Atrophy subtypes and the ATN classification scheme in Alzheimer's disease

Nira Cedres (✉ nira.cedres@ki.se)

Karolinska Institutet <https://orcid.org/0000-0002-1850-6451>

Urban Ekman

Karolinska Institutet

Konstantinos Poulakis

Karolinska Institutet

Sara Shams

Karolinska Institutet

Lena Cavallin

Karolinska Institutet

Sebastian muehlboeck

Karolinska Institutet

Tobias Granberg

Karolinska Institutet

Lars-Olof Wahlund

Karolinska Institutet

Daniel Ferreira

Karolinska Institutet

Eric Westman

Karolinska Institutet

Research

Keywords: Alzheimer's disease, heterogeneity, subtypes, ATN, CSF biomarkers, factorial analysis

Posted Date: June 19th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-35996/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

BACKGROUND

We investigated the association between atrophy subtypes of Alzheimer's disease (AD), the ATN classification scheme, and key demographic and clinical factors, in two cohorts with different source characteristics (a highly selective research-oriented cohort, ADNI; and a naturalistic heterogeneous clinically-oriented cohort, Karolinska Imaging Dementia Study (KIDS)).

METHODS

A total of 382 AD patients were included. Factorial analysis of mixed data was used to investigate associations between AD subtype based on brain atrophy patterns, ATN profiles based on cerebrospinal fluid biomarkers, and age, sex, Mini Mental State Examination (MMSE), cerebrovascular disease (CVD) (burden of white matter signal abnormalities, WMSA), and *APOE* genotype.

RESULTS

Older patients with high WMSA burden, belonging to the typical AD subtype, and showing A + T + N + or A + T + N - profiles clustered together and were mainly from ADNI. Younger patients with low WMSA burden, limbic-predominant or minimal atrophy AD subtypes, and A + T-N- or A + T-N + profiles, clustered together and were mainly from KIDS. *APOE* ϵ 4 carriers more frequently showed the A + T-N- and A + T + N- profiles.

CONCLUSIONS

Our findings align with the recent framework for biological subtypes of AD: the combination of risk factors, protective factors, and brain pathologies determines belonging of AD patients to distinct subtypes.

1. Background

Disentangling the heterogeneity within Alzheimer's disease (AD) has become an important task in order to guide personalized interventions [1, 2]. Neuropathological and neuroimaging studies have consistently identified three biological subtypes of AD: typical, limbic-predominant, and hippocampal-sparing AD. Typical AD is characterised by a balanced count of neurofibrillary tangles (NFT) or atrophy in hippocampus and association cortex. Limbic-predominant AD has NFT or atrophy predominantly in the hippocampus. Hippocampal-sparing AD has NFT or atrophy predominantly in the association cortex. Several neuroimaging studies have also identified a fourth subtype with minimal signs of brain atrophy, i.e., the minimal atrophy AD subtype [3, 4]. However, very few studies have investigated the

pathophysiological background of these subtypes *in vivo* [5], which is needed to completely disentangle the biological heterogeneity within AD.

Another way to stratify AD patients and also inform on their pathophysiological background is the ATN classification scheme, which is based on dichotomous categories (normal/abnormal) of amyloid-beta (A), tau (T), and neurodegeneration (N) biomarkers. To our knowledge, only one study investigated AD subtypes in combination with ATN profiles, and that study was performed in mild cognitive impairment (MCI) patients [6].

A task that remains to be done is the incorporation of a category for cerebrovascular (CVD, V) to the ATN scheme [7]. White matter signal abnormalities (WMSA) on magnetic resonance imaging (MRI) are a well-established marker of CVD. WMSA are implicated in AD pathogenesis [8, 9] and are commonly found in cognitively unimpaired older individuals [10, 11]. Including the V category in the scheme is important to advance our understanding of associations between ATV pathologies and their contribution to neurodegeneration. Stratifying AD patients into biological subtypes extends the N category by including a topographical dimension. The topographical dimension likely corresponds to different combinations of ATV and demographic, clinical, and genetic factors. A recent conceptual framework proposed how all these factors interrelate with each other giving rise to the biological subtypes of AD [5]. However, this framework has not been tested empirically.

The aim of this study was to investigate the association between AD subtypes, ATN profiles, and key demographic and clinical factors. We evaluated AD subtypes in combination with ATN profiles in two cohorts: a homogeneous research-oriented cohort (the ADNI study: Alzheimer's Disease Neuroimaging Initiative), and a heterogeneous clinically oriented cohort (the KIDS study: Karolinska Imaging Dementia Study). Investigating AD subtypes and ATN profiles in cohorts with different characteristics is relevant because these subtypes are thought to result from risk factors, protective factors, and comorbid brain pathologies [5] that are differently represented in research- and clinically-oriented cohorts¹². We hypothesized that the distribution of AD subtypes and ATN profiles would differ depending on cohort and demographic and clinical characteristics. We hypothesized that older patients would include a higher proportion of women with higher WMSA burden, higher proportion of A + T + N + individuals, and lower global cognitive performance; all these related with a higher proportion of individuals classified with typical or limbic-predominant AD subtypes. Younger patients would include a higher proportion of men with lower WMSA burden.

2. Material And Methods

2.1. Participants

We combined two cohorts of AD patients: ADNI-1 (N = 102) [13], and KIDS (N = 280) [14].

The ADNI (adni.loni.usc.edu) was launched in 2003 as a public-private partnership led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. ADNI diagnostic procedures are explained elsewhere [15]. Briefly, patients were clinically diagnosed as AD dementia using the NINCDS-ADRDA criteria for probable AD [16], and they were required to have memory complaints; a Clinical Dementia Rating (CDR) score ≥ 0.5 ; significant impairment on activities of daily living; MMSE scores between 20 and 26; and performance in Logical Memory II of the Wechsler Memory Scale-Revised (WMS-R) ≤ 8 for 16 years of education, ≤ 4 for 8–15 years, and ≤ 2 for 0–7 years.

Patients from the KIDS cohort underwent investigation between January 2006 and December 2011. AD diagnosis was determined in multidisciplinary clinical rounds according to the International Statistical Classification of Diseases and Related Health Problems - Tenth Revision (ICD-10), based on all available clinical information (medical history; physical, neurologic, and cognitive examinations; laboratory tests; and brain imaging).

Exclusion criteria in both ADNI and KIDS were other clinical diagnoses (dementia with Lewy bodies, vascular dementia, alcohol-related dementia, mild cognitive impairment, etc.). Further exclusion criteria for the current study were lack of an MRI scan or cerebrospinal fluid (CSF) biomarkers; insufficient MRI scan quality [17]; or a history of traumatic brain injury.

Age and sex were included as demographic variables. Clinical severity / global cognition was assessed with the MMSE.

Written informed consent was obtained from all the patients or a legal guardian, in accordance with the Declaration of Helsinki. For ADNI, study protocols were approved by the institutional review boards at each participating centre. For KIDS, ethical approval was obtained from the Regional ethics board in Stockholm, Sweden.

2.2. Implementation of the ATN classification scheme

All participants were classified into ATN groups according to CSF biomarkers for amyloid- β ("A", CSF A β_{42}), tau NFT pathology ("T", CSF phosphorylated tau), and unspecific neurodegeneration ("N", CSF total tau). Each individual was rated as either positive (+; i.e., abnormal) or negative (-; i.e., normal) on each biomarker according to cohort-specific cut-offs: ≤ 192 pg/ml for A β_{42} , ≥ 23 pg/ml for phosphorylated tau, and ≥ 93 pg/ml for total tau for the ADNI cohort [18]; and ≤ 550 pg/ml for A β_{42} , ≥ 80 pg/ml for phosphorylated tau, and ≥ 400 pg/ml for total tau for the KIDS cohort [19].

2.3. Magnetic resonance imaging

A T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence was acquired on ADNI patients. Data was acquired on 1.5T scanners with a voxel size of $1.1 \times 1.1 \times 1.2$ mm³. For the KIDS patients, a T1-weighted MPRAGE sequence (RT ~ 1700 ms., ET ~ 3 ms., TI ~ 1000 ms., slice thickness ~

1.2 mm) and a fluid attenuated inversion recovery (FLAIR) sequence (RT ~ 8000 ms., ET ~ 100 ms., TI = 2100–2500 ms., slice thickness ~ 5.0 mm) were acquired in three MRI scanners at the Radiology Department of the Karolinska University Hospital, Stockholm, Sweden: (1) 1.5T Magnetom Symphony scanner, (2) 1.5T Magnetom Avanto scanner, and (3) 3T Magnetom Trio scanner [14].

WMSA were investigated as a marker of CVD. In the ADNI cohort, WMSA were assessed through automatically segmented white matter hypointensities from FreeSurfer 6.0.0 (<https://surfer.nmr.mgh.harvard.edu/>). FreeSurfer is increasingly used to automatically segment WMSA in the form of hypointensities in the T1-weighted MRI sequence [20–24]. In KIDS, the MRI data is clinical and there is variation across patients in scanning parameters. In such data, the Fazekas visual rating scale [25] is appropriate as a measure of WMSA because it is not influenced by variation in scanning parameters [26]. Hence, in the KIDS cohort, WMSA were assessed through the Fazekas scale on FLAIR images. Importantly, hypointense WMSA from FreeSurfer and the Fazekas scale are strongly associated with each other [27]. We followed a previous study that demonstrated that hypointense WMSA from FreeSurfer and Fazekas scores can be combined together by converting hypointense WMSA into low and high CVD burden [27]. Hence, we computed a unique WMSA variable by applying a cut-off of 0.00321 on hypointense WMSA from FreeSurfer after total intracranial volume (TIV) adjustment. This creates two categories of low and high CVD burden that are analogous to Fazekas scores of 0 or 1 defined as low WMSA and Fazekas scores of 2 or 3 defined as high WMSA [27].

Regional brain atrophy was assessed with visual rating scales as detailed elsewhere [4, 28]. Briefly, medial temporal atrophy (MTA) was assessed with the Scheltens' scale [29], posterior atrophy (PA) with the Koedam's scale [30], and atrophy in the frontal lobe with the global cortical atrophy scale – frontal subscale (GCA-F) [31]. Ratings were performed by an expert neuroradiologist who showed excellent intra- and inter-rater performance: weighted κ values for intra-rater reliability: MTA-left = 0.94, MTA-right = 0.89, PA = 0.88; GCA-F = 0.83; and for inter-rater reliability: MTA-left = 0.71, MTA-right = 0.70; PA = 0.88, GCA-F = 0.79. All ratings were performed blinded to patients' information.

2.4. AD subtypes based on patterns of brain atrophy

Deviation from normality in visual ratings was determined using previously published cut-offs [28]. The MTA scores ≥ 1.5 , ≥ 1.5 , ≥ 2 , ≥ 2.5 were considered abnormal for the respective age ranges 45–64, 65–74, 75–84, and 85–94 years. Since an age-correction does not improve PA and GCA-F diagnostic performance [28], a score ≥ 1 was considered abnormal irrespectively of the age range [28]. The three AD subtypes identified in the previous literature [32, 33] were defined based on the combination of MTA, PA, and GCA-F, as in previous studies [4, 6, 34, 35]. The minimal atrophy AD subtype [3, 34, 36] was identified when AD patients displayed normal scores in MTA, PA, and GCA-F. Visual examples of the four AD subtypes can be found in Fig. 1.

2.5. Statistical analysis

The main aim of this study was to investigate the association between AD subtypes, ATN profiles, and key demographic and clinical factors. Given the nature of our data, which included both continuous and categorical variables, we applied a multivariate method for data analysis called factorial analysis of mixed data (FAMD) [37]. The main strength of FAMD is that it accommodates both quantitative and qualitative data simultaneously. FAMD works as a principal component analysis for quantitative data and as a multiple correspondence analysis for qualitative data [37]. In our FAMD model, age and MMSE scores were included as continuous variables, and the cohort (ADNI vs. KIDS), ATN categories, AD subtypes, sex (men vs. women), and WMSA burden (high vs. low) were included as categorical variables. A complementary FAMD model was conducted by adding *APOE* genotype as a categorical variable (carriers of at least one $\epsilon 4$ allele vs. non-carriers). One-way ANOVA was used for continuous variables and the chi-square test was used for categorical data. Missing data on MMSE was estimated via the MissForest algorithm [38] for six KIDS patients. All statistical analyses were conducted using the R statistical software (R Foundation for Statistical Computing, Vienna, <http://www-R-project.org>). A p -value ≤ 0.05 was deemed statistically significant.

3. Results

3.1. Cohort characteristics

Cohort characteristics are shown in Table 1a (N = 382). ADNI patients were significantly older with higher scores in MMSE and a lower frequency of women as compared with KIDS patients. Further, ADNI patients showed a significantly higher WMSA burden as well as a higher frequency of abnormal CSF A β 42 and phosphorylated tau levels, while KIDS patients showed a significantly higher frequency of abnormal CSF total tau levels. Due to the reduced number of amyloid-beta negative (A-) patients (N = 79), A- groups were excluded from subsequent analyses. The amyloid-beta positive (A+) subsample is shown in Table 1b (N = 303). In the A+ subsample, ADNI patients were significantly older with higher scores in MMSE, a higher WMSA burden, and a lower frequency of women and abnormal CSF phosphorylated tau levels, as compared with KIDS patients.

Table 1
Cohort characteristics.

	(a) whole cohort			(b) A + subsample		
	ADNI	KIDS	p-value	ADNI	KIDS	p-value
n	102	280	–	94	209	–
Age	75.0	67.5	< 0.001	74.5	67.2	< 0.001
Sex (% female)	42	58	0.008	41	59	0.007
MMSE	23.5	22.0	0.005	23.5	21.8	0.002
WMSA burden (% high)	46	20	< 0.001	45	20	< 0.001
Aβ42 (% abnormal)	92.2	76.6	< 0.001	100	100	–
p-tau (%abnormal)	87.3	55.0	< 0.001	90.4	55.5	< 0.001
t-tau (% abnormal)	54.7	69.6	< 0.001	68.1	69.4	0.927
APOE ϵ4(% carriers)	70	66	0.607	76	66	0.153
MMSE: Mini-Mental State Examination; WMSA: white matter signal abnormalities; A β Amyloid β ; p-tau: Phosphorylated tau; t-tau: total tau.						

Visual inspection of the data shows that typical AD was the most frequent subtype in both ADNI and KIDS (Fig. 2). Limbic-predominant and minimal atrophy AD were more frequent in KIDS, and hippocampal-sparing AD was more frequent in ADNI (Fig. 2). Minimal atrophy AD patients were significantly younger than patients from the other subtypes and had a lower WMSA burden than typical AD patients (Table 2). Typical AD patients had worse MMSE scores than the other subtypes. Hippocampal-sparing AD patients showed a higher proportion of abnormal CSF phosphorylated tau levels as compared with limbic-predominant AD patients.

Table 2
Demographic and clinical characteristics by AD subtype.

	typical AD	limbic predominant	Hippocampal sparing	minimal atrophy	p-value
n	131	76	45	51	
ADNI	48	18	17	11	0.070
KIDS	83	58	28	40	
Age	71.6 ^d	69.8 ^d	68.5 ^d	64.4 ^{a,b,c}	< 0.001
Sex (% female)	47	55	64	59	0.146
MMSE	20.1 ^{b,c,d}	23.2 ^a	23.1 ^a	23.8 ^a	< 0.001
WMSA burden (% high)	37 ^d	23	18	14 ^a	0.015
p-tau (%abnormal)	64	55 ^c	82 ^b	75	0.019
t-tau (% abnormal)	64	63	84	77	0.041
APOE ε4(% carrier)	76	68	56	72	0.210
MMSE: Mini-Mental State Examination; WMSA: white matter signal abnormalities; p-tau: Phosphorylated tau; t-tau: total tau. ^a Significantly different from typical AD; ^b Significantly different from limbic predominant; ^c Significantly different from hippocampal sparing; ^d Significantly different from minimal atrophy.					

The frequency of the A + T + N + profile (68%) was significantly higher ($p < 0.001$) than the frequencies of A + T + N- (22%) and A + T-N- (10%) profiles in the ADNI cohort (Fig. 2). Interestingly, none of the ADNI patients had an A + T-N+ profile. In the KIDS cohort, the frequency of the A + T + N + profile (55%) was also significantly higher ($p < 0.001$) as compared with the other ATN profiles. Interestingly, we observed a substantial proportion of A + T-N+ (15%) patients in the KIDS cohort. The A + T-N- profile accounted for 30% of the KIDS patients, and the A + T + N- profile included less than 1% of KIDS patients.

3.2. Association between AD subtypes, ATN profiles, and key demographic and clinical factors

Visual inspection of the correspondence between AD subtypes and ATN profiles showed that in ADNI, the A + T + N + was the most frequent group across all AD subtypes (Fig. 2). The A + T-N- group was present in every subtype but in minimal atrophy AD in ADNI. In KIDS, the frequency of A + T + N + was the lowest in typical and limbic-predominant AD (Fig. 2). In contrast, A + T-N+ was equally distributed across all AD subtypes. The A + T + N- was only present in the typical AD subtype in KIDS.

These visual analyses were further supported by the FAMD models, also showing the correspondence between AD subtypes, ATN profiles, WMSA burden, age, sex, MMSE, cohort, and *APOE* genotype.

The main FAMD model (N = 382) showed 3 dimensions that explained 42% of the variance (Table 3). Dimension 1 explained 19% of the variance and was mainly driven by cohort and age (although WMSA burden, ATN, AD subtype, and sex also contributed statistically significantly to Dimension 1). Dimension 2 explained 12% of the variance and was driven by AD subtype (although MMSE, ATN, and cohort also contributed statistically significantly to Dimension 2). Dimension 3 explained 12% of the variance and was driven by AD subtype, MMSE, and ATN.

Table 3
Contribution of each variable to the dimensions of the FAMD.

	Dim 1(R ² = 19%)	Dim 2(R ² = 12%)	Dim 3(R ² = 12%)
Age	25.3	1.1	2.5
MMSE	1.0	25.4	30.1
Cohort	28.9	8.2	0.9
Subtypes	7.9	42.1	31.9
ATN	14.1	17	29.6
WMSA	18.2	3.1	3.2
Sex	4.7	3.1	1.8
The values represent the percentage of contribution of each variable to the total variation captured by each dimension. MMSE: Mini-Mental State Examination; ATN: ATN classification scheme; WMSA: white matter signal abnormalities.			

Although ATN and AD subtype contributed to the three Dimensions, it was different categories within ATN and AD subtype that differently contributed to the Dimensions. To elaborate on this, Dimensions 2 and 3 were plotted against Dimension 1 (Figs. 3 and 4). Figure 3 shows that older patients have higher WMSA burden and tend to be from the ADNI cohort, clustering together. This cluster also showed a high frequency of A + T + N + and A + T + N - profiles, and a high frequency of patients with the typical AD subtype (Fig. 4). This cluster also includes a substantial proportion of patients with the hippocampal-sparing AD subtype. However, the AD subtype factor is slightly oblique to Dimension 1, hence many hippocampal-sparing AD patients fall within a second cluster including younger patients with lower WMSA burden who tended to be from the KIDS cohort (Fig. 3). This second cluster showed a high frequency of A + T-N- and A + T-N + profiles (Fig. 4), and included most of the patients with limbic-predominant and minimal atrophy AD. However, as explained above, the AD subtype factor is slightly oblique to Dimension 1, so this second cluster included patients from typical and hippocampal-sparing AD subtypes as well.

Dimension 3 separates the AD subtypes and ATN profiles more clearly and shows the effect of MMSE. When Dimension 3 is plotted against Dimension 1, it can be observed that A + T + N + and A + T + N - have lower MMSE scores, independently of the cohort, WMSA burden, and age (Fig. 4). Limbic-predominant AD patients have higher MMSE scores, and typical and hippocampal-sparing AD patients have lower MMSE scores, while minimal atrophy AD does not completely align with MMSE scores (Fig. 4).

The complementary FAMD model adding *APOE* ϵ 4 status was conducted in the subsample with available *APOE* data (N = 178). This model showed very similar results to the main FAMD model. Dimension 1 explained 19% of the variance and was mainly driven by cohort and age. Dimension 2 explained 12% of the variance and was driven by AD subtype. Dimension 3 explained 11% of the variance and was driven by ATN and *APOE* ϵ 4 status. Within Dimension 3, *APOE* ϵ 4 carriers showed a higher frequency of A + T-N- and A + T + N- profiles, and a tendency to include patients with typical AD.

4. Discussion

We investigated the association between AD subtypes and ATN classification scheme in two cohorts with different source characteristics. As hypothesised, the distribution of AD subtypes and ATN profiles differed between the research oriented cohort (i.e., ADNI) and the clinically oriented cohort (i.e., KIDS). In addition, we empirically tested the recent conceptual framework of biological subtypes of AD [5]. We applied a multivariate method for data analysis to investigate the association between AD subtypes, ATN profiles, and key demographic and clinical factors, including WMSA burden, age, sex, global cognition, and *APOE* genotype. To our knowledge, this study is the first in investigating AD subtypes in combination with ATN profiles in patients with AD dementia.

The recent conceptual framework of biological subtypes of AD proposes two dimensions: severity and typicality [5]. The severity dimension corresponds to the “N” domain of the ATN scheme and includes typical and minimal AD as the two extremes of a continuum of neurodegeneration. Typical AD is in the severe end of the continuum, and minimal atrophy AD is in the other end. In our study, typical AD was the most frequent subtype in ADNI and KIDS. This result might seem unexpected since ADNI recruited mild to moderate AD patients, while typical AD would reflect full-blown AD at the highest degree of neurodegeneration. However, ADNI is a highly selective research cohort with strict inclusion criteria [12] that aimed to recruit the prototypical amnesic presentation of AD, which correlates with the typical AD subtype in neuropathological studies [32]. In addition, ADNI recruited patients with high education, which probably positively influenced patients’ cognitive reserve, possibly explaining why patients in ADNI have overt ATN and brain atrophy profiles, yet they are at mild to moderate clinical stages. On the other hand, the clinically oriented KIDS cohort is a naturalistic memory clinic sample that includes younger patients mainly at an early clinical stage with challenging differential diagnoses. This could explain the higher frequency of patients in the minimal atrophy AD subtype in KIDS.

The typicality dimension in the conceptual framework of biological subtypes of AD includes limbic-predominant AD on the one side, and hippocampal-sparing AD on the opposite side, both deviating from

typical AD in the middle [5]. We found that limbic-predominant AD was slightly more frequent in KIDS, and hippocampal-sparing AD was slightly more frequent in ADNI. Based on previous studies [5], we hypothesised that these differences could be explained by demographic and clinical factors. To further test for this hypothesis, we investigated the association between AD subtype, ATN profiles, age, sex, cognitive status, and *APOE* genotype (discussed below).

A + T + N- and A + T + N+ profiles were more frequent in ADNI than in KIDS. The current biological definition of AD [39] postulates that A + is the first pathological change, followed by T + and, eventually, N+. Further, A + and T + reflect AD pathology, while N + is unspecific, with pathologies other than A and T contributing to neurodegeneration (N) as well. Hence, our finding of A + T + N- and A + T + N+ being more frequent in ADNI than in KIDS could be related to the stricter selection criteria of ADNI, with a special interest on the amnesic form of AD. This interpretation is further supported by our finding of a high frequency of A + T-N + in KIDS. The N + category in the presence of a T- category suggests that the neurodegeneration in these patients is due to some pathology other than tau NFT, which suggests a mixed aetiology of clinical AD. As explained above, KIDS is a heterogeneous naturalistic memory clinic sample including young patients with challenging diagnoses, as reflected by the higher frequency of the A + T-N + profile. Hence, the frequency of ATN profiles is highly dependent upon cohort, but not so much upon AD subtype.

CVD could be one of the non-AD pathologies contributing to N+. A previous study demonstrated that CVD contributes differently to AD subtypes [35]. Our current study provides novel data on the association between CVD, AD subtype, and ATN classification scheme. We found a higher WMSA burden in ADNI. This result may be unexpected since vascular risk factors (a predictor of WMSA) [40, 41] are exclusion criteria in ADNI. However, previous studies showed that WMSA burden increases with older age [41, 42], and ADNI patients are older than KIDS patients in our study, which could explain our finding of higher WMSA in ADNI. This finding aligns with the recent conceptual framework of biological subtypes of AD [5], i.e., older patients had higher WMSA burden, they more frequently had an A + T + N + profile, and included a higher proportion of typical and limbic-predominant AD cases. Further, typical AD patients showed greater cognitive impairment as compared with limbic-predominant AD [5].

Our complementary FAMD model showed that *APOE* ϵ 4 carriers tended to cluster together with patients with A + T-N- and A + T + N- profiles who belonged to the typical AD subtype. The association between the *APOE* ϵ 4 genotype and amyloid-beta pathology (A+) is a well-established finding [43]. Further, previous studies showed that the frequency of *APOE* ϵ 4 is higher in typical AD than in hippocampal-sparing AD [5]. Sex only marginally contributed to Dimension 1 in the main FAMD model. Although sex is also listed as one of the contributors to the emergence of AD subtypes [5], our current data suggest that the contribution of sex is less prominent than that of ATN profiles and other demographic and clinical factors. All in all, our findings largely support the recent conceptual framework of biological subtypes of AD [5].

AD subtype and ATN classifications are two popular approaches to disentangle disease heterogeneity in AD. An important finding in our study is that the correspondence between AD subtypes and ATN profiles is not absolute, suggesting that both approaches may capture complementary information. The FAMD model showed that AD subtype was the main driver of one of the dimensions (Dimension 2), while ATN always emerged as a secondary driver after AD subtype, MMSE, cohort, age, or WMSA burden (Dimensions 1, 2, and 3). The association of ATN with MMSE, age, and WMSA burden, as well as the ATN distribution observed in the highly selective homogeneous ADNI cohort suggest that the ATN classification scheme may be useful to assess disease staging. The capacity of AD subtype to drive a dimension by itself, partially independently of ATN and demographic and clinical factors, suggests that AD subtype classification may be less influenced by disease staging. Whether AD subtypes reflect disease staging or truly distinct subtypes is an open discussion [2, 4, 32, 44] that can only be answered in future longitudinal studies. The distinct subtypes hypothesis postulates that there are different pathophysiological pathways underlying clinical syndrome in AD [2, 5]. Current data show that these pathways seem to rely on different forms of spread of pathology across the brain [5, 32], leading to different patterns of brain atrophy in structural MRI [33]. An advantage of the AD subtype classification is the inclusion of the topographical dimension to the N category of ATN [35]. Whether AD subtype is a stronger approach to disentangle disease heterogeneity as compared with the ATN classification scheme must be confirmed in future studies.

5. Limitations

The current study has some limitations. We did not include A- individuals in the main analysis. Including A- individuals might increase the heterogeneity and shown slightly different associations between AD subtype, ATN profiles, and demographic and clinical factors. Further, the methods to assess WMSA were different in ADNI and KIDS. In the ADNI cohort we used an automatic segmentation based on white matter hypointensities while in the KIDS cohort we used visual ratings based on white matter hyperintensities. Although using different methods for WMSA could induce some noise in our analysis, we recently showed that both methods are strongly associated with each other [27]. Further, by classifying the output from both methods into high and low WMSA burden, we used a rougher measure that is less influenced by differences between the two methods and has greater clinical applicability [42]. Finally, we lacked data for several factors listed in the recent conceptual framework for biological subtypes of AD [5]. Future studies should thus extend our current analysis by including measures of education or cognitive reserve, other markers of CVD, information about disease onset or disease duration, and data on specific cognitive domains. Investigating the contribution of other comorbid brain pathologies such as Lewy body pathology or TDP-43 is challenging at present by the lack of reliable biomarkers for these two pathologies.

6. Conclusions

We conclude that the distribution of AD subtypes and ATN profiles depends on the source of the patients and it aligns with different demographic and clinical factors, depending on whether the cohort is more selective and homogeneous or more naturalistic and heterogeneous. Our findings largely support the recent conceptual framework of biological subtypes of AD [5]. This framework postulates that the combination of risk factors, protective factors, and comorbid brain pathologies will determine belonging of AD patients to distinct biological subtypes of AD. Future studies should continue testing this framework with the goal of advancing our currently limited possibilities to realize precision medicine in clinical routine.

7. Declarations

Acknowledgment

ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and several generous contributions from the following: Alzheimer's Association, Alzheimer's Drug Discovery Foundation, BioClinica Inc., Biogen Idec Inc., Bristol-Myers Squibb Co., Eisai, Inc., Elan Pharmaceuticals, Inc., Eli Lilly and Co., F. Hoffmann-La Roche Ltd and its affiliated company Genentech Inc., GE Healthcare, Innogenetics NV, IXICO Ltd., Janssen Alzheimer Immunotherapy Research & Development LLC, Johnson & Johnson Pharmaceutical Research & Development LLC, Medpace Inc., Merck & Co. Inc. Meso Scale Diagnostics LLC, NeuroRx Research, Novartis Pharmaceuticals Corp., Pfizer Inc., Piramal Imaging, Servier, Synarc Inc. and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. Data collection and sharing for in ADNI were funded by the National Institutes of Health (NIH) (Grant U01 AG024904). ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This research was also supported by NIH Grants P30 AG010129 and K01 AG030514. These sponsors did not have any involvement on the study design; collection, analysis, and interpretation of data; writing of the report; and the decision to submit the article for publication.

Ethics approval and consent to participate

Written informed consent was obtained from all the patients or a legal guardian, in accordance with the Declaration of Helsinki. For ADNI, study protocols were approved by the institutional review boards at each participating centre. For KIDS, ethical approval was obtained from the Regional ethics board in Stockholm, Sweden.

Consent for publication

Not applicable

Availability of data and materials

The dataset generated and analyzed during the current study are available in the ADNI data repository, <http://adni.loni.usc.edu/data-samples/access-data/> and at the KIDS study: Karolinska Imaging Dementia Study.

Competing interests

None

Authors' contributions

NC, DF, and EW contributed to the conception and design of the study. NC, KP, SS, LC, TG, SM, and LOW contributed to the acquisition and analysis of data. NC, UE, DF and EW contributed to drafting a significant portion of the manuscript and preparing the figures. All the authors revised the manuscript and contributed on scientific content.

Funding

This study was supported by the Swedish Foundation for Strategic Research (SSF), the Strategic Research Programme in Neuroscience at Karolinska Institutet (StratNeuro), the Swedish Research Council (VR, 2016-02282), the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet; Center for Innovative Medicine (CIMED); Demensförbundet, the Swedish Alzheimer Foundation; the Swedish Brain Foundation; the Åke Wiberg Foundation; Demensfonden; Stiftelsen Olle Engkvist Byggmästare; Birgitta och Sten Westerberg.

Authors' information

^aDivision of Clinical Geriatrics, Department of Neurobiology, Care Sciences, and Society, Karolinska Institutet, 14152 Stockholm, Sweden

Abbreviations

A
amyloidosis
AD
Alzheimer's disease
ADNI
Alzheimer's Disease Neuroimaging Initiative
APOE
Apolipoprotein E
CDR
Clinical dementia rating

CSF
cerebrospinal fluid
CVD
Cerebrovascular disease
FAMD
Factorial analysis of mixed data
FLAIR
fluid attenuated inversion recovery
GCA-F
global cortical atrophy scale – frontal subscale
ICD-10
International Statistical Classification of Diseases and Related Health Problems - Tenth Revision
KIDS
Karolinska Imaging Dementia Study
MCI
Mild Cognitive Impairment
MMSE
Mini Mental State Examination
MPRAGE
magnetization-prepared rapid gradient-echo
MRI
magnetic resonance imaging
MTA
medial temporal atrophy
N
Neurodegeneration
NFT
Neurofibrillary tangles
PA
posterior atrophy
PET
Positron emission tomography
T
tauopathy
TIV
total intracranial volume
WMS-R
Wechsler Memory Scale-Revised
WMSA
white matter signal abnormalities

References

1. Veitch DP, Weiner MW, Aisen PS, Beckett LA, Cairns NJ, Green RC, et al. Understanding disease progression and improving Alzheimer's disease clinical trials: Recent highlights from the Alzheimer's Disease Neuroimaging Initiative. *Alzheimer's Dement*. Elsevier Inc.; 2019;15:106–52.
2. 10.3389/fneur.2019.00524/full
Ferreira D, Pereira JB, Volpe G, Westman E. Subtypes of Alzheimer's Disease Display Distinct Network Abnormalities Extending Beyond Their Pattern of Brain Atrophy. *Front Neurol* [Internet]. 2019;10:524. Available from: <https://www.frontiersin.org/article/10.3389/fneur.2019.00524/full>.
3. Byun MS, Kim SE, Park J, Yi D, Choe YM, Sohn BK, et al. Heterogeneity of regional brain atrophy patterns associated with distinct progression rates in Alzheimer's disease. *PLoS One*. 2015;10:1–16.
4. Ferreira D, Verhagen C, Hernández-Cabrera JA, Cavallin L, Guo CJ, Ekman U, et al. Distinct subtypes of Alzheimer's disease based on patterns of brain atrophy: Longitudinal trajectories and clinical applications. *Sci Rep* Nature Publishing Group. 2017;7:46263.
5. 10.1212/WNL.00000000000009058
Ferreira D, Nordberg A, Westman E. Biological subtypes of Alzheimer disease. *Neurology* [Internet]. 2020;94:436–48. Available from: <http://www.neurology.org/lookup/doi/10.1212/WNL.00000000000009058>.
6. 10.1038/s41598-018-26151-8
Ekman U, Ferreira D, Westman E. The A/T/N biomarker scheme and patterns of brain atrophy assessed in mild cognitive impairment. *Sci Rep* [Internet]. Springer US; 2018;8:8431. Available from: <http://dx.doi.org/10.1038/s41598-018-26151-8>.
7. Jack C, Bennett D, Blennow K, Carrillo M, Feldman H, Frisoni G, et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology*. 2016;87:539–47.
8. 10.1016/j.dadm.2016.03.001
Kandel BM, Avants BB, Gee JC, McMillan CT, Erus G, Doshi J, et al. White matter hyperintensities are more highly associated with preclinical Alzheimer's disease than imaging and cognitive markers of neurodegeneration. *Alzheimer's Dement Diagnosis, Assess Dis Monit* [Internet]. Elsevier Inc.; 2016;4:18–27. Available from: <http://dx.doi.org/10.1016/j.dadm.2016.03.001>.
9. <http://doi.wiley.com/10.1002/ana.24647>
Lee S, Viqar F, Zimmerman ME, Narkhede A, Tosto G, Benzinger TLS, et al. White matter hyperintensities are a core feature of Alzheimer's disease: Evidence from the dominantly inherited Alzheimer network. *Ann Neurol* [Internet]. 2016;79:929–39. Available from: <http://doi.wiley.com/10.1002/ana.24647>.
10. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* [Internet]. Lancet Pub. Group; 2013;12:822–38. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23867200>.

11. Lindemer ER, Greve DN, Fischl BR, Augustinack JC, Salat DH, Neuroimaging D. NeuroImage. Clinical Regional staging of white matter signal abnormalities in aging and Alzheimer ' s disease. NeuroImage Clin The Authors. 2017;14:156–65.
12. <http://doi.wiley.com/10.1002/hipo.22721>
Ferreira D, Hansson O, Barroso J, Molina Y, Machado A, Hernández-Cabrera JA, et al. The interactive effect of demographic and clinical factors on hippocampal volume: A multicohort study on 1958 cognitively normal individuals. Hippocampus [Internet]. 2017;27:653–67. Available from: <http://doi.wiley.com/10.1002/hipo.22721>.
13. Mueller SG, Weiner MW, Thal LJ, Petersen RC, Jack CR, Jagust W, et al. Ways toward an early diagnosis in Alzheimer's disease: The Alzheimer's Disease Neuroimaging Initiative (ADNI). Alzheimer's Dement. 2005;1:55–66.
14. Shams S, Martola J, Granberg T, Li X, Shams M, Fereshtehnejad SM, et al. Cerebral microbleeds: Different prevalence, topography, and risk factors depending on dementia diagnosis-the Karolinska imaging dementia study. Am J Neuroradiol. 2015;36:661–6.
15. Petersen RC, Aisen PS, Beckett LA, Donohue MC, Gamst AC, Harvey DJ, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI): Clinical characterization. Neurology. 2010;74:201–9.
16. [10.1016/j.jalz.2011.03.005](http://dx.doi.org/10.1016/j.jalz.2011.03.005)
Mckhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer ' s disease: Recommendations from the National Institute on Aging- Alzheimer ' s Association workgroups on diagnostic guidelines for Alzheimer ' s disease. Alzheimer's Dement [Internet]. Elsevier Ltd; 2011;7:263–9. Available from: <http://dx.doi.org/10.1016/j.jalz.2011.03.005>.
17. Simmons A, Westman E, Muehlboeck S, Mecocci P, Vellas B, Tsolaki M, et al. The AddNeuroMed framework for multi-centre MRI assessment of Alzheimer's disease: Experience from the first 24 months. Int J Geriatr Psychiatry. 2011;26:75–82.
18. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarker signature in alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 2009;65:403–13.
19. Shams S, Granberg T, Martola J, Li X, Shams M, Fereshtehnejad SM, et al. Cerebrospinal fluid profiles with increasing number of cerebral microbleeds in a continuum of cognitive impairment. J Cereb Blood Flow Metab. 2016;36:621–8.
20. Pai A, Sørensen L, Darkner S, Sparring J, Rostrup E, Nielsen M. White matter hypointensity growth rate correlates with rate of brain atrophy. Alzheimer's Dement [Internet]. Elsevier Ltd; 2014;10:P75–6. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S1552526014007845>.
21. Schiffmann R, Van Der Knaap MS. Invited Article: An MRI-based approach to the diagnosis of white matter disorders. Neurology. 2009;72:750–9.
22. [10.1016/j.jneuroim.2014.06.010](http://dx.doi.org/10.1016/j.jneuroim.2014.06.010)

- Ferreira D, Voevodskaya O, Imrell K, Stawiarz L, Spulber G, Wahlund LO, et al. Multiple sclerosis patients lacking oligoclonal bands in the cerebrospinal fluid have less global and regional brain atrophy. *J Neuroimmunol* [Internet]. Elsevier B.V.; 2014;274:149–54. Available from: <http://dx.doi.org/10.1016/j.jneuroim.2014.06.010>.
23. Narayana PA, Zhou Y, Hasan KM, Datta S, Sun X, Wolinsky JS. Hypoperfusion and T1-hypointense lesions in white matter in multiple sclerosis. *Mult Scler J*. 2014;20:365–73.
 24. Leritz EC, Shepel J, Williams VJ, Lipsitz LA, Mcglinchey E, Milberg WP, et al. Associations Between T1 White Matter Lesion Volume and Regional White Matter Microstructure in Aging. *Hum Brain Mapp*. 2015;35:1085–100.
 25. Fazekas F, Chawluk JB, Zimmerma A, June M. MR Signal Abnormalities at 1.5 T in Alzheimer's Dementia and Normal Aging deficiency. *AJR*. 1987;149:351–6.
 26. Kapeller P, Barber R, Vermeulen RJ, Adèr H, Scheltens P, Freidl W, et al. Visual rating of age-related white matter changes on magnetic resonance imaging: Scale comparison, interrater agreement, and correlations with quantitative measurements. *Stroke*. 2003;34:441–5.
 27. Cedres N, Ferreira D, Machado A, Shams S, Sacuiu S, Waern M, et al. Predicting Fazekas scores from automatic segmentations of white matter signal abnormalities. *Aging (Albany NY)* [Internet]. 2020;12:894–901. Available from: <http://www.aging-us.com/article/102662/text>.
 28. <http://doi.wiley.com/10.1111/joim.12358>
Ferreira D, Cavallin L, Larsson E-M, Muehlboeck J-S, Mecocci P, Vellas B, et al. Practical cut-offs for visual rating scales of medial temporal, frontal and posterior atrophy in Alzheimer's disease and mild cognitive impairment. *J Intern Med* [Internet]. 2015;278:277–90. Available from: <http://doi.wiley.com/10.1111/joim.12358>.
 29. Scheltens P, Leys D, Barkhof F, Huglo D, Weinstein HC, Vermersch P, et al. Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. *J Neurol Neurosurgery Psychiatry*. 1992;55:967–72.
 30. Koedam ELGE, Lehmann M, van der Flier WM, Scheltens P, Pijnenburg Y, Fox L. N, et al. Visual assessment of posterior atrophy development of a MRI rating scale. *Eur Radiol*. 2011;21:2618–25.
 31. [10.1007/s00330-015-4101-9](http://dx.doi.org/10.1007/s00330-015-4101-9)
Ferreira D, Cavallin L, Granberg T, Lindberg O, Aguilar C, Mecocci P, et al. Quantitative validation of a visual rating scale for frontal atrophy: associations with clinical status, APOE e4, CSF biomarkers and cognition. *Eur Radiol* [Internet]. 2016;26:2597–610. Available from: <http://link.springer.com/10.1007/s00330-015-4101-9>.
 32. Murray ME, Graff-Radford NR, Ross OA, Petersen RC, Duara R, Dickson DW. Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. *Lancet Neurol* [Internet]. 2011;10:785–96. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S1474442211701569>.
 33. Whitwell JL, Dickson DW, Murray ME, Weigand SD, Tosakulwong N, Senjem ML, et al. Neuroimaging correlates of pathologically defined subtypes of Alzheimer's disease: a case-control study. *Lancet*

- Neurol. 2012;11:868–77.
34. Persson K, Eldholm RS, Barca ML, Cavallin L, Ferreira D, Knapskog AB, et al. MRI-assessed atrophy subtypes in Alzheimer's disease and the cognitive reserve hypothesis. *PLoS One*. 2017;12:1–15.
 35. 10.1016/j.neurobiolaging.2018.05.028
Ferreira D, Shams S, Cavallin L, Viitanen M, Martola J, Granberg T, et al. The contribution of small vessel disease to subtypes of Alzheimer's disease: a study on cerebrospinal fluid and imaging biomarkers. *Neurobiol Aging* [Internet]. Elsevier Inc; 2018;70:18–29. Available from: <https://doi.org/10.1016/j.neurobiolaging.2018.05.028>.
 36. Poulakis K, Pereira JB, Mecocci P, Vellas B, Tsolaki M, Kłoszewska I, et al. Heterogeneous patterns of brain atrophy in Alzheimer's disease. *Neurobiol Aging*. 2018;65:98–108.
 37. Lê S, Josse J, Husson F, FactoMineR: An R Package for Multivariate Analysis. *J Stat Softw* [Internet]. 2008;25:253–8. Available from: <http://www.jstatsoft.org/v25/i01/>.
 38. Stekhoven DJ, Bühlmann P. Missforest-Non-parametric missing value imputation for mixed-type data. *Bioinformatics*. 2012;28:112–8.
 39. Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeblerlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's Dement* [Internet]. Elsevier Inc.; 2018;14:535–62. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S1552526018300724>.
 40. Lechner H, Schmidt R, Bertha G, Justich E, Offenbacher H, Schneider G. Nuclear magnetic resonance image white matter lesions and risk factors for stroke in normal individuals. *Stroke*. 1988;19:263–5.
 41. DeBette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: Systematic review and meta-analysis. *BMJ*. 2010;341:288.
 42. Hilal S, Mok V, Youn YC, Wong A, Ikram MK, Chen CLH. Prevalence, risk factors and consequences of cerebral small vessel diseases: Data from three Asian countries. *J Neurol Neurosurg Psychiatry*. 2017;88:669–74.
 43. Kanekiyo T, Xu H, Bu G. ApoE and A β in Alzheimer's Disease: Accidental Encounters or Partners? *Neuron* [Internet]. 2014;81:740–54. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0896627314000993>.
 44. Zhang X, Mormino EC, Sun N, Sperling RA, Sabuncu MR, Yeo BTT. Bayesian model reveals latent atrophy factors with dissociable cognitive trajectories in Alzheimer's disease. *Proc Natl Acad Sci* [Internet]. 2016;113:E6535 LP-E6544. Available from: <http://www.pnas.org/content/113/42/E6535.abstract>.

Figures

Figure 1

AD subtypes based on patterns of brain atrophy. Regional atrophy was measured with the MTA, PA and GCA-F visual rating scales based only on T1-weighted images. In the three visual rating scales, a score of zero denotes no atrophy, whereas scores from one to three (PA and GCA-F) or four (MTA) indicate an increasing degree of atrophy. The typical AD subtype was defined as abnormal MTA together with abnormal PA and/or abnormal GCA-F. The limbic-predominant subtype was defined as abnormal MTA alone with normal PA and GCA-F. The hippocampal-sparing subtype included abnormal PA and/or abnormal GCA-F, but normal MTA. The minimal atrophy subtype was defined as normal scores in MTA, PA, and GCA-F. The figure shows examples of each subtype. AD = Alzheimer's disease; MTA = medial temporal atrophy scale; PA = posterior atrophy scale; GCA-F = global cortical atrophy scale – frontal subscale; A = anterior part of the brain; P = posterior part of the brain; R = right; L = left.

Figure 2

Frequency of ATN profiles by cohort and AD subtype. Panel A shows the frequency of ATN profiles within the ADNI (top) and KIDS (bottom) cohorts. Panel B shows the frequency of ATN profiles across AD subtypes in the ADNI (top) and KIDS (bottom) cohorts. AD = Alzheimer's disease; A- = normal CSF A β biomarker; A+ = abnormal CSF A β biomarker; T- = normal CSF phosphorylated tau biomarker; T+ = abnormal CSF phosphorylated tau biomarker; N- = normal CSF total tau biomarker; N+ = abnormal CSF total tau biomarker.

Figure 3

Factorial analysis of mixed data (FAMD): scatterplots for cohort and WMSA burden. Dots represent individual AD patients. In panels A, B, C and D, the x-axis represents Dimension 1. In panels A and B the y-axis represents Dimension 2. In panels C and D the y-axis represents Dimension 3. AD = Alzheimer's Disease; WMSA = white matter signal abnormalities; A + = CSF A β abnormal; T - = CSF p-tau normal; T + = CSF p-tau abnormal; N- = CSF t-tau normal; N + = t-tau abnormal. MMSE = mini-mental state examination.

Figure 4

Factorial analysis of mixed data (FAMD): scatterplots for AD subtypes and ATN profiles. Dots represent individual AD patients. In panels A, B, C and D, the x-axis represents Dimension 1. In panels A and B the y-axis represents Dimension 2. In panels C and D the y-axis represents Dimension 3. AD = Alzheimer's Disease; WMSA = white matter signal abnormalities; A + = CSF A β abnormal; T - = CSF p-tau normal; T + = CSF p-tau abnormal; N- = CSF t-tau normal; N + = t-tau abnormal. MMSE = mini-mental state examination.