

# PPMI Biochemical Biomarker Working Group

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# October 2011: Kickoff Call

- Reviewed inventory and QC issues of PPMI biosamples
- Reviewed alpha-synuclein Round Robin study
- Reviewed initial  $A\beta_{1-42}$ , t-tau & p-tau<sub>181</sub> data



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# Identification of biomarker candidates for inclusion is critical to PPMI

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**Five biomarker candidates are being actively tracked in PPMI**

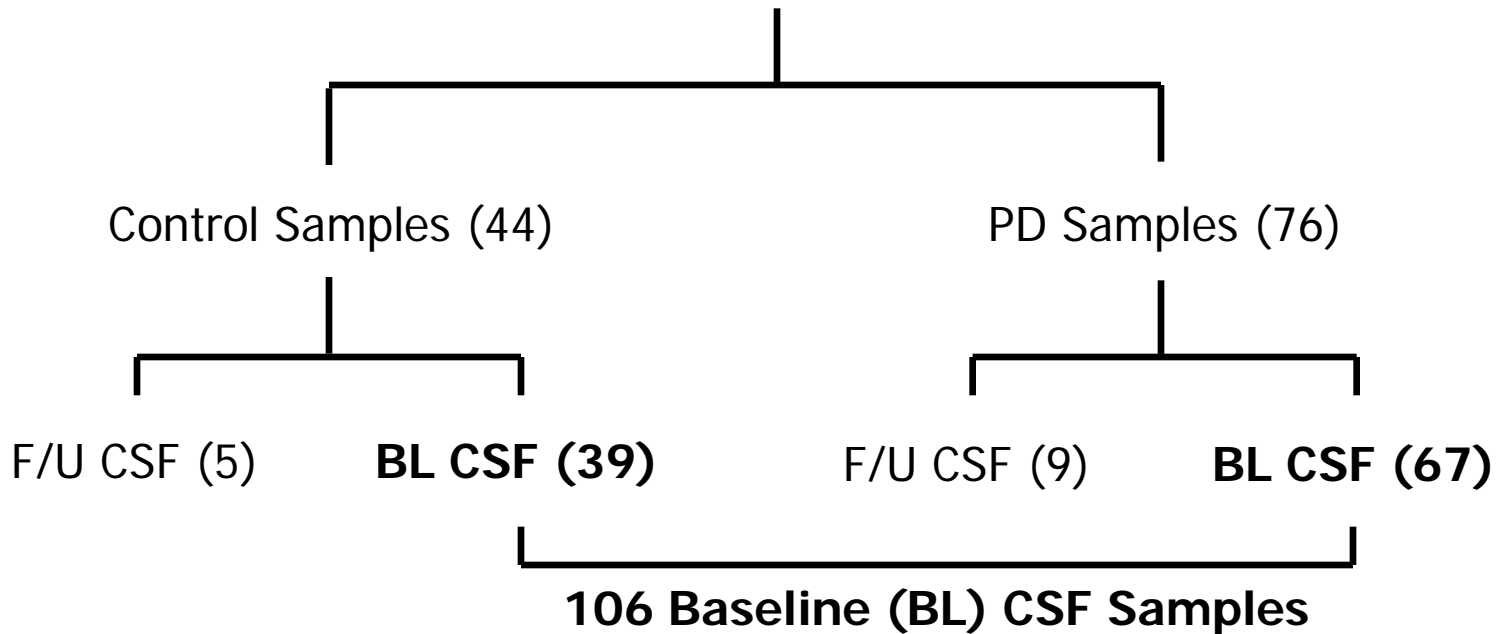


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# Transfer of 120 PPMI CSF Samples



# Analysis of CSF biomarkers

**AD biomarkers analyses : Luminex- platform with AlzBio3 kit,**

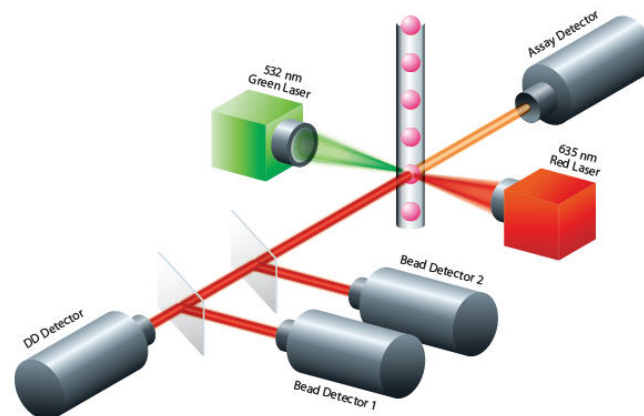
**Total of 4 plates (4 runs)**

1<sup>st</sup> plate : Jul. 14 – 15, 2011

2<sup>nd</sup> plate : Jul. 18 – 19, 2011

3<sup>rd</sup> plate : Jul. 19 – 20, 2011

4<sup>th</sup> plate : Jul. 20 – 21, 2011



**$\alpha$ -synuclein analyses : ELISA methodology by Covance Inc.**

Nov. 15 – Nov. 29, 2011

# Characteristics of samples and Analytical performance

Number of subjects	Control				PD	
	40				67	
Number of samples (Total = 120)	Baseline		F/U		Baseline	
	39		5 (VT02)		67  (ST: 5, VT02: 4)	
Analytical performance	T-tau (%CV)		A $\beta$ <sub>1-42</sub> (%CV)		p-tau <sub>181</sub> (%CV)	
Aqueous QC controls (n = 4)	ConA	ConB	ConA	ConB	ConA	ConB
	7.45	3.77	3.60	3.99	3.67	5.17
CSF pool QC samples (n = 4)	Pool48	Pool52	Pool48	Pool52	Pool48	Pool52
	5.09	7.69	9.67	5.37	3.29	2.72

# Demographics of the PPMI subjects

	HC (N = 39)	PD (N = 63)	P value	SWEDD (N= 4)
Age, years (95% C.I.)	59 ± 13 (55 – 63)	62 ± 10 (60 – 65)	0.2781	67 ± 7 (55 – 78)
Sex, F/M (% of Male)	18/21 (53.8)	24/39 (61.9)	0.4216 <sup>#</sup>	2:2 (50.0)
Education, years (95% C.I.)	16.9 ± 2.4 (16.1 – 17.6)	16.4 ± 2.5 (15.8 – 17.0)	0.1421	14.3 ± 2.1 (11.0 – 17.5)
Age at onset, years (95% C.I.)	-	59.5 ± 10.8 (56.8 – 62.2)	-	63.5 ± 8.2 (50.5 – 76.5)
Mean duration of symptoms, median years (range)	-	1.8 (0.3 – 20.8)	-	2.0 (0.0 – 2.9)
Number of subjects with CSF Hb > 200 ng/mL	6	18	0.1271 <sup>#</sup>	1

<sup>#</sup>Chi-square test

# Clinical characteristics of the PPMI subjects<sup>#</sup>

	HC (N = 39)				PD (N = 63)				p value <sup>*</sup>	SWEDD (N= 4)			
H & Y stage	0.03 ± 0.16				1.65 ± 0.51				< 0.0001	1.50 ± 0.58			
UPDRS III motor score	1.6 ± 2.7				22.6 ± 7.6				< 0.0001	17.3 ± 6.2			
Mean tremor score	0.05 0.13				0.46 0.27				< 0.0001	0.50 ± 0.20			
Mean PIGD score	0.01 0.04				0.24 0.26				< 0.0001	0.00 ± 0.00			
UPSIT score	35.1 ± 3.4				21.9 ± 8.1				< 0.0001	33.0 ± 2.9			
Striatal binding ratios (Mean values)	PR	PL	CR	CL	PR <sup>†</sup>	PL	CR	CL	<0.0001	PR	PL	CR	CL
	1.38	1.39	2.06	2.05	0.61	0.64	1.34	1.33		1.39	1.56	2.00	2.02
MoCA (95% C.I.)	28.4 ± 1.0 (28.0 – 28.7)				27.2 ± 2.0 (26.7 – 27.7)				0.0039	27.3 ± 2.4 (23.5 – 31.0)			
Semantic fluency	53.8 ± 12.1				49.5 ± 10.6				0.0578	40.8 ± 4.1			
WMSIII-LNS test score	12.1 ± 2.8				11.0 ± 2.0				0.0510	10.0 ± 1.4			
SDMT <sup>§</sup>	48.6 ± 11.2				41.9 ± 8.9				0.0051	44.8 ± 7.7			
HVLT_total recall	9.0 ± 1.6				8.2 ± 1.5				0.0077	7.8 ± 2.4			
HVLT delayed recall	9.9 ± 2.3				8.3 ± 2.3				0.0004	9.3 ± 4.2			

<sup>\*</sup>Mann-Whitney U test

<sup>†</sup>PR: Right putamen, PL: Left putamen, CR: Right caudate, CL: Left caudate, N=39 for HC, N=62 for PD, N=4 for SWEDD.

<sup>#</sup>Data were updated based on PPMI database (04. 12. 2012); Diagnoses of 2 patients were changed.



# Comparison of CSF Biomarker levels between HC and PD #

Biomarkers	HC (N = 39)	PD (N = 63)	P value <sup>#</sup>	SWEDD (N = 4)
A $\beta_{1-42}$ (pg/mL) (95% C.I.)	242.8 $\pm$ 49.95 (226.7 – 259.0)	228.7 $\pm$ 45.63 (217.2 – 240.2)	0.0466	276.0 $\pm$ 22.99 (239.4 – 312.6)
t-tau (pg/mL) (95% C.I.)	53.9 $\pm$ 19.33 (47.6 – 60.1)	46.1 $\pm$ 24.71 (39.8 – 52.3)	0.0276	55.0 $\pm$ 25.47 (14.47 – 95.53)
p-tau <sub>181</sub> (pg/mL) (95% C.I.)	24.9 $\pm$ 8.45 (22.2 – 27.6)	21.0 $\pm$ 7.83 (19.0 – 23.0)	0.0093	23.5 $\pm$ 8.35 (10.22 – 36.78)
t-tau/A $\beta_{1-42}$ ratio (95% C.I.)	0.240 $\pm$ 0.141 (0.195 – 0.286)	0.215 $\pm$ 0.157 (0.176 – 0.255)	0.0451	0.196 $\pm$ 0.083 (0.063 – 0.329)
p-tau <sub>181</sub> /A $\beta_{1-42}$ ratio (95% C.I.)	0.113 $\pm$ 0.075 (0.089 – 0.138)	0.099 $\pm$ 0.063 (0.084 – 0.115)	0.1482	0.084 $\pm$ 0.023 (0.047 – 0.121)
p-tau <sub>181</sub> /t-tau ratio (95% C.I.)	0.491 $\pm$ 0.160 (0.439 – 0.543)	0.543 $\pm$ 0.263 (0.477 – 0.609)	0.6820	0.495 $\pm$ 0.230 (0.130 – 0.860)
$\alpha$ -syn (pg/mL) (95% C.I.)	1264 $\pm$ 425.7 (1126 – 1403)	1082 $\pm$ 611.1 (928 – 1235)	0.0120	1413 $\pm$ 750.6 (219 – 2608)
$\alpha$ -syn (pg/mL) (95% C.I.) *	1267 $\pm$ 443.5 (1109 – 1424)	1020 $\pm$ 456.7 (883 – 1158)	0.0175	1359 $\pm$ 909.8 (-901 – 3619)
$\alpha$ -syn (pg/mL) (95% C.I.) **	1269 $\pm$ 435.2 (1124 – 1414)	1019 $\pm$ 474.8 (886 – 1151)	0.0073	1359 $\pm$ 909.8 (-901 – 3619)

\*Subjects with CSF Hb < 200 ng/mL. N=33 for HC, N=45 for PD, N=3 for SWEDD.

\*\*Subjects with CSF Hb < 500 ng/mL. N=37 for HC, N=52 for PD, N=3 for SWEDD.

#Mann-Whitney U test

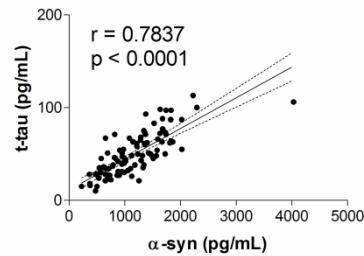
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# Summary of multivariate regression analyses

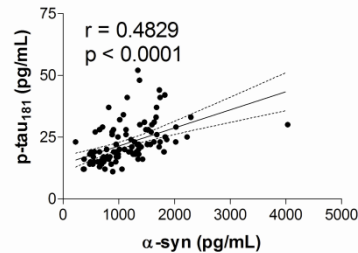
- Multivariate regression analysis:  $\alpha$ -SYN is significantly associated with PD diagnosis ( $p=0.0019$ ), but other biomarkers or their ratios are not.
- For clinical variables in PD, p-tau is significantly associated with UPDRS III motor score, ( $p=0.0140$ ), but we could not observe any significant associations between other biomarkers or ratios and other clinical variables.

## Correlation between AD biomarkers and $\alpha$ -synuclein

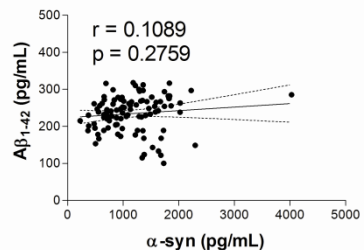
t-tau



p-tau



$A\beta_{1-42}$



# Association of CSF biomarkers with clinical Phenotype of PD

- ✓ Tremor dominant, or Postural instability and gait disturbance (PIGD) dominant phenotype
- ✓ These phenotypes were classified by tremor and PIGD score derived from motor subsection of the UPDRS (Zetuský WJ et al, 1985; Jacovic J et al., 1990)
  - Ratio of tremor/PIGD score  $\geq 1.5$ : Tremor dominant
  - Ratio of tremor/PIGD score  $\leq 1.0$ : PIGD dominant
  - Others: Mixed type

# UPDRS Subsection used to classify Tremor or PIGD-dominant phenotype

- ✓ Mean tremor score (10 items)

  - : UPDRS II – 1) Tremor

  - : UPDRS III – 2, 3) Postural tremor (both hands), 4, 5) Kinetic tremor (both hands), 6-10) Resting tremor (4 extremities and lip/jaw)

- ✓ Mean PIGD score (5 items)

  - : UPDRS II – 1) Walking and balance, 2) Freezing

  - : UPDRS III – 3) Gait, 4) Freezing of gait, 5) Postural stability

# CSF biomarkers according to clinical phenotype in PD patients

Biomarkers	PIGD (N = 22)	Tremor (N = 18)	<i>p</i> value*	Mixed (N = 27)	HC
A $\beta$ <sub>1-42</sub> (pg/mL)	213.9 ± 40.5	241.7 ± 28.1	<b>0.0081</b>	239.1 ± 55.8	<b>242.8 ± 49.95<sup>#</sup></b>
t-tau (pg/mL)	38.7 ± 25.5	49.6 ± 16.1	<b>0.0187</b>	51.0 ± 27.7	<b>53.9 ± 19.33<sup>#</sup></b>
p-tau <sub>181</sub> (pg/mL)	19.5 ± 8.0	22.0 ± 6.6	0.1697	22.0 ± 8.4	<b>24.9 ± 8.45<sup>#</sup></b>
$\alpha$ -syn (pg/mL)	905.8 ± 482.1	1149.0 ± 490.1	0.0553	1229.0 ± 758.8	<b>1264 ± 425.7<sup>#</sup></b>
$\alpha$ -syn (pg/mL), CSF Hb < 500 ng/mL	857.5 ± 495.3	1068.0 ± 447.6	0.0824	1150.0 ± 517.8	<b>1269 435.2<sup>#</sup></b>
$\alpha$ -syn (pg/mL), CSF Hb < 200 ng/mL	778.9 ± 363.0	1098.0 ± 449.0	<b>0.0291</b>	1186.0 ± 532.9	<b>1267 443.5<sup>#</sup></b>
t-tau/A $\beta$ <sub>1-42</sub> ratio	0.197 ± 0.176	0.208 ± 0.072	0.0503	0.232 ± 0.175	<b>0.240 ± 0.141<sup>#</sup></b>
p-tau/A $\beta$ <sub>1-42</sub> ratio	0.096 ± 0.052	0.092 ± 0.030	0.5960	0.105 ± 0.082	0.113 ± 0.075
p-tau/t-tau ratio	0.622 ± 0.338	0.466 ± 0.113	0.2769	0.523 ± 0.248	0.49 ± 0.16

\*PIGD vs. Tremor; Mann-Whitney U test

<sup>#</sup>Significant different vs PIGD, but not vs Tremor group

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# January 2012 Call: Review of PD biomarker literature

- Alpha-synuclein
  - Post translational modifications: phosphorylated, nitrated, aggregated, etc
- DJ-1 modifications (Oxidized)
- MicroRNAs
- Isoprostanes/Oxidative markers
- Neurofilament markers
- Ft3 and Fractalkines
- Neuromelanin



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# Next Biologics WG call

- Revisit criteria and data required for inclusion in PPMI
- Invite Jing Zhang to discuss his data around novel PD biomarkers



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