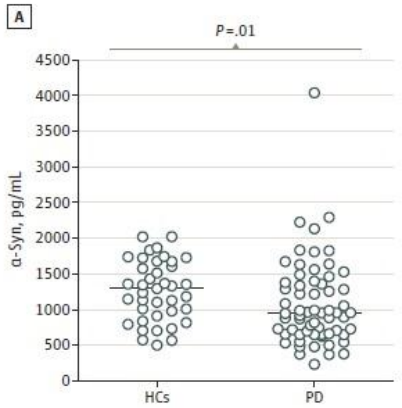
PARKINSON'S PROGRESSION MARKERS INITIATIVE

Play a Part in Parkinson's Research

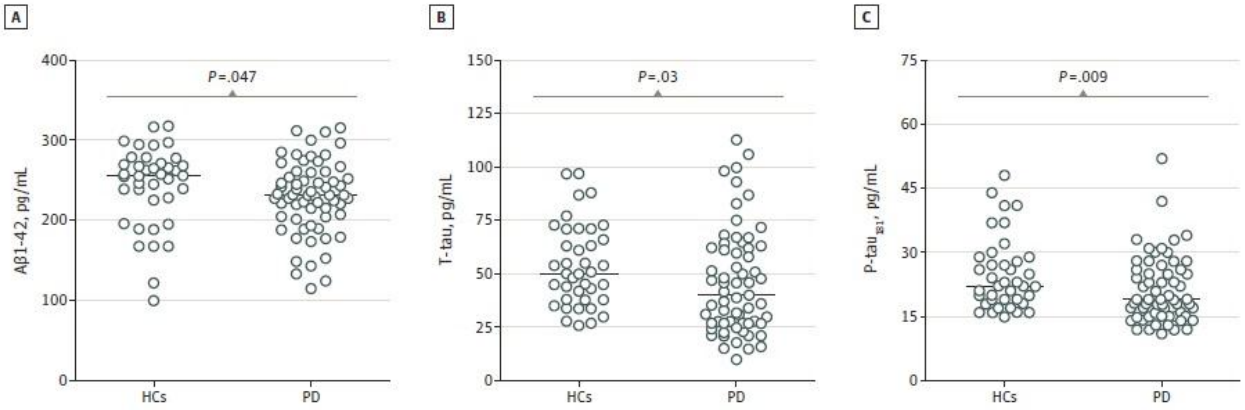
PPMI CSF ASSAY VARIABILITY ANALYSES

PILOT STUDY: CSF BIOMARKERS IN HC AND PD

Covance ELISA



AlzBio3 xMAP



* *P*-value assessed by Mann-Whitney *U* test

Baseline CSF samples from 39 HC and 63 PD subjects were analyzed in 2011 as part of a preliminary baseline biologics publication.

JAMA Neurol, 2013; 70(10):1277-87.

FULL-BASELINE STUDY: CSF BIOMARKERS IN PPMI COHORT

- Full-baseline study (completed late 2013) included 666 CSF aliquots from 24 sites – 191 HC, 415 PD and 60 SWEDD
  - Baseline CSF samples from the 102 subjects included in the 2011 pilot study were re-analyzed
  - 6 and 12 month-visit longitudinal CSF samples from 300 subjects were analyzed in parallel with the baseline samples with **low intra-subject variability** (*data not shown; ~1300 samples total run in 2013*)

Analyte (data in pg/ml)	**HC 2011** (Ave ± SD)	**HC 2013** (Ave ± SD)	**PD 2011** (Ave ± SD)	**PD 2013** (Ave ± SD)	***p*-value: HC vs. PD 2013**
αSyn	1264 ± 426	2191 ± 1086	1082 ± 611	1851 ± 790	**0.0005**
$A\beta_{1-42}$	243 ± 50	378 ± 113	229 ± 46	370 ± 100	0.3116
t-tau	54 ± 19	52 ± 27	46 ± 25	45 ± 19	**0.0019**
$p\text{-tau}_{181}$	25 ± 8	18 ± 12	21 ± 8	16 ± 10	**0.0005**

* *P*-value assessed by Mann-Whitney *U* test

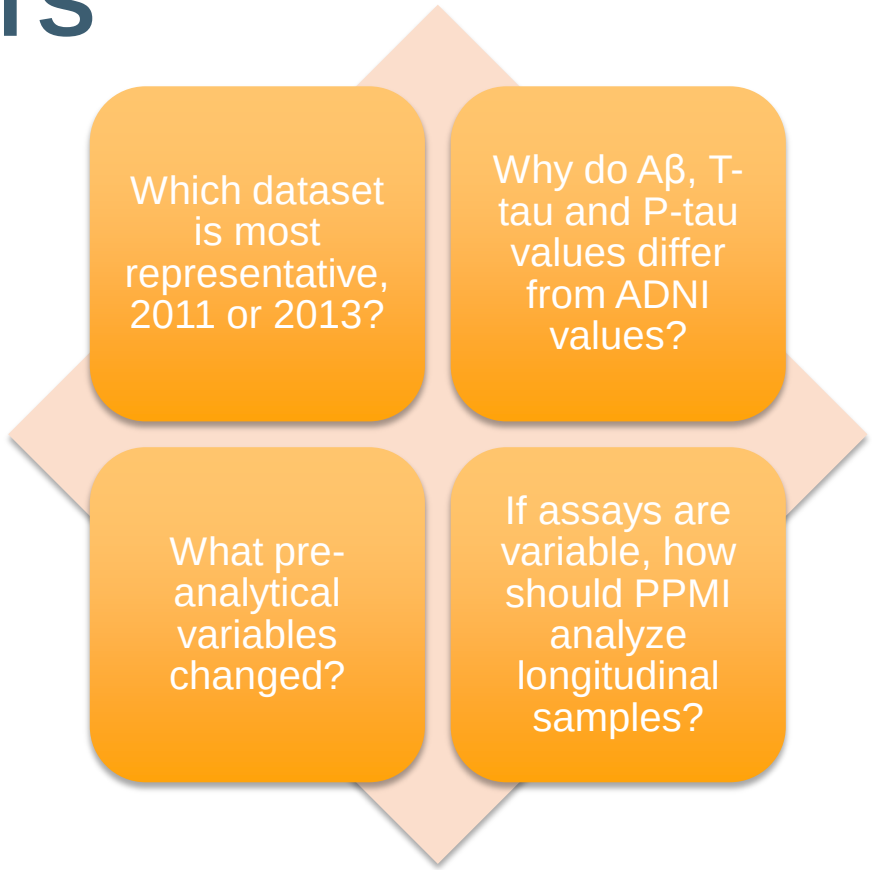
The variance and mean concentrations of αSyn and $A\beta_{1-42}$ were significantly higher in 2013 compared with 2011. Importantly, αSyn, t-tau and p-tau measurements in PD were still significantly lower than HC.

Courtesy of the PPMI Bioanalytics Core

CONCLUSIONS

- **Conclusions**
  - αSyn, t-tau and p-tau measurements in CSF from PD subjects were significantly lower than HC
  - Effects of sample storage duration on analyte concentration could not definitively be ruled-in or out
  - Pre-analytical variables, including aliquoting SOP, did not substantially contribute to discrepancies in αSyn and $A\beta_{1-42}$ values between 2011 and 2013
  - Discordant measurements for lipophilic proteins (αSyn and $A\beta_{1-42}$) are primarily due to assay variability
  - QC and reference standards will improve assay calibration

CORE QUESTIONS ON BASELINE CSF DATASETS



Beginning in early January 2014 and lasting through March 2014, the PPMI biologics teams began convening weekly to answer these and other related questions.

POST-HOC ANALYSES: SOURCES OF PREANALYTICAL VARIANCE

Correlation with PPMI site(s)?

Correlation with repository source?
(Coriell vs. BioRep)

POST-HOC ANALYSES: COMPARISON BY SITE LOCATION

Variable	Enrolled Subjects		*p*-value
	US Sites (N = 508)	Non-US Sites (N = 111)	
$A\beta_{1-42}$			0.32
Mean (SD)	376.44 (109.04)	357.72 (76.19)	
(Min, Max)	(88.80, 879.50)	(160.60, 512.50)	
t-Tau			0.26
Mean (SD)	47.56 (22.27)	44.60 (18.38)	
(Min, Max)	(15.40, 223.10)	(14.40, 110.50)	
p-Tau			0.28
Mean (SD)	16.64 (10.74)	15.03 (8.43)	
(Min, Max)	(4.70, 94.10)	(5.00, 64.70)	
αSyn			0.18
Mean (SD)	1982.47 (932.23)	1827.32 (764.64)	
(Min, Max)	(332.93, 8608.91)	(581.17, 4709.78)	

PPMI sites in the US submit samples to Coriell, whereas sites outside the US submit samples to BioRep. No differences in CSF analyte values were observed based on repository source.

POST-HOC ANALYSES: SOURCES OF PREANALYTICAL VARIANCE

- No correlation with PPMI site(s)
- No correlation with repository source (Coriell vs. BioRep)
- No issues with sample storage at the repositories
- No change in vendors (tubes, shippers, etc.)

POST-HOC ANALYSES: COMPARISON OF ALIQUOTING SOP (ALPHA-SYN)

Criteria	2011 Run SOP 1241 Rev A	2013 Run SOP 1241 Rev A - F	2013 Run SOP 1241 Rev G - I
αSyn HC subjects			
n	43	204	217
Mean (pg/ml)	1287.43	2229.52	2130.69
Sd Dev	427.65	899.21	1095.44
% CV	33%	40%	51%
MIN (pg/ml)	491.9	592.6	641.9
MAX (pg/ml)	2025.1	5237.7	8608.9
% mean change 2011 to 2013	___	73%	65%
αSyn PD subjects			
n	71	280	489
Mean (pg/ml)	1073.55	1878.63	1853.01
Sd Dev	583.23	704.11	821.64
% CV	54%	37%	44%
MIN (pg/ml)	227.4	332.9	472.9
MAX (pg/ml)	4032.2	5174.2	6694.6
% mean change 2011 to 2013	___	75%	73%

SOP 1241 Rev A – F specified a thaw at 4° C or on wet ice with aliquoting over wet ice

SOP 1241 Rev G – I specified a that at 4° C with aliquoting over dry ice

Courtesy of Shirley Lasch, IND

POST-HOC ANALYSES: COMPARISON OF ALIQUOTING SOP (A-BETA)

Criteria	2011 Run SOP 1241 Rev A	2013 Run SOP 1241 Rev A - F	2013 Run SOP 1241 Rev G - I
$A\beta_{1-42}$ All subjects (PD, HC, SWEDD)			
n	119	470	829
Mean (pg/ml)	235.13	356.94	385.47
Sd Dev	47.67	92.58	106.27
% CV	20%	26%	28%
MIN (pg/ml)	100.0	88.8	95.2
MAX (pg/ml)	318.0	688.0	879.5
% mean change 2011 to 2013	—	52%	64%

SOP 1241 Rev A – F specified a thaw at 4° C or on wet ice with aliquoting over wet ice

SOP 1241 Rev G – I specified a that at 4° C with aliquoting over dry ice

Courtesy of Shirley Lasch, IND

POST-HOC ANALYSES: COMPARISON OF ALIQUOTING SOP (T-TAU)

Criteria	2011 Run SOP 1241 Rev A	2013 Run SOP 1241 Rev A - F	2013 Run SOP 1241 Rev G -K
t-Tau HC subjects			
n	44	171	248
Mean (pg/ml)	54.64	50.94	52.83
Sd Dev	20.05	24.02	27.50
% CV	37%	47%	52%
MIN (pg/ml)	26.0	16.8	18.1
MAX (pg/ml)	103.0	188.2	223.1
% mean change 2011 to 2013	___	7%	3%
t-Tau PD subjects			
n	72	268	497
Mean (pg/ml)	45.75	43.22	44.04
Sd Dev	23.61	17.47	18.06
% CV	52%	40%	41%
MIN (pg/ml)	10.0	15.6	14.4
MAX (pg/ml)	113.0	134.7	121.0
% mean change 2011 to 2013	___	6%	4%

SOP 1241 Rev A – F specified a thaw at 4° C or on wet ice with aliquoting over wet ice

SOP 1241 Rev G – I specified a that at 4° C with aliquoting over dry ice

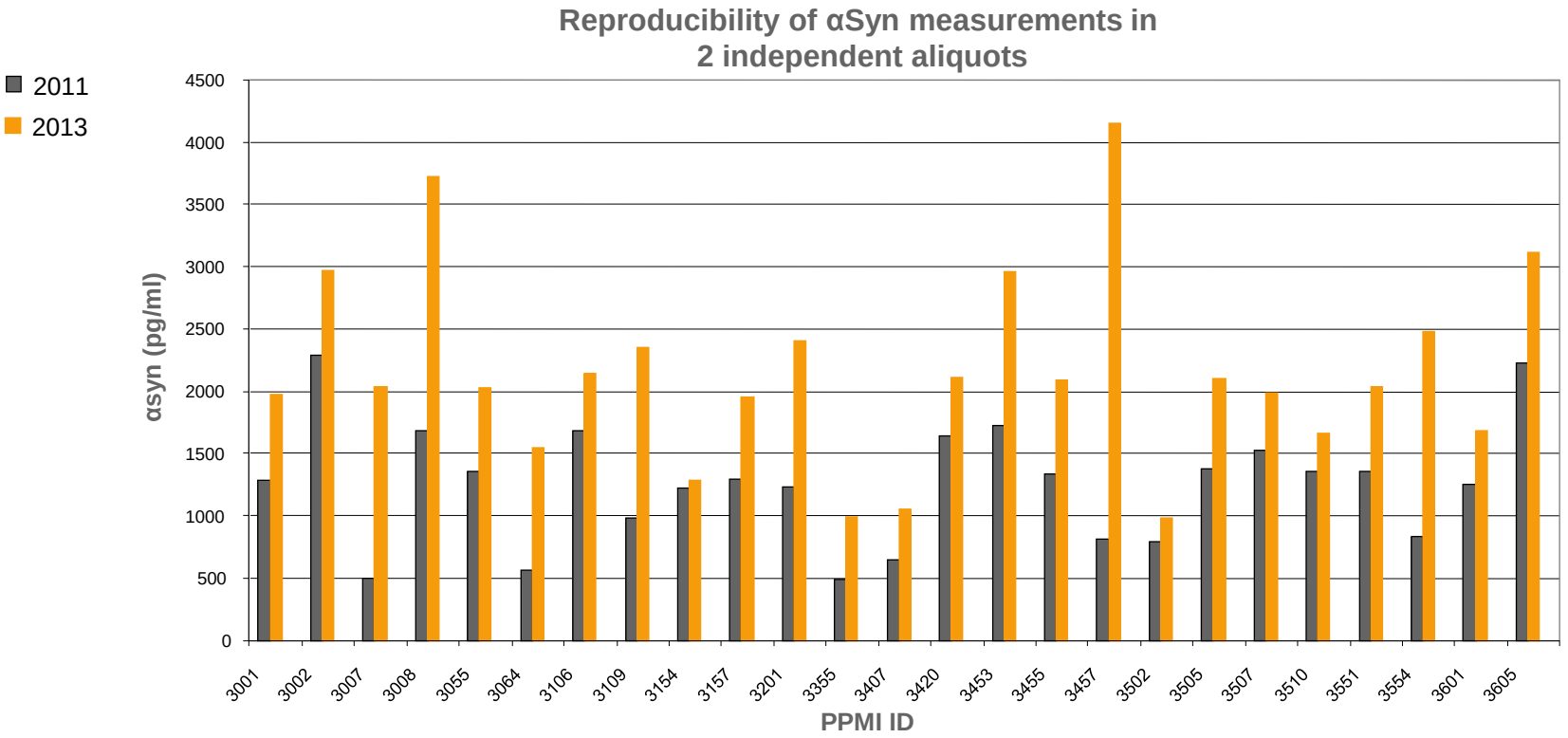
Courtesy of Shirley Lasch, IND

POST-HOC ANALYSES: SOURCES OF PREANALYTICAL VARIANCE

- No correlation with PPMI site(s)
- No correlation with repository source (Coriell vs. BioRep)
- No issues with sample storage at the repositories
- No change in vendors (tubes, shippers, etc.)
- Minor change to CSF aliquoting SOP at repositories
  No changes in SOP at Covance or UPenn

The only identifiable change in sample processing between 2011 and 2013 was the repository SOP for aliquoting CSF over dry ice vs. on wet ice. Subsequent analyses ruled-out SOP as a substantial contributor to the discrepant αSyn and $A\beta_{1-42}$ results.

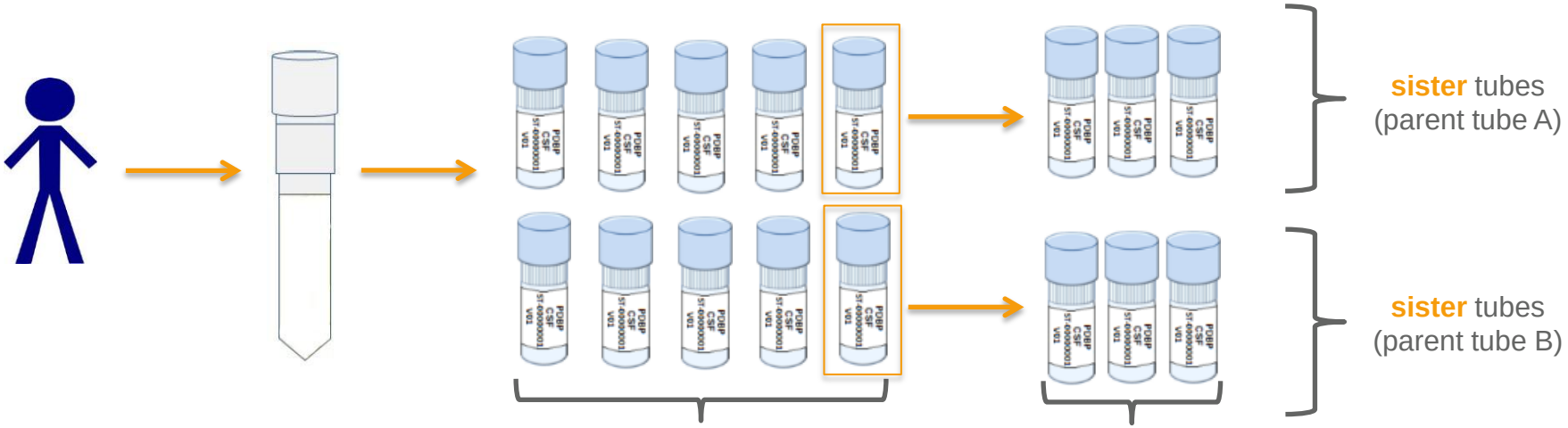
POST-HOC ANALYSES: COMPARISON OF SAMPLES RE-RUN IN 2013



Aliquots run in 2013 were consistently higher than in 2011. Variation in reported values between the 2 measurements is greater in some subjects than others.

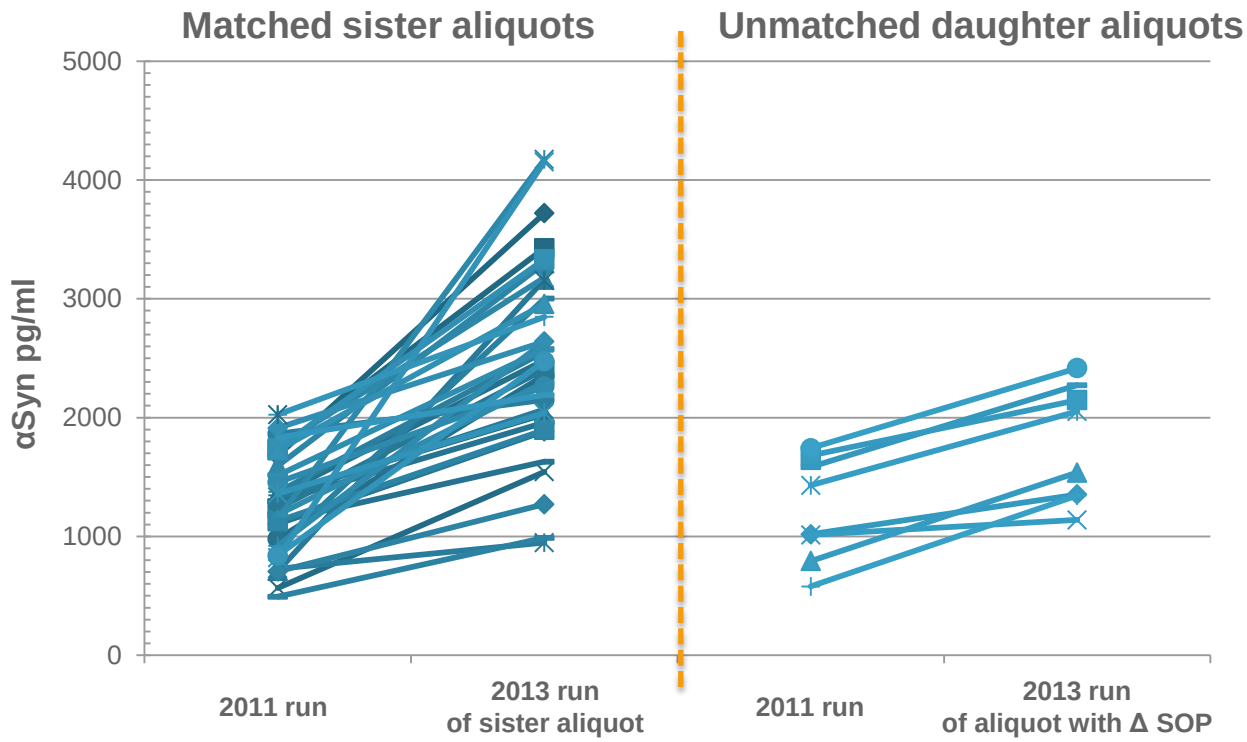
Courtesy of Peggy Taylor, Covance

POST-HOC ANALYSES: COMPARISON OF SISTER CSF ALIQUOTS



Matched sister tubes are aliquots prepared from the same parent tube. Unmatched daughter aliquots are prepared from different parent tubes, although all parent tubes are derived from the same original CSF sample.

POST-HOC ANALYSES: COMPARISON OF ALPHA-SYN IN SISTER TUBES

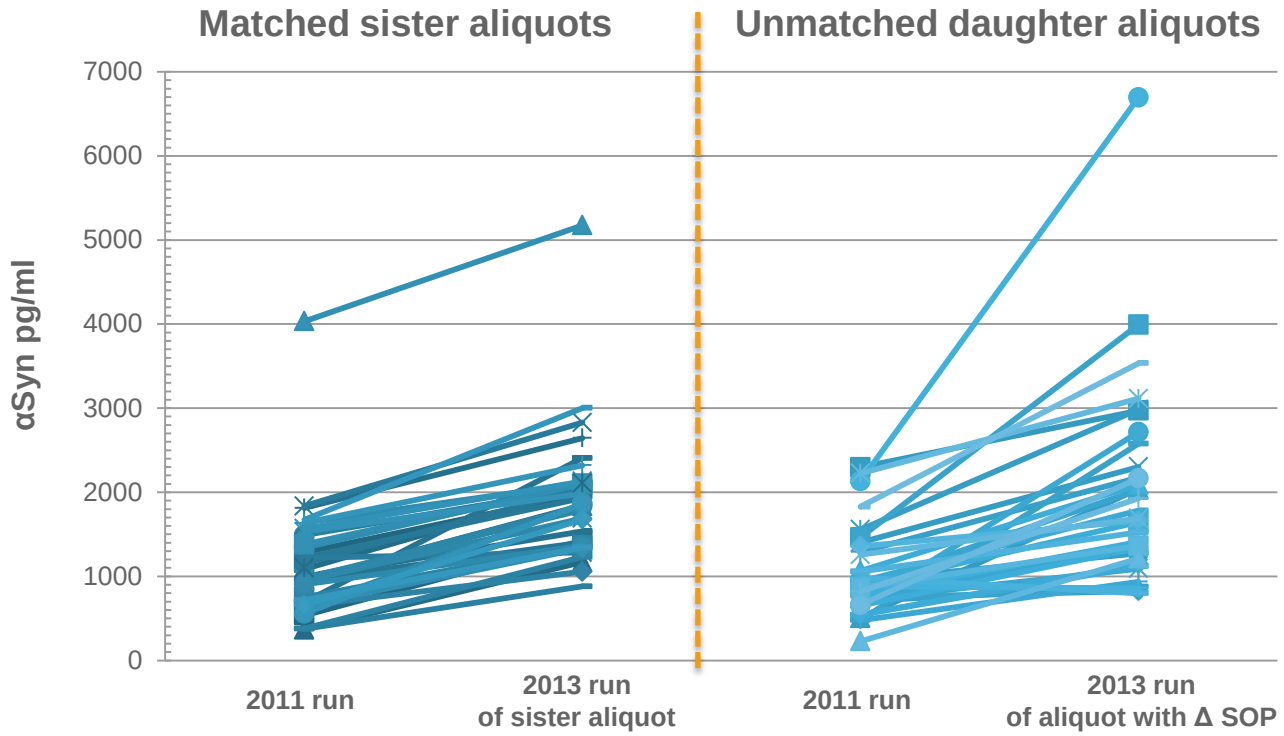


NOTE–Data from HC subjects only

In samples run in both 2011 and 2013, αSyn values increased in a majority of samples. The average percent increase from 2011 to 2013 values was 93% for sister aliquots and 45% for samples from different parent tubes (*HC samples only*).

Courtesy of Shirley Lasch, IND

POST-HOC ANALYSES: COMPARISON OF ALPHA-SYN IN SISTER TUBES

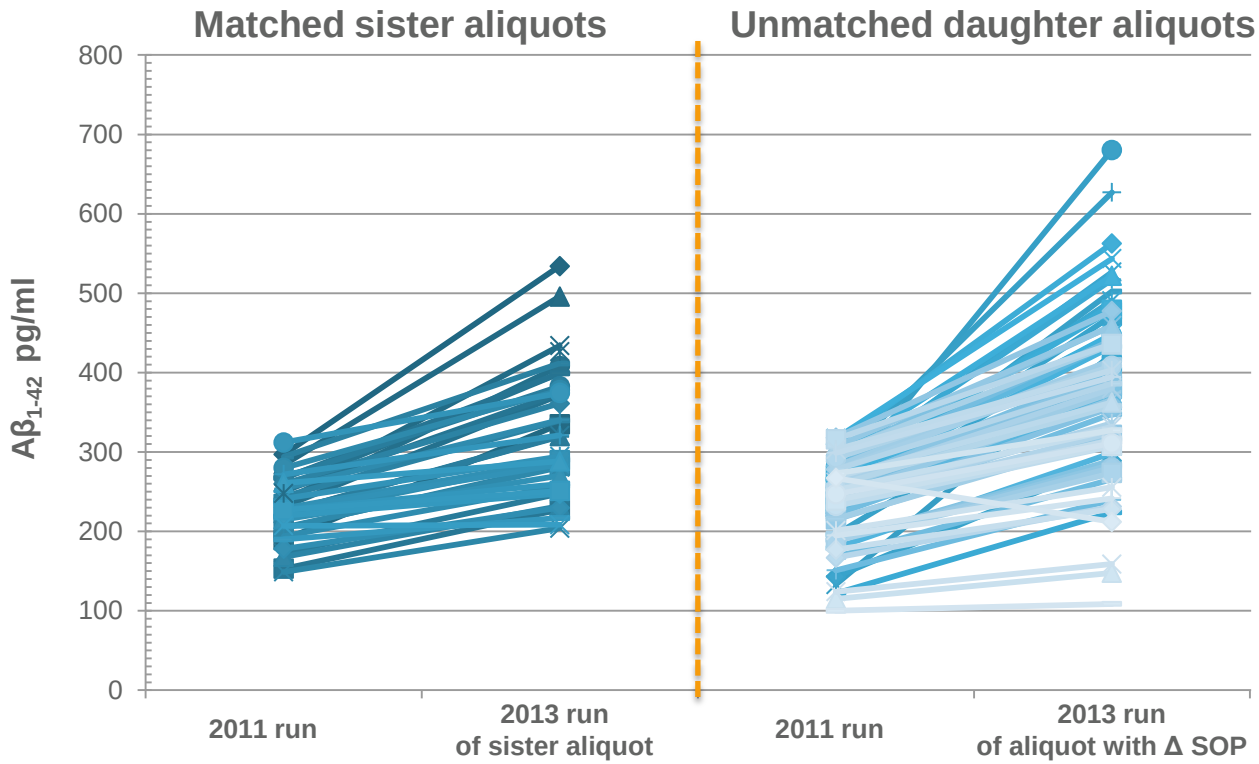


NOTE–Data from PD subjects only

In samples run in both 2011 and 2013, αSyn values increased in a majority of samples. The average percent increase from 2011 to 2013 values was 50% for sister aliquots and 62% for samples from different parent tubes (*PD samples only*).

Courtesy of Shirley Lasch, IND

POST-HOC ANALYSES: COMPARISON OF A-BETA$_{1-42}$ IN SISTER TUBES

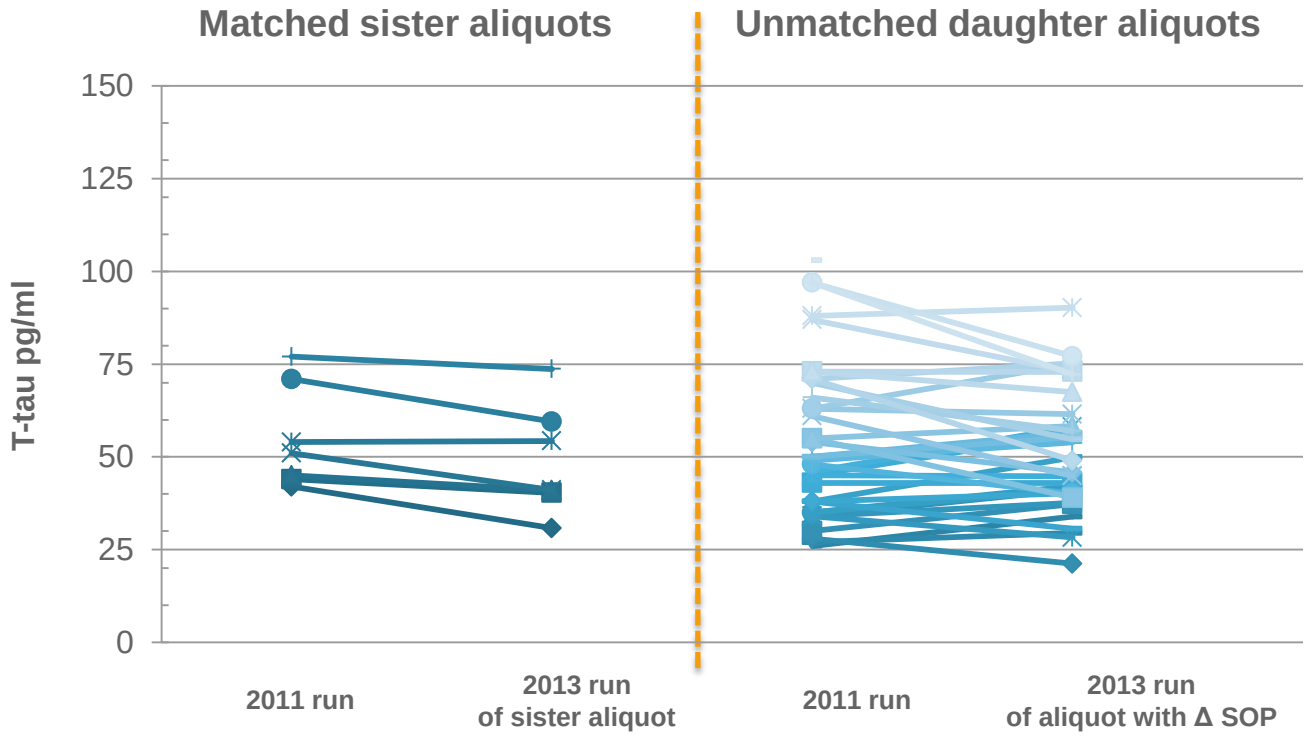


NOTE–Data from all subjects (HC, PD, SWEDD)

In samples run in both 2011 and 2013, Aβ$_{1-42}$ values increased in a majority of samples. The average percent increase from 2011 to 2013 values was 39% for sister aliquots and 59% for samples from different parent tubes.

Courtesy of Shirley Lasch, IND

POST-HOC ANALYSES: COMPARISON OF T-TAU IN SISTER TUBES

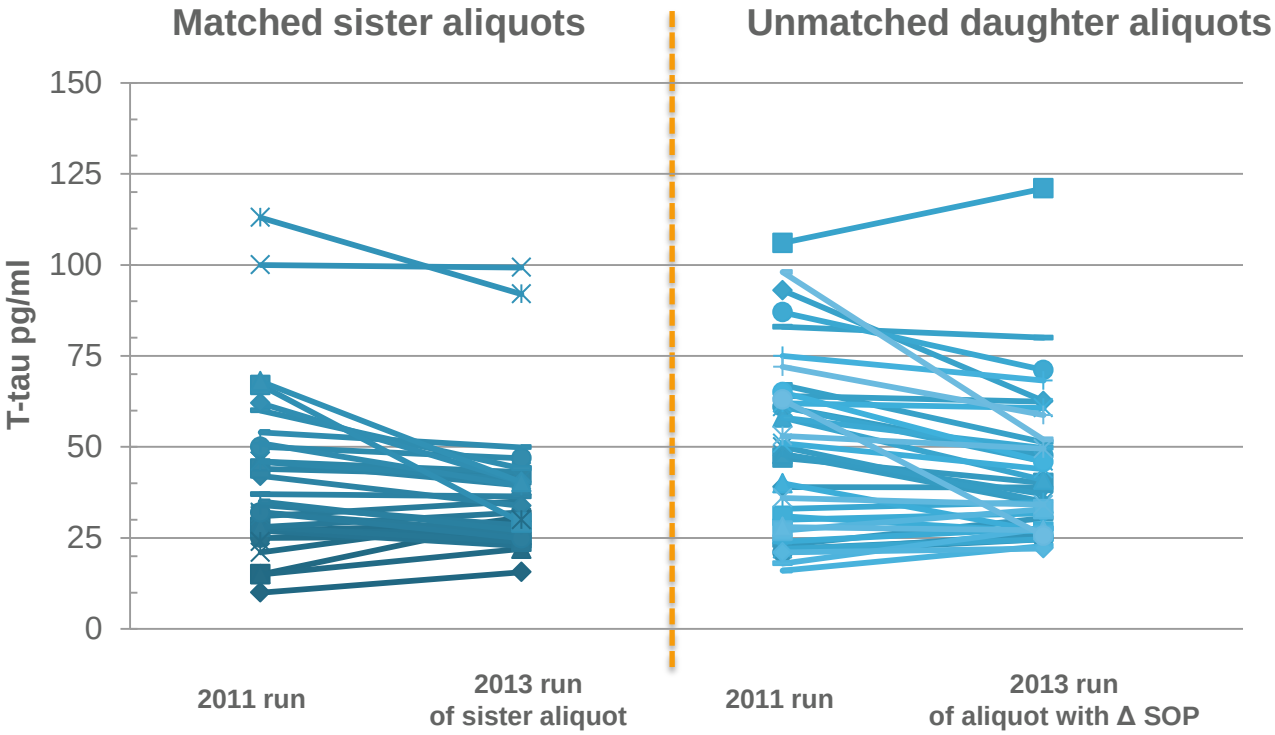


NOTE–Data from HC subjects only

In samples run in both 2011 and 2013, t-Tau values decreased in a majority of samples. The average percent increase from 2011 to 2013 values was 11% for sister aliquots and 6% for samples from different parent tubes (*HC samples only*).

Courtesy of Shirley Lasch, IND

POST-HOC ANALYSES: COMPARISON OF T-TAU IN SISTER TUBES



NOTE–Data from PD subjects only

In samples run in both 2011 and 2013, t-Tau values decreased in a majority of samples. The average percent increase from 2011 to 2013 values was 12% for sister aliquots and 13% for samples from different parent tubes (*PD samples only*).

Courtesy of Shirley Lasch, IND

POST-HOC ANALYSES: RANK-BASED ANALYSES OF 2011 AND 2013 DATA

Pearson Correlation Coefficients between Ranks of 2011 and 2013 baseline CSF values

Biomarker	All Subjects (N = 102) Correlation (*p*-value)	PD (N = 63) Correlation (*p*-value)	HC (N = 38) Correlation (*p*-value)
$A\beta_{1\text{-}42}$	0.7587 (<.0001)	0.6954 (<.0001)	0.8256 (<.0001)
t-tau	0.8665 (<.0001)	0.8754 (<.0001)	0.8459 (<.0001)
p-tau	0.4601 (<.0001)	0.4664 (<.0001)	0.3068 (0.0575)
αSyn	0.6670 (<.0001)	0.6801 (<.0001)	0.5676 (0.0002)

While the mean values of these biomarkers differ in a non-uniform manner due to assay variability, there is a strong correlation of the ranks for the 2011 and 2013 values emphasizing the importance of presenting nonparametric rank-based analyses vs. mean differences.

Courtesy of the PPMI Statistics Core

CONCLUSIONS AND RECOMMENDATIONS

- **Conclusions**
  - αSyn, t-tau and p-tau measurements in CSF from PD subjects were significantly lower than HC
  - Effects of sample storage duration could not definitively be ruled-in or out
  - Pre-analytical variables, including aliquoting SOP, did not substantially contribute to discrepancies in αSyn and $A\beta_{1\text{-}42}$ values between 2011 and 2013 although the current SOP may improve protein preservation
  - Discordant measurements for lipophilic proteins are primarily due to assay variability
  - QC and reference standards require optimization to improve assay calibration

- **Recommendations**
  - PPMI SC will utilize the 2013 data set in analyses and external investigators will be similarly advised
  - 2013 CSF data will be posted to LONI with 2011 datasets remaining as a separate file for historical reference
  - Longitudinal studies of these biomarkers in PPMI may include a strategy for reanalyzing baseline samples