Symptomatic clusters related to amyloid positivity in cognitively unimpaired individuals

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Abstract

**Background:** The NIA-AA Research Framework on Alzheimer’s Disease (AD) proposes a transitional stage (stage 2) between the fully asymptomatic stage 1 and mild cognitive impairment (stage 3) in the evolution of symptoms over the disease course. Proposed features of stage 2 include subtle cognitive dysfunction, subjective cognitive decline (SCD) and mild neurobehavioral symptoms. Here, we aimed to identify specific clusters of participants based on these features and assess the association with amyloid positivity in cognitively unimpaired individuals.

**Methods:** We used baseline data of \( n = 338 \) participants from the German DZNE Longitudinal Cognitive Impairment and Dementia (DELCODE) study without objective evidence of cognitive impairment and with available data on cerebrospinal fluid biomarkers for AD. Specifically, healthy controls \( (n=90) \), participants with SCD \( (n=202) \) and first-degree relatives of AD patients \( (n=46) \) were included. Classification into the Alzheimer’s continuum (i.e., amyloid positivity, A+) was based on Aβ42/40 status. Neuropsychological test data were used to assess subtle objective cognitive dysfunction (OBJ), the subjective cognitive decline interview (SCD-I) was used to detect SCD, and the Neuropsychiatric Inventory Questionnaire (NPI-Q) was used to assess neurobehavioral changes (NPS). A two-step cluster analysis was carried out and differences in AD biomarkers between clusters were analysed.

**Results:** We identified three distinct participant clusters based on presented symptoms. The highest rate of A+ participants (47.6%) was found in a cluster characterized by both OBJ and SCD. A cluster of participants that presented with SCD and NPS (A+:26.6%) and a cluster of participants with overall few symptoms (A+:19.7%) showed amyloid positivity in a range that was not higher than the expected A+ rate for the age group. Across the full sample, participants with a combination of SCD and OBJ in the memory domain showed a lower Aβ42/ptau181 ratio compared to those with neither SCD nor OBJ.

**Conclusion:** In this study, we identified three distinct clusters of participants based on symptoms associated with the NIA-AA stage 2. The cluster characterized by OBJ and concomitant SCD was associated with an increased A+ frequency, suggesting that this combination is enriched for stage 2 of the Alzheimer’s continuum.

**Trial registration**

German Clinical Trials Register DRKS00007966. Registered 4 May 2015.

**INTRODUCTION**

The neuropathological changes of Alzheimer’s disease (AD) start years or even decades before clinical symptoms appear, solidifying the concept of AD as a continuum [1]. In light of the importance of early treatment and prevention, AD research has shifted towards preclinical disease phases, highlighting the relevance of biological disease markers to identify AD prior to or at the very early stage of symptom onset. As a consequence of these developments, the National Institute on Aging and Alzheimer’s
Association (NIA-AA) updated their 2011 diagnostic guidelines and proposed a research framework in 2018 that puts the biological definition of AD and the role of biomarkers in defining AD at its center [2].

The updated NIA-AA research framework introduced a numeric clinical staging scheme of the development of symptoms applicable to individuals on the Alzheimer's continuum as characterized by biomarker evidence of abnormal beta amyloid (Aß) with or without pathologic tau biomarkers. By incorporating the features of objective cognitive performance, subjective report of cognitive decline, neurobehavioral changes and functional impact on daily life, six clinical stages were created that relate to pre-dementia (stages 1–3) and dementia (stages 4–6). Stage 2 is defined as a stage of initial symptom manifestation and transitional cognitive decline from the fully asymptomatic stage (stage 1) to the stage of MCI (stage 3).

Aside from objective evidence of subtle transitional cognitive decline, classification into stage 2 may also be based on the subjective report of cognitive decline. Self-experienced cognitive decline in the absence of objective impairment (SCD) is considered an at-risk state for dementia [3–8] and SCD severity has been shown to further increase the risk for developing MCI or dementia. Among others, these include SCD in the memory domain [11], worry about the perceived decline [7, 10–12] and persistence of SCD [7, 13]. Consequently, the stage 2 criteria specify that the subjective report of cognitive decline should be of concern to the participants and persist for at least six months. Confirmation of perceived decline by an informant may be provided, but it is not required [2].

In addition, the criteria for stage 2 also acknowledge that early changes may not necessarily present as cognitive deficits, but rather as neurobehavioral changes. There is growing evidence that neuropsychiatric symptoms (NPS) such as depression, anxiety or irritability may precede cognitive decline in AD [14–18]. As such, they might be regarded as early disease manifestations [6, 19]. These findings led to the introduction of mild behavioral impairment (MBI) as a diagnostic construct that is distinct from MCI [19]. In a recent longitudinal cohort study of cognitively unimpaired individuals, the authors reported that the presence of SCD and MBI each increased the risk for cognitive and functional decline compared to participants without SCD or MBI, and a combination of both SCD and MBI led to the highest risk of progression [6].

In this study, we aimed to identify clusters of participants based on these features associated with the NIA-AA stage 2 (i.e., subtle objective cognitive dysfunction, SCD, NPS) and assess which symptoms are specifically related to AD pathology in cognitively unimpaired individuals.

**METHODS**

**Participants**

We analyzed baseline data from cognitively unimpaired participants of the German DZNE Longitudinal Cognitive Impairment and Dementia (DELCODE) observational multicenter study. A detailed description of
the study design with inclusion and exclusion criteria has been published earlier [20, 21]. For the current analysis, we included baseline data of \( N = 338 \) participants that were enrolled as healthy controls \( (n = 90) \), participants with SCD \( (n = 202) \) or cognitively healthy first-degree relatives of patients with a documented AD dementia diagnosis \( (n = 46) \). All participants had available cerebrospinal fluid (CSF) AD biomarker information and complete data on objective cognition, SCD and NPS. All were 60 years or older, had no current major depressive episode and no history of major psychiatric disorders. Study partners acted as informants to provide information about the participant on several clinical measures.

Participants with SCD were recruited from the ten memory clinics participating in DELCODE. SCD was defined by the presence of self-experienced cognitive decline with concerns expressed towards the clinician at the memory clinic. In addition, a performance better than \( \sim 1.5 \) standard deviations (SD) on all subtests of the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) test battery was required to rule out objective cognitive deficits. The cognitive assessment was completed prior to enrollment into DELCODE within the clinical diagnostic work-up of the respective memory clinics.

Healthy controls and the group of first-degree relatives of AD dementia patients were recruited via newspaper advertisements and screened for concerns of cognitive decline by phone. Healthy controls were only included if they did not express concerns about their memory and considered it normal for their age. First-degree relatives were included regardless of potential worry about cognitive decline. In addition, participants in both groups had to achieve unimpaired cognitive performance according to the same criteria as the SCD group, which was verified at the DELCODE baseline assessment.

All participants provided written informed consent prior to inclusion. The study was approved by the institutional review boards and ethical committees of each of the participating DELCODE sites.

**CSF AD biomarker assessment**

All participants in the reported sample underwent lumbar puncture at the baseline visit. The collection and processing of CSF samples was carried out according to standardized operating procedures of the German Center for Neurodegenerative Diseases (DZNE), and has been previously described in detail [20]. The cut-offs for normal and abnormal concentrations of CSF biomarkers were calculated based on a mixture modeling approach of the whole DELCODE sample by the Institute for Medical Biometry, Informatics and Epidemiology (IMBIE) at the University of Bonn Medical Center. In line with the most recent NIA-AA guideline AT(N) system, classification into the Alzheimer’s continuum was based on amyloid biomarker status. A cut-off of 0.08 in the A\( \beta \)42/\( A \beta \)40 ratio was used to divide the sample into Alzheimer’s continuum positive (A+) and negative (A-) participants [21].

**Assessment and operationalization of symptoms**

The NIA-AA numerical clinical staging scheme names clinical features of each stage, but does not specify particular clinical or neuropsychological measures. To assess the clinical features or symptoms that reflect stage 2, we used the following assessments.
**Subtle Objective Cognitive Dysfunction (OBJ).** All participants underwent thorough clinical examination and neuropsychological testing. A detailed description of the DELCODE neuropsychological assessment battery has been provided earlier [22]. Five cognitive domain scores were derived from confirmatory factor analysis: memory (MEM), executive function (EXEC), visuospatial abilities (VIS), working memory (WM) and language (LANG) [22]. For the present analysis, the cognitive domain scores were rescaled by z-transformation with mean and SD taken from healthy controls with normal Aβ42/40 ratio biomarker status. The NIA-AA clinical staging proposes transitional cognitive decline as a measure for subtle changes in cognition. Our analysis focuses on the cross-sectional baseline data of DELCODE. As such, a cut-off to reflect objective subtle dysfunction (OBJ) not yet meeting MCI-criteria was chosen and defined by a performance below −1SD on the rescaled cognitive domain score. Note that none of the participants fulfilled MCI criteria as outlined above.

**Subjective Cognitive Decline (SCD).** The presence and extent of SCD was measured with the SCD-Interview (SCD-I), a structured clinical interview that assesses subjective decline in five cognitive domains (memory, language, planning, attention, any other cognitive decline) [11]. For each domain, a potential subjective decrease in function was recorded and, if confirmed, additional questions addressed associated worries, onset of decline, performance in comparison to peers and if the perceived cognitive decline had been previously discussed with a physician. The presence of SCD in any cognitive domain was defined by subjective report of self-perceived cognitive difficulties on the SCD-I with concerns and persistence for at least 6 months.

**Neurobehavioral Changes (NPS).** Neurobehavioral changes were assessed using the Neuropsychiatric Inventory Questionnaire (NPI-Q) [23]. The NPI-Q evaluates 12 neuropsychiatric symptoms: delusions, hallucinations, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behavior, sleep disturbance, and changes in appetite. For each item, the informant rates the presence of the symptom in the past month (“yes” or “no”) and indicates its severity (1-mild, 2-moderate, 3-severe). Here, we focused on the presence or absence of symptoms as a binary characteristic.

**Statistical analysis**

All statistical analyses were performed with IBM SPSS Statistics 28.0 (IBM Corp., Armonk, NY) for Windows.

A two-step cluster analysis (TSCA) using log-likelihood distance measure was applied to the whole sample to identify subgroups of patients based on the symptomatic features. We dichotomized and included the five cognitive domain scores and five SCD-I domains. All NPI-Q domains that were present in more than 5% of cases – namely, agitation (13.6%), depression (15.1%), anxiety (12.4%), disinhibition (6.2%), irritability (20.3%), appetite changes (5.9%) and sleep disturbances (23.4%) were included as absent or present. The CSF Aβ42/40 status was also dichotomized (i.e., A+ or A-). The two-step cluster analysis is suited to analyze binary data in large samples and does not require a pre-defined number of clusters. Instead, the optimal number of clusters is determined by the Schwarz's Bayesian Information Criterion (BIC) as a statistical measure of fit. In the first step, the cases are pre-clustered by a sequential
approach. These pre-clusters are then grouped according to a hierarchical clustering method. To avoid order effects, the data were randomly sorted prior to the analysis [24]. The silhouette measure of cohesion and separation is reported as a goodness-of-fit measure [25].

In a second step, the emerging clusters were compared with regard to demographic and symptomatic characteristics and amyloid status using one-way analysis of variance (ANOVA) and Chi-square analyses. The eta-squared measure ($\eta^2$) and Cramer's $V$ were computed to estimate effect size [26] and Bonferroni-corrected post-hoc comparisons were carried out to identify differences between clusters.

Building on results from the TSCA, a classification of participants into groups was performed based on those features that discriminated the clusters most strongly. We compared the resulting groups with regard to $\text{A}\beta_{42}/\text{A}\beta_{40}$, and the additional CSF markers total tau, phosphorylated tau 181 (ptau181), and the ratio of $\text{A}\beta_{42}$ and ptau181 using ANOVA and ANCOVA to control for the effects of age. For exploratory reasons, the analysis was repeated for group classification based on OBJ and SCD in the memory domain only due to its specific association with AD [12, 27]. Post-hoc comparisons between groups were corrected for multiple comparisons.

**RESULTS**

Participants had a mean age of 69.5 years ($SD = 5.8$) and were highly educated (years of education $M = 14.7$, $SD = 2.9$). The sex distribution was well balanced (47.9% female participants). Based on $\text{A}\beta_{42}/\text{A}\beta_{40}$, 103 (30.5%) participants with abnormal $\text{A}\beta_{42}/\text{A}\beta_{40}$ ratio were classified into the A+ group and 235 (69.5%) participants with normal $\text{A}\beta_{42}/\text{A}\beta_{40}$ ratio into the A- group.

**Cluster analysis**

The TSCA yielded three different clusters. The silhouette measure of cohesion and separation was 0.3, indicating a fair cluster solution [25]. Cluster 1 ($n = 103$) was characterized by a high frequency of participants with OBJ in all cognitive domain scores, as well as SCD and NPS to a lesser degree. SCD was predominantly reported in the memory (75.7%) and language (62.1%) domain, while irritability (24.3%) and agitation (18.5%) were the most frequently reported NPS in this cluster.

In cluster 2 ($n = 113$), SCD and NPS were most prominent, while OBJ occurred rarely. 85.8% of participants in this cluster reported SCD in the memory domain and the most frequent NPS were sleep disturbances (41.6%), followed by irritability (35.4%) and depression (24.8%).

Cluster 3 ($n = 122$) comprised participants who experienced neither SCD nor NPS. Less than 20% of participants showed OBJ in either one of the five cognitive domains - a rate that was lower compared to cluster 1, but higher compared to cluster 2.

The rate of A+ participants was highest in cluster 1 (47.6%) compared to cluster 2 (26.6%, $p = .002$) and cluster 3 (19.7%, $p < .0005$). Participants in cluster 1 had fewer years of education compared to cluster 2
(p = .017) and were significantly older than participants in cluster 2 (p < .0005) and cluster 3 (p < .0005). Clusters 2 and 3 did not differ in the rate of A+ participants, age and education. There were no sex differences between the clusters.

A graphic representation of the clusters’ symptomatic distribution is shown in Fig. 1. Differences between clusters regarding demographic characteristics and stage 2 characteristics are provided in Table 1.
Table 1
Differences in demographic characteristics and symptoms related to NIA-AA stage 2 between clusters

<table>
<thead>
<tr>
<th></th>
<th>Cluster 1 (n = 103)</th>
<th>Cluster 2 (n = 113)</th>
<th>Cluster 3 (n = 122)</th>
<th>F / ( \chi^2 )</th>
<th>( \eta^2 / \nu )</th>
</tr>
</thead>
<tbody>
<tr>
<td>age, M(SD)</td>
<td>72.46 (6.16)</td>
<td>68.65 (5.35)***</td>
<td>67.72 (4.79)***</td>
<td>23.25, ( p &lt; .001 )</td>
<td>.12</td>
</tr>
<tr>
<td>sex (female), n(%)</td>
<td>44 (39.81)</td>
<td>58 (51.33)</td>
<td>63 (51.64)</td>
<td>3.92, ( p = .141 )</td>
<td>.11</td>
</tr>
<tr>
<td>education, M(SD)</td>
<td>14.15 (2.83)</td>
<td>15.23 (2.97)</td>
<td>14.64 (2.71)</td>
<td>3.96, ( p = .020 )</td>
<td>.02</td>
</tr>
<tr>
<td>A+, n(%)</td>
<td>49 (47.57)</td>
<td>30 (26.55)**</td>
<td>24 (19.67)***</td>
<td>21.75, ( p &lt; .001 )</td>
<td>.25</td>
</tr>
<tr>
<td>SCD memory, n(%)</td>
<td>76 (73.79)</td>
<td>97 (85.84)</td>
<td>8 (6.56)***†††</td>
<td>172.63, ( p &lt; .001 )</td>
<td>.72</td>
</tr>
<tr>
<td>SCD language, n(%)</td>
<td>64 (62.14)</td>
<td>74 (65.49)</td>
<td>6 (4.92)***†††</td>
<td>111.12, ( p &lt; .001 )</td>
<td>.57</td>
</tr>
<tr>
<td>SCD planning, n(%)</td>
<td>20 (19.42)</td>
<td>22 (19.47)</td>
<td>0 (0)***†††</td>
<td>37.23, ( p &lt; .001^a )</td>
<td>.28</td>
</tr>
<tr>
<td>SCD attention, n(%)</td>
<td>36 (34.95)</td>
<td>45 (39.82)</td>
<td>0 (0)***†††</td>
<td>82.57, ( p &lt; .001^a )</td>
<td>.42</td>
</tr>
<tr>
<td>SCD other, n(%)</td>
<td>23 (22.33)</td>
<td>28 (24.87)</td>
<td>1 (0.82)***†††</td>
<td>40.74, ( p &lt; .001^a )</td>
<td>.31</td>
</tr>
<tr>
<td>OBJ memory, n(%)</td>
<td>78 (75.73)</td>
<td>13 (11.50)***</td>
<td>20 (16.39)***</td>
<td>124.19, ( p &lt; .001 )</td>
<td>.61</td>
</tr>
<tr>
<td>OBJ language, n(%)</td>
<td>85 (82.52)</td>
<td>5 (4.42)***</td>
<td>22 (18.03)***†††</td>
<td>175.37, ( p &lt; .001^a )</td>
<td>.71</td>
</tr>
<tr>
<td>OBJ executive function, n(%)</td>
<td>80 (77.67)</td>
<td>2 (1.77)***</td>
<td>18(14.75)***†††</td>
<td>175.88, ( p &lt; .001^a )</td>
<td>.71</td>
</tr>
<tr>
<td>OBJ working memory, n(%)</td>
<td>77 (74.76)</td>
<td>3 (2.65)***</td>
<td>15 (12.30)***†</td>
<td>163.80, ( p &lt; .001^a )</td>
<td>.69</td>
</tr>
<tr>
<td>OBJ visuospatial abilities, n(%)</td>
<td>52 (50.49)</td>
<td>8 (7.08)***</td>
<td>19 (15.57)***</td>
<td>60.52, ( p &lt; .001^a )</td>
<td>.43</td>
</tr>
<tr>
<td>NPS Agitation, n(%)</td>
<td>19 (18.45)</td>
<td>25 (22.12)</td>
<td>2 (1.64)***†††</td>
<td>29.32, ( p &lt; .001^a )</td>
<td>.27</td>
</tr>
</tbody>
</table>

Bonferroni-adjusted post hoc p-values in comparison to cluster 1: *\( p < .025 \), **\( p < .005 \), ***\( p < .0005 \).
Bonferroni-adjusted post hoc p-values in comparison to cluster 2: †\( p < .025 \), ††\( p < .005 \), †††\( p < .0005 \).

\( a \)Fisher’s exact test used. A+ Classified into the Alzheimer’s continuum, NPS Neurobehavioral changes, OBJ Subtle Objective Cognitive Dysfunction, SCD subjective cognitive decline.
<table>
<thead>
<tr>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>$F / \chi^2$</th>
<th>$\eta^2 / V$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPS Depression, $n(%)$</td>
<td>17 (16.50)</td>
<td>28 (24.78)</td>
<td>6 (4.92)*†††</td>
<td>18.29, $p &lt; .001$</td>
</tr>
<tr>
<td>NPS Anxiety, $n(%)$</td>
<td>16 (15.53)</td>
<td>25 (22.12)</td>
<td>1 (0.82)**†††</td>
<td>31.95, $p &lt; .001^a$</td>
</tr>
<tr>
<td>NPS Disinhibition, $n(%)$</td>
<td>5 (4.85)</td>
<td>15 (13.27)</td>
<td>1 (0.82)**††</td>
<td>15.94, $p &lt; .001^a$</td>
</tr>
<tr>
<td>NPS Irritability, $n(%)$</td>
<td>25 (24.27)</td>
<td>40 (35.40)</td>
<td>1 (0.82)**†††</td>
<td>58.89, $p &lt; .001^a$</td>
</tr>
<tr>
<td>NPS Sleep, $n(%)$</td>
<td>17 (16.50)</td>
<td>47 (41.59)**</td>
<td>15 (12.30)**††</td>
<td>32.02, $p &lt; .001$</td>
</tr>
<tr>
<td>NPS Appetite, $n(%)$</td>
<td>6 (5.83)</td>
<td>11 (9.73)</td>
<td>3 (2.46)</td>
<td>5.52, $p = .056^a$</td>
</tr>
</tbody>
</table>

Bonferroni-adjusted post hoc $p$-values in comparison to cluster 1: *$p < .025$, **$p < .005$, ***$p < .0005$.
Bonferroni-adjusted post hoc $p$-values in comparison to cluster 2: †$p < .025$, † †$p < .005$, † † †$p < .0005$.
aFisher’s exact test used. A + Classified into the Alzheimer’s continuum, NPS Neurobehavioral changes, OBJ Subtle Objective Cognitive Dysfunction, SCD subjective cognitive decline.

Percentages relate to the proportion of participants who are classified as A + and/or show the clinical features that are associated with NIA-AA stage 2 per cluster. A + Classified into the Alzheimer’s continuum, Att attention, Exec executive function, Lan language, Mem memory, OBJ subtle objective cognitive dysfunction, Oth other, Pln planning, SCD subjective cognitive decline, VIS visuospatial abilities, WM working memory.

**Differences in AD biomarkers between groups**

The symptoms that most strongly differentiated the clusters were OBJ in memory, language, executive function and working memory as well as SCD in the memory domain. We therefore classified all participants into four different groups based on the presence (+) or absence (-) of these features: 1) SCD-OBJ- ($n = 84$), 2) SCD + OBJ- ($n = 88$), 3) SCD- OBJ+ ($n = 73$) and 4) SCD + OBJ+ ($n = 93$).

The groups differed significantly with regard to ptau181 ($F(3) = 2.85, p = .037, \eta^2 = .03$) and Aß42/ptau181 ratio ($F(3) = 2.95, p = .033, \eta^2 = .03$). Tukey-adjusted post-hoc tests revealed that the group of SCD + OBJ + had a significantly lower Aß42/ptau181 ratio compared to the SCD- OBJ- group ($p = .028$). However, after correcting for age, the effects lost significance (ptau181: $F(3) = 1.28, p = .282, \eta^2 = .01$; Aß42/ptau181 ratio: $F(3) = 1.03, p = .380, \eta^2 = .01$). There were no group differences for Aß42/40 and total tau. The age-corrected results are plotted in Fig. 2.
Boxplots with jitter points depicting age-corrected differences between the groups SCD- OBJ-, SCD + OBJ-, SCD- OBJ+ and SCD + OBJ+ with regard to 2A) Aβ42/40 ratio; 2B) levels of total tau; 2C) levels of ptau181 and 2D) Aβ42/ptau181 ratio. Note. Classification into groups is based on OBJ in memory, language, executive function or working memory and SCD in the memory domain.

When classifying the groups based on SCD and OBJ in the memory domain only, the observed group differences were more robust, indicating the relevance of subjective and objective impairment specifically in the memory domain. Prior to correction for age, groups differed in terms of Aβ42/40 ratio (F(3) = 4.49, p = .004, η² = .04), with post-hoc tests revealing that the group of SCD + OBJ+ had significantly lower levels of Aβ42/40 compared to the SCD- OBJ- (p = .004) and SCD + OBJ- (p = .038) groups. However, this effect lost significance after correcting for age (F(3) = 1.67, p = .173, η² = .01). There were age-corrected group differences for ptau181 (F(3) = 3.32, p = .020, η² = .03), with Tukey-corrected post-hoc tests revealing that the group of SCD + OBJ- had significantly lower levels of ptau181 compared to the SCD- OBJ+ group (p = .028). With regard to the ratio of Aβ42/ptau181 (F(3) = 3.63, p = .013, η² = .03), the group of SCD- OBJ- showed significantly higher levels compared to SCD + OBJ+ (p = .047). The results are shown in Fig. 3. In general, visual inspection revealed a pattern of increasing pathology with increasing symptomatology.

**DISCUSSION**

Based on our operationalization of the symptomatic features of OBJ, SCD and NPS, we were able to identify three distinct participant clusters. In cluster 1, the rate of individuals on the Alzheimer's continuum was highest. Close to 50% of A+ cases is at the upper limit of prevalence rates observed in unselected and unimpaired samples in that age range. A recent meta-analysis by Jansen et al. reported CSF amyloid abnormality prevalence rates of approximately 20%-44% for cognitively healthy individuals aged 60–75 and approximately 20%-49% for individuals with subjective cognitive decline in the same age range [28]. The majority of participants in cluster 1 showed OBJ mainly in the domains of memory, language, executive functioning and working memory as well as SCD in the memory domain. These findings highlight the particular combination of OBJ and SCD as indicative of classification into the Alzheimer's continuum, where SCD reflects the individual experience of OBJ. Associations between OBJ in the observed domains and AD-related pathologic changes have also been observed in other studies in cognitively unimpaired individuals [29, 30].

Cluster 2 was characterized by SCD and, to a lesser degree, NPS, while OBJ was rare. With 26% A+ cases, there was no clear overrepresentation of individuals in the Alzheimer's continuum in this cluster compared to what is expected in cognitively unimpaired individuals in the respective age range [28]. This
suggests that SCD in this cluster might be more associated with behavioral symptoms (NPS) and not with A+. While the rate of A+ was lower compared to cluster 1 and did not differ from cluster 3, 26% A+ cases is still a relevant proportion that may have driven symptom manifestation. The most prevalent NPS in this cluster were irritability and sleep disturbances.

Cluster 3 grouped a sample with a low rate of participants who showed SCD, OBJ and NPS and with an A+ rate that is expected for that age range in unimpaired controls [28].

When classifying participants by OBJ in either memory, language, executive function or working memory and SCD in the memory domain - the symptomatic features mainly differentiating the clusters - we observed a lower ratio of Aβ42/ptau181 in participants who showed OBJ and SCD simultaneously compared to those who showed neither. However, this effect was confounded by age. According to the NIA-AA workgroup's definition of stage 2, OBJ as well as SCD may occur in any cognitive domain, not exclusively in the memory domain. However, there is evidence that both, objective decline in memory and subjective decline in memory are specifically associated with AD pathology [11, 12, 27, 31, 32]. When comparing groups based on SCD and OBJ in the memory domain only, we observed a higher level of ptau-181 in participants showing OBJ only compared to those with SCD only. The ratio of Aβ42/ptau181 was lower in participants with OBJ and SCD combined compared to participants showing neither SCD nor OBJ. We observed a general visual trend for increasing pathology with increasing symptomatology for all biomarkers.

There has been a longstanding debate about whether SCD indicates very early effects of AD pathology on cognition, as proposed by the NIA-AA stage 2 concept, or whether SCD is mainly a correlate of psychiatric symptoms. The argument for the latter stems from the frequently observed cross-sectional correlation of SCD with depressive symptoms rather than with objective cognitive performance [33–35]. Our data provide evidence for both by identifying two clusters that each support one of the two assumptions. Cluster 1 appears to reflect the stage 2 concept through the combination of SCD with OBJ and A+ enrichment, whereas cluster 2 supports the association with behavioral features, but not with OBJ or A+ enrichment. Nevertheless, there was a substantial rate of A+ cases in cluster 2, which may have at least partly driven the manifestation of SCD and NPS observed in this cluster. This further highlights the heterogeneity of causes underlying SCD. Unfortunately, in our data, there seems to be no distinction of SCD domain patterns between these two clusters. As such, the level of the objective cognitive performance discriminates between SCD in relation to A+ and SCD in relation to other causes.

By definition, SCD requires objective performance in the unimpaired range, and identification of OBJ on cross-sectional testing in an individual person is challenging. Either very robust and sensitive tests, which provide a reliable measure for cross-sectionally detecting performance in the lower range of normal; or longitudinal testing with reliable identification of an individual downward slope are required [36–38]. However, since biomarker technologies are moving to easily accessible plasma, the sensitivity of SCD [39] can be used in combination with biologically obtained specificity.
Limitations

Our study has limitations. First, it needs to be stressed that the majority of the sample was enrolled into the DELCODE study because of the presence of SCD, leading to an overrepresentation in comparison to samples unselected for SCD. Further validation of the findings from our cluster analysis will be necessary in other samples of cognitively healthy individuals to evaluate the generalizability of our findings.

Additionally, we used cross-sectional data only. We operationalized the criterion of OBJ by choosing a cut-off that allows for the detection of subtle, but observable dysfunction within a common range of normal performance. The advantage of this approach is that longitudinal testing is not required. Replication of our results with a longitudinal criterion of transitional cognitive decline will increase comparability with previous stage 2 operationalization approaches [40, 41].

Another limitation is the assessment of NPS using the NPI-Q, which was originally developed to measure NPS in patients with dementia [23]. It is possible that subtle changes in behavior are not detected by this instrument. In addition, the NPI-Q inquires about the presence of NPS in the past month, which is a short timeframe that can be influenced by external events or current health status.

Conclusion

This analysis focused on symptomatic features that characterize NIA-AA stage 2 in a sample of cognitively healthy individuals aged 60 or older with and without biomarker evidence for AD. In conclusion, our results indicate that the combination of SCD and OBJ, particularly in the memory domain, was associated with enrichment for AD pathology.

Abbreviations

AD Alzheimer’s disease

A+ Individuals with evidence of AD pathology according to CSF Aβ42/Aβ40 ratio

A- Individuals without evidence of AD pathology according to CSF Aβ42/Aβ40 ratio

CERAD Consortium to Establish a Registry for Alzheimer’s Disease

DELCODE DZNE-Longitudinal Cognitive Impairment and Dementia Study

CSF Cerebrospinal fluid

EXEC Executive function factor score derived from confirmatory factor analysis

DZNE German Centre for Neurodegenerative Diseases (Deutsches Zentrum für Neurodegenerative Erkrankungen)

IMBIE Institute for Medical Biometry, Informatics and Epidemiology
LANG Language factor score derived from confirmatory factor analysis

MBI Mild behavioural impairment

MCI Mild cognitive impairment

MEM Memory factor score derived from confirmatory factor analysis

NIA-AA National Institute on Aging and Alzheimer's Association

NPI-Q Neuropsychiatric Inventory

NPS Neuropsychiatric symptoms

OBJ Subtle objective cognitive dysfunction

SCD Subjective cognitive decline

SCD-I Subjective cognitive decline - Interview

SPSS Statistical Package for the Social Sciences – 28th edition

TSCA Two-step cluster analysis

VIS Visuospatial abilities factor score derived from confirmatory factor analysis

WM Working memory factor score derived from confirmatory factor analysis

**Declarations**

**Ethics approval and consent to participate**

The study protocol was approved by the ethical committees of the medical faculties of all participating sites: the ethical committees of Berlin (Charité, Universitätsmedizin Berlin), Bonn, Cologne, Göttingen, Magdeburg, Munich (Ludwig-Maximilians-University), Rostock, and Tübingen. The process was led and coordinated by the ethical committee of the medical faculty of the University of Bonn. The registration number of the trial at the ethical committee in Bonn is 117/13. All participants provided written informed consent.

**Consent for publication**

Not applicable.

**Availability of data and materials**
The data that support this study are not publically available, but may be provided upon reasonable request.

**Competing interests**

The authors declare that they have no competing interests with regard to the content of the manuscript.

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**Contributions**

FJ, KB, MH, CL, OP, JP, RP, NRK, AS, AS, ST, JW, SW, MW, and ED contributed to the overall design and implementation of the study. FB, CB, IF, KF, SDF, WG, DJ, IK, LK, MM, BSR, AR, LS, AKS, LSS, EJS, EJS and MW were responsible for the conduction of the study at the respective sites. FJ, FB, ED, IF, KF, MH, LK, NRK, AS, AS, MW and SW were responsible for methodological core central data management and data analyses. LS was responsible for statistical analyses in this manuscript. All authors contributed to the drafting of the manuscript and approved the final version.

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**References**


**Figures**
Figure 1

Distribution of symptomatic features per cluster.
Figure 2

Biomarker differences between symptomatic groups

Figure 3
Biomarker differences between symptomatic groups based on the memory domain