

The Neurobiology of Suicide: Blood-Brain Barrier Breakdown as a Novel Suicide-Risk Biomarker

INTRODUCTION

- Over 1 million suicide deaths annually: No biomarkers for suicide ideation, attempt, risk, or completion exist (National Institute of Mental Health #1 priority is biomarker identification)
 - Lack of effective psychopharmacological medications identification due to the lack of novel molecular targets
- Blood-Brain Barrier (BBB) Breakdown: Claudin-5 degradation
 - Allows for perivascular intrusion of albumin and immunoglobulin- γ (IgG), the most predominant protein and antibody in the blood, into the brain parenchyma (Fig. 1)
- Claudin-5: Candidate biomarker in other neurological disorders but role in suicide neuropathology remains unelucidated

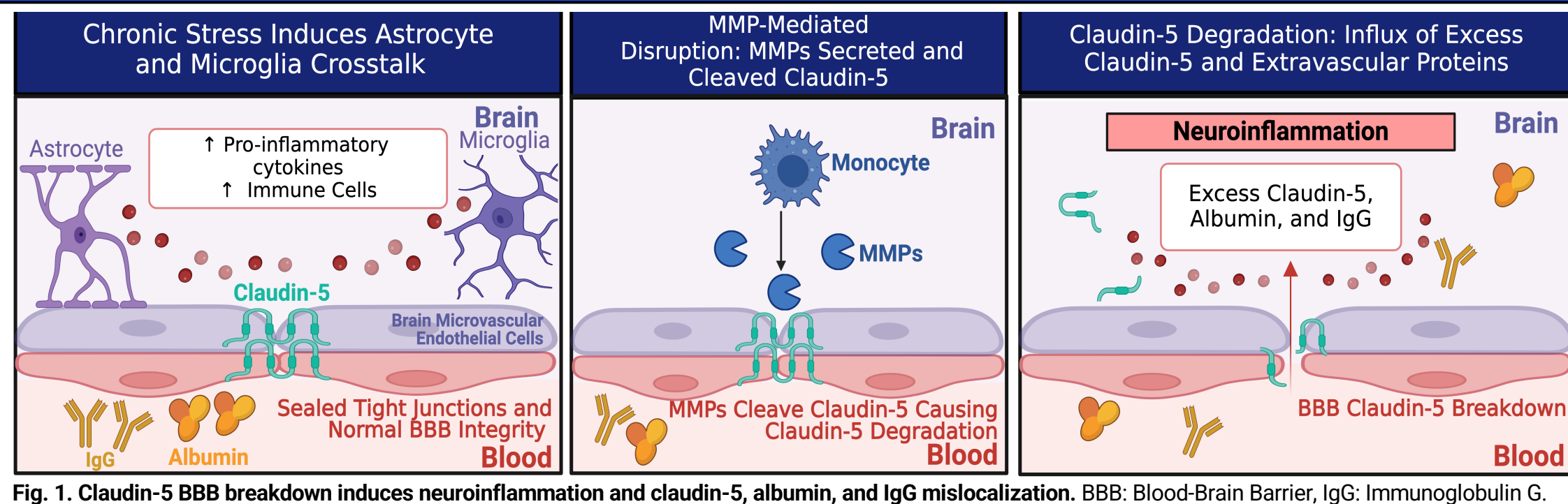


Fig. 1. Claudin-5 BBB breakdown induces neuroinflammation and claudin-5, albumin, and IgG mislocalization. BBB: Blood-Brain Barrier, IgG: Immunoglobulin G.

METHODOLOGY

- Determine claudin-5, albumin, and IgG alterations *in situ*: ELISA Assay ($n=100$) of human postmortem dorsolateral prefrontal cortex (dlPFC) brain tissue
- Identify claudin-5-related gene expression alterations in suicide completion: methylGSA DNA methylation and STAR Alignment Tool for RNA-sequencing transcription profiling
- Assess molecular compatibility of claudin-5 with antidepressants and anti-inflammatory medications: PyRx Autodock Vina and BIOVIA Drug Discovery Studio

RESULTS/DISCUSSIONS

Increased Claudin-5 in Suicide Decedents

- ELISA identified increased claudin-5 levels in dlPFC of suicide decedents (1.33 mg) than in controls (1.01 mg; $*p<0.05$; Fig. 2)
- Increased claudin-5 expression in the dlPFC exacerbates cerebral perfusion deficits impair neuronal function

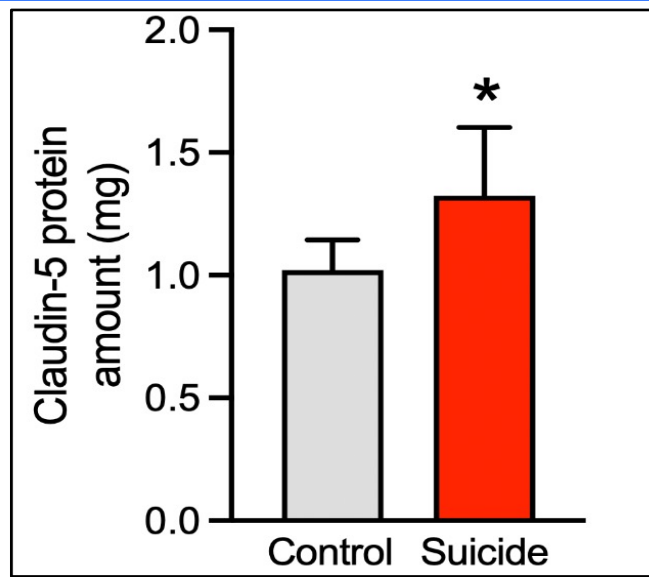


Fig. 2. Increased claudin-5 in suicide ($n=20$). Error Bars indicate standard error mean \pm 1. $*p<0.05$.

Identifying claudin-5 localization *in situ* is vital to understanding neurovascular dysfunction contributing to suicide neuropathology

Claudin-5 Mislocalization in Suicide

- Claudin-5 is normally expressed in the brain microvascular endothelial (BMEC) lining of blood vessels
- Controls: Claudin-5 (Fig. 3A) and blood vessel staining (Fig. 3B) merged to visualize claudin-5 in the blood vessels of BMECs (Fig. 3C)
- Suicide: Claudin-5 (Fig. 3D) and neuron staining (Fig. 3E) merged to visualize excess claudin-5 residue in neurons (Fig. 3F)
- Investigating the association between claudin-5 alteration and stress-induced neuroinflammation in suicide can establish synergistic associations of physiological alterations with behavioral attributes

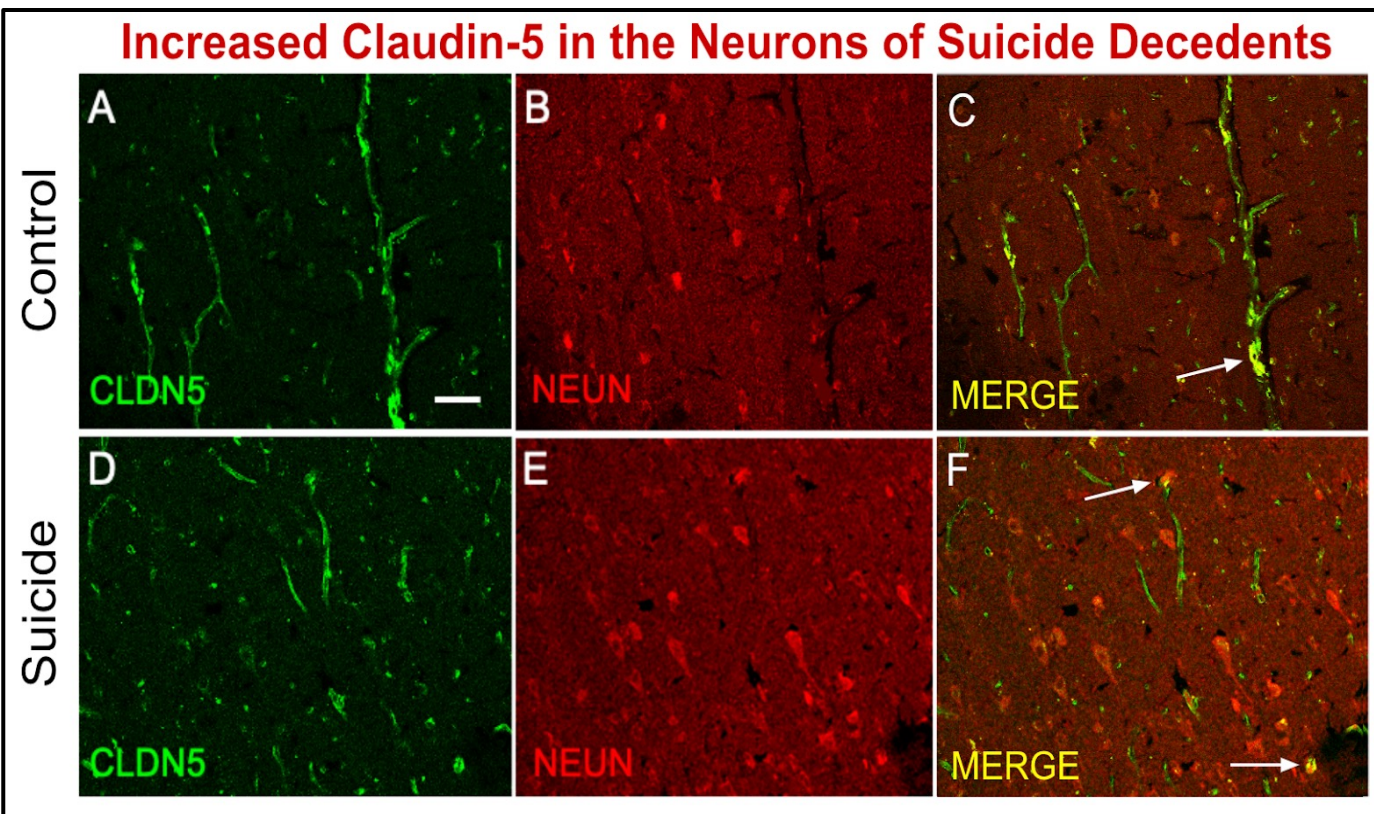


Fig. 3. IDISCO and NeuN immunofluorescence microscopy of claudin-5 localization in the dorsolateral prefrontal cortex. Representative images showing controls (A-C) and suicide decedents (D-F): A) Claudin-5, B) NeuN staining, and C) Merged image indicating proper co-localization of claudin-5 in the blood-microvessel controls; D) Claudin-5, E) NeuN staining, F) Claudin-5 is mislocalized in the neurons of the dlPFC in suicide decedents instead of localized in the blood vessels. White arrows indicate claudin-5. The scale bar represents 80 microns. Photomicrographs at 400x were captured with a Leica TCS SP8 confocal scanning microscope.

CLDN5 is Downregulated in Suicide Decedents

- Maintenance of the BBB gene ontology group in suicide decedents ($p<0.05$; Table 1)
- CLDN5 manipulation is an optimal way to modulate BBB paracellular permeability: CpG methylation regulates gene repression

Table 1. *CLDN5* was downregulated in suicide decedents. *CLDN5*: Claudin-5, *JAM3*: Junctional adhesion molecule C, *TJP2*: Tight junction protein 2, *LAMA2*: laminin subunit alpha 2, *LSR*: Lipopolysaccharide-stimulated lipoprotein receptor, *MBP*: Myelin basic protein. $*p<0.05$.

Gene Ontology Group	Size	Downregulated Genes	p-value
GO:0035633 (Maintenance of the Blood-Brain Barrier)	35	<i>CLDN5</i> , <i>JAM3</i> , <i>TJP2</i> , <i>LAMA2</i> , <i>LSR</i> , <i>MBP</i>	0.0269

- CLDN5 manipulation is an optimal way to modulate BBB paracellular permeability: CpG methylation regulates gene repression

CLDN5 CpG Sites Targets

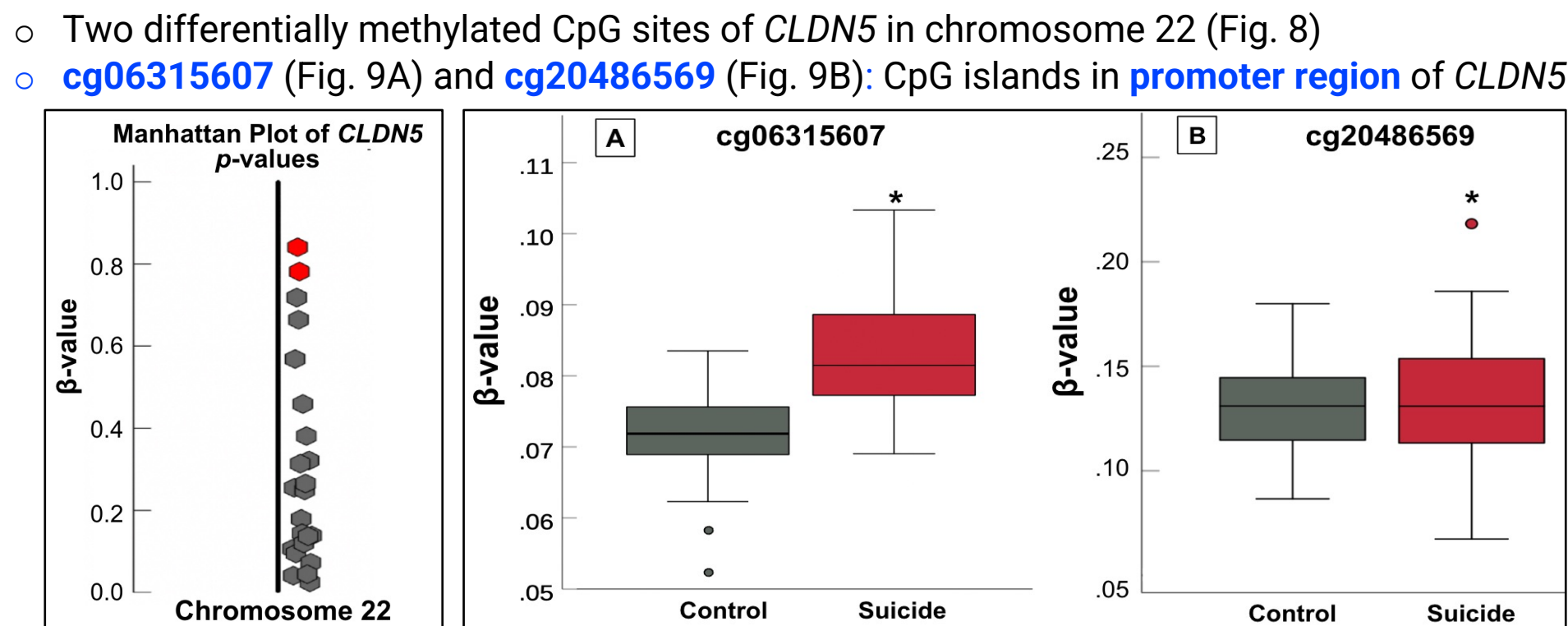


Fig. 8. Two differentially methylated CpG sites of *CLDN5* in chromosome 22 (Fig. 8)

- Two differentially methylated CpG sites of *CLDN5* in chromosome 22 (Fig. 8)
- cg06315607 (Fig. 9A) and cg20486569 (Fig. 9B): CpG islands in promoter region of *CLDN5*

- CpG alterations are reversible: Non-invasive biopsies can allow for early interventions

Differentially Expressed Genes

- AQP1*, *AGPAT1*, *GLDN*, *MMP-1*, *TGM4*, *CHRN4*: Neurodegeneration (Fig. 10)
- MMP-1* (logFC=4.10, $p<0.001$) and *AQP1* (logFC=-2.31, $p<0.001$): risk loci associated with suicide
- AQP1* downregulation: Decreased water transport results in mechanical compression of BMEC appositions
- MMP-1* upregulation: Induces extracellular matrix breakdown
- Examining therapeutics that can modulate *AQP1* and *MMP-1*: alternative medications for suicide prevention

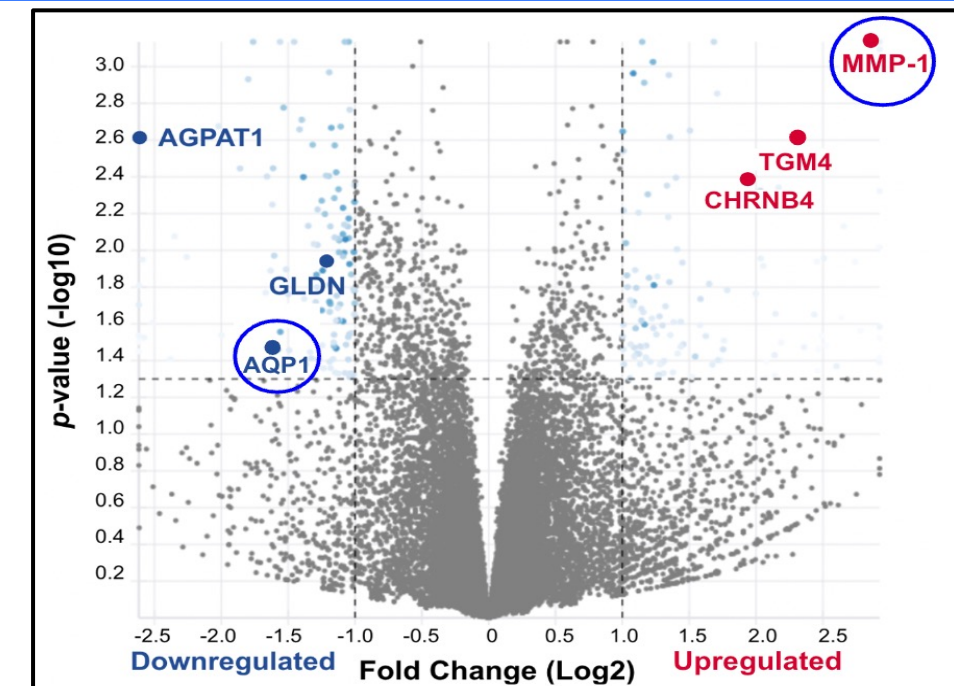


Fig. 10. Volcano plot of differentially expressed genes. Downregulated genes in blue, upregulated genes in red, genes associated with blood-brain barrier breakdown circled; *MMP-1*: Matrix metalloproteinase-1, *TGM4*: Transglutaminase 4, *CHRN4*: Neuronal Acetylcholine beta-4, *AGPAT1*: 1-acyl-sn-glycerol-3-phosphate acyltransferase, *GLDN*: Gliomedin, *AQP1*: Aquaporin 1.

Neuroinflammation and Claudin-5 Breakdown Association

- In suicide decedents, increased recent-life stress was associated with greater claudin-5 residue in the dlPFC ($*p<0.05$; Fig. 4)
- Claudin-5: Promising objective index that can bridge the gap between behavioral assessments and neuroanatomic anomalies

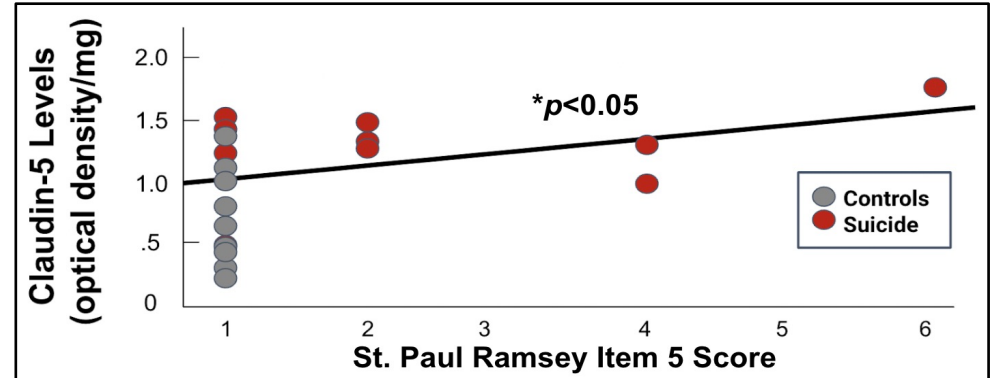


Fig. 4. Claudin-5 levels and Recent-Life Stress-Induced Neuroinflammation are correlated. $*p<0.05$, Item 5 of the St. Paul Ramsey Life Experience Scale assesses neuroinflammatory health disorders exacerbated by stress; Stress Severity Rating: 1=None, 2=Minimal, 3=Mild, 4=Moderate, 5=Severe, 6=Extreme.

Increased Peripheral Proteins in Suicide Decedents

- Elevated IL-6 and IL-8 in suicide ($*p<0.05$; Fig. 5)
- IL-6 and IL-8 induce transcriptional downregulation of claudin-5
- BBB compromise: Influx of large peripheral proteins
- Increased albumin (70 kDa) ($*p<0.01$; Fig. 6)
- 33% increase IgG (150 kDa) but not significant ($p=0.066$; Fig. 7)
- BBB breakdown in suicide: Greater risk for neurotoxic molecules into the brain increasing vulnerability to altered neurocognition

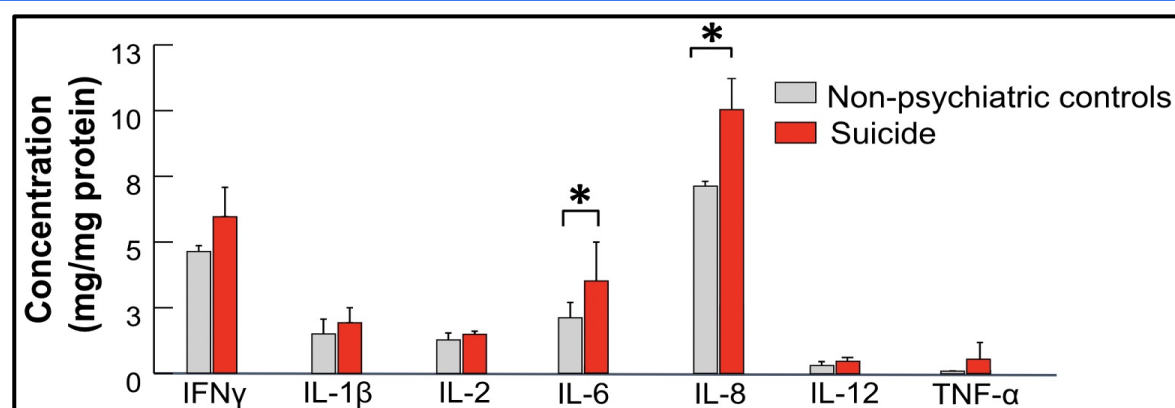


Fig. 5. Increased pro-inflammatory cytokines in suicide. Error Bars indicate standard error mean \pm 1. $*p<0.05$.

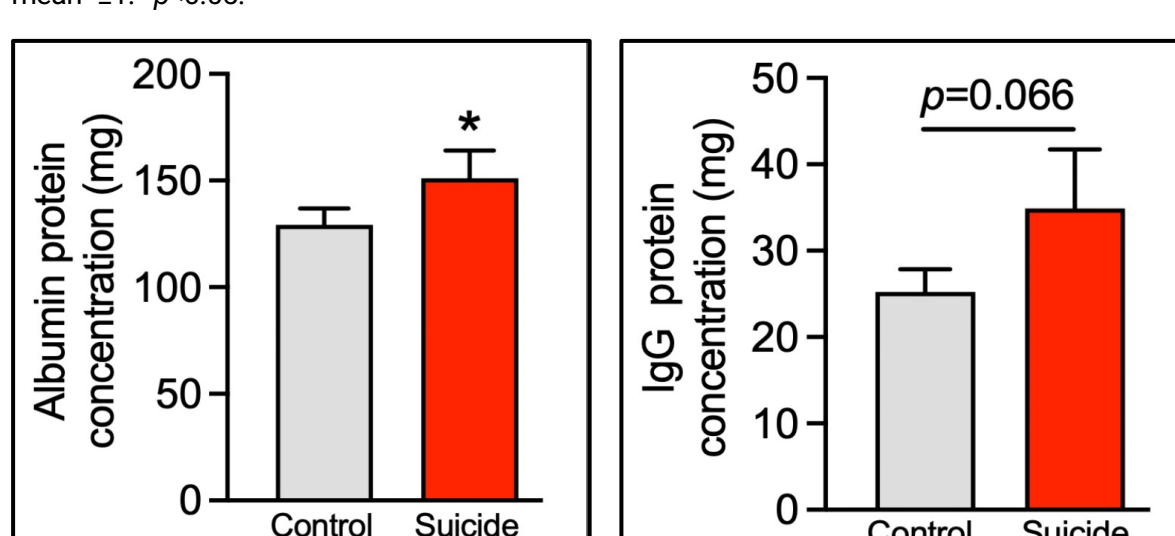


Fig. 6. Increased albumin in suicide ($n=40$). Error bars indicate standard error mean \pm 1. $*p<0.01$. Fig. 7. Increased IgG in suicide ($n=40$). Error bars indicate standard error mean \pm 1. $*N.S.$

Molecular Docking

- Escitalopram and Benzodiazepine: Present drugs to treat suicide ideation have low affinity with claudin-5
- Escitalopram (Fig. 11A) and Benzodiazepine (Fig. 11B) not effective in treating suicide: not effective in targeting claudin-5 levels
- Doxycycline (Fig. 12A), Prinomastat (Fig. 12B), and Wortmannin (Fig. 12C) as alternative medications: restore claudin-5 BBB integrity

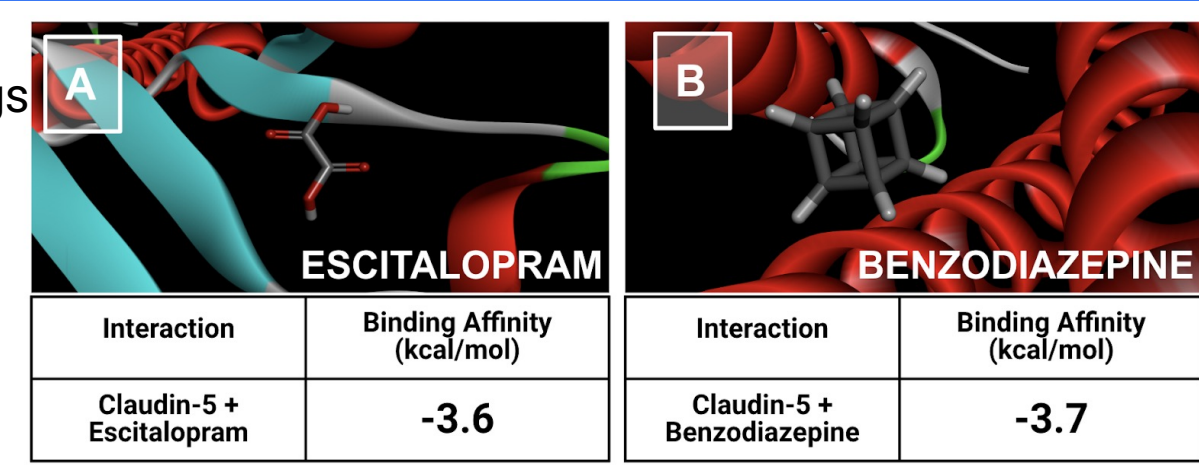


Fig. 11. Predicted affinities of claudin-5 to antidepressants used to treat suicidal ideation A) Binding affinity of escitalopram to claudin-5 is -3.6 kcal/mol. B) Binding affinity of benzodiazepine to claudin-5 is -3.7 kcal/mol. Binding affinity greater than -6.00 kcal/mol not significant.

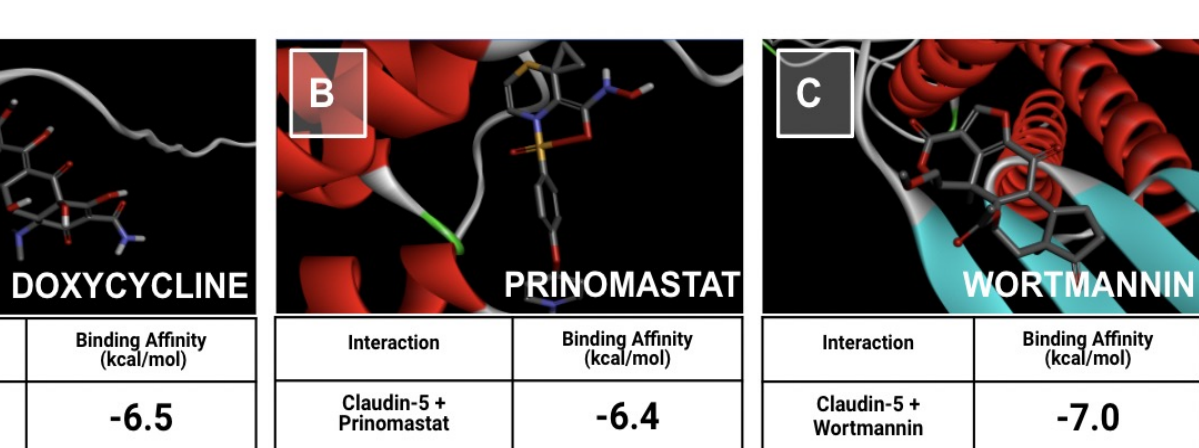


Fig. 12. Predicted affinities of claudin-5 to anti-inflammatory compounds A) Binding affinity of doxycycline to claudin-5 is -6.5 kcal/mol. B) Binding affinity of prinomastat to claudin-5 is -6.4 kcal/mol. C) Binding affinity of wortmannin to claudin-5 is -7.0 kcal/mol. Binding affinity less than -6.00kcal/mol was significant.

CONCLUSIONS/FUTURE RESEARCH/APPLICATIONS

- First human postmortem study to assess BBB disruption through increased claudin-5 and albumin in the dlPFC of suicide decedents and genomic alterations of *CLDN5*
- Future *in vitro* assessments should evaluate novel therapeutics promoting claudin-5 for suicide prevention and genetic manipulation of *CLDN5* to restore BBB integrity
- Claudin-5 and albumin can serve as novel biomarkers to discern high-risk individuals with the severity of suicide risk allowing for earlier interventions

*All images/graphics/figures were created by the finalist