Brain Atrophy Subtypes and the ATN Classification Scheme in Alzheimer's Disease

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Keywords

Alzheimer's disease · Heterogeneity · Subtypes · Amyloidosis tauopathy neurodegeneration · Cerebrospinal fluid biomarkers · Factorial analysis

Abstract

Introduction: We investigated the association between atrophy subtypes of Alzheimer's disease (AD), the ATN classification scheme, and key demographic and clinical factors in 2 cohorts with different source characteristics (a highly selective research-oriented cohort, the Alzheimer's Disease Neuroimaging Initiative [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 subtypes based on brain atrophy patterns, ATN profiles based on cerebrospinal fluid biomarkers, and age, sex, Mini Mental State Examination (MMSE), cerebrovascular disease (burden of white matter signal abnormalities, WMSAs), 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 ε4 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.

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Background

Disentangling the biological heterogeneity in Alzheimer's disease (AD) has become an important task in order to guide personalised interventions [1, 2]. Neuro-

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pathological and neuroimaging studies have consistently identified 3 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 the 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, that is, 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 further 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 patients with mild cognitive impairment (MCI) [6].

A task that remains to be done is the incorporation of a category for cerebrovascular disease (CVD, V) to the ATN scheme [7]. White matter signal abnormalities (WMSAs) on magnetic resonance imaging (MRI) are a well-established marker of CVD. WMSAs are implicated in AD pathogenesis [8, 9] and are commonly found in cognitively unimpaired older individuals [10, 11]. Hence, including the V dichotomous category in the scheme is important to advance our understanding of associations between amyloid, tau, and vascular 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 amyloid, tau, and vascular 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 2 cohorts: a homogeneous research-oriented cohort (the Alzheimer's Disease Neuroimaging Initiative [ADNI]) and a heterogeneous clinically oriented cohort (the KIDS: 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 cohorts [5, 12]. We hypothesised that the distribution of AD subtypes and ATN profiles would differ depending on cohorts and demographic and clinical characteristics. We hypothesised 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 to 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.

Material and Methods

Participants

We combined 2 cohorts of AD patients: ADNI-1 ($N = 102$) [13] and KIDS ($N = 280$) [14]. 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, 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, Mini-Mental State Examination (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).

The exclusion criteria in both ADNI and KIDS were other clinical diagnoses (dementia with Lewy bodies, vascular dementia, alcohol-related dementia, MCI, 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.

Fig. 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 3 visual rating scales, a score of zero denotes no atrophy, whereas scores from 1 to 3 (PA and GCA-F) or 4 (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 in axial and coronal sections of the brain. 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.

Implementation of the ATN Classification Scheme

Lumbar puncture for CSF sampling was conducted for both cohorts following standard procedures. For ADNI, amyloid- β_{42} ($A\beta_{42}$), phosphorylated-tau (P-tau), and total tau (T-tau) were measured using the multiple xMAP Luminex platform (Luminex Corp, Austin, TX, USA) with INNO-BIA AlzBio3 immunoassay kit-based reagents (Ghent, Belgium; for research use-only reagents) [18]. For KIDS, biomarkers were measured using a sandwich-type ELISA; $A\beta_{42}$ was measured with Innostest b-amyloid (1–42), T-tau with Innostest hTau-Ag, and P-tau with Innostest Phospho-tau (181 P) (Innogenetics, Ghent, Belgium). The unit used for biomarkers is ng/L. All participants were classified into ATN groups according to CSF biomarkers for amyloid- β (“A,” CSF $A\beta_{42}$), tau NFT pathology (“T,” CSF P-tau), and unspecific neurodegeneration (“N,” CSF T-tau). Each individual was rated as either positive (+; i.e., abnormal) or negative (–; i.e., normal) on each biomarker according to previously published cohort-specific cut-offs: ≤ 192 pg/mL for $A\beta_{42}$, ≥ 23 pg/mL for P-tau, and ≥ 93 pg/mL for T-tau for the ADNI cohort [18]; and ≤ 550 pg/mL for $A\beta_{42}$, ≥ 80 pg/mL for P-tau, and ≥ 400 pg/mL for T-tau for the KIDS cohort [19].

Magnetic Resonance Imaging

A T1-weighted magnetization-prepared rapid gradient-echo sequence was acquired on ADNI patients (repetition time [RT] $\sim 2,300$ ms, echo time [ET] ~ 3.6 ms, inversion time [TI] $\sim 1,000$ ms, slice thickness = 1.2 mm; <http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/>). Data were 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 magnetization-prepared rapid gradient-echo sequence (RT $\sim 1,700$ ms, ET ~ 3 ms, TI $\sim 1,000$ ms, slice thickness ~ 1.2 mm) and a fluid-attenuated inversion recovery sequence (RT $\sim 8,000$ ms, ET ~ 100 ms, TI = 2,100–2,500 ms, slice thickness ~ 5.0 mm) were acquired in 3 MRI scanners at the Radiology Department of the Karolinska University Hospital, Stockholm, Sweden: (1) a 1.5T Magnetom Symphony scanner, (2) 1.5T Magnetom Avanto scanner, and (3) 3T Magnetom Trio scanner [14]. TheHiveDB was used for data management in this study [20].

WMSAs were investigated as a marker of CVD. In the ADNI cohort, WMSAs were assessed through automatically segmented white matter hypointensities from FreeSurfer 6.0.0 (<https://surfer>).

Table 1. Cohort characteristics

	(a) Whole cohort			(b) A+ subsample		
	ADNI	KIDS	<i>p</i> value	ADNI	KIDS	<i>p</i> value
<i>N</i>	102	280	–	94	209	–
Age, years	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
A β 42, % 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 ϵ 4, % carriers	70	66	0.607	76	66	0.153

MMSE, mini-mental state examination; WMSAs, white matter signal abnormalities; A β , amyloid β ; P-tau, phosphorylated tau; T-tau, total tau; KIDS, Karolinska imaging dementia study; ADNI, Alzheimer's disease neuroimaging initiative.

nmr.mgh.harvard.edu/). FreeSurfer is increasingly used to automatically segment WMSAs in the form of hypointensities in the T1-weighted MRI sequence [21–25]. In KIDS, the MRI data are clinical, and there is variation across patients in scanning parameters. In such data, the Fazekas visual rating scale [26] is appropriate as a measure of WMSAs because it is not influenced by variation in scanning parameters [27]. Hence, in the KIDS cohort, WMSAs were assessed through the Fazekas scale on fluid-attenuated inversion recovery images. Importantly, hypointense WMSAs from FreeSurfer and the Fazekas scale are strongly associated with each other [28]. We followed a previous study that demonstrated that hypointense WMSAs from FreeSurfer and Fazekas scores can be combined together by converting hypointense WMSA into low and high CVD burden [28]. Hence, we computed a unique WMSA variable by applying a cut-off of 0.00321 on hypointense WMSAs from FreeSurfer after total intracranial volume adjustment. This creates 2 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 [28].

Regional brain atrophy was assessed with visual rating scales, as detailed elsewhere [4, 29]. Briefly, medial temporal atrophy (MTA) was assessed with Scheltens' scale [30], posterior atrophy (PA) with Koedam's scale [31], and atrophy in the frontal lobe with the global cortical atrophy scale – frontal subscale (GCA-F) [32]. 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.

AD Subtypes Based on Patterns of Brain Atrophy

Deviation from normality in visual ratings was determined using previously published cut-offs [29]. The MTA scores ≥ 1.5 , ≥ 1.5 , ≥ 2 , and ≥ 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

[29], a score ≥ 1 was considered abnormal irrespective of the age range [29]. The 3 AD subtypes identified in the previous literature [33, 34] were defined based on the combination of MTA, PA, and GCA-F, as in previous studies [4, 6, 35, 36]. The minimal atrophy AD subtype [3, 35, 37] was identified when AD patients displayed normal scores in MTA, PA, and GCA-F. Visual examples of the 4 AD subtypes can be found in Figure 1.

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) [38]. 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 [38]. The main objective of FAMD is to simplify multiple data features in latent dimensions (or components), allowing to reduce data and identify association patterns. 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 1 ϵ 4 allele vs. non-carriers). One-way ANOVA was used for continuous variables, and the χ^2 test was used for categorical data. Missing data on MMSE were estimated via the MissForest algorithm [39] for 6 KIDS patients. All statistical analyses were conducted using R statistical software (R Foundation for Statistical Computing, Vienna, <http://www-R-project.org>). FactoMineR and Factoextra packages were used for FAMD analysis [40, 41]. *p* values in all principal and post hoc analyses were adjusted with Benjamini-Hochberg's correction for multiple comparisons [42]. A *p* value ≤ 0.05 was deemed statistically significant.

Fig. 2. Frequency of ATN profiles by cohort and AD subtype. **a** Frequency of ATN profiles within the ADNI (top) and KIDS (bottom) cohorts. **b** 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 P-tau biomarker; T+, abnormal CSF P-tau biomarker; N-, normal CSF T-tau biomarker; N+, abnormal CSF total tau biomarker; KIDS, Karolinska Imaging Dementia Study; ADNI, Alzheimer's Disease Neuroimaging Initiative; P-tau, phosphorylated tau; T-tau, total tau; CSF, cerebrospinal fluid.

Results

Cohort Characteristics

Cohort characteristics are shown in Table 1 ($N = 382$). 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 and a higher frequency of abnormal CSF A β 42 and P-tau levels, while KIDS patients showed a significantly higher frequency of abnormal CSF T-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 1 ($N = 303$). 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 P-tau levels, as compared with KIDS patients.

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 P-tau levels than limbic-predominant AD patients.

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

Table 2. Demographic and clinical characteristics by AD subtype

	Typical AD	Limbic predominant	Hippocampal sparing	Minimal atrophy	<i>p</i> value
<i>N</i>	131	76	45	51	
ADNI	48	18	17	11	0.070
KIDS	83	58	28	40	
Age, years	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

None of the paired comparisons for AD subtypes in the T-tau measure was significant after the Benjamini-Hochberg correction for multiple comparisons. MMSE, mini-mental state examination; WMSAs, white matter signal abnormalities; P-tau, phosphorylated tau; T-tau, total tau; AD, Alzheimer's disease; KIDS, karolinska imaging dementia study; ADNI, Alzheimer's disease neuroimaging initiative. ^a Significantly different from typical AD. ^b Significantly different from limbic predominant. ^c Significantly different from hippocampal sparing. ^d Significantly different from minimal atrophy.

higher ($p < 0.001$) than 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.

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– group was only present in the typical AD subtype in KIDS. These visual analyses were further supported by the FAMMD models, also showing the correspondence among AD subtypes, ATN profiles, WMSA burden, age, sex, MMSE, cohort, and APOE genotype.

The main FAMMD model ($N = 382$) showed 3 dimensions that explained 42.3% of the variance (Table 3). Dimension 1 explained 18.6% 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 12.2% of the variance and was driven by AD subtype (although MMSE, ATN, and cohort also contributed statis-

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

	Dimension 1 (R ² = 18.6%)	Dimension 2 (R ² = 12.2%)	Dimension 3 (R ² = 11.5%)
Age, years	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
WMSAs	18.2	3.1	3.2
Sex	4.7	3.1	1.8

Values represent the percentage of contribution of each variable to the total variation captured by each dimension. MMSE, mini-mental State examination; WMSA, white matter signal abnormalities; FAMMD, factorial analysis of mixed data.

tically significantly to dimension 2). Dimension 3 explained 11.5% of the variance and was driven by AD subtype, MMSE, and ATN.

Although ATN and AD subtypes contributed to the 3 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. 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

Fig. 3. FAMD: scatterplots for cohort and WMSA burden. Dots represent individual AD patients. In **a–d**, the x-axis represents dimension 1. In **a, b**, the y-axis represents dimension 2. In **c, 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; FAMD, factorial analysis of mixed data; P-tau, phosphorylated tau; T-tau, total tau; CSF, cerebrospinal fluid.

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 previously, 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, independent 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* $\epsilon 4$ 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* $\epsilon 4$ status.

Fig. 4. FAMD: scatterplots for AD subtypes and ATN profiles. Dots represent individual AD patients. In **a–d**, the x-axis represents dimension 1. In **a, b**, the y-axis represents dimension 2. In **c, d**, the y-axis represents dimension 3. AD, Alzheimer's disease; WMSAs, 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; FAMD, factorial analysis of mixed data; P-tau, phosphorylated tau; T-tau, total tau; CSF, cerebrospinal fluid.

Within dimension 3, *APOE* $\epsilon 4$ carriers showed a higher frequency of A+T–N– and A+T+N– profiles and a tendency to include patients with typical AD.

Discussion

We investigated the association between AD subtypes and ATN classification scheme in 2 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 sub-

types 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 2 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 2 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 [33]. 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 this hypothesis, we investigated the association among the 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 [43] 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 earlier, 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 the 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 [36]. 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) [44, 45] are exclusion criteria in ADNI. However, previous studies showed that WMSA burden increases with older age [45, 46], and ADNI patients are older than KIDS patients in our study, which could explain our finding of higher WMSA in the ADNI. This finding aligns with the recent conceptual framework of biological subtypes of AD [5], that is, 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 than limbic-predominant AD [5].

Our complementary FAMD model showed that *APOE* ε4 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* ε4 genotype and amyloid-beta pathology (A+) is a well-established finding [47]. Further, previous studies showed that the frequency of *APOE* ε4 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].

The AD subtype and ATN classifications are 2 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 the AD subtype was the main driver of one of the dimensions (dimension 2), while ATN always emerged as a secondary driver after the 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 suggests that the

ATN classification scheme may be useful to assess disease staging. The capacity of the AD subtype to drive a dimension by itself, partially independent 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, 33, 48] that can only be answered in future longitudinal studies. The distinct subtype 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, 33], leading to different patterns of brain atrophy in structural MRI [34]. An advantage of the AD subtype classification is the inclusion of the topographical dimension to the N category of ATN [36]. Whether the AD subtype is a stronger approach to disentangle disease heterogeneity than the ATN classification scheme must be confirmed in future studies.

The current study has some limitations. We did not include A- individuals in the main analysis. Including A- individuals might increase the heterogeneity and show slightly different associations between the 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 [28]. 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 2 methods and has greater clinical applicability [46]. Finally, we lacked data for several factors listed in the recent conceptual framework for biological subtypes of AD, including disease duration [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 2 pathologies.

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 ho-

mogeneous or more naturalistic and heterogeneous. Our findings largely support the recent conceptual framework of biological subtypes of AD. 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 realise precision medicine in clinical routine.

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Statement of Ethics

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 the Karolinska Imaging Dementia Study (KIDS), ethical approval was obtained from the Regional Ethics Board in Stockholm, Sweden (DNR:2011/1987-31/4).

Conflict of Interest Statement

The authors declare no conflict of interest.

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Author Contributions

N.C., D.F., and E.W. contributed to the conception and design of the study. N.C., K.P., S.S., L.C., T.G., S.M., and L.O.W. contributed to the acquisition and analysis of data. N.C., U.E., D.F., and E.W. contributed to drafting a significant portion of the manuscript and preparing the figures. All the authors revised the manuscript and contributed on scientific content.

Availability of Data and Materials

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

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