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Biomarker Discovery and Validation for Major Depressive Disorder (MDD)

Alexander M. Buko PhD

VP Business and Product Development



Human Metabolome Technologies America, Inc.

Over 12 years of growth and discovery

- **Over 12 years of growth**
- **~ 3 years in USA (Boston)**
- **Unique metabolic data based upon *CE-MS platform***
- ***Proprietary data analysis software***
- ***Large metabolomic data library* of over 1600 compounds**
- ***Accurate quantitation profiling* on over 400 metabolites**
- **Over 500 projects a year (2014)**





One of the largest metabolomics research center (>50 MS systems)

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Pipeline to Discovery/Validation

The Process

- SOP
- Validation
- QC
- Quantitative
- Internal standards
- Documentation
- Algorithm generation
- Statistical analysis
- PCA, OPLS-DA, HCA
- Diagnostic plan

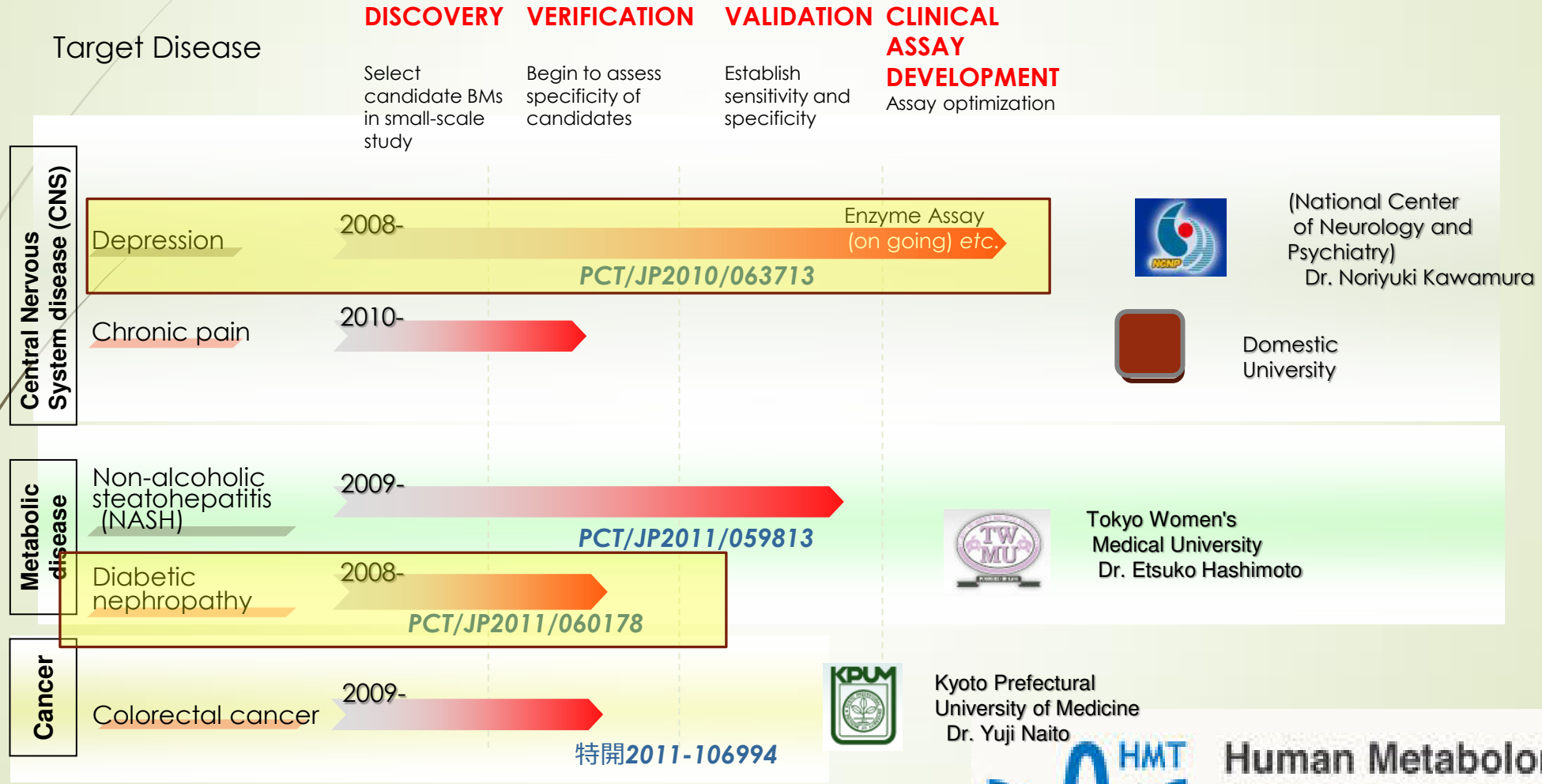
Samples

- Documentation
- SOP
- Sufficient group size
- Proper control groups
- Clinical data

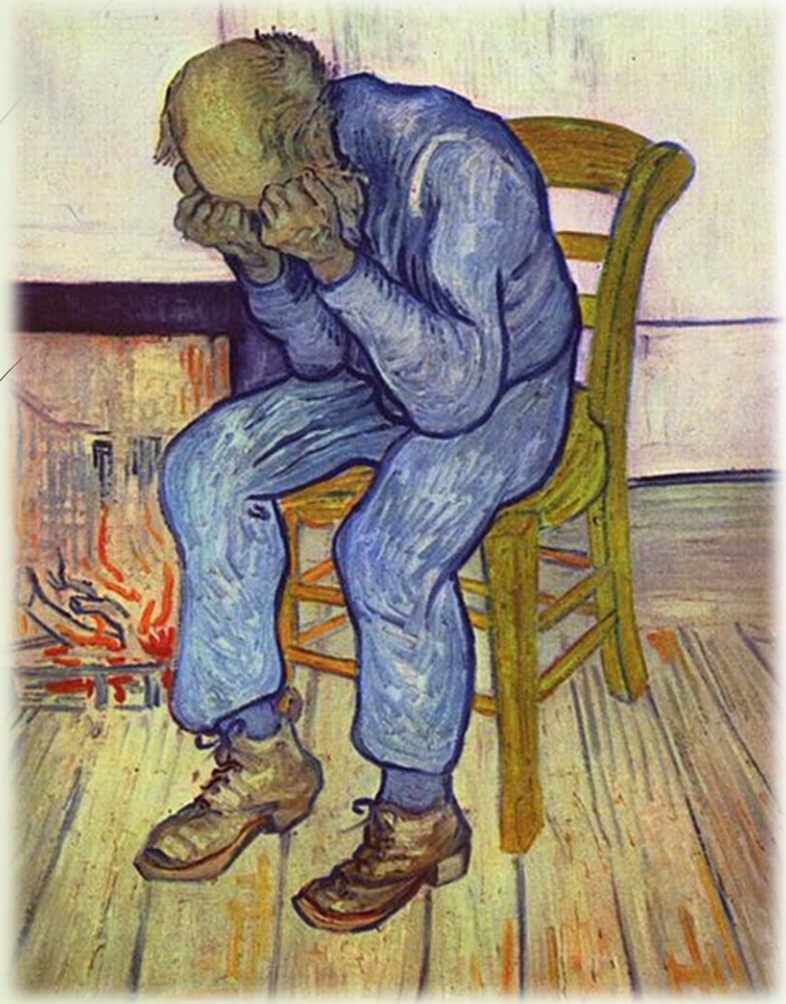
HMT Biomarker Process

- **Profiling** – Significant changes within limited number of samples and controls using high performance platform
- **Metabolite Identification** – Validated reference material
- **Validation** – Using larger number of samples to account for natural and biological variation to validate previously identified metabolites – working with other omic and phenotype data…
- **Biology** –Connection between the validated metabolite biomarkers and their biological processes or pathways
- **Clinical plan** – How such biomarker would be used in field
  - Type 0: disease progression, history, outcome
  - Type 1: therapeutic effect or intervention
  - Type 2: Surrogate: clinical end point

HMT Biomarker Pipeline



Major Depressive Disorder (MDD)



うつ病の血液診断、簡便に

血液中に含まれるリン酸の濃度を測り、うつ病を診断する検査法を、慶応大の研究成果をもとにしたベンチャー企業が開発した。従来、研究されてきた血液による診断法に比べ簡便なことが特徴。健康診断で使うことで早期発見につながる可能性がある。

開発したのは「ヒューマン・メタボローム・テクノロジーズ」（山形県鶴岡市）。22日に、東京都で開かれる日本生物学的精神医学会で発表する。

同社は、国立精神・神経医療研究センターで「大うつ病性障害（うつ病）」と診断された31人と、年齢や性別の構成が近い健康な35人の血液を分析。うつ病患者は、血漿中の「エタノールアミンリン酸」の濃度が低いことを見つけた。このリン酸の濃度を調べて、うつ病患者を正しく診断できた確率は82%、健康な人をうつ病でないと診断できた確率は95%だった。

血液でうつ病を診断する方法は複数の研究グループが開発している。しかし、約20種類の白血球の遺伝子を調べたりするなど、簡便な測定は難しかった。

同社は、このリン酸の濃度を数分で測れる試薬を開発中。1年以内に完成する見込みで、臨床試験を行い、医療機器としての承認を目指す。

厚生労働省研究班の調査では、一般医の9割は経験則でうつ病を診断しているという。うつ病で受診している人は70万人を超えるが、治療が必要な人の4分の1程度しか受診しておらず、早期発見が難しい。

（岡崎明子）

ベンチャーが開発

リン酸の濃度測り、数分で判定

US Patent: 8951739

PCT/JP2010/063713

ZL201080046087.6



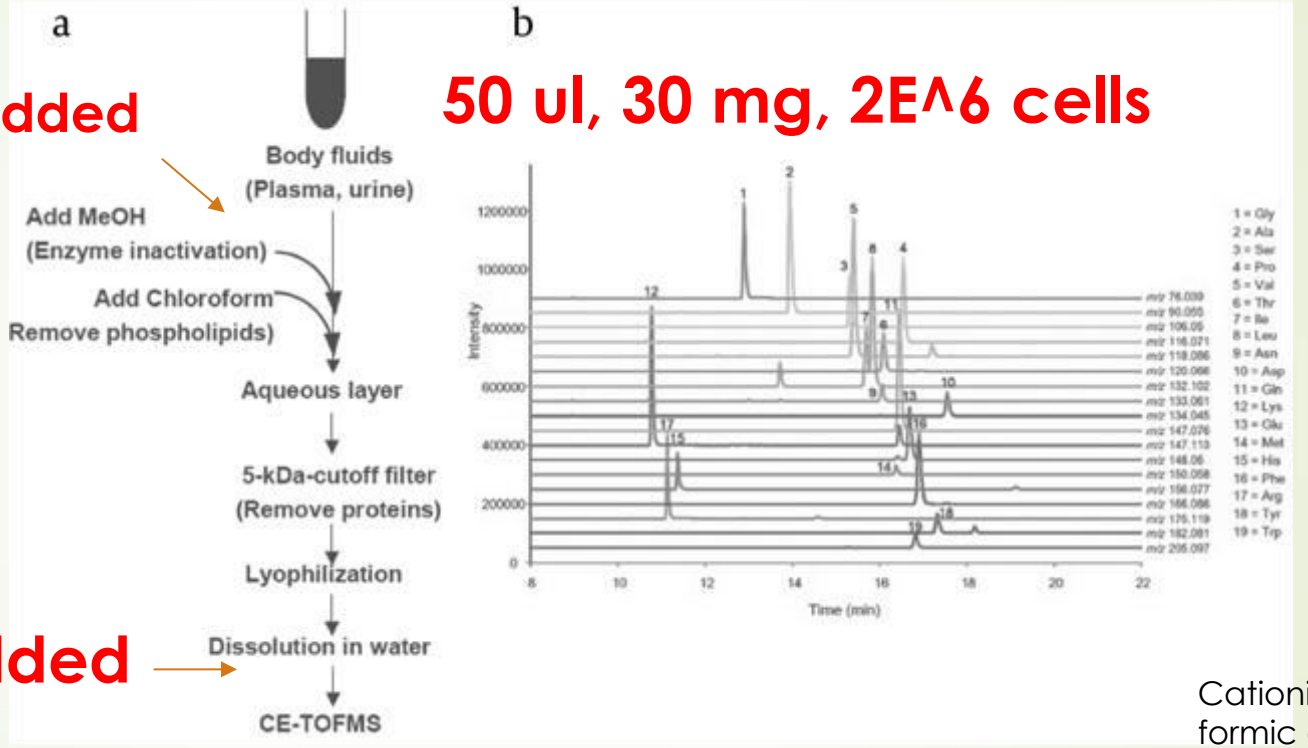
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Plasma Biomarker Discovery

- EDTA-treated **plasma** was collected by vacuum blood tubes .
- Plasma was prepared **within 2 hours**, and stored at **-80 degree Celsius.**
- Metabolites were extracted using (**H_2O:MeOH:$CHCl_3$**).
- Metabolites in the **aqueous layer** were analyzed by capillary electrophoresis-time-of-flight mass spectrometry system (**CE-TOFMS**: Agilent) by anion (negative) and cation (positive) modes.
- Yielded plasma metabolome data include **538 metabolites.**
- Metabolite levels were **compared** across patient cohorts.

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Normalization and Quality Assessment



Cationic Metabolites: MSD-TOF
formic acid
Anionic Metabolites: TSQ AmAc
Uncoated Fused Silica Capillaries

Hayashi K, Sasamura H, Hishiki T, Suematsu M, Ikeda S, Soga T, et al. Use of serum and urine metabolome analysis for the detection of metabolic changes in patients with stage 1-2 chronic kidney disease. Nephro-Urol Mon. 2011;3(3):164-171.

HMT Publication

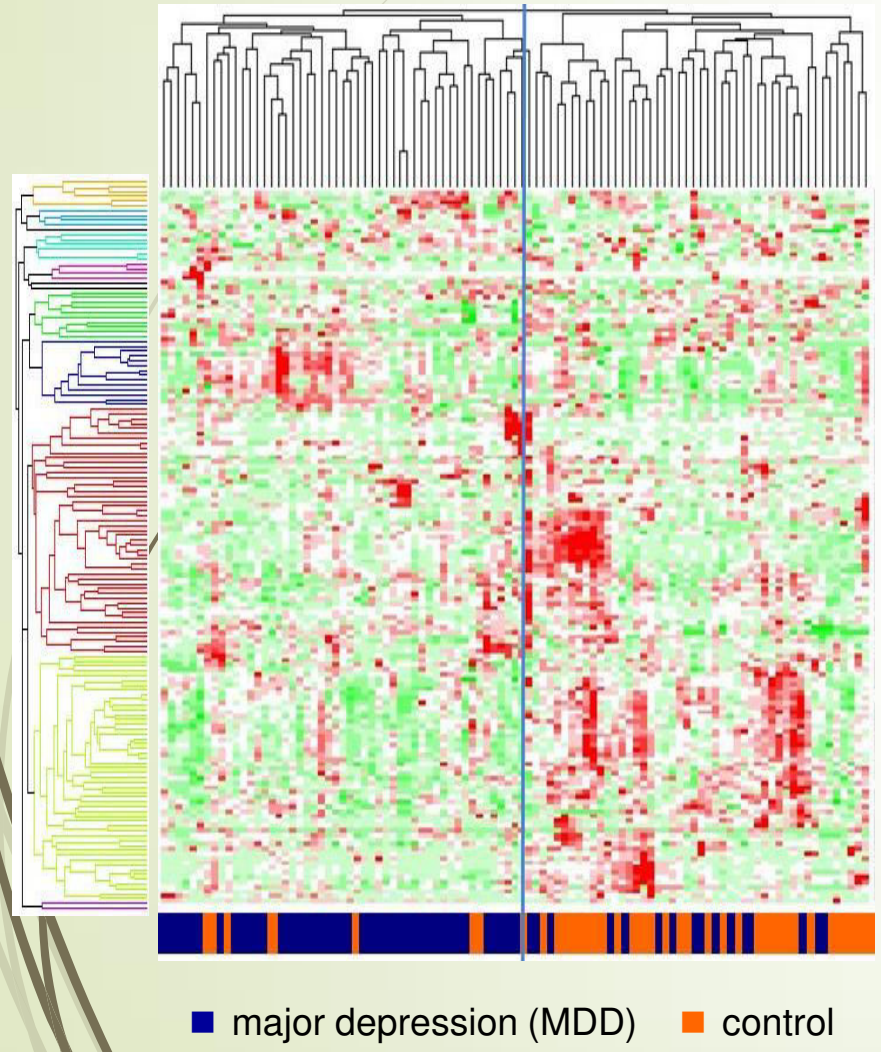
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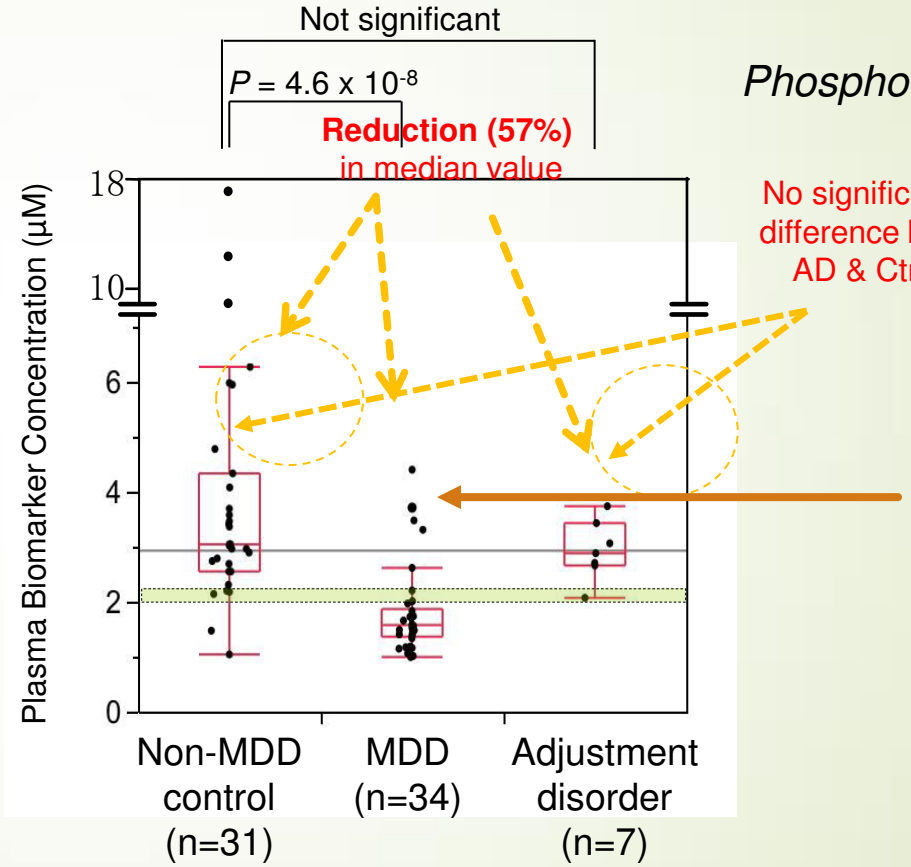
Early Discovery

		Non-MDD Group	MDD Case Group
	Healthy	Adjustment Disorder	MDD
Subjects	31	7	34
Age	37.8 (20-63)	49.4 (27-78)	38.9 (20-70)
Gender	M 15, F 16	M 2, F 5	M 14, F 20
BMI	21.8 (16.6-30.4)	23.7 (18.7-33.3)	22.9 (18.6-31.2)
CES-D (Score)	7.7 (0-17)	21.6 (13-34)	31.6 (8-50)

- Hospital/IRB: National Center of Neurology and Psychiatry, JAPAN (NCNP)
- Diagnosis: DSM-IV-TR. SCID. Depression scale was estimated by the CES-D.
- Non-MDD group: Subjects answered a news paper advertisement.
- All subjects are Asians living in Japan (mainly in Tokyo).
- CES-D: Center for Epidemiologic Studies Depression Scale (a simple questionnaire, 18>MDD for Japanese). This score is used to be reference to diagnose MDD.

Promising Results



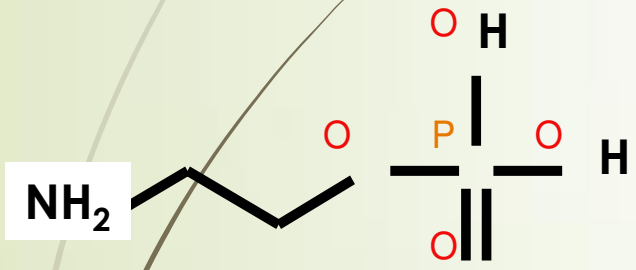


Phosphoethanolamine (PEA)

"Outliers" in whisker plot are "out of season" patients

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Single Molecule - ROC



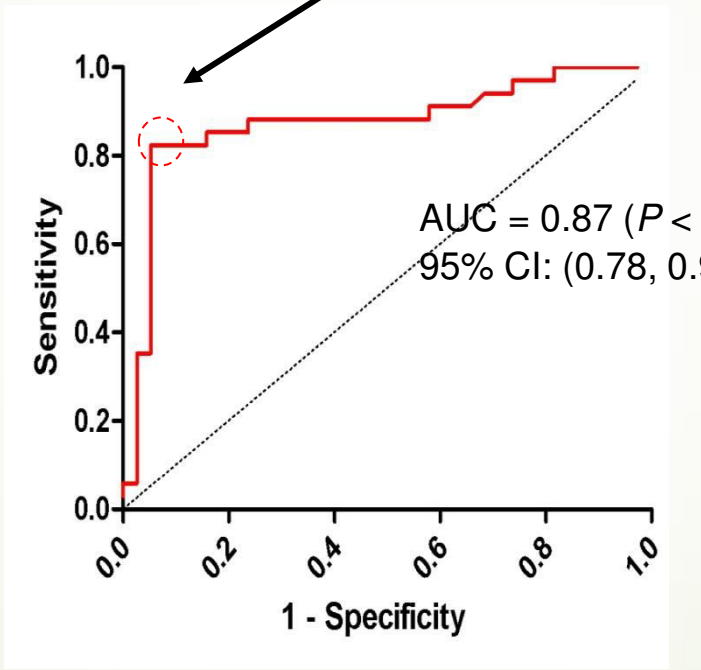
Phosphoethanolamine (PEA)

Molecular formula:

$C_2H_8NO_4P$

Exact mass: 141.019097

Sensitivity: 82%, Specificity: 95%

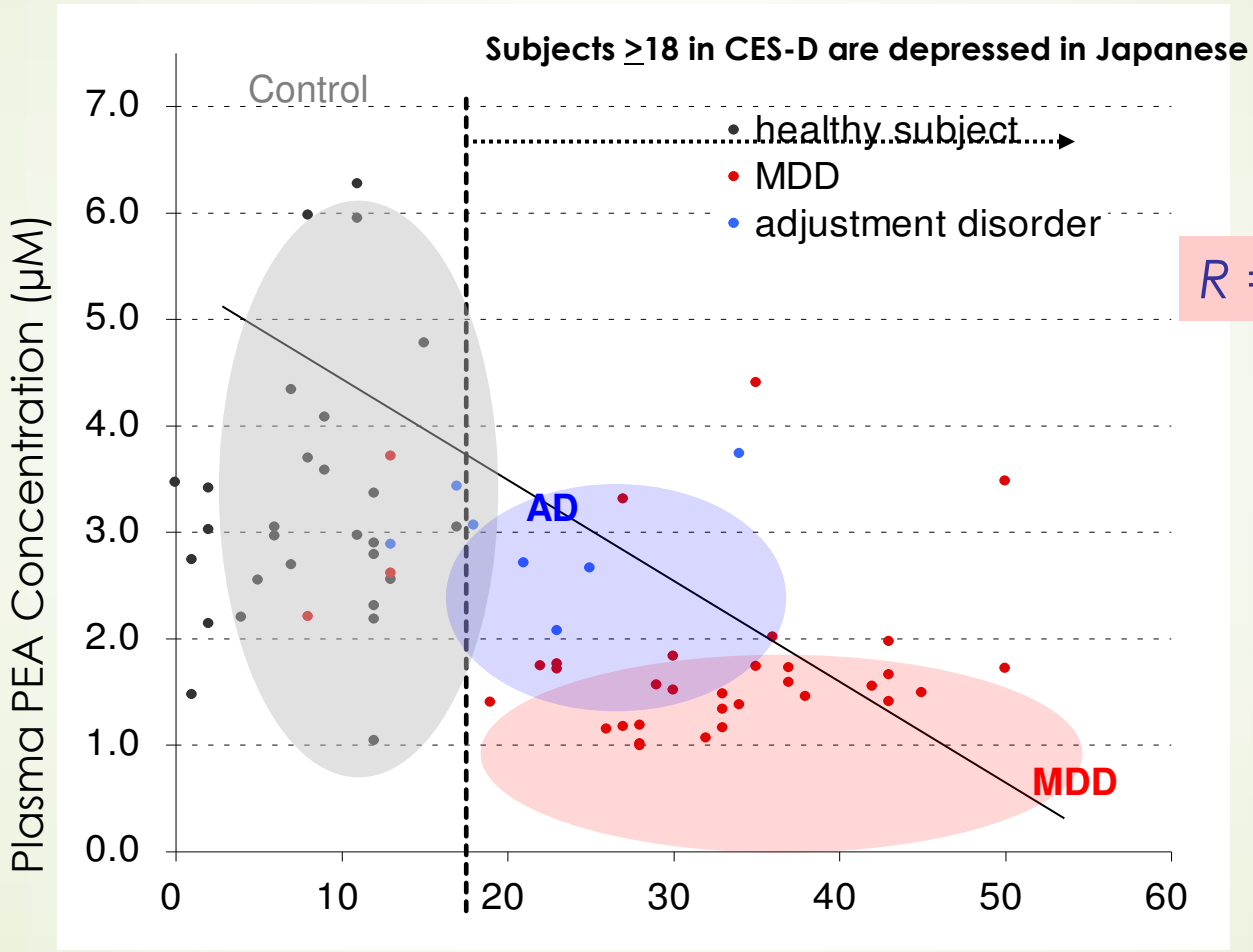


- Receiver operator characteristic (ROC) curve for biomarkers observed in 72 plasma samples
- (34 MDD, 38 non-MDD individuals)

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Correlates with CES-D Score

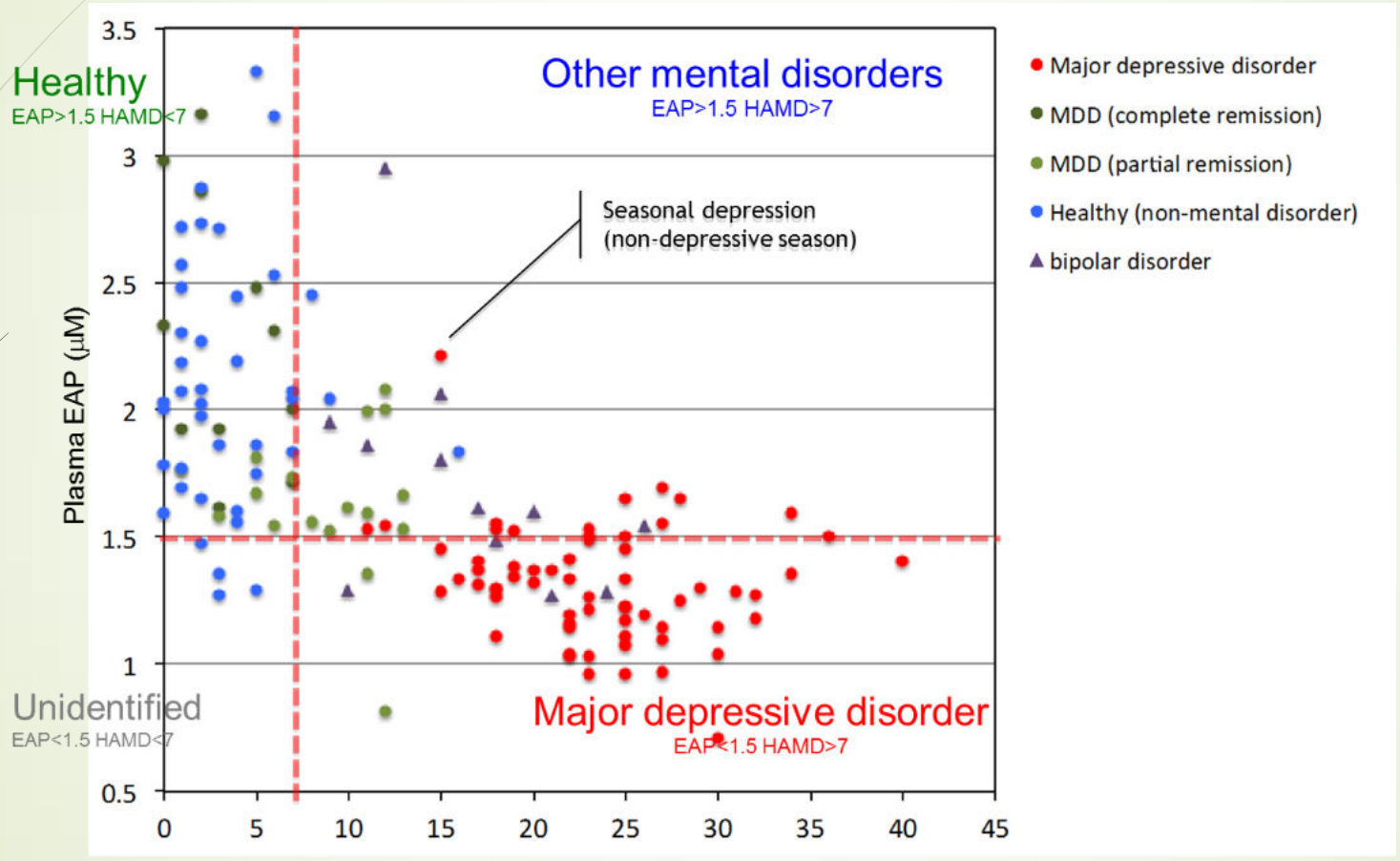
Plasma PEA level correlates significantly with the popular depression rating scale.



$R = -0.433$ ($P = 0.0001$)

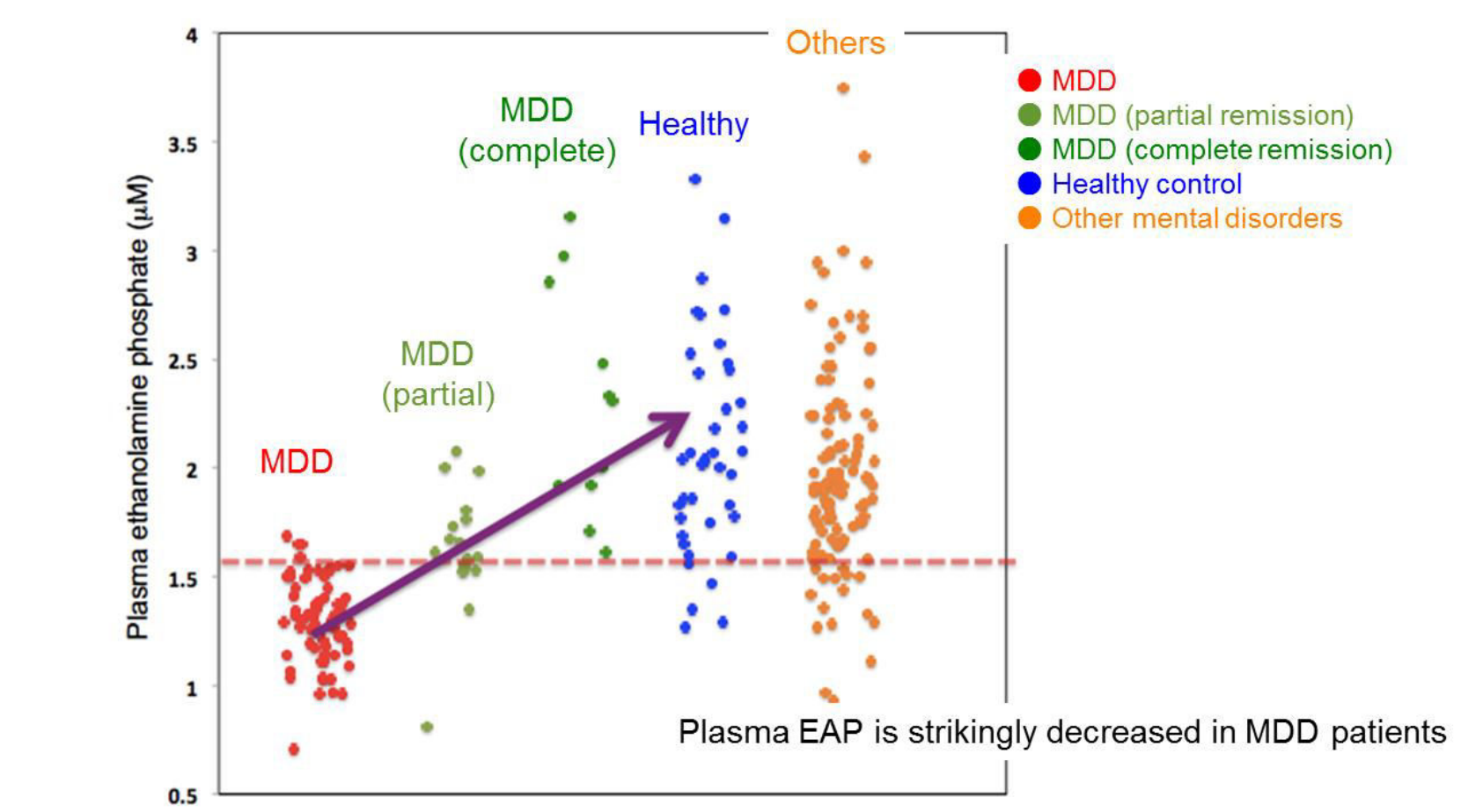
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Correlates with HAMD-17 Score



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Correlates with Remission



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Clinical Features

- Correlation with HAMD-17 and CES-D good
- PEA levels restored with remission
  - Medication can be reduced when levels rebound over 1.8 uM
- Depression with high PEA suggests other diagnoses
- In Bipolar Disease, PEA is low when depressed, high when manic

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Assay Transfer

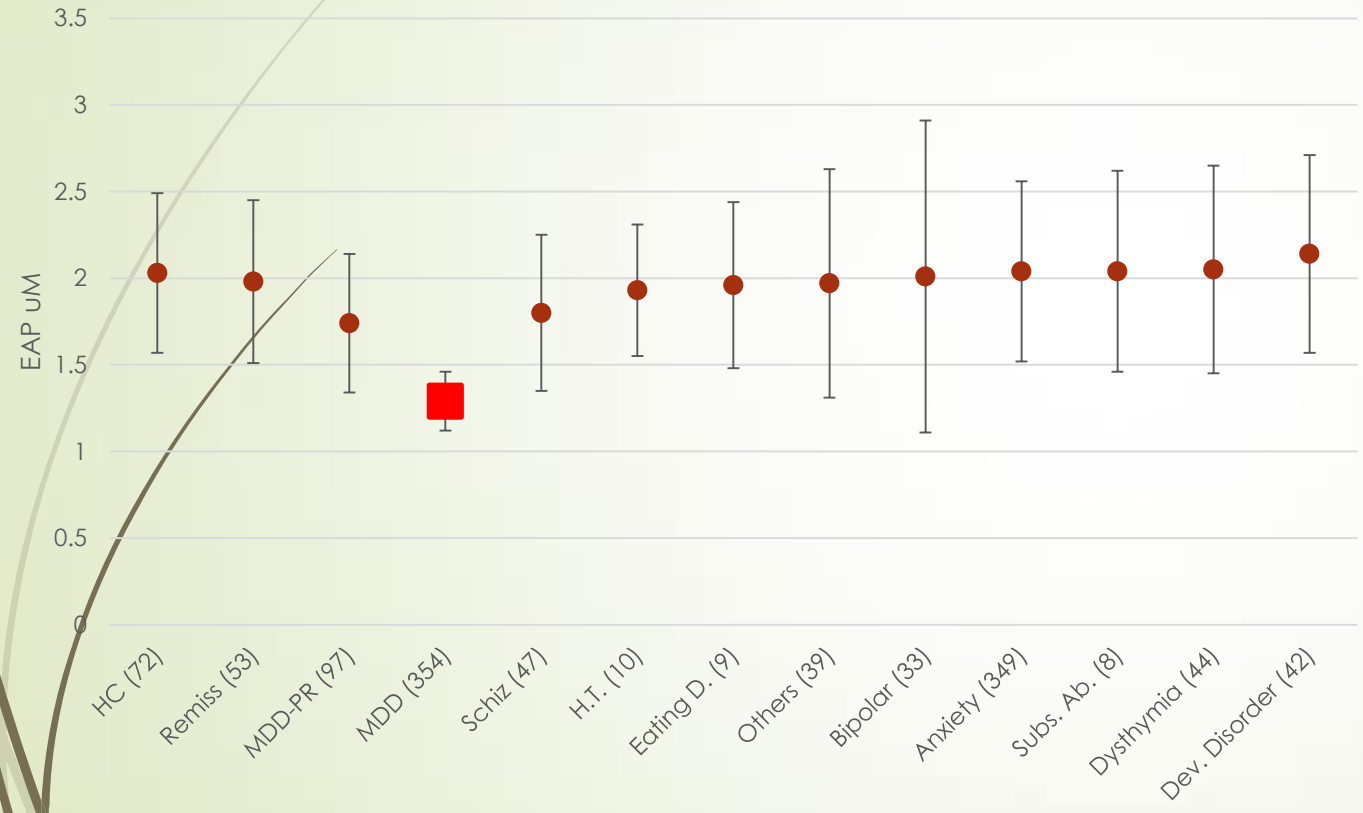
- Initial Discovery and early validation
- CE-MS advance scan – relative data
- CE-MS QQQ – quantitative data

- IC-FLD





In Clinical Validation (> 1,100 tests)



- **Expand comparisons to other disorders (PTSD)**
- **Exercise**
- **Circadian Rhythm**
- **Alcohol**
- **Eating, Smoking**
- **Drug use**
- **Other nationalities**

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ROC Improves – Onto Larger Studies

	Sensitivity (%)	Specificity (%)
BDI-II > 10	73.3	84.4
BDI-II > 18	40	100
PHQ-9	76	81
PHQ-2	76	82
CED-D > 16	86.7	76.6
CES-D > 21	73	96.1
PEA < 1.50 uM	94.4	92.8
	% finds MDD	% Hit is MDD

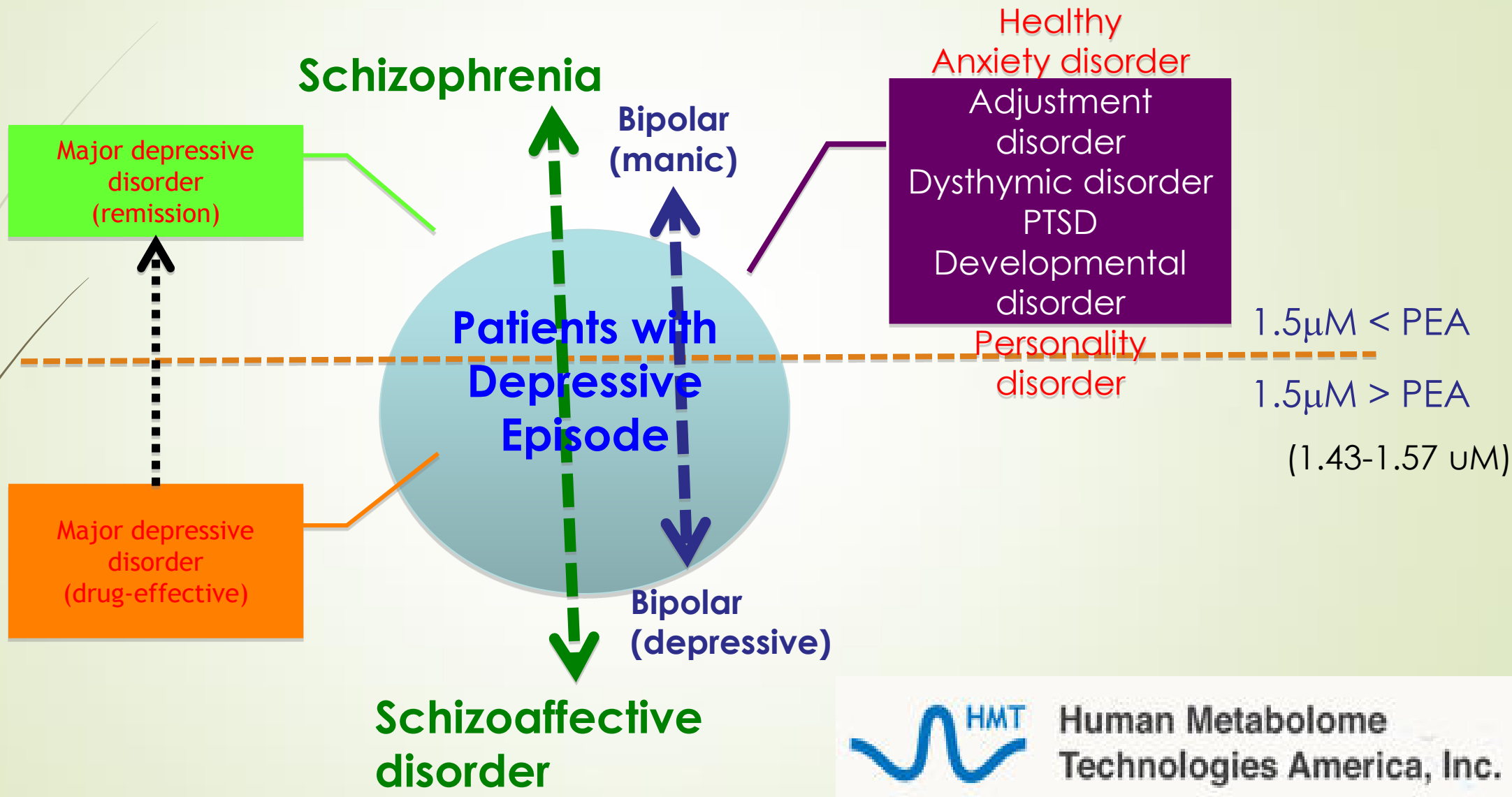
Large Scale Validation studies (2015-)

- Toyoko-Keiai Hospital of St. Marianna Assoc.
- Shinjuku Mental Clinic

Initial Large scale validation study was done in the Dr. Kawamura's clinic (formally designated as "Kawamura Clinic for General Practice, Gyokikai Medical Corporation".

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Diagnostics Plan



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Effect of SSRI and SNRI Treatment

- Studies showed imbalances in dopamine, norepinephrine, and serotonin in patients with depression. An SSRI and an SNRI both affect absorption of serotonin, but an SNRI also affects noradrenaline levels in the brain
- SSRI: Selective Serotonin Reuptake Inhibitors (Paxil, Prozac, Zoloft)
- SNRI: Serotonin Noradrenaline Reuptake Inhibitors (Effexor, Pristiq)

Observations



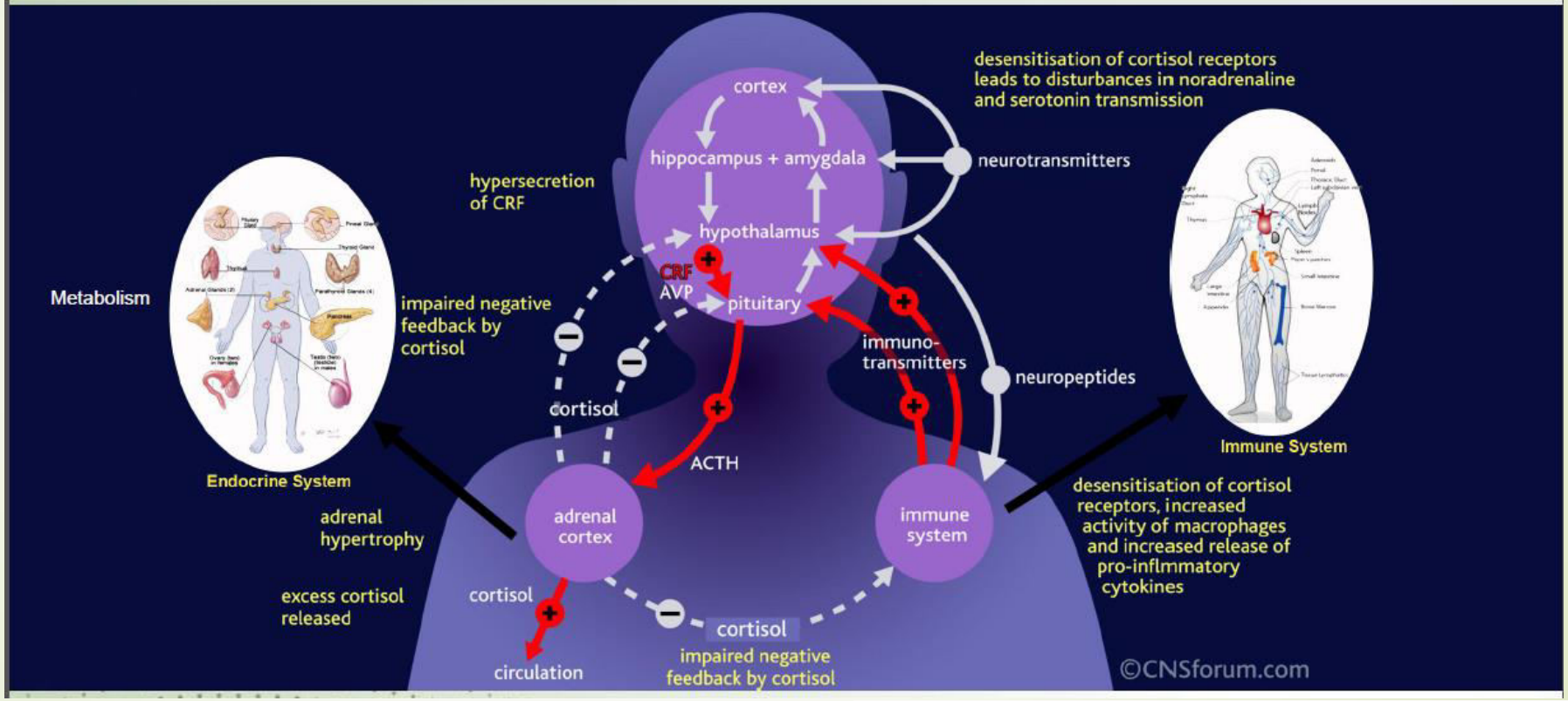
- With **SSRI treatment**, PEA decreases. Perhaps **anxiety** is an excited brain, SSRI is effective with anxiety. Lower PEA may be calming down brain.



- With **SNRI treatment**, PEA increases. Perhaps **depression** due to decline in noradrenaline function. SNRI activates brain, stimulates dopamine and PEA increases

Depression is Not Solely a Brain Disorder- It has a Complex Systemic Impact

Depression can be assessed by measuring multiple biological mediators within the Neuroendocrine, Hypothalamic, Pituitary, Adrenal, Metabolic and Immune Systems.

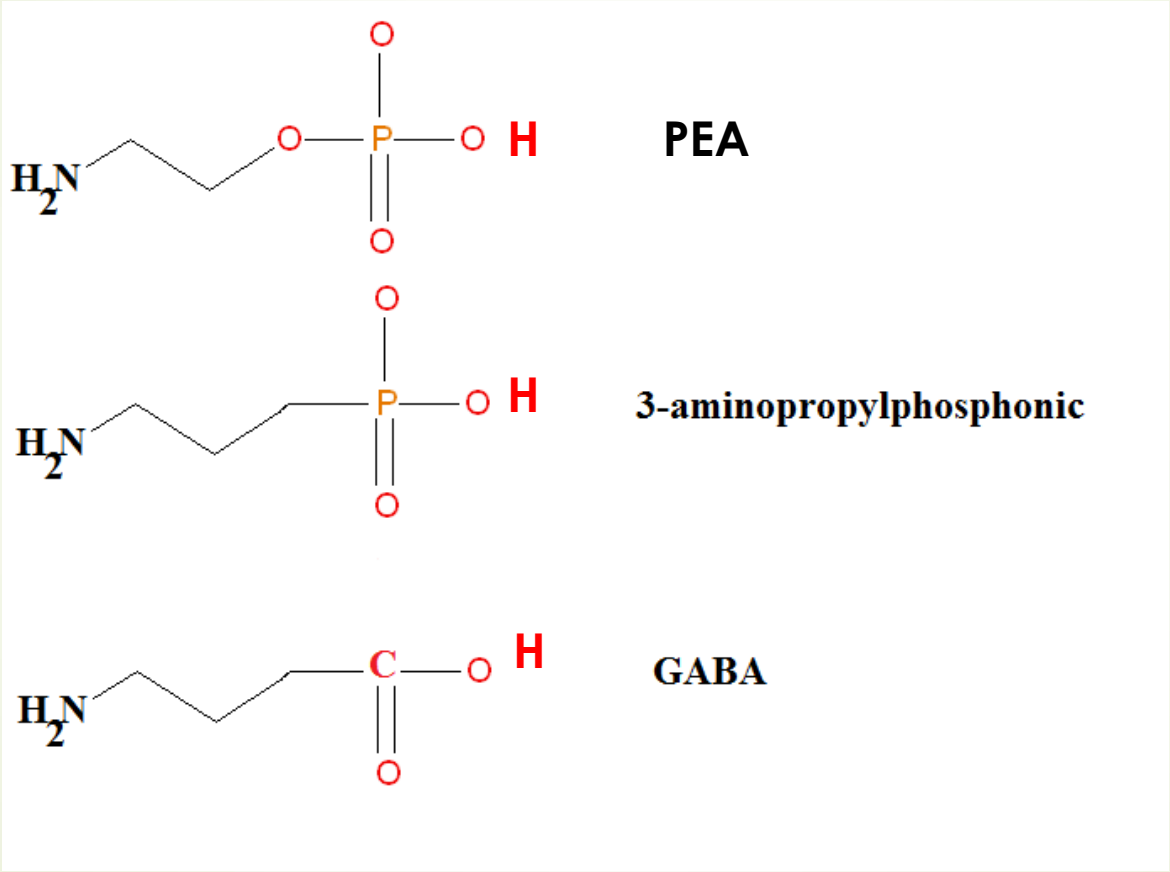


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What is PEA?

- A phosphomonoester metabolite of phospholipid metabolism
- In developing brain, phosphomonoesters are normally elevated during the period of neuritic proliferation
- Role of phosphomonoesters in membrane biosynthesis
- It is NOT beta-phenylethylamine (PEA); a naturally occurring trace amine neurotransmitter and neuroregulator that is normally synthesized in the brain from the amino acid phenylalanine.

Biosimilars to PEA



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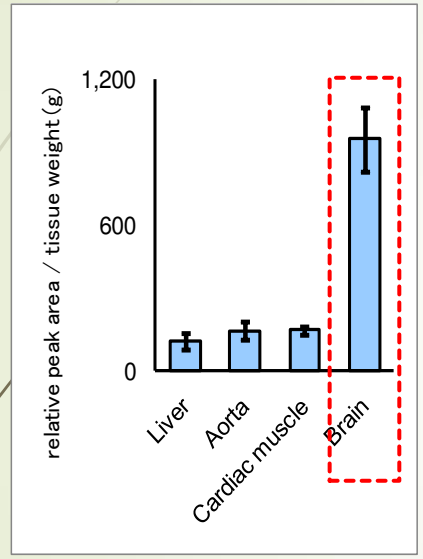
PEA bioactivity

- PEA showed little activity at any of the **GABA** binding sites.
- PEA was most potent at **GABAB** sites.
- The GABAB receptor 1 gene is mapped to chromosome 6p21.3 within the HLA class I region close to the HLA-F gene. Susceptibility loci for **multiplesclerosis, epilepsy, and schizophrenia** have also been mapped in this region.
- The efficient exclusion of PEA from GABA binding sites may be an important physiologic mechanism in the control of **inhibitory neurotransmission**.

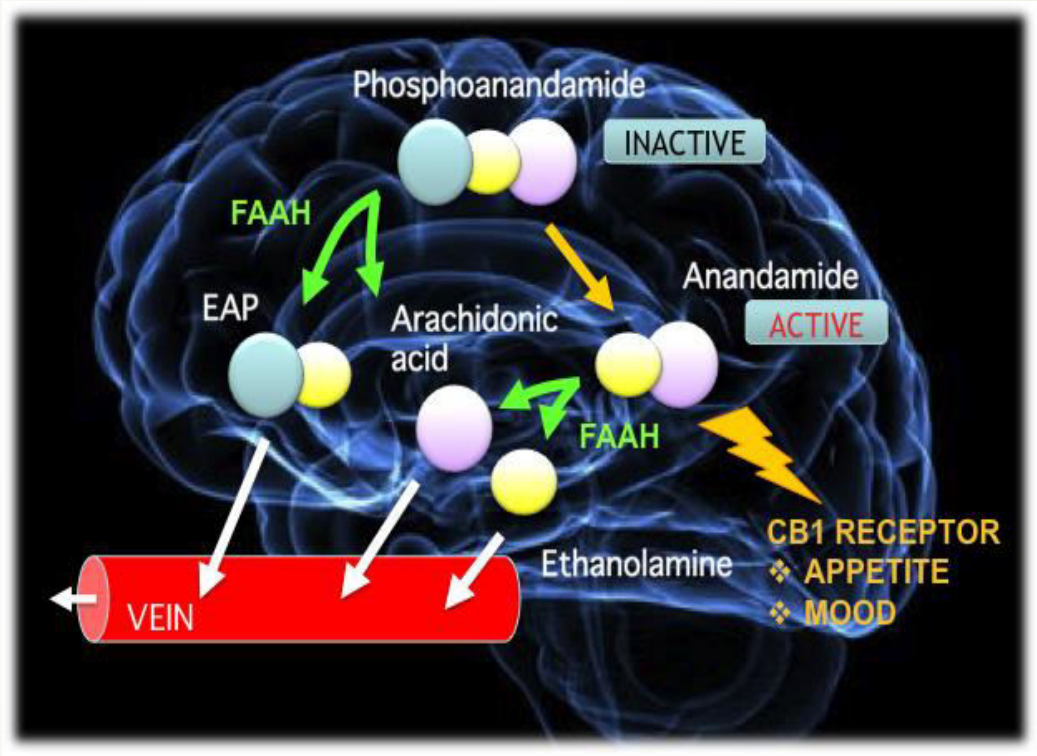
Life Sci. 1995;56(26):2377-83.
Inactivity of phosphoethanolamine, an endogenous GABA analog decreased in Alzheimer's disease, at GABA binding sites.
Klunk WE[1], Debnath ML, McClure RJ, Pettegrew JW.

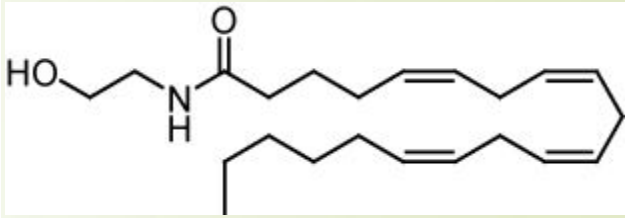
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Link to Reward Pathway



Tissue specific accumulation of free PEA (rabbit)

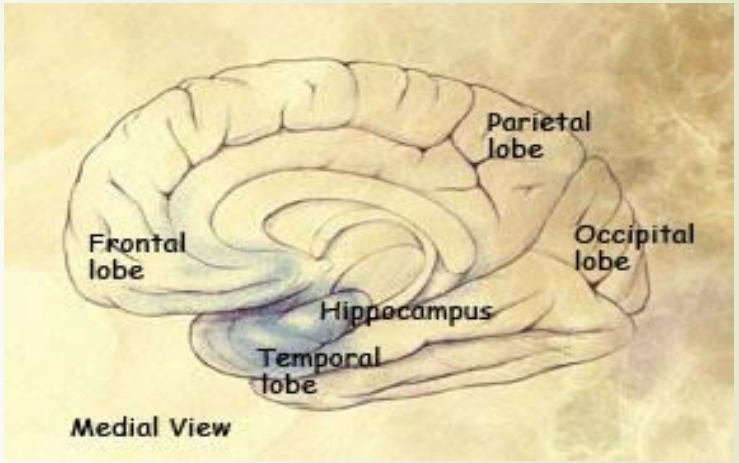




NOTE: Anandamide is an endogenous cannabinoid neurotransmitter. It was isolated in 1992. It is important in the regulation of feeding behavior, and the neural generation of motivation and pleasure. In addition, anandamide injected directly into the forebrain reward-related brain structure nucleus accumbens enhances the pleasurable responses of rats to a rewarding sucrose taste, and enhances food intake as well.

PEA and Alzheimer's Disease

- PEA stable post mortem brain
- 5 – 10 samples, umol/g PEA:
- Phospholipid turnover
- Precursor to phosphatidylcholine
- Released during some depolarizing events
- Often followed by taurine release



	CTRL	AD	% Decr.
Temporal Cortex	1.21	0.43	64%
Frontal Cortex	0.97	0.5	48%
Parietal Cortex	0.78	0.52	33%
Occipital Cortex	0.9	0.8	12%
Hippocampus	0.95	0.57	40%

Brain Res. 1987 Aug 11;417(2):389-92.
Phosphoethanolamine and ethanolamine are decreased in Alzheimer's disease and Huntington's disease.
Ellison DW[1], Beal MF, Martin JB.

Other Pipeline Developments

- Diabetic Nephropathy

Biomarkers Diabetic Nephropathy (DN)

- 78 Type 2 DM patients
  - Without nephropathy and albuminuria (**non-DN**) – 20 patients
    - UACR* 12.1 +/- 6.7 mg/g eGFR 81.9 +/- 24.0
  - Early DN with micro-albuminuria (**Micro-DN**) – 32 patients
    - UACR 103.9 +/- 77.8 mg/g eGFR 70.5 +/- 21.9
  - Overt DN with macro-albuminuria (**Macro-DN**) – 26 patients
    - UACR 1055.3 +/- 741.3 mg/g eGFR 47.2 +/- 25.6

Anal Bioanal Chem. 2012 Dec;404(10):3101-9
Metabolic profiling reveals new serum biomarkers for differentiating diabetic nephropathy.
Hirayama A[1], Nakashima E, Sugimoto M, Akiyama S, Sato W, Maruyama S, Matsuo S, Tomita M, Yuzawa Y, Soga T.

***Urine Albumin-to-Creatinine Ratio** (**UACR**).

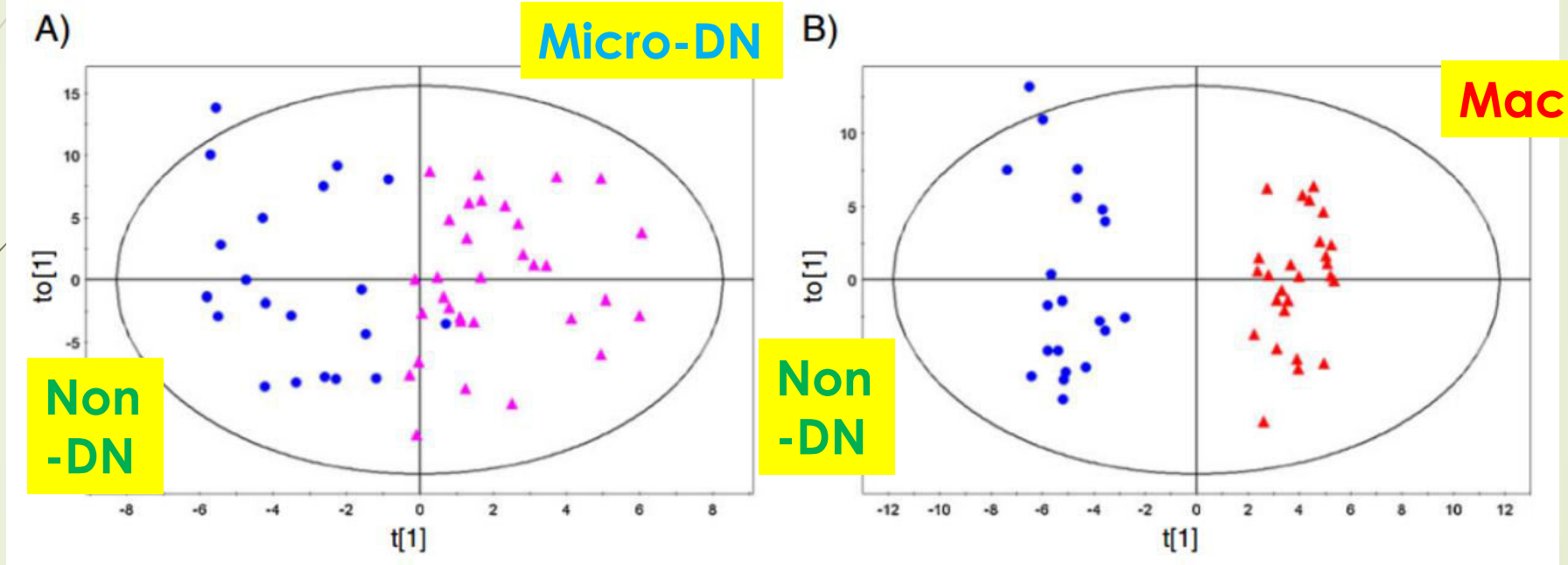
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Discovery

- CE-TOFMS analysis of serum
- Relative metabolite ratios
- Spearman's rank correlation to UACR, eGFR
- Multiple logistic regression
- PCA analysis

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OPLS-DA* (0.95 range)



*Orthogonal Projections to Latent Structures- discriminant analysis

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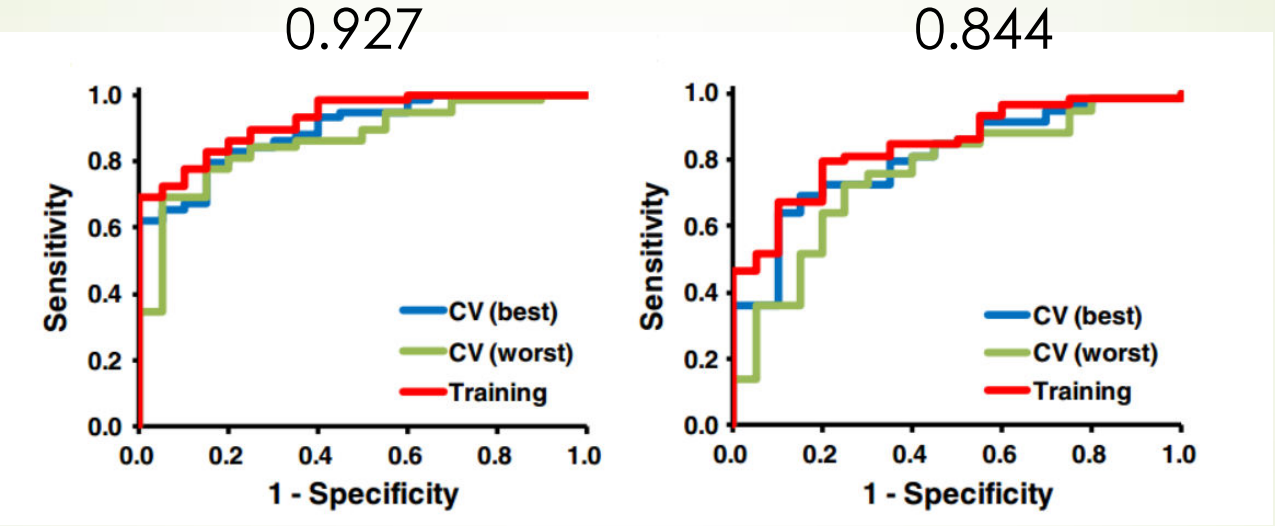
19 candidates separate DN stages

MID	Mode[a]	*m/z*	Non-DN	Micro-DN	Macro-DN	*p* value	Formula	Metabolite
11	C	114.067	0.501±0.153	0.612±0.209	1.052±0.506	<0.0001	$C_4H_7N_3O$	Creatinine
17	C	129.067	0.046±0.035	0.065±0.049	0.125±0.079	<0.0001	$C_5H_8N_2O_2$	
29	C	134.046	0.126±0.025	0.141±0.041	0.170±0.036	<0.0001	$C_4H_7NO_4$	Aspartic acid
39	C	146.118	0028±0.007	0.032±0.008	0.039±0.010	<0.0001	$C_7H_{16}NO_2$	γ-Butyrobetaine
51	C	156.139	0.006±0.005	0.005±0.004	0.001±0.003	0.0005	$C_9H_{17}NO$	
52	C	158.154	0.063±0.020	0.055±0.022	0.040±0.012	0.0007	$C_9H_{19}NO$	
69	C	176.104	0.194±0.053	0.199±0.060	0.275±0.081	0.0005	$C_6H_{13}N_3O_3$	Citrulline
78	C	203.150	0.006±0.002	0.007±0.002	0.010±0.005	0.0004	$C_8H_{18}N_4O_2$	SDMA
82	C	209.093	0.010±0.004	0.011±0.005	0.016±0.005	0.0002	$C_{10}H_{12}N_2O_3$	Kynurenine
96	C	243.184	0.026±0.013	0.019±0.012	0.015±0.007	0.0301		
97	C	244.106	0.0006±0.001	0.0009±0.001	0.002±0.002	0.0003		
114	C	276.128	0.008±0.006	0.006±0.004	0.003±0.003	0.0104		
127	C	302.197	0.106±0.050	0.079±0.047	0.062±0.028	0.0372		
134	C	316.213	0.010±0.009	0.006±0.007	0.003±0.002	0.0158		
152	A	96.960	0.216±0.063	0.243±0.063	0.341±0.103	<0.0001		
158	A	103.014	0.003±0.002	0.004±0.002	0.005±0.002	0.0001	$C_2H_4N_2O_3$	
202	A	149.049	0.030±0.006	0.034±0.009	0.051±0.019	<0.0001	$C_6H_6N_4O$	
232	A	187.098	0.020±0.013	0.017±0.011	0.009±0.004	<0.0001	$C_9H_{16}O_4$	Azelaic acid
246	A	209.031	0.029±0.012	0.024±0.009	0.016±0.013	<0.0001	$C_6H_{10}O_8$	Galactaric acid

Correlation to clinical measurements

MID	Metabolite	UACR		eGFR	
		Coefficients	*p* value	Coefficients	*p* value
11	Creatinine	0.5701	<0.0001[a]	−0.8832	<0.0001[a]
17		0.4968	<0.0001[a]	−0.5808	<0.0001[a]
29	Aspartic acid	0.4993	<0.0001[a]	−0.3912	0.0004[a]
39	γ-Butyrobetaine	0.4942	<0.0001[a]	−0.6492	<0.0001[a]
51		0.4728	<0.0001[a]	0.2204	<0.0001[a]
52		−0.4871	<0.0001[a]	−0.0678	0.053
69	Citrulline	0.4300	<0.0001[a]	−0.6531	<0.0001[a]
78	SDMA	0.4820	<0.0001[a]	−0.7111	<0.0001[a]
82	Kynurenine	0.5351	<0.0001[a]	−0.5627	<0.0001[a]
96		−0.3085	0.006[b]	0.1975	0.083
97		0.5223	<0.0001[a]	−0.7651	<0.0001[a]
114		−0.3638	0.001[b]	0.1392	0.224
127		−0.2961	0.009[b]	0.2035	0.074
134		−0.3669	0.001[a]	0.2397	0.035[c]
152		0.5336	<0.0001[a]	−0.7687	<0.0001[a]
158		0.4980	<0.0001[a]	−0.6302	<0.0001[a]
202		0.6352	<0.0001[a]	−0.7455	<0.0001[a]
232	Azelaic acid	−0.5210	<0.0001[a]	0.3739	0.0007[a]
246	Galactaric acid	−0.4596	<0.0001[a]	0.4152	0.0002[a]

ROC analysis – non-DN / DN patients



- γ-butyrobetaine, SDMA, azelaic acid, **MID 114, MID 127**
- Best Panel, highest AUC

- Aspartic acid, SDMA, azelaic acid, galactaric acid
- Best panel, only known metabolites

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Conclusions

- Biomarker discovery and validation is a pipeline
- Starts with accurate, quantitative data
- Builds increasing group size, additional controls
- Assay development
- Single (PEA) or multiple analyte panel (DN)
- Biological / pathway analysis
- Clinical plan (Type 0, 1, 2)
  - Algorithm based (combination clinical / blood metabolites)
  - Classifier, scoring

HMT Human Metabolome Technologies America, Inc.

- Quantitative
- High value metabolic space
- Unique resolution CE-MS
- Experienced



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