellum)	34	-74	-28	10404	13.5	R
	Ant. Et post. cingulate Gyrus	-2	-16	42	346	3.73	L

IC9	Right V (Cerebellum)	20	-38	-24	2985	7.2	R
	Left V (Cerebellum)	-18	-42	-24	2978	5.89	L
	Postcentral Gyrus. prefrontal gyrus	-42	-24	56	1392	6.16	L
	sup. Lateral Occipital Cortex	20	-60	60	850	4.5	R
	Left Crus II (Cerebellum)	-6	-88	-42	415	3.63	L
	Precentral Gyrus	32	-16	68	368	4.5	R
	Right Amygdala	32	-2	-18	164	3.83	R

IC 10	Precuneous Cortex	-2	-62	52	13148	14.2	L
	Middle Frontal Gyrus	34	0	52	604	5.39	R
	Left Crus I (Cerebellum)	-40	-76	-34	415	4.98	L
	Frontal Orbital Cortex. Right Amygdala	26	6	-16	244	3.58	R
	Right VI (Cerebellum)	36	-34	-32	233	4.2	R
	Right Crus I (Cerebellum)	42	-78	-36	226	4	R

IC 12	Precuneous Cortex	2	-64	32	6790	9.64	R
	Right VIIIa (Cerebellum)	22	-62	-54	1167	4.5	R
	Precentral Gyrus	28	-8	52	422	5.16	R
	Occipital Fusiform Gyrus. Right crus I (Cerebellum)	22	-78	-22	145	3.41	R
	Superior Frontal Gyrus	-24	-2	74	118	3.77	L

IC 15	Left Crus I (Cerebellum)	-40	-66	-32	3419	8	L
	Left Caudate	-6	14	0	230	4.33	L
	ant. Temporal Fusiform Cortex	-30	-8	-44	223	3.94	L
	Cingulate Gyrus. posterior division	2	-30	48	220	3.85	R
	Postcentral Gyrus	36	-32	50	123	3.59	R

IC 16	Insular Cortex. right putamen	36	0	-2	4749	7.9	R
	Insular Cortex	-36	-8	2	1686	5.79	L
	Left Thalamus	-4	-16	2	1270	5.63	L
	sup. Lateral Occipital Cortex	-46	-62	38	180	3.2	L
	Right Caudate	18	12	16	145	4.56	R

IC 17	Frontal Pole. Middle Frontal Gyrus	32	38	28	8784	12.8	R
	Angular Gyrus. sup. Lateral Occipital Cortex	32	-56	34	1170	11.1	R
	Precentral Gyrus	54	8	14	507	7.02	R
	ant. Supramarginal Gyrus	-54	-36	36	352	5.79	L
	Precentral Gyrus	12	-20	72	292	5.84	R
	inf. Lateral Occipital Cortex	38	-78	-8	148	3.57	R
	ant. Temporal Fusiform Cortex	-38	2	-38	137	4.04	L
	Precuneous Cortex	-20	-54	24	123	4.54	L
	ant. Superior Temporal Gyrus	68	-2	2	119	5.12	R
	Precentral Gyrus	40	-18	70	115	3.44	R

IC18	Pre- and Postcentral Gyrus	28	-24	58	2402	7.1	R
	Pre- and postcentral Gyrus	-32	-26	58	636	4.04	L
	Parietal Operculum Cortex	32	-28	20	271	3.93	R
	Intracalcarine Cortex	-10	-86	6	211	4.18	L
	sup. Lateral Occipital Cortex	-34	-70	18	191	3.25	L
	Precentral Gyrus	-52	-6	36	153	3.32	L

IC 19	Supracalcarine Cortex	2	-78	18	2350	7.41	R
	Frontal Pole	6	60	-24	2015	5.28	R
	inf. Lateral Occipital Cortex	-46	-72	-4	406	4.11	L

Table A- 1: Anatomical description of the identified structural covariance networks. Each IC represents a structural covariance network. Structures were identified using the Harvard-Oxford Cortical and Subcortical structural atlas and the Cerebellar Atlas in MNI152 space, all integrated in FSL. MNI (Montreal Neurological Institute 152 standard space image) x-, y-, and z-coordinates, number of voxels, maximum intensity of a cluster (Max) and hemispheric localization of a cluster (left or right) are indicated. We exclusively described clusters above a size of100 voxels. Underlined ICs show a statistical significant group difference in structural covariance between patients and controls. Abbreviations: ant. =anterior division, post. = posterior division, inf. = inferior division, sup. =superior division

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