Blood-Brain molecular alterations in depressed suicides

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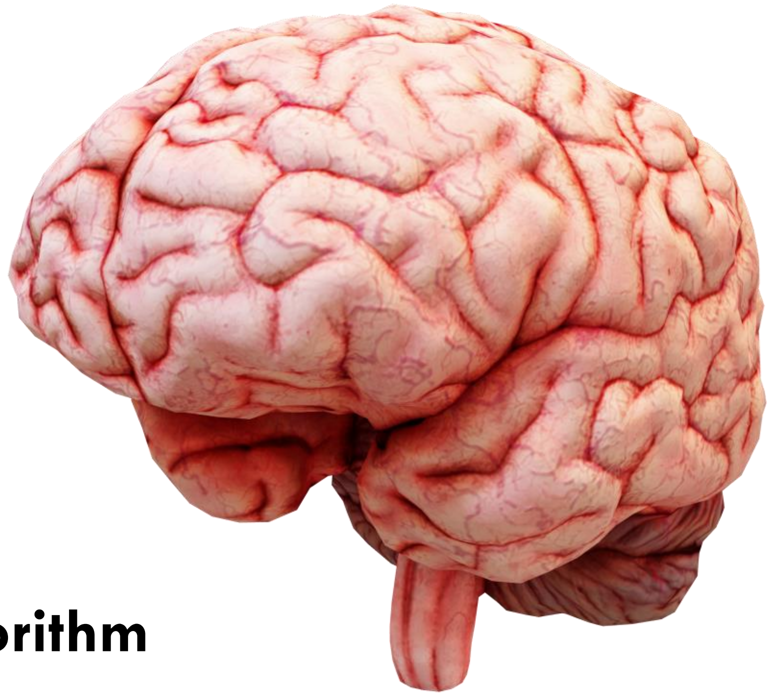
The suicide epidemic

- 3rd cause of death in men between 18-44 in the US
- 1.4 million attempts and 48,000 deaths per year in the US
- **Men die by suicide four times more often than women**
- **1 week** after patients leave psychiatric care suicide death rate is increased by **300%**
- ~45% of those who die by suicide see a clinician in the **month** prior to death and a third see a healthcare professional within the **week** of dying by suicide

Risk factors for suicide

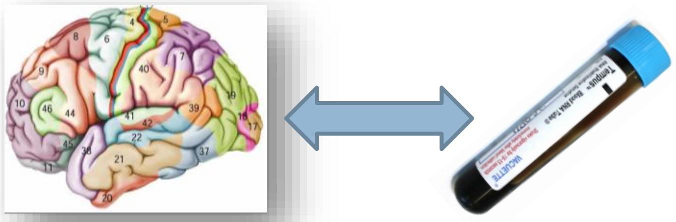
- **Previous attempt**
- **Family history** of suicide
- History of **depression or other mental illness**
- History of **alcohol or drug abuse**
- **Stress**ful life event or loss
- Easy **access to lethal methods**
- **Aggression and impulsivity**
- **Hopelessness**
- High **cortisol** levels
- **Psychological pain**



- **Currently no risk prediction algorithm**

Suicide specific biomarkers in MDDs

- **A high proportion of patients visit a health-care provider in the month prior to dying by suicide but there is currently no accurate way to predict who is at risk for suicide**
- GWAS and functional studies (methylation, gene expression) often compared MDD-Suicides to Controls or studied suicidal behaviors
- Most studies to date have looked at Brain or Blood independently
- Post-mortem Blood
  - Difficult to collect when PMI is high
  - High levels of coagulation and cell lysis
  - Not always collected in blood tubes with a preservation buffer
  - RNA often too degraded for qPCR, microarrays or RNA-Seq



- AFSP: Can we combine brain and blood expression signatures **to develop a transcriptional (mRNA) blood test to assess suicide risk?**

Novel target identification and validation

- Postmortem brain gene expression data:
  - Proprietary (McGill, Pritzker) and public (PsychENCODE, Allen Institue, GTEx, etc.)
- Analysis of data using commercial and proprietary Bioinformatics tools
- Integration of proprietary/public genetic and other relevant biological data
  - (UK Biobank, GWAS database extraction of nominally significant associated SNPs)
- Classification on GO, Ingenuity pathway analysis, KEGG
- Selection of targets based on:
  - Differential expression in tissue or circuit of interest
  - Genetic association
  - Drug-Target specificity
- Translational **biomarkers** of response (SNPs, expression, methylation) tested in humans

Global Brain Gene Expression Analysis Links Glutamatergic and GABAergic Alterations to Suicide and Major Depression

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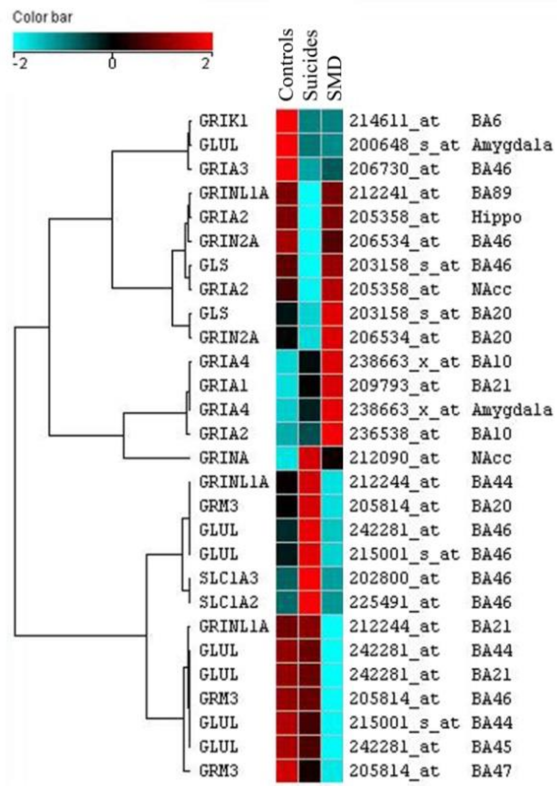
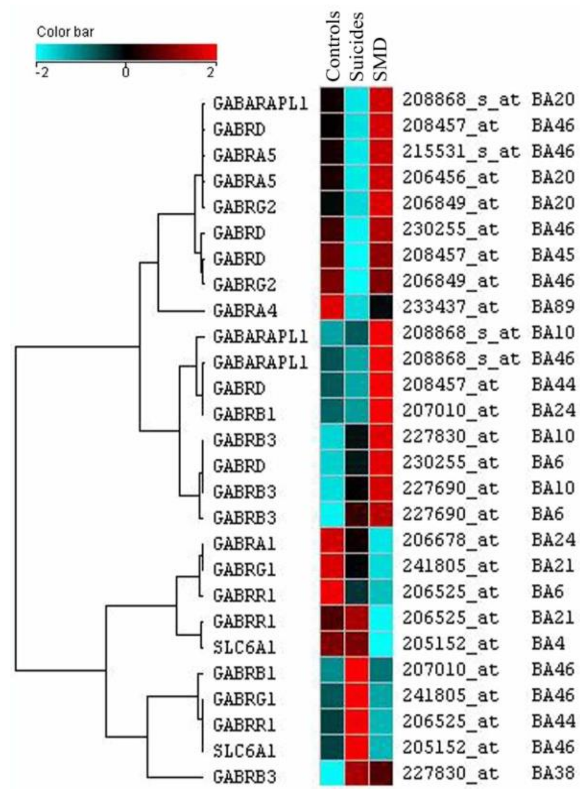
1 McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada, **2** Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada, **3** Ste Justine Hospital, Université de Montréal, Montreal, Quebec, Canada, **4** Department of Psychiatry and Human Behavior, School of Medicine, University of California Irvine, Irvine, California, United States of America





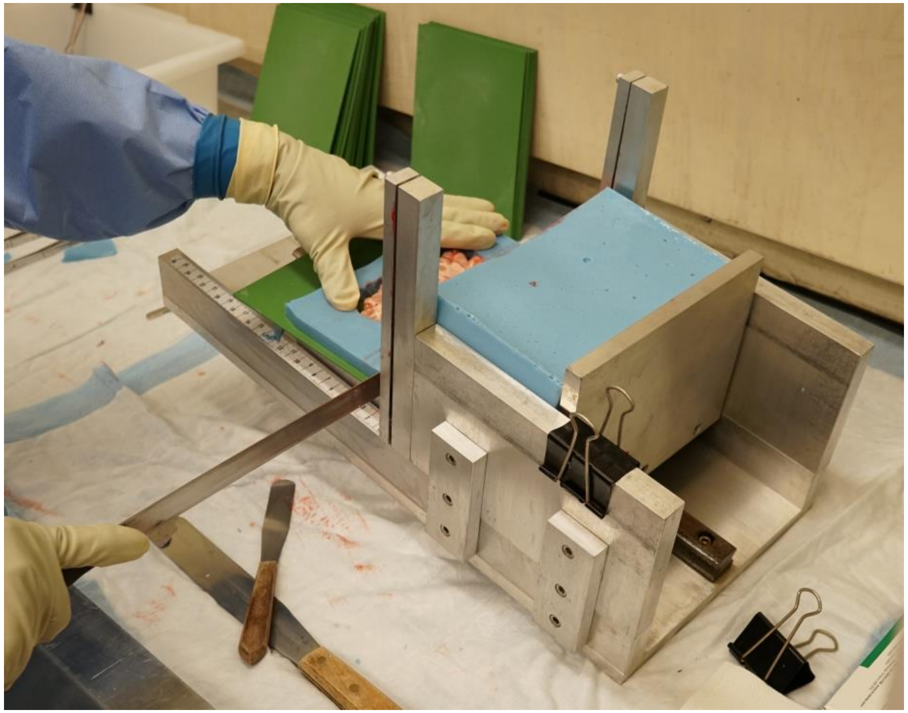
The vast majority of gene expression (from 16 brain regions) changes associated with depression and suicide involve GABA and glutamate receptors and transporters.

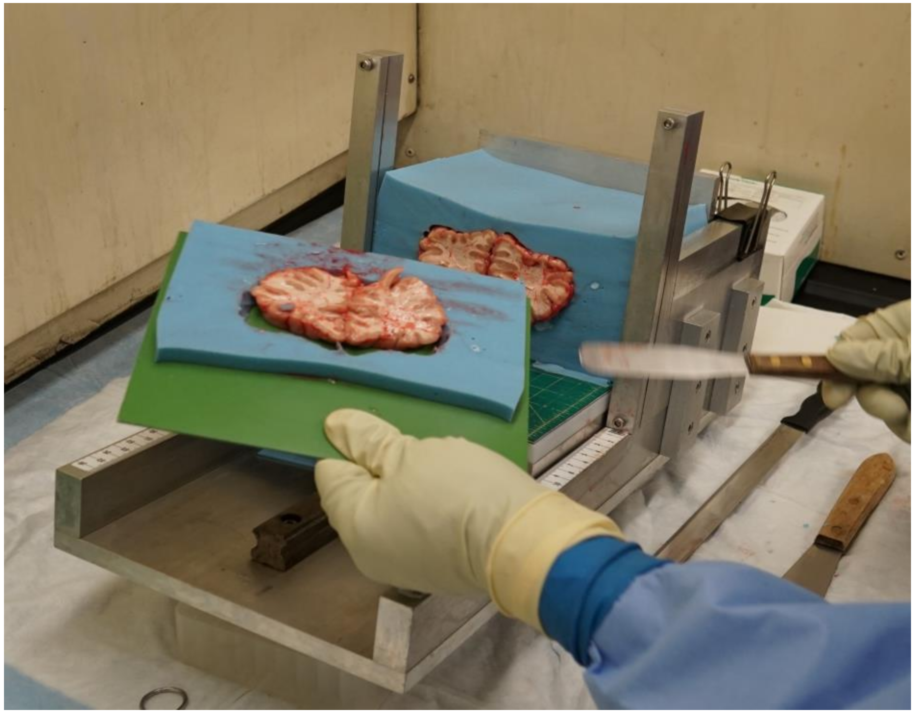
Sequeira et al., Plos One, 2009

Post-mortem psychological autopsy

- Interviews with family and friends
- Medical files
- Coroner notes/investigation
- Toxicological reports
- Hospital files
- 144 item questionnaire based on the DSM-IV and the SCID

Coronal brain slicing

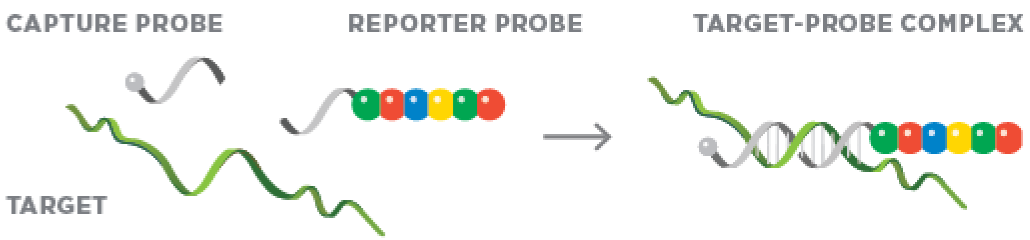




- Brain is encased in alginate to keep the shape/integrity and to prevent deformation/warping while slicing
- Coronal slicing (1 cm) is done using a precise guide with the ventral surface up to avoid damage to ventral areas

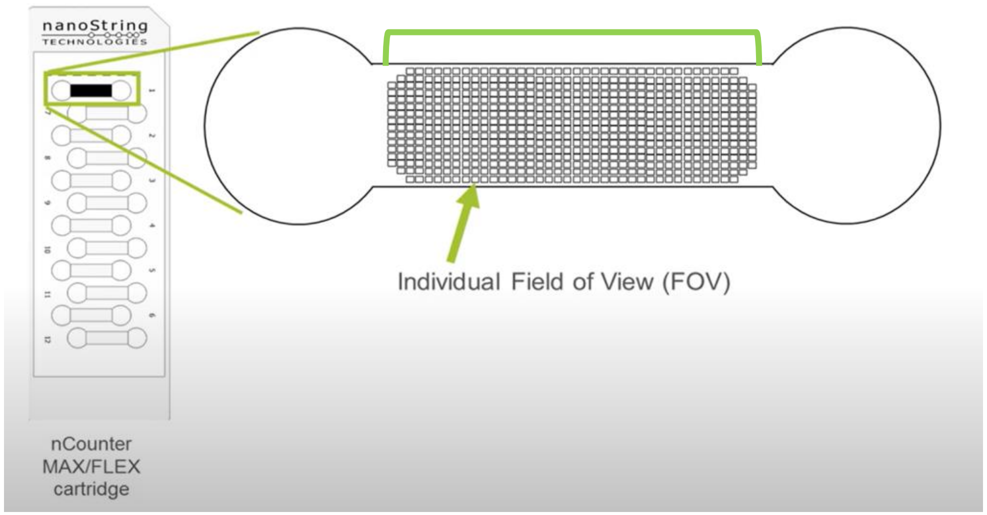
NanoString Platform

The NanoString platform allows for the direct counting of mRNA molecules (no cDNA) using a barcode-probe system



- RNA degradation (<1000 bp) is a problem for cDNA production (qPCR, microarrays) and for RNA-Seq.
- Some degradation in post-mortem blood still allows the extraction of enough RNA (>100 bp) to reliably detect changes in gene expression using NanoString
- QC was improved by loading more RNA for blood samples compared to brain samples

NanoString Platform



Imaging QC = $\frac{\text{FOV Counted}}{\text{Total FOV}}$
(data robustness-RNA degradation)

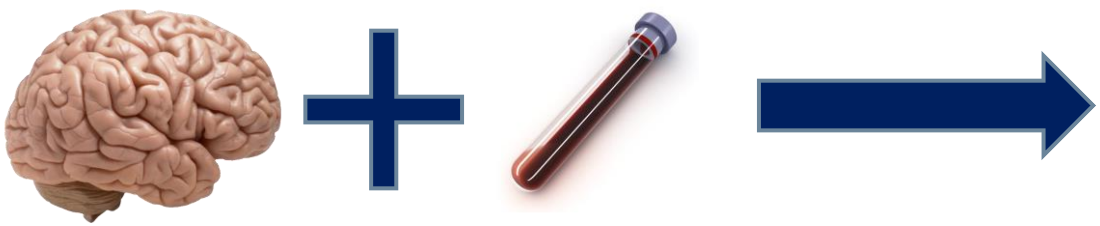
Binding density = Fluorescent spots/μ^2
(saturation-RNA degradation)

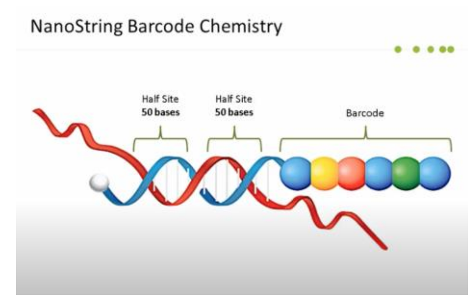
- Field of view count (FOV) or total number of FOVs imaged per lane is used to control for possible RNA quality differences

	Average FOV	FOV Stdev	% FOV	Min % FOV	Max % FOV
Brain	533.94	10.84	96.21	85.23	98.74
Blood	540.06	8.27	97.31	92.43	99.64

Biomarker Signature for Suicide

- Pilot study in post-mortem **BRAIN and BLOOD** using NanoString technology to study 117 genes relevant for suicide:





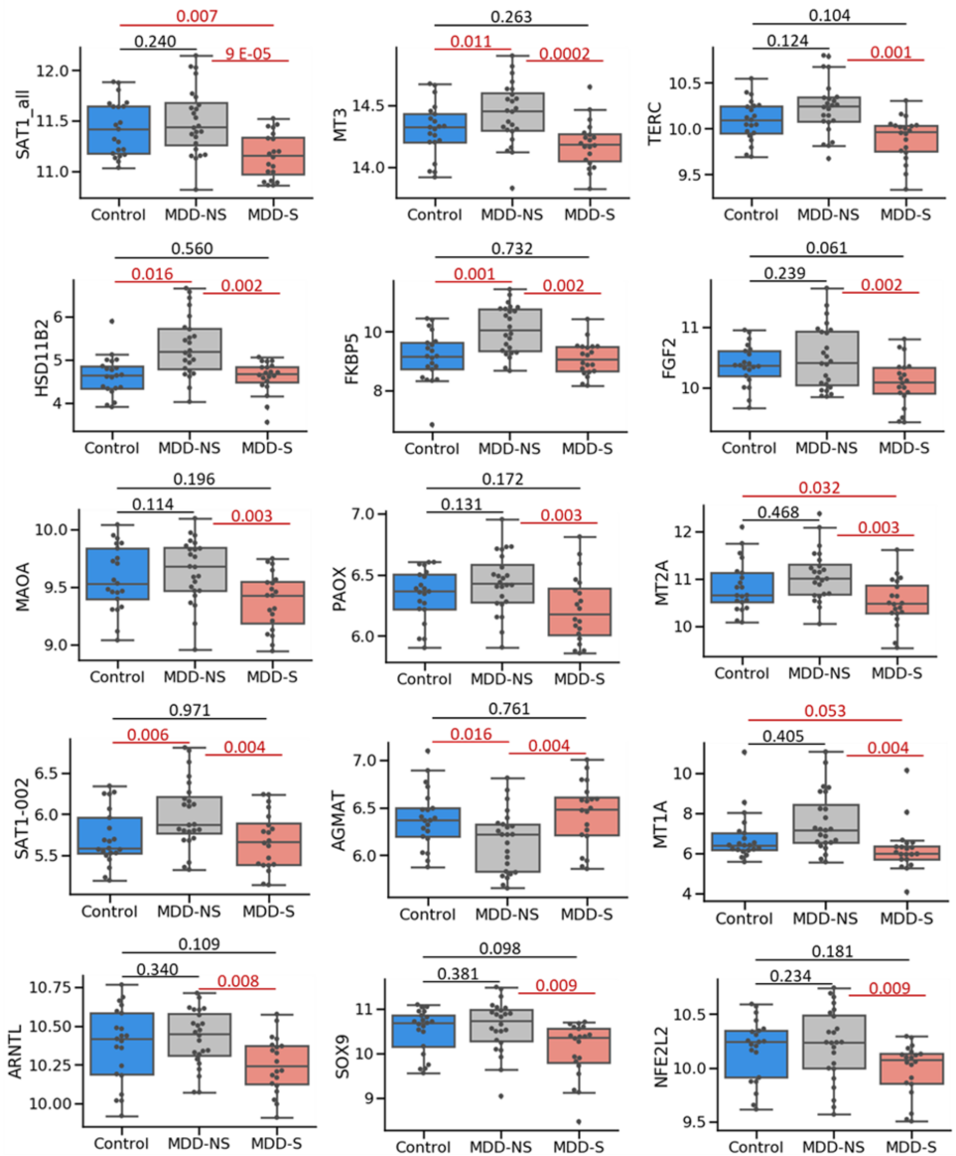
tim.blog/onepager

Sample	Diagnosis	N	Gender		Age	
			Males	Females	Males	Females
Brain	Control	21	17	4	45.71	47.50
	MDD-NS	24	11	13	50.73	50.92
	MDD-S	24	11	13	42.82	37.83
Blood	Control	16	12	4	43.08	45.20
	MDD-NS	18	11	7	51.45	55.57
	MDD-S	19	12	7	42.83	36.57

- Highly significant differences in peripheral gene activity were identified in **MDD-Suicides versus MDD Non-Suicides** for eleven genes ($Q \leq 0.1$, FDR corrected)

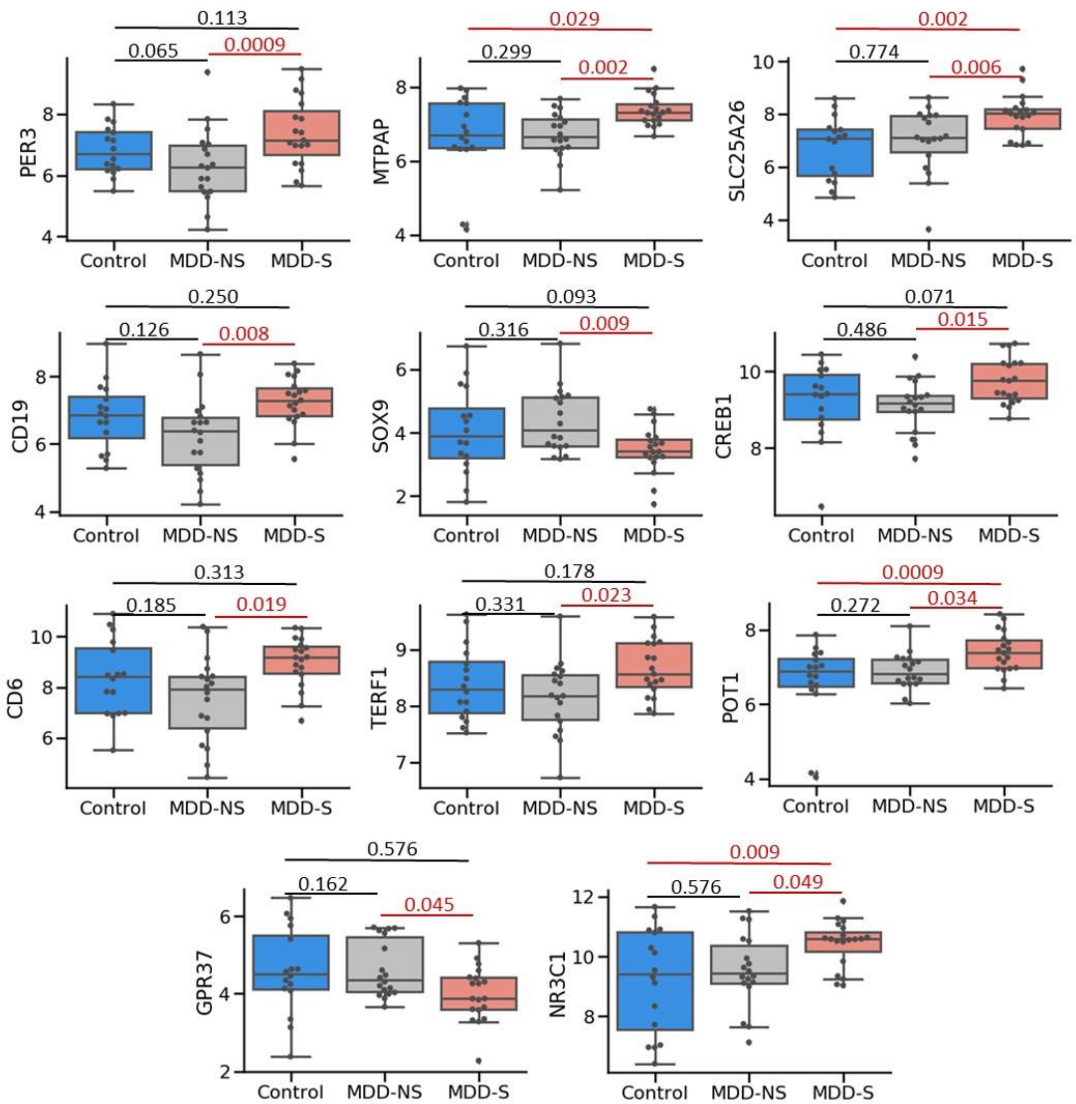
Mamdani et al, Translational Psychiatry, 2022

Gene expression changes in suicide - DLPFC



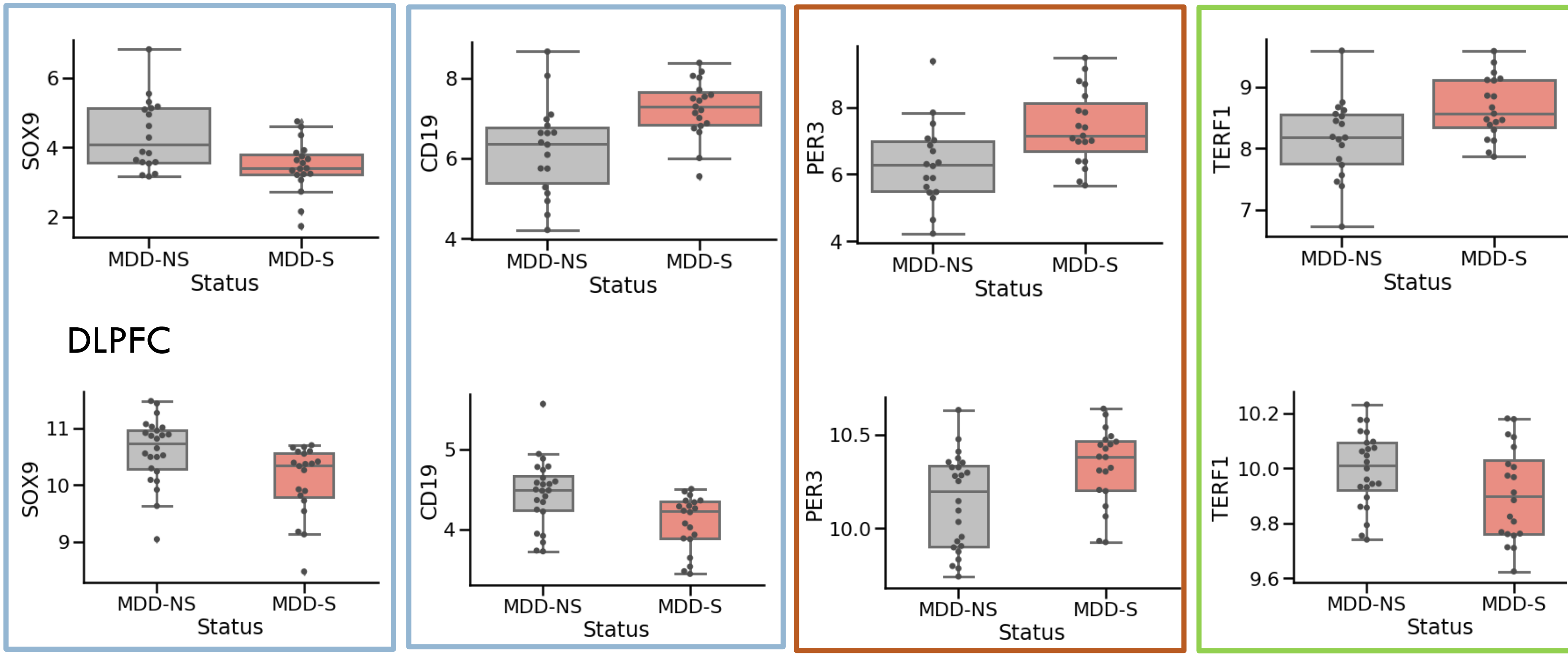
Mamdani et al, Translational Psychiatry, 2022

Gene expression changes in suicide - Blood



Mamdani et al, Translational Psychiatry, 2022

Biomarker Signature for Suicide



SOX9:
Activated B cells (Blood)
Astrocytes (Brain)

CD19:
B-Cell (Blood)
Mural cell (Brain)

PER3:
Delayed sleep phase
syndrome gene

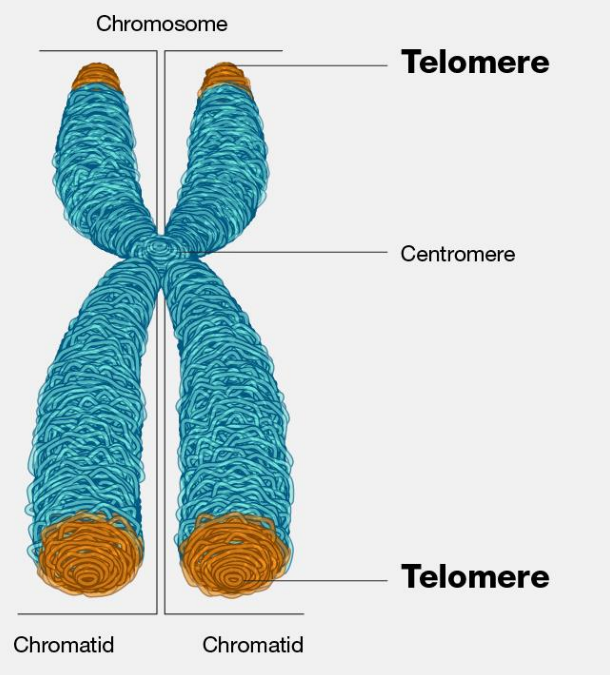
TERF1:
Telomere Repeat-
Binding Factor 1

Immune

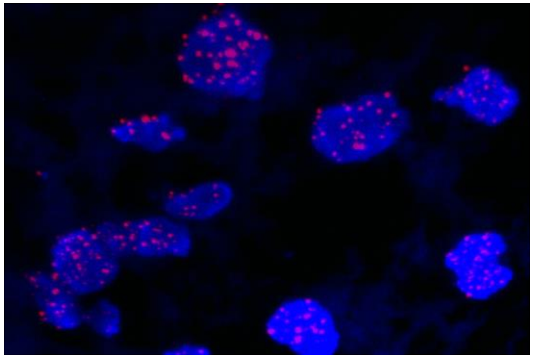
Circadian

Telomeres

Telomeres



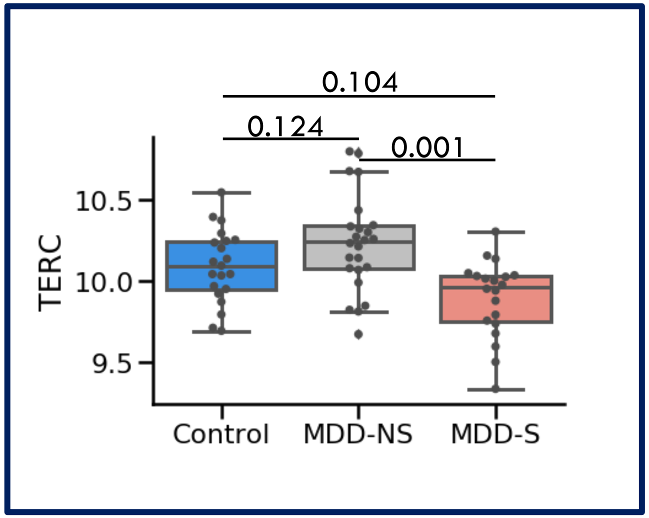
Telomeres in brain cells



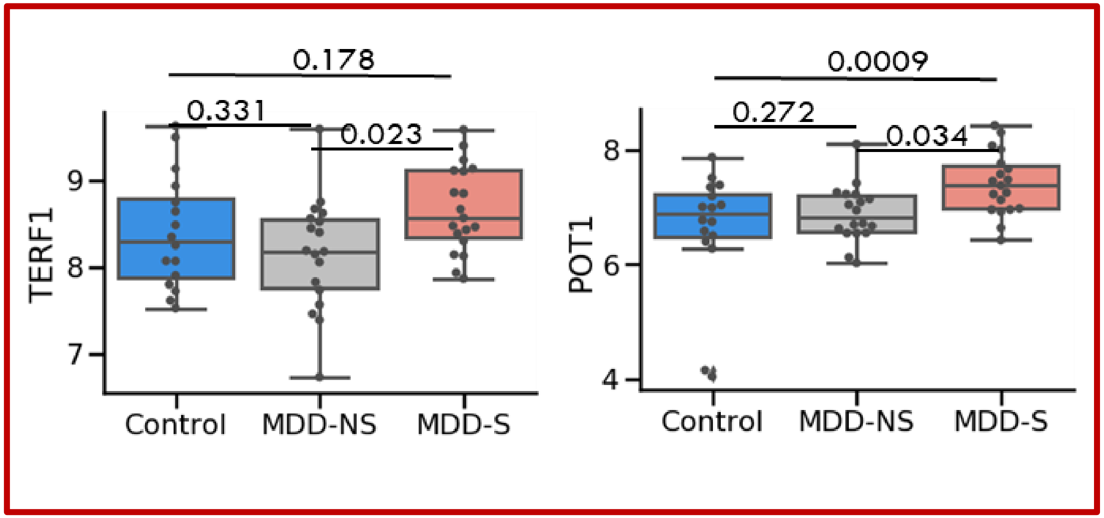
Stan Watson (University of Michigan)

Telomere

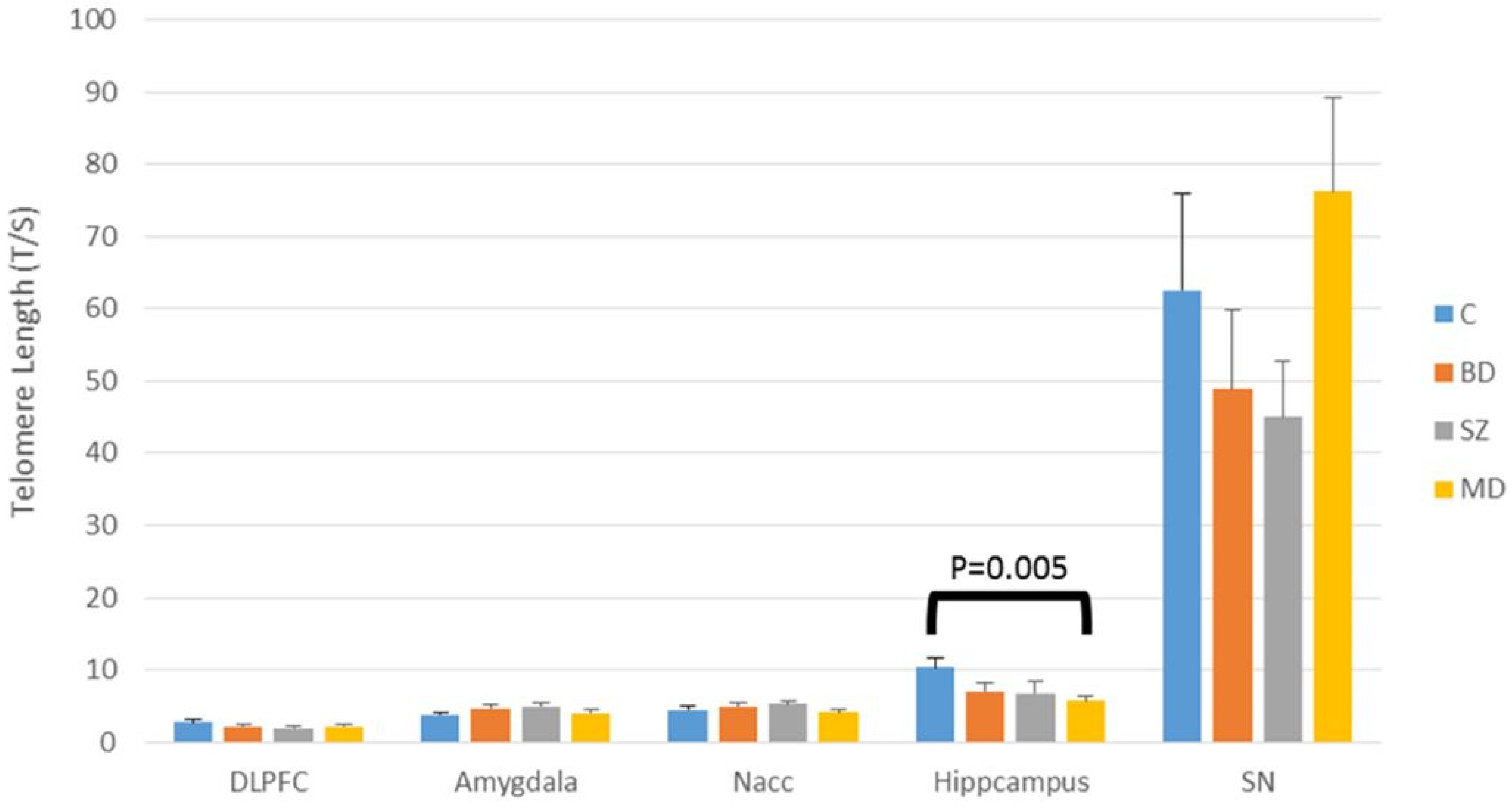
DLPFC



Blood



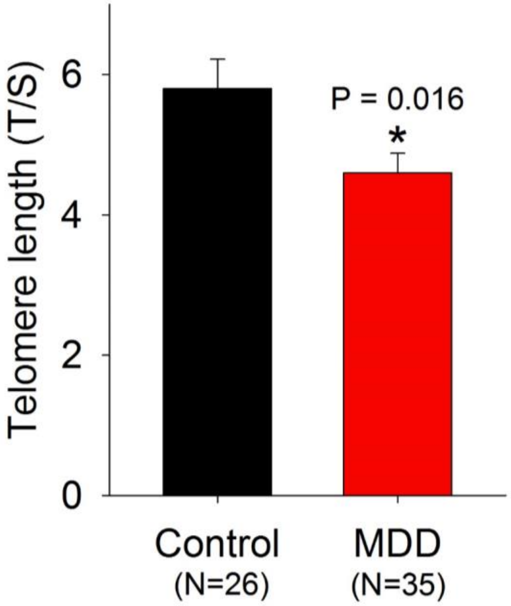
Depression specific shortening of telomeres in the hippocampus



Mamdani et al, Translational Psychiatry, 2015

Hippocampal telomere shortening in depression

- Protective caps at the end of chromosomes formed by a repetitive sequence (TTAGGG).
- **MDDs have shorter telomeres in the hippocampus.**
- In future studies suicide specific effects and cellular differences will be investigated.



Circadian changes

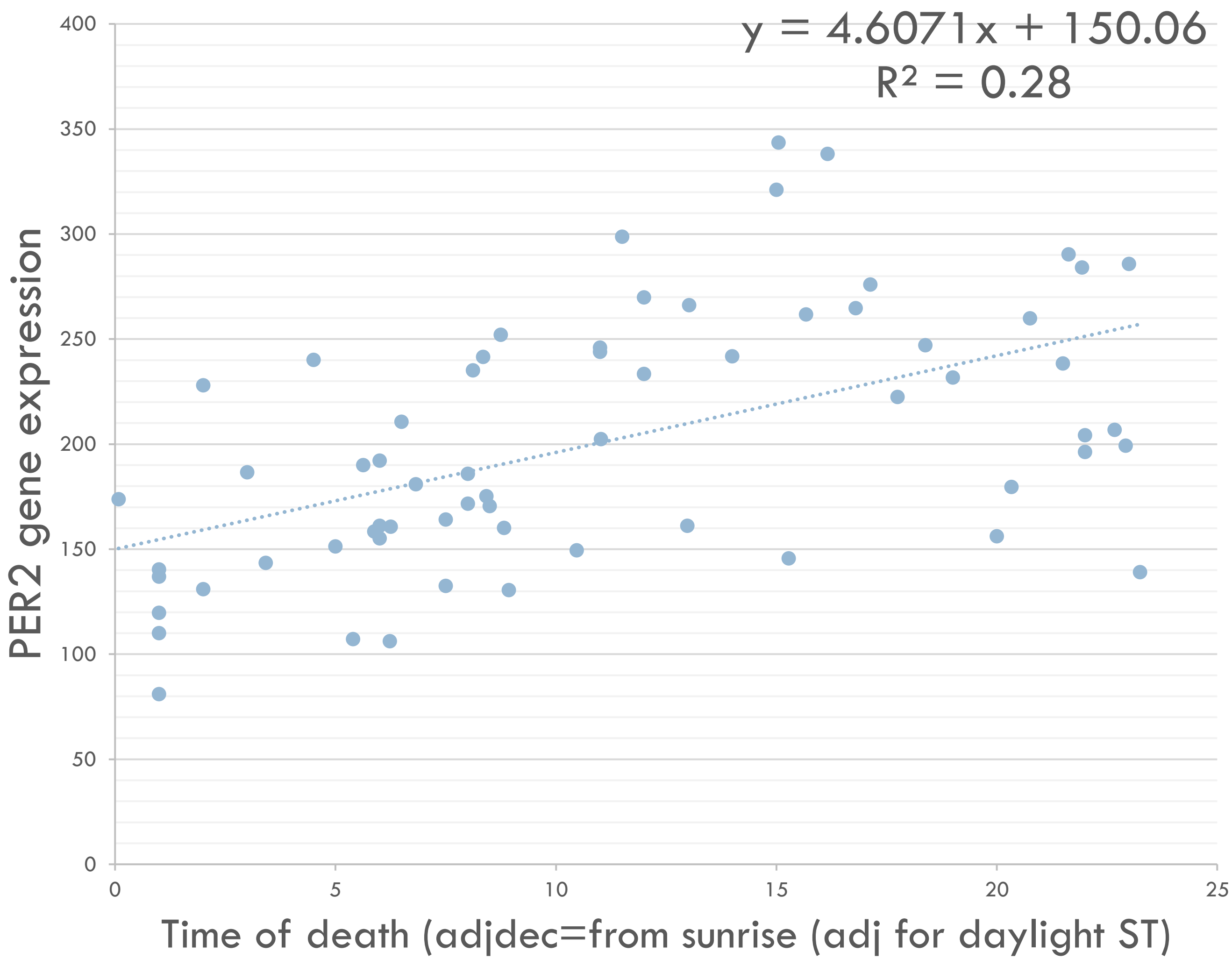
Top three genes significantly affected by adjusted* time of death in the DLPFC

Column ID	p (Sex)	p (Age)	p (Status)	**p (adjdec*)**	p (MDD-S vs. MDD-NS)	FC(MDD-S vs. MDD-NS)
PER2	0.00030212	0.0572825	0.204982	**0.00251788**†	0.158159	1.19292
CRY1	0.0126436	0.0338407	0.503564	**0.0155696**†	0.880263	1.01376
CRH	0.00149369	0.0148091	0.0644279	0.0298922	0.0216211	1.3836

* adjusted time of death from sunrise corrected by daylight savings time

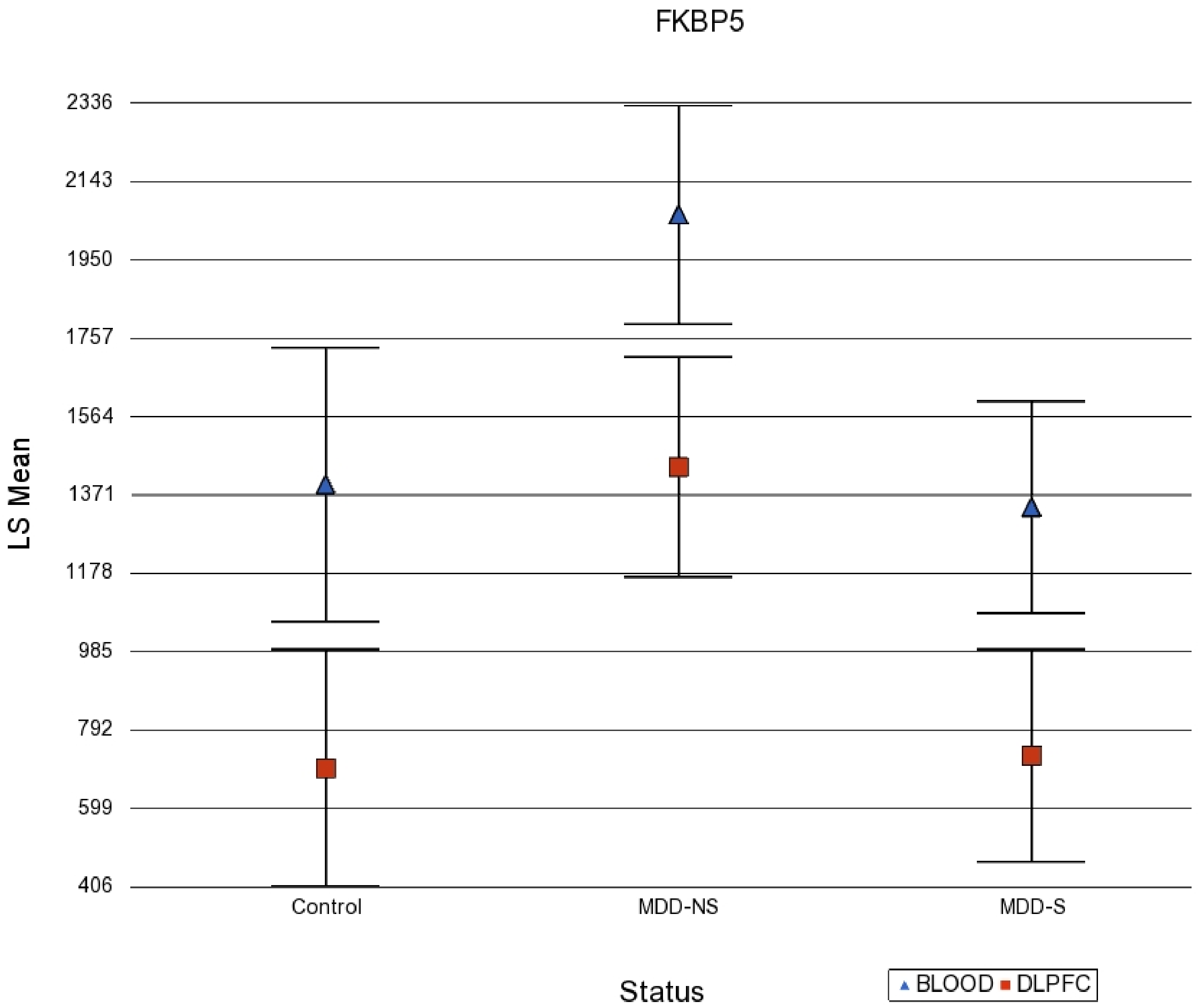
† significant after FDR correction

PER2 gene expression correlated with "time of death" in the brain (DLPFC)



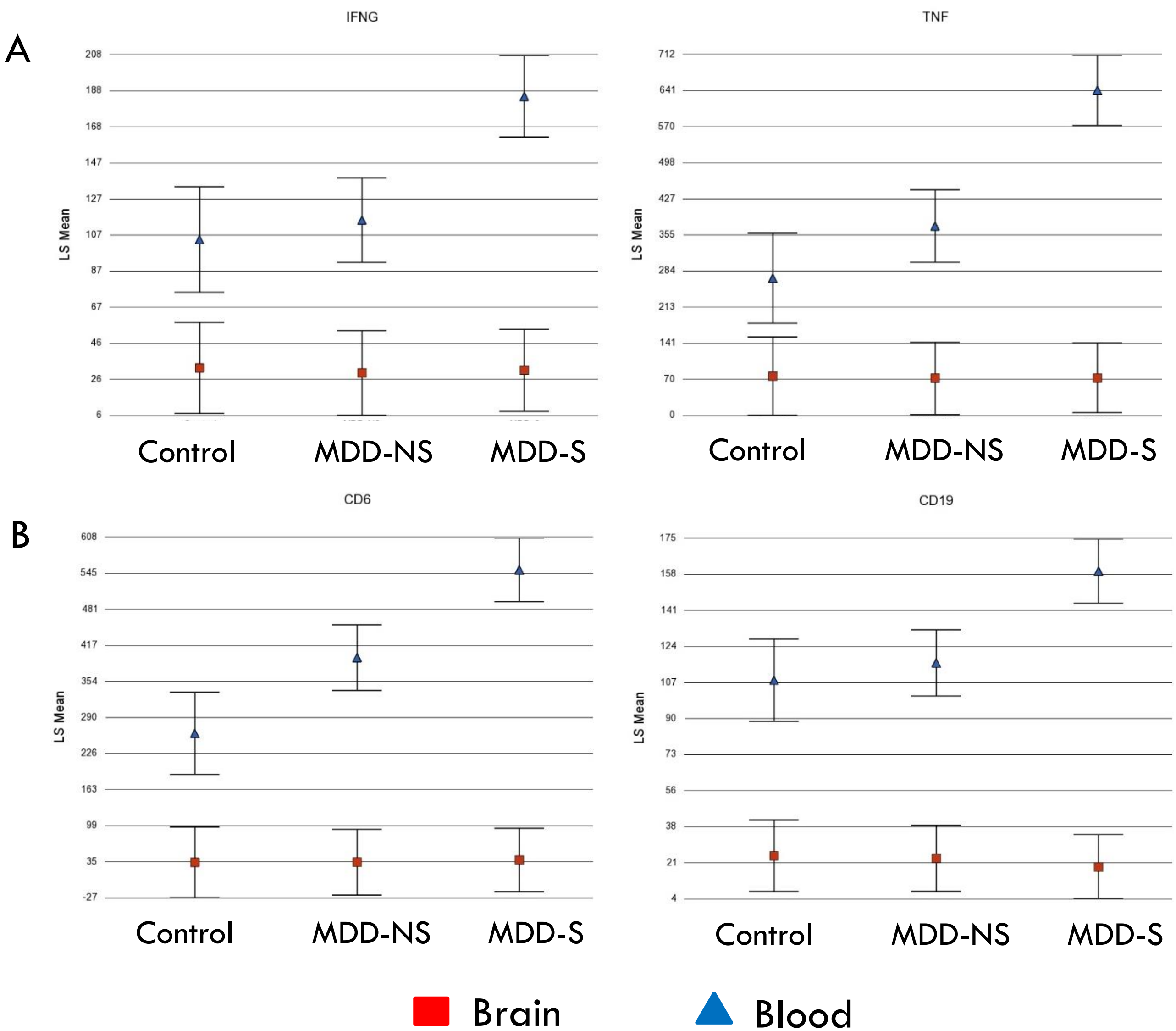
Inflammation-stress response

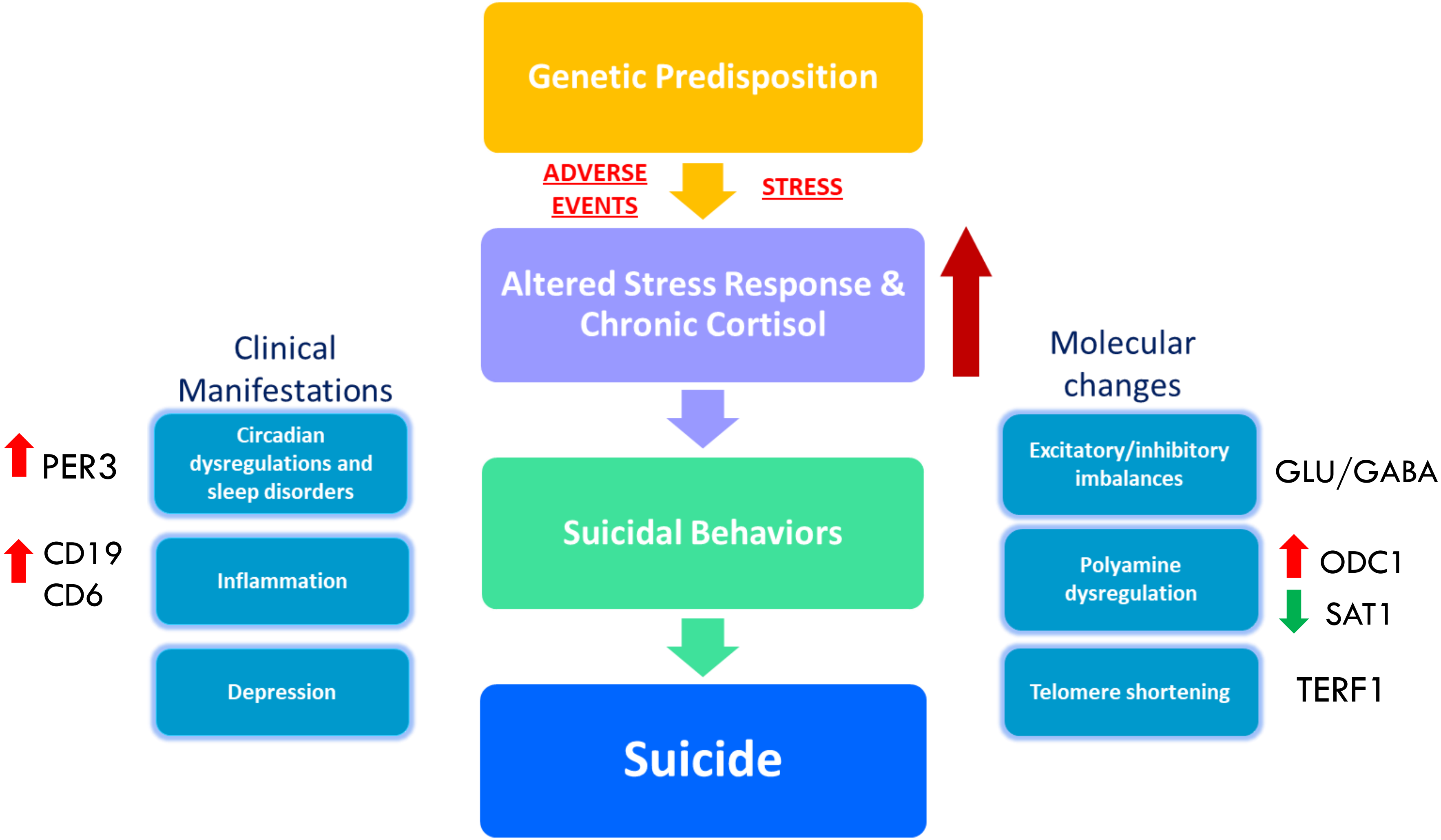
Stress - FKBP5



Blood-P=0.014
DLPFC-P=0.035

Blood specific inflammation in suicide



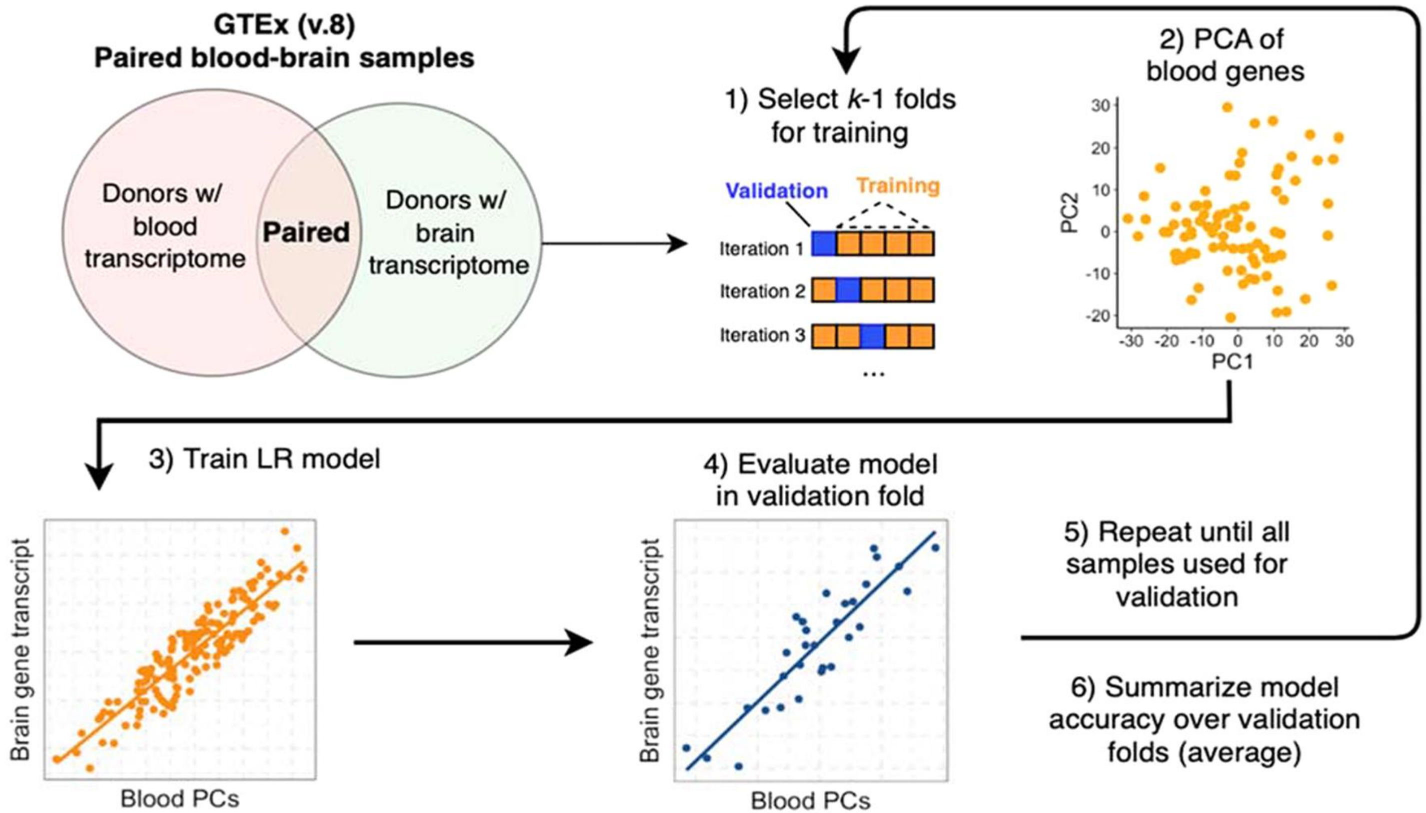


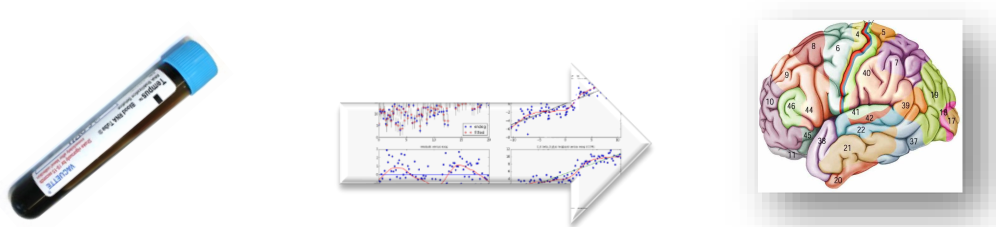
- Suicide biomarker signature that can be evaluated in non-preserved blood
- We think some of these **clinical manifestations** and **molecular changes** are involved in the transition from thinking about suicide to attempting and dying by suicide

Future directions

- **Brain circuits** involved in stress, depression and suicide: DLPFC-Hippocampus-NAcc-Amygdala.
- Single nuclei/cell RNA-Seq
- Telomere changes in depression, association with the stress hormone cortisol and suicide risk
- **Test a suicidal behaviors "blood test"** (expression/metabolomics) to identify at-risk patients for suicide in a clinical sample followed over time (baseline, 1 and 2 year follow-ups)

BrainGENIE using paired blood–brain transcriptome data from the GTEx dataset





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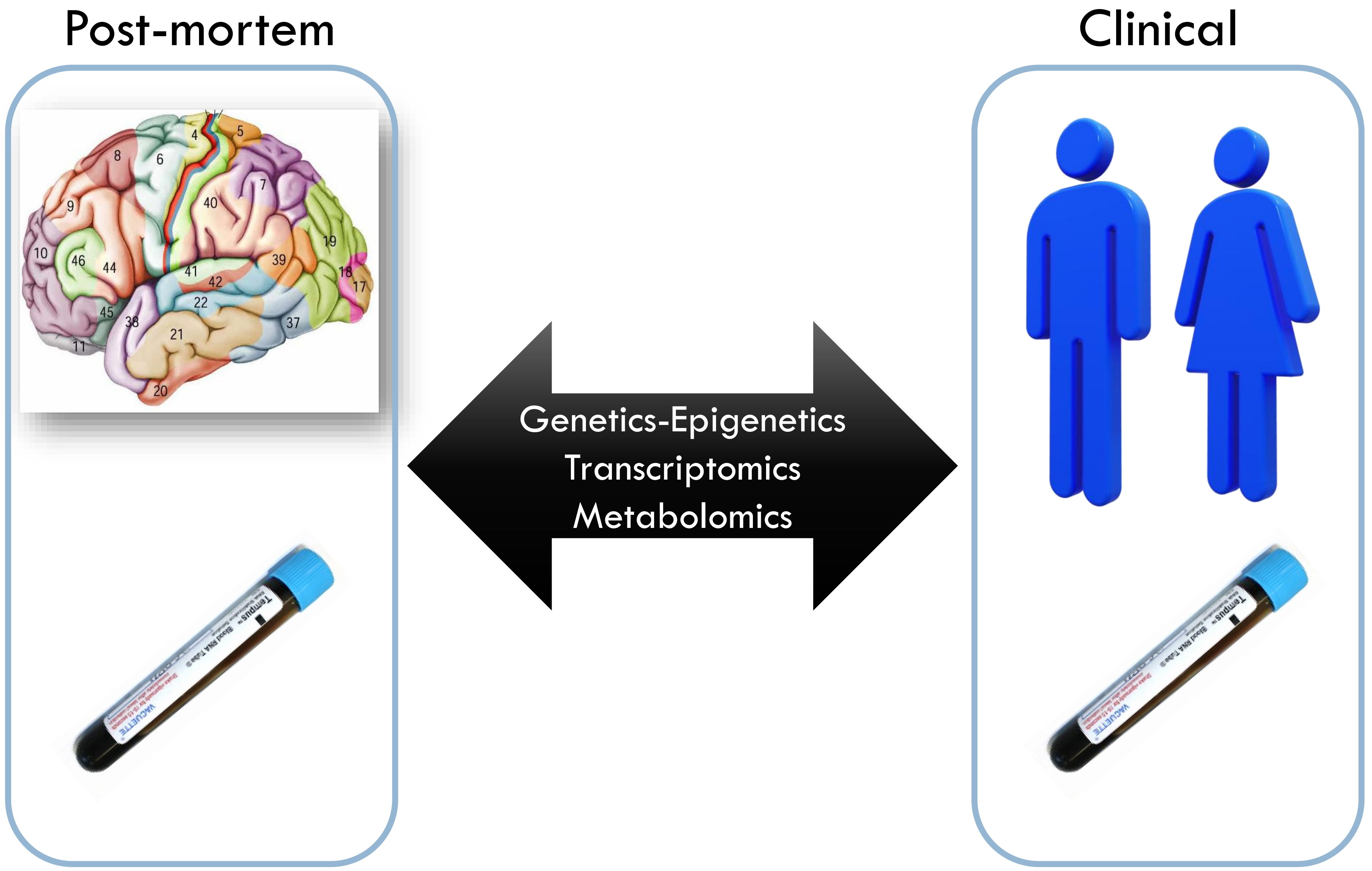
Post-mortem
Clinical
Genetics-Epigenetics
Transcriptomics
Metabolomics

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Diagnosis & Treatment
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HudsonAlpha
University of California at Irvine
Cornell University

BRAIN & BEHAVIOR RESEARCH FOUNDATION
Awarding NARSAD Grants

NIH National Institute of Mental Health

Depression

Differentially expressed genes between MDDs (regardless of the suicide status) and controls

Blood

Gene	Mean(Control)	Mean(MDD-NS)	Mean(MDD-S)	P (MDD vs. Control)	Q Value (MDD vs. Control)	FC (MDD vs. Control)	FC Direction
ODC1	7.747	8.723	8.643	0.007	**0.070**	1.878	UP
ACD	8.306	7.556	7.724	0.013	**0.103**	-1.543	DOWN
POT1	6.624	6.886	7.403	0.013	0.117	1.511	UP
EIF5A	9.528	9.873	10.272	0.013	**0.103**	1.505	UP
TERC	11.089	10.318	10.666	0.019	**0.103**	-1.466	DOWN
HTR2A	3.361	2.843	2.209	0.024	**0.103**	-1.783	DOWN
MT-ND6	13.714	13.262	12.791	0.029	**0.103**	-1.664	DOWN
PMFBP1	5.426	4.690	4.781	0.032	**0.103**	-1.552	DOWN
SRM	7.273	7.429	7.761	0.038	0.191	1.346	UP
MT1H	5.345	4.760	4.071	0.040	**0.103**	-1.929	DOWN
SAT1-002	6.361	7.195	7.177	0.040	0.124	1.706	UP
NOP10	8.876	9.439	9.482	0.042	**0.103**	1.412	UP

Brain

Gene	Mean(Control)	Mean(MDD-NS)	Mean(MDD-S)	P (MDD vs. Control)	Q Value (MDD vs. Control)	FC (MDD vs. Control)	FC Direction
CRY2	10.478	10.382	10.377	0.013	0.548	-1.121	DOWN
SMOX	9.355	9.551	9.444	0.017	0.283	1.127	UP
ODC1	9.388	9.553	9.436	0.017	0.283	1.092	UP
FKBP5	9.071	10.055	9.085	0.037	0.283	1.327	UP