Serum Beta-Synuclein Is Higher in Down Syndrome and Precedes Rise of pTau181

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This exploratory case–control study investigates the synaptic marker beta-synuclein in serum and plasma pTau181 in adults with Down syndrome (DS) with (sDS, $n = 14$) and without (aDS, $n = 47$) clinical symptoms of Alzheimer disease (AD) as well as euploid controls ($n = 23$). Beta-synuclein was higher in aDS and more pronounced in sDS ($p < 0.0001$), whereas pTau181 was only higher in sDS ($p < 0.0001$). Both markers showed good discriminatory power (area under the curve > 0.90) to distinguish symptomatic from asymptomatic AD. The data indicate that synaptic alterations belong to the earliest AD-associated events in DS and highlight the value of serum beta-synuclein as a potential early marker of AD.

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Down syndrome (DS) is characterized by developmental intellectual disability and originates from the triplication of the defining sequences on chromosome 21. In most cases of DS, the triplication includes the Alzheimer disease (AD)-associated amyloid precursor protein gene (*APP*). Thus, DS represents a form of genetic early onset AD, and most adults with DS develop AD after the age of 40 years.[1] AD is the main cause of death in adult individuals with DS.[2] The study of brain-derived biomarkers in peripheral venous blood is a minimally invasive procedure and enables the characterization of AD-associated pathophysiological processes in both DS and AD in general. An improved understanding of these processes, especially in the asymptomatic phase, is of fundamental importance to develop early diagnostic biomarkers and treatment strategies for AD. Recently, the measurement of tau protein phosphorylated at T181 (pTau181) and neurofilament light chain (NfL) in blood, markers of amyloid/tau pathology and neurodegeneration, has been shown to reflect AD-associated alterations in DS and has shown good diagnostic performance to diagnose symptomatic AD in DS.[3,4] Alterations of pTau181 seem to appear with symptom onset, whereas NfL already changes at an age of 30 years.[3,4] Synaptic degeneration is another early pathological hallmark of AD strongly related to cognitive dysfunction,[5] but information on synaptic markers in DS and their relation to other AD-related pathological alterations is scarce.

Beta-synuclein is a presynaptic protein and a synaptic biomarker candidate in body fluids. Its expression is reduced in AD brains, and we recently observed higher beta-synuclein levels in both cerebrospinal fluid (CSF) and blood of AD patients.[6–8] Because CSF collection is ethically difficult to justify in DS individuals, the possibility to measure beta-synuclein in blood makes it ideally suited to study synaptic alterations in DS.

In this explorative case–control study, we investigated the synaptic marker beta-synuclein in serum samples of adults with DS in comparison with euploid healthy controls (HC). We divided the DS cohort into individuals without clinical signs of AD (asymptomatic DS [aDS]) to uncover early alterations and DS with symptomatic AD (sDS). Beta-synuclein levels were compared with plasma pTau181 as an already established marker of AD-related

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amyloid/tau pathology. We used correlation analyses to investigate associations with age and cognitive performance and receiver operating characteristic curve analysis to determine diagnostic performance.

Subjects and Methods

Adults with DS were recruited at the Department of Neurology, Ludwig Maximilian University of Munich (LMU Munich) between 2017 and 2021 and euploid HCs without neurological disease at the Departments of Neurology, LMU Munich (2020) and University Hospital Ulm (2012–2019).

Trisomy 21 was confirmed by chromosome analysis. Cognitive performance of adults with DS was assessed by trained neuropsychologists using the Cambridge Cognitive Examination for Older Adults with Down Syndrome (CAMCOG-DS).[9] Four individuals with DS did not complete the whole CAMCOG-DS test battery. We therefore calculated in all individuals the achieved number of points relative to the number that could maximally be reached (CAMCOGDS%). Intellectual disability (ID) was stratified according to Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V) criteria into mild, moderate, severe, and profound based on the individuals' best-ever level of functioning as obtained from detailed interviews with caregivers, neuropsychological assessment, behavioral observation, and review of previous medical records.

AD diagnosis was reached independently by two neurologists adhering to a predefined diagnostic algorithm.[10] In brief, changes in cognition, behavior, and activities of daily living were assessed based on patient and caregiver information (including the Cambridge Examination for Mental Disorders of Older People with Down Syndrome and Others with Intellectual Disabilities). Subsequently, differential diagnoses were excluded via neurological and neuropsychological examination and laboratory blood tests (full blood count; thyroid, liver, and kidney function; vitamins D, B1, B6, and B12; homocysteine; folate). Then, cognitive abilities were quantified via a standardized neuropsychological examination (CAMCOG-DS, highest and current level of disability according to DSM-V), which led to the syndromal diagnosis of dementia. AD was verified using A/T/N criteria (A, amyloid; T, tau; N, neurodegeneration) where possible (CSF: tau, phospho-tau, Abeta1–42/1–40 ratio; magnetic resonance imaging: regional atrophy, florbetaben and PI-2620 positron emission tomography).

All individuals or their legal proxies provided written informed consent for their samples to be used in this study, and it was approved by the local ethics committees (Munich #17–126, Ulm #20/10). Characteristics of individuals with DS and HC are listed in the Table.

Beta-synuclein levels were measured in serum with immunoprecipitation–mass spectrometry as recently described.[8] Plasma pTau181 was determined using the Quanterix Simoa pTau-181 V2 Advantage Kit (Quanterix, Billerica, MA) according to the manufacturer's instructions. We used plasma instead of serum for pTau181 for better comparability with other publications mainly reporting plasma pTau181.

Statistical analyses were performed with Prism 8.3.0 (GraphPad Software, San Diego, CA) and SPSS Statistics 26.0 (IBM, Armonk, NY). Normal distribution of data was tested by Shapiro–Wilk test. If not stated otherwise, correlation analysis was performed with Spearman rank correlation coefficient. Statistical tests are described in the Table and the figure legend. Age and sex were included as covariates in group comparisons. A p value < 0.05 was regarded as significant.

Results and Discussion

We investigated a total of 61 individuals with DS comprising 47 with aDS and 14 with sDS and 23 controls (for demographics, see Table). There was a trend toward lower frequency of female individuals among sDS subjects compared with the other groups ($p = 0.06$). Individuals with aDS were significantly younger than sDS subjects and HC (see Table). Both age and sex were included as covariates in statistical analyses. As expected, sDS individuals showed a lower performance on the CAMCOG-DS than aDS subjects ($p = 0.006$). Cognitive performance in individuals with DS has previously been shown to change already within the fifth decade of life.[4] The distribution of ID levels was comparable between aDS and sDS ($p = 0.81$). Serum beta-synuclein and plasma pTau181 levels did not correlate with age in HC ($r = -0.04$, $p = 0.85$ and $r = 0.28$, $p = 0.19$). In agreement, no or weak positive correlation of beta-synuclein and pTau181 with age has been described in previous publications,[8,11,12] indicating a low contribution of age-related compared with disease-related changes. Both markers correlated strongly with each other in sDS individuals ($r = 0.82$, $p = 0.0005$) but not in aDS subjects ($r = 0.10$, $p = 0.52$) and HC ($r = 0.41$, $p = 0.05$).

We observed higher serum beta-synuclein levels in aDS subjects (11.3pg/ml, range = 10.1–14.3pg/ml, $p < 0.0001$), which was more pronounced in sDS individuals (23.7pg/ml, range = 17.8–33.4pg/ml, $p < 0.0001$ vs HC and aDS), compared with HC (7.3pg/ml, range = 6.6–9.1pg/ml; Fig). In contrast, plasma pTau181 levels were higher in sDS only (5.88pg/ml, range = 3.34–7.38pg/ml vs 1.37pg/ml,

TABLE. Characteristics of Individuals with Down Syndrome and Controls

	n (% F)	Age, yr[a]	CAMCOG-DS(%)[a]	Level of ID				Serum Beta-Synuclein, pg/ml[a]	Plasma pTau181, pg/ml[a]
				Mild	Moderate	Severe	Profound		
HC	23 (69.6%)	41.1 (32.4–54.2)	n.a.	n.a.	n.a.	n.a.	n.a.	7.34 (6.63–9.10)	1.37 (0.89–1.68)
aDS	47 (51.1%)	28.0 (23.0–31.0)[b]	61.5 (52.3–72.5)	24 (52%)	21 (46%)	1 (2%)	0 (0%)	11.3 (10.1–14.3)[b]	1.42 (1.04–1.81)
sDS	14 (28.6%)	55.0 (50.0–58.0)[c]	45.0 (26.3–61.9)	6 (46%)	7 (53%)	0 (0%)	0 (0%)	23.7 (17.8–33.4)[b,c]	5.88 (3.34–7.38)[b,c]
p	0.06	<0.0001	0.006	0.81				<0.0001	<0.0001

Sex distribution and level of ID between groups was compared with Fisher exact test and age distribution with Kruskal-Wallis test and Dunn post hoc test. CAMCOG-DS is given as a percentage of the maximal achievable score and was compared with Mann–Whitney test. Serum beta-synuclein and plasma pTau181 levels were log2-transformed, and groups were compared with multiple linear regression including age and sex as covariates. Missing values: CAMCOG-DS: sDS, n = 1; aDS, n = 4; level of ID: sDS, n = 1; aDS, n = 1.

[a]Median (interquartile range).

[b]$p < 0.0001$ vs HC.

[c]$p < 0.0001$ vs aDS.

AD = Alzheimer disease; aDS = Down syndrome without AD (asymptomatic); CAMCOG-DS = Cambridge Cognitive Examination for Older Adults with Down Syndrome; F = female; HC = healthy controls; ID = intellectual disability; n.a. = not available; sDS = Down syndrome with AD (symptomatic).

range = 0.89–1.68pg/ml in HC, $p < 0.0001$) but not aDS (1.42pg/ml, range = 1.04–1.81pg/ml). Higher beta-synuclein and pTau181 levels in sDS are consistent with previous observations for both markers in sporadic AD patients[6–8,11] and for pTau181 in sDS.[3] The higher levels of beta-synuclein but not pTau181 in aDS indicate that alterations of beta-synuclein precede pTau181. To estimate a time course of changes, we correlated beta-synuclein and pTau181 levels with age in DS subjects and HC. The regression lines of plasma pTau181 levels in HC and DS subjects diverged at the age of 39 years, which is in good agreement with data for plasma and CSF pTau181 from previous reports in DS consistently demonstrating alterations at approximately 40 years of age.[3,4,13] Serum beta-synuclein levels seem to increase already at the age of approximately 27 years. These data could indicate that synaptic alterations are one of the earliest AD-related events in DS, coinciding with amyloid pathology, which is also estimated to appear within an age range of 20 to 30 years[3,4,14] and also possibly precedes elevation of NfL levels in CSF and blood as a marker of neurodegeneration.[4,13]

Blood levels of pTau181 have been characterized as a very specific marker for AD pathology, correlating with both amyloid and tau pathology.[15–17] The observed changes of plasma pTau181 in sDS in our study are therefore expected to reflect mainly AD-related alterations. This is further supported by the degree of pTau181 changes in sDS (4.3-fold), which is in the same range as for sporadic AD (≈3.5-fold)[11,16] and sDS in other studies (3.5-fold).[3] Synaptic alterations, however, might not only be caused by AD pathology. Serum beta-synuclein levels in our study showed a much more pronounced increase in sDS (3.2-fold) than in sporadic AD (1.4-fold) as compared to healthy controls.[8] Moreover, beta-synuclein was already higher in aDS (1.5-fold). This could indicate a cumulative effect of AD-related mechanisms and synaptic alterations caused by triplication of chromosome 21. Beta-synuclein is highly expressed in the hippocampus,[18] and hippocampal differences are described in DS at an early age,[4,19] which might be accompanied with altered beta-synuclein release from hippocampal synapses. Our previous observations in sporadic AD patients, showing beta-synuclein changes already in the early disease phase,[7,8] point to beta-synuclein as a prodromal AD marker in aDS rather than a developmental alteration. However, beta-synuclein is still a relatively new synaptic biomarker candidate, and its relation to different pathological changes needs to be studied in more detail to confirm this hypothesis. Especially the question of whether the beta-synuclein changes originate from developmental

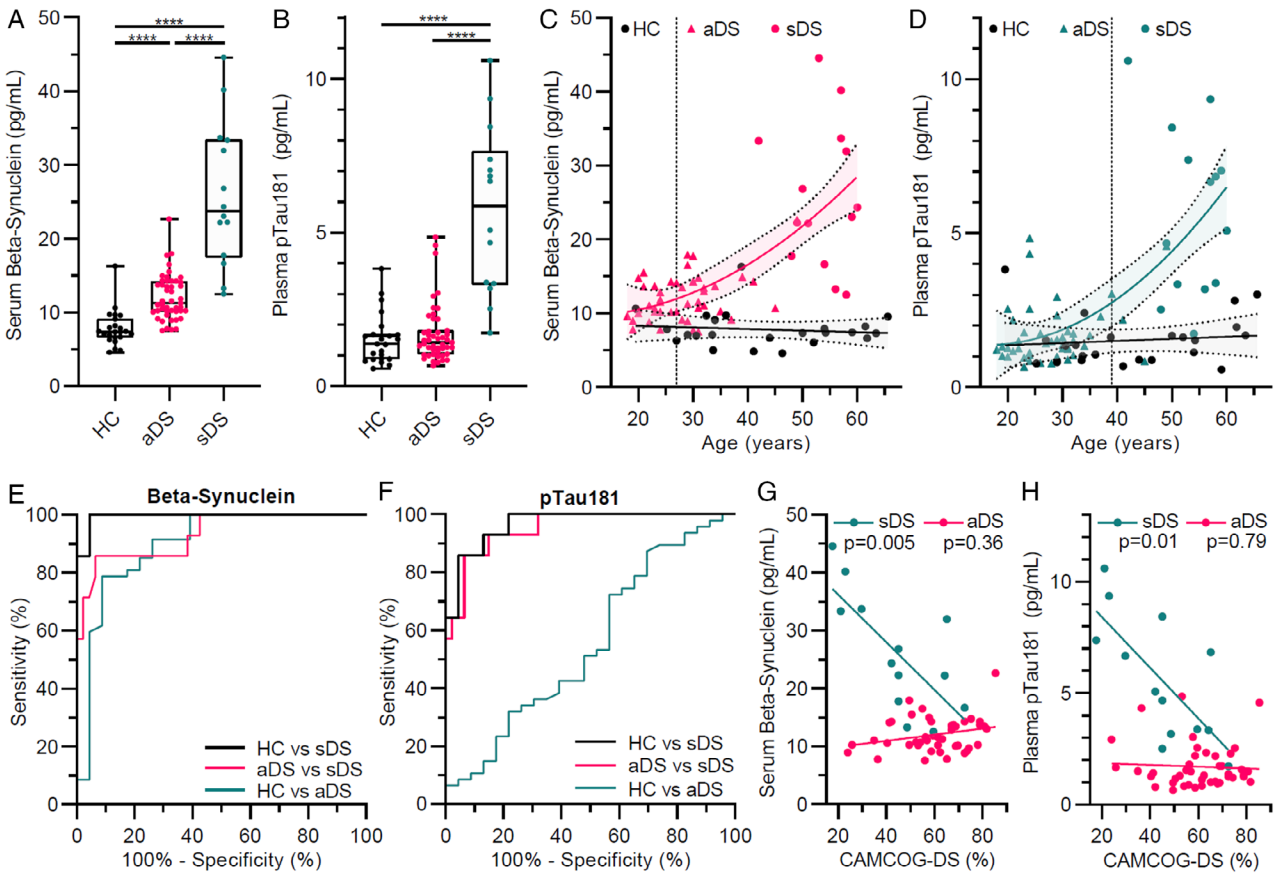

FIGURE: Group comparisons, correlation analyses, and diagnostic performance of serum beta-synuclein and plasma pTau181. (A) Serum beta-synuclein and (B) plasma pTau181 levels were compared between euploid healthy controls without neurological disease (HC, *n* = 23) and adults with Down syndrome (DS) diagnosed with Alzheimer disease (symptomatic, sDS, n = 14) or without AD (asymptomatic, aDS, n = 47). Groups were compared with multiple linear regression after log2 transformation of biomarker levels and with age and sex as covariates. Boxes are median and interquartile range; whiskers are minimum and maximum. ****$p < 0.0001$. (C) Scatter plot of serum beta-synuclein and (D) plasma pTau181 levels with age. Linear (HC) and second order polynomial regression (DS) was performed to estimate the time course of biomarker changes indicated by the solid lines (with 95% confidence interval [CI]). The dotted vertical line indicates the age where the two regression lines diverge (point where the 95% CIs no longer overlap). Dots and triangles are individual values. (E, F) Receiver operating characteristic curve analysis of (E) beta-synuclein and (F) pTau181 levels for discrimination of the different groups. Beta-synuclein: HC vs sDS, area under the curve (AUC) = 0.99, 95% CI = 0.98–1.00; aDS vs sDS, AUC = 0.94, 95% CI = 0.85–1.00; HC vs aDS, AUC = 0.90, 95% CI = 0.81–0.99. pTau181: HC vs sDS, AUC = 0.97, 95% CI = 0.92–1.00; aDS vs sDS, AUC = 0.95, 95% CI = 0.90–1.00; HC vs aDS, AUC = 0.55, 95% CI = 0.40–0.70. (G, H) Spearman partial correlation analysis of (G) beta-synuclein and (H) pTau181 levels with achieved number of points relative to the number that could maximally be reached on the Cambridge Cognitive Examination for Older Adults with Down Syndrome (CAMCOG-DS), including age as covariate. Beta-synuclein: aDS, $r = 0.15$; sDS, $r = -0.75$. pTau181: aDS, $r = 0.04$; sDS, $r = -0.69$. Dots are individual values, and lines are from linear regression.

differences or from AD pathology requires the study of very young individuals with DS, in whom AD pathology is not yet present.

Both beta-synuclein and pTau181 showed very good discriminatory power to distinguish DS subjects with symptomatic AD from asymptomatic individuals (area under the curve [AUC] = 0.94 and 0.95) and from HC (AUC = 0.99 and 0.97; see Fig E, F). This is in agreement with data reported by Lleó et al for plasma pTau181.[3] In addition, serum beta-synuclein levels could also distinguish aDS subjects from controls with high accuracy (AUC = 0.90; see Fig E). The stepwise increase of beta-synuclein might enable the definition of different stages of AD development in DS that might be helpful in future clinical practice to adjust treatment regimens when preventive or disease-modifying drugs are available. Additional studies are required to define robust cutoff values, which we did not calculate here due to the exploratory design and low number of individuals.

There was a strong correlation of serum beta-synuclein ($r = -0.75$) and plasma pTau181 ($r = -0.69$) with cognitive performance in sDS individuals measured by the CAMCOG-DS (see Fig G, H) but not in asymptomatic individuals. This is in agreement with the finding that CAMCOG-DS performance in nondemented individuals is primarily based on the degree of ID.

The limitations of this exploratory study are the low number of HC and sDS individuals and the cross-sectional design, which makes assumptions to temporal changes difficult. However, temporal estimates from genetically determined neurodegenerative diseases have successfully been proven in the past,[20,21] and our data definitely represent a time point in aDS where beta-synuclein but not pTau181 is elevated.

In conclusion, our data indicate that synaptic alterations belong to the earliest AD-associated events in DS and precede elevation of pTau181. They clearly show the added value of serum beta-synuclein to the already existing panel of central nervous system–derived blood markers. Beta-synuclein and pTau181 show equally high performance in diagnosing symptomatic AD in DS, and beta-synuclein might also be helpful to define presymptomatic stages of AD development. The temporal estimates on synaptic alterations in our study are also of great relevance for sporadic and autosomal dominant AD and should be confirmed in longitudinal studies.

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Author Contributions

P.O., O.W., J.L., and M.O. contributed to the conception and design of the study; all authors contributed to the acquisition and analysis of data; P.O., O.W., J.L., and M.O. contributed to drafting the text or preparing the figure.

Potential Conflicts of Interest

M.O., P.O., and S.H. are coapplicants on a filed patent application for beta-synuclein measurement in blood.

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