Imaging and plasma biomarkers for pathological accumulation in Down syndrome

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Abstract

Down syndrome is characterized by triplication of chromosome 21, leading to early-onset Alzheimer disease pathology, with nearly all individuals with Down syndrome developing amyloid and tau pathology. In the new era of amyloid modifying therapies, it is vital to identify early biomarkers for Alzheimer disease (AD) pathology in Down syndrome. Striatal amyloid may begin to accumulate sooner than cortical amyloid in Down syndrome. Tau phosphorylation at specific sites, including 217, can be quantified in plasma and may represent an important mechanistic step in the development of tau pathology. This study had two aims: 1. To compare the relative age at increase of multiple biomarkers (cortical amyloid, striatal amyloid, plasma pTau217 and summary tau pathology) 2. To test whether plasma pTau217 can identify both the current presence and likely future accumulation of amyloid and tau pathology.

To identify optimal biomarkers for early intervention, we examined longitudinal cortical and striatal amyloid PET, plasma pTau217, and tau PET in 328 individuals with Down syndrome enrolled in the Alzheimer Biomarker Consortium – Down Syndrome study. To compare the timing of biomarker changes, we modeled longitudinal biomarkers using generalized additive mixed models relative to age. We used receiver operating characteristic curve analysis to identify thresholds for both current and likely future accumulation of amyloid and tau pathology. For all comparisons, we used age as the null model, performing Delong tests to evaluate the performance of age relative to biomarker-based prediction.

Imaging biomarkers increased around 40 years old, with plasma pTau217 increasing somewhat later than the three PET biomarkers. Striatal amyloid increased before cortical amyloid in some participants; however, this was not uniform across individuals. If an individual was classified as a reliable accumulator with one biomarker, he or she was likely to be a reliable accumulator in other biomarkers. Age was as sensitive as plasma pTau217 in its ability to both detect preclinical Alzheimer disease pathology and predict near future accumulation of both amyloid and tau.

These results suggest that all adults with Down syndrome should be screened for Alzheimer disease pathology starting shortly before age 40 and considered for clinical trials. Age alone was as effective at detecting both current pathology and likely future accumulation as plasma pTau217. Because this disease is so closely concurrent with age in individuals with Down Syndrome, plasma pTau217 may not provide more diagnostic benefits than age.

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Introduction

Down syndrome is primarily caused by chromosome 21 triplication, which includes the genes encoding the amyloid precursor (*APP*) protein and dual-specificity tyrosine-phosphorylated and regulated kinase 1A (*DYRK1A*). The extra copy of the *APP* gene causes amyloid overproduction throughout the lifespan, leading to the canonical pattern of Alzheimer Disease pathology[1–3]. Tau aggregation may be promoted by a dose-dependent response to *DYRK1A*[4]. Nearly all individuals with Down syndrome have the hallmark Alzheimer Disease pathology – amyloid plaques and tau tangles by age 40[2,5], with nearly full penetrance of dementia reported in individuals over 60[6]. This genetic manifestation of Alzheimer Disease yields a pattern of pathological spread that is relatively concordant with age[1,7–9].

Understanding the temporal nature of Alzheimer Disease pathological spread is critical, as clinical interventions must be timed for maximum efficacy[2]. Successful amyloid removal therapy trials outside of Down syndrome have highlighted that earlier intervention yields greater clinical benefit[10,11]. Clinical intervention is highly important in this population, as Alzheimer Disease is currently the primary limitation for extending the lifespan and quality of life of people with Down syndrome[6]. Thus, a crucial focus for individuals with Down syndrome is identifying biomarkers that have the greatest utility for detecting early Alzheimer Disease pathology and determining the optimal window for clinical screening and therapeutic interventions.

In individuals with Down syndrome, amyloid accumulates first in the striatum and frontal lobe[12–15], although this initial striatal binding is only observed with Pittsburgh Compound B (PiB) as the PET (positron emission tomography) radiotracer[13,14]. Individuals with Down syndrome also develop substantial cortical amyloid burden, similar to observations in autosomal dominant

forms of Alzheimer Disease (ADAD), and sporadic Alzheimer Disease [16,17], but at a relatively young age[12,15,18–21]. Cross-sectional studies in adults with Down syndrome report elevation in cortical amyloid compared to neurotypical controls between the ages of 35 and 42 years[12,19,22–24]. Longitudinal studies with PiB suggest striatal amyloid increases roughly 3 years prior to cortical amyloid in Down syndrome[13,15]. Striatal amyloid accumulates at nearly twice the rate as observed in neurotypical individuals[14]. Cross-sectional measures of burden and longitudinal rates of change in both cortical amyloid and striatal amyloid are promising biomarkers for both identifying adults with Down syndrome early in the Alzheimer Disease pathological continuum and quantifying intervention efficacy[25].

Amyloid accumulation is thought to trigger a cascade of events leading to tau pathology, including the hyperphosphorylation of tau, which disrupts its normal function in stabilizing microtubules[26]. In Down syndrome, the overexpression of *DYRK1A*, the gene for which is on chromosome 21, may promote tau hyperphosphorylation by directly phosphorylating tau at key residues, priming it for further phosphorylation by *GSK-3β*, and contributing to a feedback loop involving amyloid and *RCAN1* that amplifies tau dysregulation and aggregation[4]. This hyperphosphorylation promotes tau aggregation into neurofibrillary tangles[27].

Tau can be phosphorylated at more than 40 amino acid sites, many of which can now be quantified in plasma[28]. Notably, *DYRK1A* phosphorylates tau specifically at sites 212 and 217, which may lead to elevated levels of pTau212 and pTau217 in individuals with Down syndrome[19,29]. In sporadic Alzheimer Disease, longitudinal work suggests that approximately 70% of the association between amyloid PET rate of change and tau PET rate of change is mediated by soluble tau[26], suggesting that tau phosphorylation provides an important mechanistic step in the Alzheimer Disease pathological continuum. Cross-sectional work in Down syndrome identifies a stronger correlation between plasma pTau217 and tau PET in amyloid positive individuals than between plasma pTau217 and amyloid PET[30], which is opposite to what has been reported in sporadic Alzheimer Disease[31]. It is possible that mechanistic differences in the development of Alzheimer Disease pathology exist due to the triplication of chromosome 21 in Down syndrome, leading to differences in the differential utility of specific tau phosphorylation sites[32]. This suggests that the choice of biomarker for both inclusion criterion and study endpoints may be dependent on the form of Alzheimer Disease.

Although it is possible to quantify many different tau phosphorylation sites, plasma pTau217 has seen wide adoption in recent years[27,28,31,33–38]. Plasma pTau217 demonstrates high efficacy at detecting both the presence of amyloid plaques[30,38] and tau tangles[39] across forms of Alzheimer Disease. In individuals with Down syndrome, cross-sectional studies suggest it increases between ages 36 and 40 years[19,40]. When targeting clinical intervention for Alzheimer Disease, plasma pTau217 may represent a powerful and relatively non-invasive approach to determining both the current and future state of Alzheimer Disease pathology.

Studies of tau PET in Down syndrome highlight the relative temporal proximity of tau accumulation to cortical amyloid accumulation[7,19,41–43]. At autopsy, the pattern of spatial spread of tau follows Braak staging, with early entorhinal, hippocampal and subcortical tau deposition in individuals around age 35 years, and full neocortical coverage by the mid-50's[21]. I*n vivo* work suggests that tau PET increases in individuals with Down syndrome between ages 37 and 41[7,19,41], often within 2 years of converting to amyloid positivity[42,43]. The apparent condensed timeframe of tau aggregation following amyloid deposition highlights the potentially narrow timeframe for which therapies that solely target amyloid may be beneficial for individuals with Down syndrome. Thus, the earliest possible identification of individuals who are likely to accumulate future amyloid is of the utmost importance.

To determine the optimal biomarker(s) for identification and the optimal window to start clinical intervention, we used longitudinal data to characterize the temporal pattern of cortical amyloid PET, striatal amyloid PET, plasma pTau217 and summary tau burden from tau PET relative to a participant's age. Given the importance in identifying individuals with early amyloid accumulation for clinical intervention and the potential benefits of using a relatively low-burden approach (plasma instead of PET imaging), we evaluated the ability of plasma pTau217 to identify both the current presence and likely future accumulation of amyloid and tau pathology, as measured by PET imaging. Because of the strong association between age and pathological development of Alzheimer Disease in Down syndrome[1,7–9], we compared the use of plasma pTau217 relative to a participant's age in assessing the likelihood of future pathology accumulation.

Materials and methods

The Alzheimer Biomarker Consortium – Down Syndrome (ABC-DS) is a multi-site study that enrolls adults with Down syndrome (≥25 years) and collects longitudinal clinical, imaging, and fluid biomarker data. For this longitudinal study, we included 328 people with Down syndrome (ABC-DS Data Release July 2024). In order to maximize sample size, we included all enrollees in ABC-DS who had completed at least one of the measures of interest (amyloid PET, tau PET, plasma pTau217). Individuals had completed some combination of amyloid PET (Longitudinal PiB = 84, Longitudinal AV45 = 49; Cross-Sectional PiB = 179, Cross-Sectional AV45 = 87), tau PET (Longitudinal = 50, Cross-Sectional = 220), and longitudinal plasma (N = 225). Detailed information about the overlapping nature of these biomarkers is presented as Supplemental Figure 1. We defined our reference population as individuals with Down syndrome below age 35 without detectable amyloid pathology on amyloid PET (< 18 Centiloids [CL]) and/or plasma pTau217 (< 0.478 pg/mL) rather than using sibling controls, as some biomarker measures can be systematically elevated or depressed in individuals with Down syndrome. Informed consent was obtained directly from participants when possible. If not possible, assent was obtained and informed consent was obtained from the participant's legally authorized representative. Study protocols were approved by centralized and local institutional review board of all ABC-DS sites.

Clinical Evaluation

Participants with Down syndrome visit ABC-DS sites every 16 months, at which point they receive a clinical status via consensus conference. Consensus conferences are based on neuropsychological assessments, medical and psychiatric history and interviews with informants[12]. Individuals receive a status of cognitively stable, mild cognitive impairment-Down syndrome (MCI-DS), dementia due to Alzheimer Disease, or unable to determine.

Plasma Sampling and Analysis

Plasma pTau217 concentration was measured using immunoassay on a Mesoscale Discovery platform (Lilly) using methods that have previously been described[30]. Biotinylated-IBA493 was used as a capture antibody and SULFO-TAG-4G10-E2 (anti-Tau) as the detector and samples

were diluted 1:2. The assay was calibrated with a synthetic P-tau217 peptide. *APOE* genotype was derived from the blood samples using KASP genotyping assays (LGC Genomics, Beverly, MA).

Amyloid- and Tau-PET Imaging and Processing

Amyloid PET was collected using either [^{11}C]-Pittsburgh Compound B (PiB) (N = 179) or [^{18}F]-AV45 (Florbetapir) (N = 87). Tau PET was collected using [^{18}F]-AV1451 (Flortaucipir) (N = 220). For PET registration, all participants also completed a 3T MRI scan within 2 years of the corresponding PET image.

PET images were aligned to FreeSurfer MR segmentations and then processed using a publicly available pipeline (PET Unified Pipeline; https://github.com/ysu001/PUP)[44,45]. Regional standard uptake value ratios (SUVRs) were calculated using the cerebellar cortex as reference region. Cortical amyloid burden was calculated as the arithmetic mean of the partial volume corrected SUVRs from the precuneus, superior frontal, rostral middle frontal, lateral orbitofrontal, medial orbitofrontal, superior temporal and middle temporal regions, and then standardized across tracers using the Centiloid scale[46]. The primary threshold for amyloid positivity was set at 18 CL[7,18], although we considered a range of values as many groups rely on different thresholds between 10 and 30 CL. Striatal amyloid burden was only considered in these analyses from amyloid PET scans obtained using PiB, as harmonization for this region has not been validated and the striatal binding pattern has been most acutely detected with PiB[13]. No published value for striatal positivity exists, so we considered a range of values from 1.10 – 1.55 SUVR. A summary measure of tau burden was calculated from tau PET based on the arithmetic mean of the partial volume corrected SUVRs from the amygdala, entorhinal cortex, inferior temporal region and lateral occipital cortex[44]. Tau positivity was set at 1.22 SUVR[44]

Statistical Analysis

Because our objective was to identify the optimal biomarker(s) to target individuals with Down syndrome who would most benefit from clinical intervention, we characterized the temporal pattern of cortical amyloid PET, striatal amyloid PET, plasma pTau217 and summary tau PET relative to a participant's age and then investigated the ability of each of these biomarkers to

forecast future pathological accumulation. We summarized participant demographics using the R package tableone[47]. All analyses were performed in R (v4.0).

Age at typical biomarker elevation

To compare the timing of amyloid PET, plasma pTau217 and tau PET changes, longitudinal biomarker levels were assessed relative to age and estimated years to symptom onset (EYO)[12,19,41]. EYO was calculated by subtracting participant age from the average age of symptom onset in Down syndrome (52.5 years) and is displayed for consistency with prior work[12,19,41]. Generalized additive mixed effect models with cubic regression splines (maximum 4 knots) were fitted for each biomarker, with age as the independent variable, an interaction with group membership (either reference population or older individual with Down syndrome), and a random effect for the individual. Super-threshold accumulation timing was estimated using 10,000 iteration bootstrap (resampling 80% of the data, with replacement), with confidence intervals based on the bootstrap distribution. The divergence age between the reference population, which included individuals with DS under the age of 35 and pathology negative, and aging individuals with DS was identified as the earliest age where 95% confidence intervals no longer overlapped. We conducted a supplemental analysis, performing robust linear regression and extracting weights with the package MASS before applying the weights to the generalized additive models and then proceeding with the bootstrap procedure (Supplemental Figure 3). To compare the measure variability, we performed a Levene's Test with post hoc Tukey test on baseline biomarker values normed to the reference population. A sensitivity analysis restricted modeling to only individuals who had received the PiB tracer for cortical amyloid PET uptake (Supplemental Figure 2).

Plasma pTau217 to predict PET positivity

Associations between plasma pTau217 and the three PET biomarkers of interest (cortical amyloid burden, striatal amyloid burden, summary tau) were evaluated using Spearman correlations and receiver operating characteristic curve (ROC) analysis. Because for some measures, wide ranges of positivity thresholds are employed[48], and for others, no published cut points for visual reads exist (e.g. striatal amyloid), we evaluated the ability of plasma pTau217 to predict PET positivity for a range of thresholds for each biomarker. As a null model comparison,

we applied the same approach using age instead of plasma pTau217. A combination of Youden index and visual inspection for multiple Youden peaks was used to identify the optimal cutpoint.

Defining Reliable Accumulation

To define a minimum noise threshold for each biomarker where individuals are likely to have higher levels of that biomarker at subsequent visits[25] we calculated the annualized rate of change for each biomarker in all amyloid negative individuals with Down syndrome under age 35 (our reference population) using linear regression. We defined reliable accumulation as the 95th percentile of the mean annualized rate of change, consistent with a previous study[25]. To visualize the relationship between annualized rate of change and baseline biomarker level, we generated scatter plots, fitting a generalized additive model with cubic regression spline and a maximum of four knots to the relationship between these two parameters.

Predicting Future Reliable Accumulation

To identify which individuals were reliable accumulators of the biomarker of interest, we first used ROC analysis with the baseline biomarker value as the variable of interest and the earlier derived classification of a reliable accumulator as the outcome variable. We evaluated the Youden index, again performing a visual inspection of Youden index relative to biomarker threshold to select the optimal threshold. We then evaluated the ability of plasma pTau217 to predict future reliable accumulation for all three PET biomarkers of interest, again applying ROC analysis. As a null model comparison, we applied the same approach using age as the variable of interest. To visualize the relationship between annualized rate of change and plasma pTau217 level / age at baseline, we generated scatter plots, fitting a generalized additive model with cubic regression spline and a maximum of four knots to the relationship between these two parameters. We compared the quality of prediction between plasma pTau217 and age using a Delong test. This analysis allowed us to infer the minimum threshold at which individuals are likely to accumulate more pathology in the future.

Results

Study participants (N = 328) were all diagnosed with Down syndrome and between the ages of 25 and 72 (mean/median = 43) years; 57.9% male. Comparing longitudinal imaging and plasma data relative to age between the reference population (amyloid negative and young [< 35 years],

N = 61) and the population of interest (N = 267), the population of interest had significantly more cortical amyloid than the reference population at age 39.6 (Figure 1A). On average, they reached amyloid positivity at 42.2 years (95% CI: 38.2, 47.0). These results using pooled data across tracers were similar to the results on the limited cohort of individuals who had received the PiB tracer (mean age = 41.1 years, 95% CI: 39.4, 45.3 years) (Supplemental Figure 2). Aging (Affected) participants who received PiB tracer had significantly greater striatal amyloid binding than the reference population (unaffected) at 39.2 years (Figure 1B). Striatal amyloid had statistically lower variability than cortical amyloid burden (Difference = 3.81 Z, 95% CI: 2.34, 5.28, $p_{adj} < 0.001$), summary tau (Difference = 1.73 Z, 95% CI: 0.20, 3.25, $p_{adj} = 0.019$), and plasma pTau217 (Difference = 2.22, 95% CI: 0.71, 3.73, $p_{adj} = 0.001$), suggesting the strength of signal change in response to the presence of pathological development is lower for striatal amyloid than the other biomarkers. On average, aging participants reached a striatal threshold of 1.25 SUVR at 38.4 years, but the 95% confidence interval exceeds the age range of the study (95% CI: <25, >65). The population of interest had significantly elevated plasma pTau217 relative to the reference population at 46.1 years (Figure 1C). They reached a plasma pTau217 level of 0.478 pg/mL at 47.4 (95% CI: 38.7, >65) years. Aging participants also had significantly elevated summary tau burden relative to the reference population at 42.4 years (Figure 1D). They reached tau positivity (SUVR>1.22) at an average age of 40.6 (95%CI: 33.1, 47.8) years. Results generated through the supplemental robust analysis were largely similar (Supplemental Figure 3), although plasma pTau217 elevation occured marginally earlier (45.9 years), but there was no significant difference in estimated age at plasma pTau217 level of 0.478 (48.6 years, 95% CI: 43.5, 52.9 years). All three PET measures were significantly elevated over the reference population around age 40, but cortical amyloid PET has the greatest variability (based on Levene's test), suggesting cortical amyloid burden has the highest signal-to-noise ratio among the biomarkers examined. The variability of plasma pTau217 levels was significantly less than cortical amyloid variability (Difference = 1.59 Z, 95% CI: 0.20, 2.97, $p_{adj} = 0.017$) but not summary tau variability (Difference = 0.50 Z, 95% CI: -0.94, 1.93, p = 0.811) or striatal amyloid variability.

Prediction of PET positivity

Plasma pTau217 and age both had moderate to strong correlations with PET-based pathological burden, showing comparable performance in detecting both amyloid and tau positivity. For cortical amyloid burden, plasma pTau217 (ρ = 0.573, 95% CI: 0.455–0.671) (Figure 2A) and age (ρ = 0.599, 95% CI: 0.476–0.704) exhibited similar correlations. The predictive performance of plasma pTau217 was highest at higher amyloid cutoffs and outperformed age-based thresholds at a cutoff of 50 CL ($AUC_{pTau217}$ = 0.923, AUC_{Age} = 0.838, $p_{Delong's\ Test}$ = 0.022), but was indistinguishable from age-based thresholds for lower amyloid cutoffs ($p_{Delong's\ Test}$ > 0.30 in all presented cases) (Figure 2B, 2C).

For striatal amyloid binding, plasma pTau217 (ρ = 0.405, 95% CI: 0.233–0.560) (Figure 2D) showed a stronger correlation than age (ρ = 0.195, 95% CI: 0.009–0.358). The predictive performance of plasma pTau217 was highest at higher striatal amyloid cutoffs but did not outperform age-based thresholds at a cutoff of 1.55 SUVR ($AUC_{pTau217}$ = 0.741, AUC_{Age} = 0.779, $p_{Delong's\ Test}$ = 0.559) or any other cutoff (Figure 2E, 2F).

For summary tau, plasma pTau217 (ρ = 0.425, 95% CI: 0.281–0.559) (Figure 2G) and age (ρ = 0.440, 95% CI: 0.309–0.556) had similar correlations. At advanced tau burden (1.58 SUVR), plasma pTau217 (threshold = 0.555 pg/mL) out-performed age-based thresholds ($AUC_{pTau217}$ = 0.965, AUC_{Age} = 0.827, $p_{Delong's\ Test}$ < 0.001), but was indistinguishable from age-based thresholds for lower tau cutoffs ($p_{Delong's\ Test}$ > 0.15 in all presented cases) (Figure 2H, 2I).

Definition of Reliable Accumulation

Reliable accumulation, defined as the 95th percentile of pathological accumulation in the reference population for each biomarker were as follows: cortical amyloid was 3.8 CL/year, striatal amyloid was 0.14 SUVR/year, plasma pTau217 was 0.26 pg/mL/year, and PET tau was 0.053 SUVR/year (Figure 3). Individuals were labeled as reliable accumulators if they exceeded this rate of change. A total of 38% of participants with longitudinal data were reliable accumulators of cortical amyloid, 13% were reliable accumulators of striatal amyloid, 9% were reliable accumulators of plasma pTau217, and 26% of participants were reliable accumulators of tau. For all biomarkers, age 40 is a critical turning point for adults with Down syndrome in the

development of Alzheimer Disease pathology as the greatest proportion of reliable accumulators were aged 40 and older. Accumulator profiles were compared across paired biomarkers (Supplemental Figure 4). The greatest degree of discordance was between cortical amyloid burden and plasma, where 39% of the participants with paired cortical amyloid and plasma pTau217 values were reliable accumulators in cortical amyloid but not plasma. Excluding the relationship between cortical amyloid and plasma, all other paired accumulator profiles were concordant in more than 75% of cases, suggesting that if an individual with Down syndrome is a reliable accumulator with one biomarker, he or she is likely to be a reliable accumulator in other biomarkers.

Prediction of Future Accumulation

To determine optimal thresholds for predicting future pathological accumulation, we conducted two cut point analyses. First, we classified individuals as accumulators or non-accumulators based on their longitudinal biomarker data. We performed within-individual linear regressions for each respective biomarker. Participants with positive associations between the biomarker of interest and time were deemed "accumulators" for that biomarker. Then we performed two cutpoint analyses, one for the threshold that maximized the baseline pathology measure's ability to detect whether an individual was an accumulator or not, and one for the identification of the baseline plasma pTau217 value that best differentiated between accumulators of cortical amyloid, striatal amyloid, or tau and an age-based null model.

Threshold for Reliable Accumulation based on Baseline Biomarker

Individuals surpassing 11.7 CL were more likely to be classified as reliable accumulators for cortical amyloid (Figure 4A). This means that if an individual had a baseline cortical amyloid value of 11.7 CL or greater, they were likely to have higher cortical amyloid values (>11.7 CL) in subsequent visits. Individuals exceeding 1.31 SUVR were likely be considered reliable accumulators for striatal amyloid (Figure 4B). A plasma pTau217 level above 0.482 pg/mL predicted reliable accumulator status for pTau217 (Figure 4C), and individuals exceeding 1.22 SUVR were likely to be reliable accumulators of tau (Figure 4D) (Table 2).

Threshold for Reliable Accumulation based on Baseline plasma pTau217

Age over 38 years was more predictive of future cortical amyloid accumulation than a baseline plasma pTau217 level above 0.270 pg/mL (Figure 5A–C), although the quality of these predictions did not differ significantly ($p_{Delong\ Test} = 0.070$). This threshold had a low specificity (0.286) with high sensitivity (0.929). A second threshold of 0.478 was identified based on visual inspection of Youden peaks, and it had considerably higher specificity (0.667) but lower sensitivity (0.500). This threshold also did not significantly differ from the age-based threshold ($p_{Delong\ Test} = 0.466$), suggesting that restricting the cutoff based on specificity criteria did not enhance the ability of plasma pTau217 to predict future cortical amyloid accumulation beyond age. For striatal amyloid accumulation**,** plasma pTau217 and age performed similarly (Figure 5D–F) ($p_{Delong\ Test} = 0.559$). Similarly, plasma pTau217 and age showed comparable accuracy in predicting future tau burden (Figure 5G–I) ($p_{Delong\ Test} = 0.668$). In brief, plasma pTau217 provides insight into future pathological accumulation, but age alone is a stronger predictor, particularly for cortical amyloid accumulation (Table 2).

Discussion

This study aimed to determine the most useful biomarkers for identifying early signs of Alzheimer Disease neuropathology accumulation in a longitudinal cohort of individuals with Down syndrome. As Alzheimer Disease is strongly associated with age, particularly in Down syndrome, the ability of age to predict future pathological accumulation was also evaluated. Amyloid and tau positivity were both detectable around age 40 in people with Down syndrome, consistent with prior studies[7,12,19,24,41]. Based upon longitudinal data, the prevalence of individuals who began to reliably accumulate Alzheimer Disease pathology as assessed by all four biomarkers considered (cortical amyloid, striatal amyloid, plasma pTau217 and summary tau burden), started around age 40. Age and plasma pTau217 were essentially equivalent in detecting both current pathology and the likelihood of future pathology accumulation. These results highlight that Alzheimer Disease in Down syndrome is strongly associated with age[1,7–9], seemingly to a much greater extent than ADAD[49]. Additionally, individuals that reliably accumulated one biomarker were likely to be a reliable accumulator of other biomarkers.

This aligns with previous cross-sectional studies reporting cortical amyloid accumulation in individuals with Down syndrome between the ages of 35 and 42[12,15,19,22–24]. We detected significant increases in cortical amyloid burden over the reference population at 39.6 years, within the previously reported range. While previous literature suggests striatal amyloid accumulates before cortical amyloid in Down syndrome[13,15], we observed striatal amyloid accumulates, on average, at the same time as cortical amyloid (39.2 years). However, there was greater variability in striatal PET ligand binding. Our results suggest that on average, individuals reach a striatal amyloid threshold of 1.25 SUVR at 38.4 years and 1.55 SUVR at 42.2 years, indicating that in some cases, striatal accumulation precedes cortical amyloid. The non-uniformity of pathological accumulation in striatal amyloid may provide evidence for subtypes of spatial amyloid distribution, which has previously only been reported in sporadic Alzheimer Disease [50,51]. This warrants additional investigation in future studies in individuals with Down syndrome.

Prior work in this cohort using only cross-sectional data reported elevation of plasma pTau217 over sibling controls at 38.9 years of age[19] but the apparent intra-individual variability in longitudinal data resulted in wider confidence intervals, indicating that participants with Down syndrome had elevated plasma pTau217 levels over the reference population at age 46.1. This corresponds roughly to the age at which we expect individuals to reach an average of 50 CL of cortical amyloid burden (Figure 2C) and is consistent with autopsy research in sAD. Salvado and colleagues compared plasma pTau217 (Lilly MSD) with neuropathological findings, observing that plasma pTau217 did not substantially increase until individuals had intermediate-to-high scores for Alzheimer Disease Neuropathic Change (ADNC) [52]. Efforts to translate ADNC scores to quantitative PET CL values suggest that intermediate ADNC corresponds to an average value of 49.4 CL[53]. Our findings that plasma pTau217 (Lilly MSD) only elevates significantly around 50 CL is aligned with these post-mortem studies and highlights that it may not be sensitive to emerging cortical amyloid burden but is highly accurate once widespread cortical amyloid has developed.

This was later than our estimated elevated tau PET threshold of 42.4 years. This discrepancy could be due to multiple factors including (1) the critical location of tau hyperphosphorylation in Down syndrome is not at amino acid 217 (although there is work to suggest that *DYRK1A* promotes phosphorylation at this site[29]). Perhaps, instead, tau phosphorylated at 212, as

promoted by *DYRK1A*[4], may be a more useful biomarker in this population[29]; (2) the relatively high intra-individual variability of this assay[54] as plasma pTau217 performance is highly assay dependent. A different assay may detect earlier changes[38]. A performance comparison of both assays and sites of tau phosphorylation is needed in this population similar to work in neurotypical populations [55].

Plasma pTau217's ability to both detect existing pathology and predict future accumulation in Down syndrome were comparable to age alone, except in the case of relatively high thresholds. While plasma pTau217 is widely used for detecting amyloid plaques[31,38,56] and tau tangles[27,39] in other causes of Alzheimer Disease, the strong age dependence of Alzheimer Disease pathology in Down syndrome[1,7,8] may limit its value in this population, particularly when we are interested in early detection of pathology. In contrast, baseline PET measures (cortical amyloid, striatal amyloid, and tau burden) were more reliable predictors of future amyloid and tau accumulation measured compared to age**.** Our results align with prior studies in sporadic Alzheimer Disease that found that a baseline amyloid PET threshold of 15.7 CL predicts future accumulation at 3 CL/year (sensitivity = 0.61, specificity = 0.83)[25]. In our Down syndrome cohort, a lower baseline threshold of 11.7 CL predicted accumulation at ≥3.8 CL/year (sensitivity = 0.766, specificity = 0.707). These similarities likely speak more to the level of noise inherent in amyloid PET tracers than biological differences between different Alzheimer Disease etiologies. The AMYPAD consortium recommends that clinicians consider 10 – 30 CL as the range of "emerging amyloid pathology" where pathology is evolving toward positivity[48]. Our finding that 11.7 CL predicts accumulation is consistent with this range and supports the notion that amyloid PET positivity is likely imminent for individuals above this threshold. Work has not yet been conducted in sporadic Alzheimer Disease to investigate thresholds for reliable accumulation of tau PET, but here, in this first longitudinal study of tau PET in Down syndrome, we observe that a baseline tau burden of 1.22 SUVR is sufficient. This result is consistent with the published threshold for tau PET positivity[44] and predicts future accumulation of at least 0.17 SUVR/year with a similar degree of accuracy as cortical amyloid burden (sensitivity = 0.818, specificity = 0.795).

This study has limitations. First, multiple commercial assays exist for the quantification of plasma pTau217 and these assays differ[55]. The generalizability of our findings to assays beyond those used in this study remains unknown. Second, a conceptual mismatch between plasma-based measures of tau phosphorylation and PET-based measures of Alzheimer Disease

pathology exists. Measures derived from biofluids reflect a dynamic state of clearance, while PET-binding reflects the cumulative deposition of the pathology of interest. Thus, plasma pTau217 may exhibit a saturation effect where individuals with advanced pathology do not show proportionally higher concentrations due to physiological limits on clearance. In contrast, PET signal continues to rise until substantial cerebral atrophy limits detectability, often beyond the point at which imaging is still feasible for the individual participant. These differences may complicate interpretation of the association between these two types of biomarkers at higher levels of pathology. Third, the existence of an amyloid probability score (APS) has been shown to have even better predictive capacity that plasma pTau217 alone [57]. While the age- and APOE-associated risks of increased cortical amyloid burden are distinct in Down Syndrome and cannot be generalized from published work in sporadic Alzheimer Disease, future work with larger sample size could independently construct and validate appropriate model weights for a Down Sydnrome-specific APS score that may reduce discordance in classification by plasma-based biomarkers as compared to PET. Finally, as with any neuroimaging study in individuals with Down Syndrome, challenges related to scan tolerability and motion introduce selection bias. Participants able to complete imaging protocols are likely healthier and have higher baseline cognitive function. Work exclusively in fluid biomarkers is vitally important in this population to expand access and generalizability of findings, and the results of this study facilitate future biofluid-only work.

For clinicians interested in determining when preclinical Alzheimer Disease pathology is present in the brain of individuals with Down syndrome, PET imaging is currently the best tool to use. Amyloid and tau PET offer the best *in vivo* quantification of current plaque and tangle load in the brain and have the greatest sensitivity and specificity in predicting near future accumulation of amyloid. If PET imaging is not feasible, age is the next best measure, acknowledging that there is heterogeneity in pathological progression. Prior work in Down syndrome identified that 5% of individuals aged 35 – 39 are amyloid positive while 90% of individuals aged 55 – 59 are amyloid positive -- a 25-year range over which individuals with Down syndrome are likely to develop amyloid[8] -- and our study found that plasma pTau217 (Lilly MSD) does not provide substantially more predictive value than participant age. Future research should explore alternative plasma biomarker assays, cerebrospinal fluid-derived biomarkers, and investigate the biological mechanisms underlying the observed variability in striatal and plasma markers. Validation

relative to gold standard pathological diagnoses from postmortem examination also remains to be completed.

Data availability

The data used in this analysis are available on request. Applications are reviewed by the ABC-DS publications committee. The data request application is available at https://www.nia.nih.gov/research/abc-ds#available-data.

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Competing interests

BLH has received research funding from Roche and Autism Speaks; receives royalties from Oxford University Press for book publications; and is the chair of the data safety and monitoring board for the Department of Defense-funded study, "Comparative Effectiveness of EIBI and MABA". BTC receives research funding from the National Institutes of Health. EH receives research funding from the National Institutes of Health and the BrightFocus Foundation. FL is supported by grants from the National Institute on Aging. HDR has received funding from the National Institutes of Health and is on the scientific advisory committee for the Hereditary Disease Foundation. JHL has received research funding from the National Institutes of Health and the National Institute on Aging. BMA receives research funding from the National Institutes of Health and has a patent ("Markers of Neurotoxicity in CAR T patients"). MSR has received consulting fees from AC Immune and Ionis, Alzheon, Alnylam, Biohaven, Embic, Positrigo and Prescient Imaging. He has received research support from the National Institutes of Health, Eisai and Lilly.. All other authors declare no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Comparison of longitudinal Alzheimer Disease (biomarkers) in people with Down Syndrome (DS) relative to the reference population (individuals with DS under age 35 and

pathology negative) as a function of age/estimated years to symptom onset. (A) People with DS have significantly elevated levels of cortical amyloid burden at 39.6 years of age. (B) People with DS have significantly elevated levels of striatal amyloid binding (PiB-only) at 39.2 years. (C) Individuals with DS had significantly elevated plasma pTau217 relative to healthy individuals with DS at age 46.1 years. (D) People with DS have significantly elevated levels of summary tau burden at an average age of 42.4.

Figure 2 Evaluation of Plasma pTau217 to detect current super-threshold biomarker levels. The correlations between baseline measurements of plasma pTau217 and cortical amyloid burden (A), striatal amyloid burden (E) and summary tau burden (I) are statistically significant and fairly similar ($r = 0.40 – 0.57$) We identified the optimal age-based and plasma pTau217 threshold to predict pathological positivity for cortical amyloid burden (C), striatal amyloid burden (F), and summary tau burden (I). We compared the utility of plasma pTau217 and age, finding that for individuals with DS, plasma pTau217 (solid lines) does not have greater predictive ability of pathological positivity than age (dashed lines) for cortical amyloid burden (B) or striatal amyloid burden (E). There may be a marginal advantage offered by plasma pTau217 (solid lines) in predicting tau positivity off relative to low (1.1 SUVR) or high (1.58 SUVR) summary tau cutoffs (H), but there is no difference in performance by age or plasma pTau217 for the previously published summary tau burden cutoff of 1.22 SUVR. For each threshold, the corresponding color is given first in the plots comparing the optimal threshold for plasma pTau217 and age (C, F, I) and then applied consistently to the ROC curve (B, E, H), such that red always corresponds to the lowest biomarker threshold and blue always corresponds to the highest biomarker threshold.

Figure 3 Comparison of Annualized Rates of Biomarker Accumulation Across Age Groups. After defining a normative group for individuals with DS as people younger than 35, we estimated the 95th percentile for the annualized rate of change from this cohort. We then compared this rate of accumulation for the four biomarkers of interest (Cortical amyloid burden [A], Striatal amyloid burden [B], plasma pTau217 [C], and summary tau burden [D]) to the observed data, stratifying by age. Visual inspection suggests very few individuals under the age

of 35 exceed the abnormal rate of accumulation for each biomarker, while many, but not all, individuals over 35 do. Individuals are most likely to be reliable accumulators for all biomarkers between the ages of 41 and 50, while individuals both older and younger than that at baseline have lower frequencies of reliable accumulation.

Figure 4 Evaluation of baseline biomarker levels to forecast future pathological accumulation. Vertical dashed lines on scatter plots indicate the optimal threshold for reliable accumulation status prediction. After 11.7 CL, individuals are most likely to accumulate future amyloid (A), although there is a relatively high degree of variability (B). After 1.31 SUVR, individuals are likely to accumulate future striatal amyloid (C). This baseline threshold is highly reliable (D). After 0.652 pg/mL, individuals are likely to accumulate higher plasma pTau217 levels (E). This threshold is highly sensitive, but with moderate specificity (F). After 1.22 SUVR, individuals are likely to accumulate additional tau burden (G). In this cohort of primarily cognitively intact individuals with DS, there are relatively few instances where individuals are likely to accumulate future tau burden, but for available samples, this baseline threshold offers good sensitivity and specificity (H).

Figure 5 Evaluation of baseline plasma pTau217 and age to forecast future pathological accumulation. Vertical dashed lines on scatter plots indicate the optimal threshold for reliable accumulation status prediction. Participants over the age of 38 (A) and with plasma pTau217 values over 0.277 pg/mL (B) are most likely to reliably accumulate cortical amyloid in future visits. Age is a better predictor of future cortical amyloid accumulation than plasma pTau217 (C). Participants over the age of 41 (D) and with plasma pTau217 values over 0.383 pg/mL (F) are most likely to accumulate future striatal amyloid, and the performance of these two markers are roughly equivalent at predicting future striatal amyloid accumulation (E). Participants over the age of 35 (G) and with plasma pTau217 values over 0.513 pg/mL (I) are most likely to accumulate future tau burden, and the performance of these two markers are roughly equivalent (H).

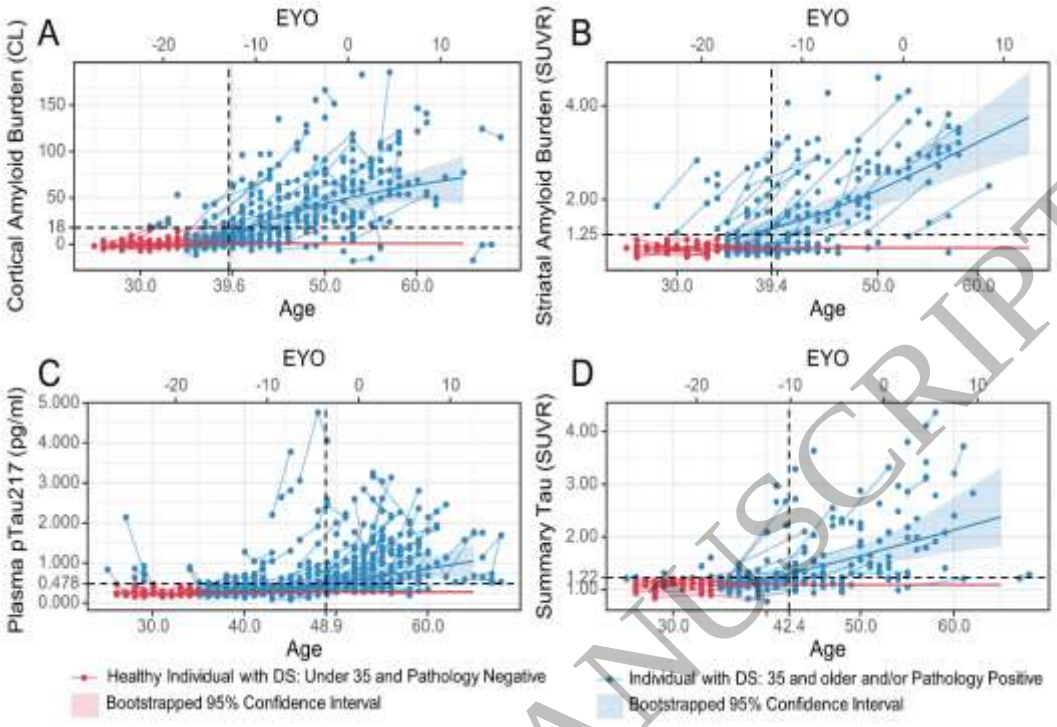

Figure 1
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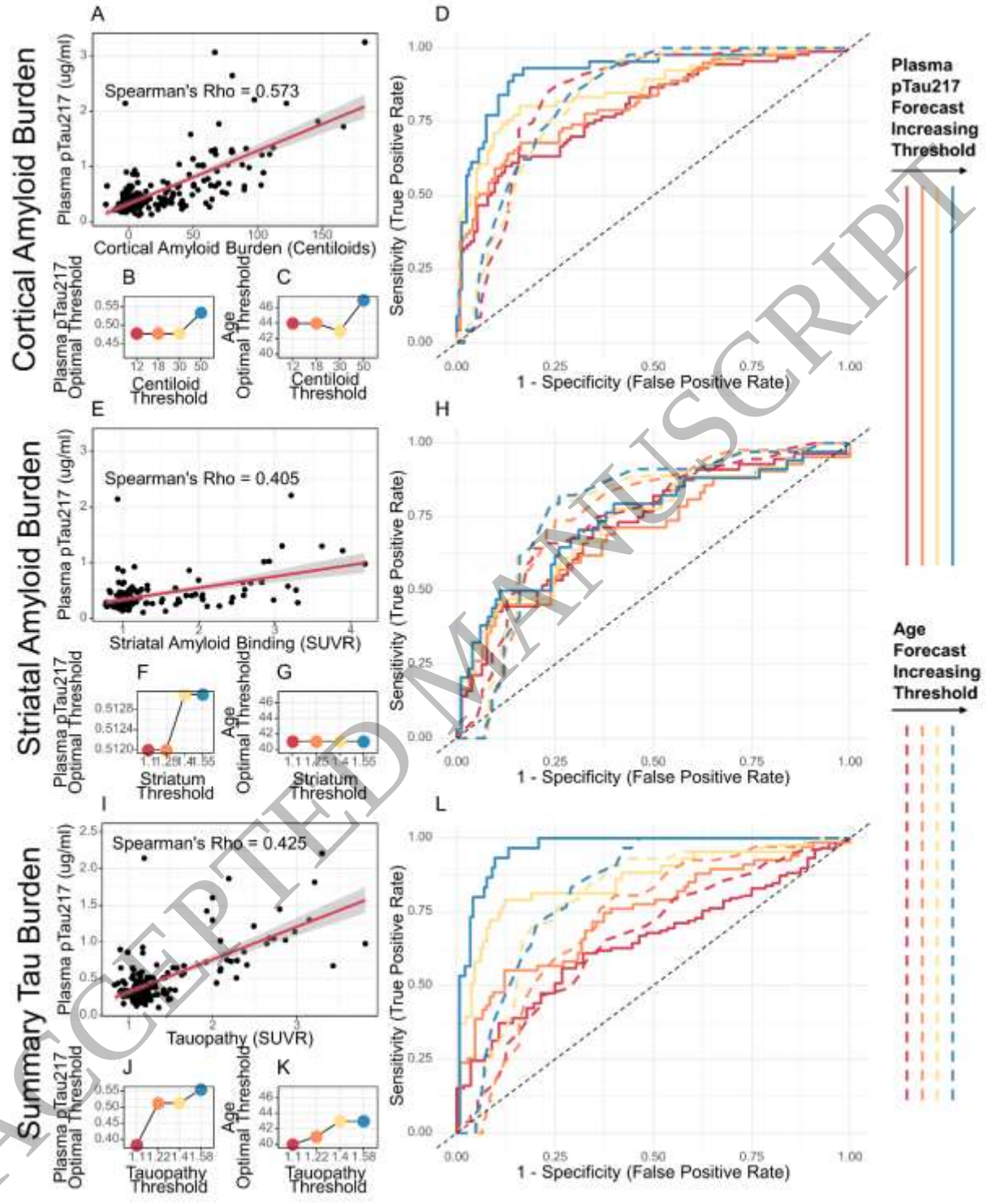

Figure 2
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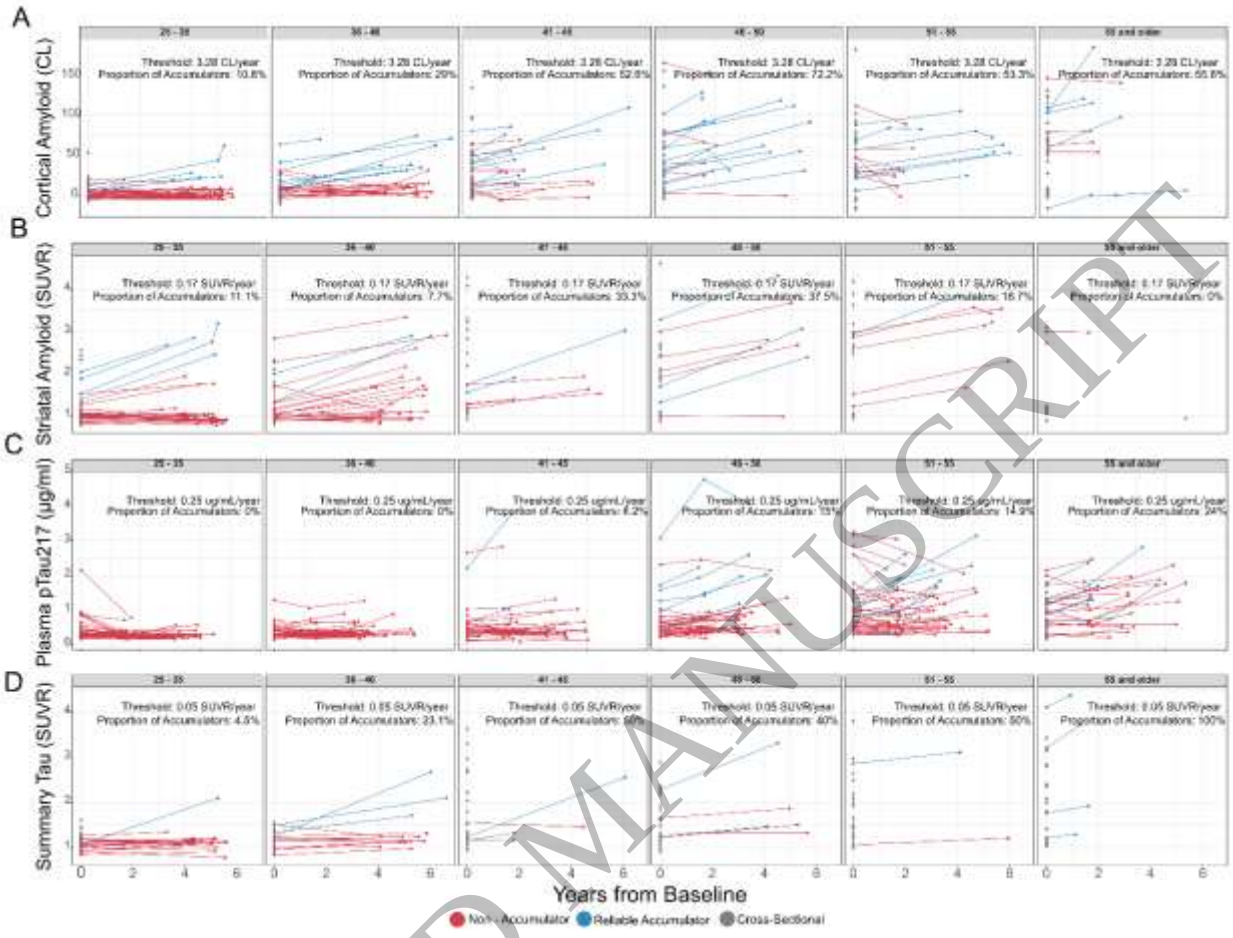

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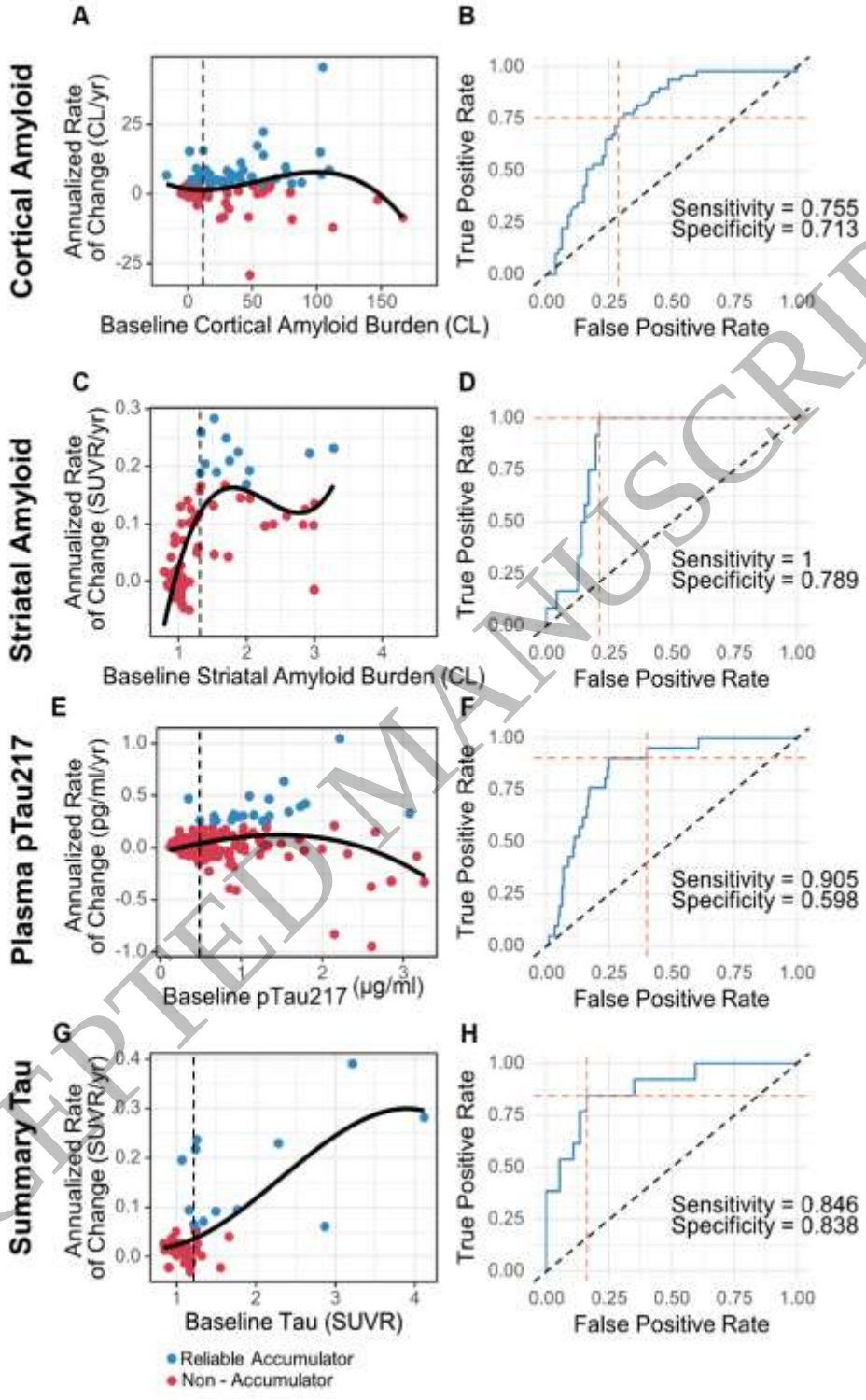

Figure 4
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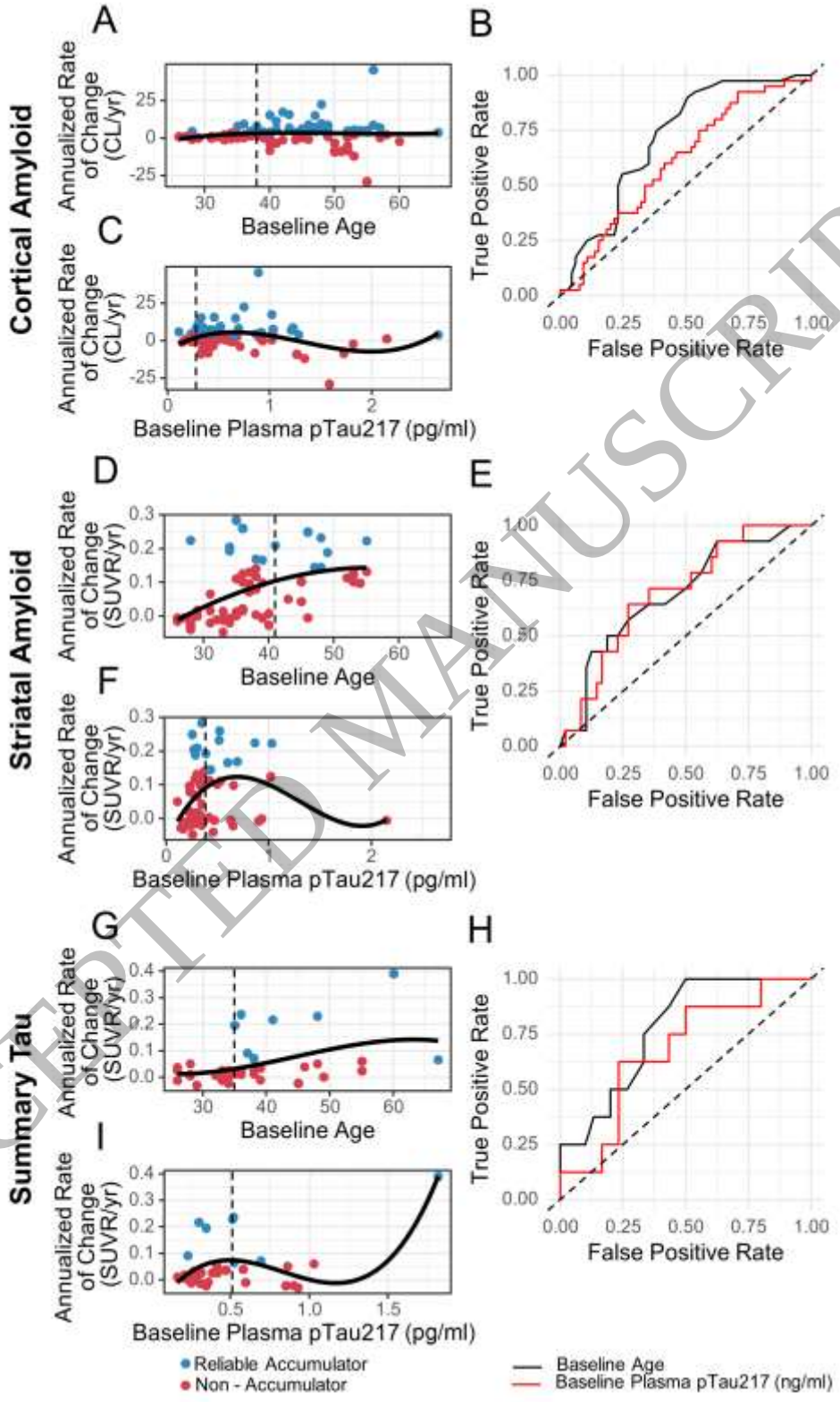

Figure 5
148x242 mm (x DPI)

Table 1 Participant demographics

	All Participants with DS	Reference Population	Aging Adults with DS	p
n	328	61	267	
Age at Baseline, mean (SD)	43.13 (9.54)	30.31 (2.83)	46.06 (7.98)	<0.001
Gender, *n* (% female)	138 (42.1)	23 (37.7)	115 (43.1)	0.534
Self-reported Race, *n* (%)				0.473
American Indian/Alaskan Native	1 (0.3)	0 (0.0)	1 (0.4)	
Asian	5 (1.5)	1 (1.6)	4 (1.5)	
Black/African-American/African/Caribbean	3 (0.9)	0 (0.0)	3 (1.1)	
Not Reported	4 (1.2)	2 (3.3)	2 (0.7)	
White	315 (96.0)	58 (95.1)	257 (96.3)	
APOE4 Carrier, *n* (%)				0.523
0 copies	250 (77.4)	45 (73.8)	205 (76.8)	
1 copy	67 (20.7)	12 (19.7)	55 (20.6)	
2 copies	6 (1.9)	2 (3.4)	4 (1.5)	
Consensus Cognitive Diagnosis, *n* (%)				<0.001
Mild Cognitive Impairment	34 (10.4)	0 (0.0)	34 (12.7)	
No Consensus	11 (3.4)	1 (1.6)	10 (3.7)	
Dementia	29 (8.8)	0 (0.0)	29 (10.9)	
Cognitively Stable	254 (77.4)	60 (98.4)	194 (72.7)	
Living Situation, *n* (%)				<0.001
Group Home	103 (31.6)	3 (4.9)	100 (37.7)	
Independent Living	45 (13.8)	11 (18.0)	34 (12.8)	
With Family/Caregiver	178 (54.6)	47 (77.0)	131 (49.4)	
Longitudinal Amyloid PET, *n* (%)	133 (40.5)	33 (54.1)	100 (34.7)	0.570
Cross-Sectional Amyloid PET, *n* (%)	266 (81.1)	59 (96.7)	207 (77.5)	0.001
Longitudinal Tau PET, *n* (%)	50 (15.2)	19 (31.1)	31 (11.6)	<0.001
Cross-Sectional Tau PET, *n* (%)	220 (67.1)	58 (95.1)	162 (60.7)	<0.001
Longitudinal Plasma pTau217, *n* (%)	225 (68.6)	32 (52.4)	193 (72.3)	0.004

Table 2 Thresholds and measures of prediction accuracy associated with age and plasma pTau217-associated predictions of pathological accumulation

Prediction of Cortical Amyloid Accumulation	**Age Threshold**	**Age Sensitivity**	**Age Specificity**
	38 years	0.881	0.492
	pTau217 Threshold	**pTau217 Sensitivity**	**pTau217 Specificity**
	0.27	0.929	0.286
Prediction of Striatal Amyloid Accumulation	**Age Threshold**	**Age Sensitivity**	**Age Specificity**
	34 years	0.909	0.353
	pTau217 Threshold	**pTau217 Sensitivity**	**pTau217 Specificity**
	0.513	0.454	0.824
Prediction of Tauopathy Accumulation	**Age Threshold**	**Age Sensitivity**	**Age Specificity**
	35 years	1	0.517
	pTau217 Threshold	**pTau217 Sensitivity**	**pTau217 Specificity**
	0.513	0.667	0.793