Antibody-Mediated Detection of Claudin-5 as a Biomarker Associated with Suicidal Behavior

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Claudin 5 is a protein in the blood-brain barrier (BBB) tight junction. Recently, its breakdown has been linked to a biomarker for suicide. The presence of Claudin 5 in blood could be an indicator that could predict or diagnose the risk of suicide. Antibodies are specialized proteins that bind to foreign substances, bacteria, viruses, or toxins. We hypothesize that Claudin 5 has a specific region on its surface to which antibodies can bind, serving as a detection tool. In the current study, we performed computational modeling simulations to identify antibodies that can strongly bind to the Claudin 5 protein. Specifically, we first modeled the 3D structure of the Claudin 5 protein using AlphaFold 3, and nine antibodies were downloaded from the Protein Data Bank (PDB). These nine antibodies were separately docked on Claudin 5 protein to form Claudin 5-antibody complex utilizing the HDOCK2.0 software. The output from the HDOCK software was downloaded and were analyzed. Claudin 5-antibody complexes were primarily visually and analyzed to ensure that the antibody binds to the binding site predicted by the graph neural network by using the GrASP web server. Secondly, the binding energy between Claudin 5 and antibodies was calculated using the PRODIGY software, and finally, hydrogen bonds were determined using an in-house script. Based on the above analysis, Antibody IX showed the most suitable binding and could be used for detecting Claudin-5. The current investigation could lead to new diagnostic tools that help in early detection and intervention and could potentially save lives from suicide.

1. INTRODUCTION

Suicide is a significant public health concern, especially for young people in the US, where it ranks as the second leading cause of death among those aged 15 to 24 [1]. Current risk assessments focus on psychological factors like depression and life events, but despite available tools and treatments, suicide rates have risen [1]. Recent neurobiological research has discovered how the blood-brain barrier (BBB) controls substances entering the brain, and its protein Claudin-5 plays a role [2]. Claudin-5 is crucial for BBB function, and disruptions in it may increase suicide risks by allowing harmful substances into critical brain areas, affecting decision-making and behavior control [2]. This research aims to find new ways to detect and intervene in the early stages of suicidal behavior by understanding Claudin-5 and BBB better, potentially leading to improved prevention methods [2].

Antibodies or serology tests check for antibodies in the blood to see if someone has a past infection or vaccination (Xiang et al., 2020). They don't find current infections but show the body's immune response (Xiang et al., 2020). One standard test is called ELISA [3]. ELISA detects and measures antibodies by using their reaction with antigens [3]. Because it is very accurate and reliable, ELISA is useful for diagnosing infections like HIV, hepatitis, and COVID-19 [3]. These tests help people understand how diseases spread and make informed health decisions more straightforward [3].

This bar graph illustrates the suicide rates per 100,000 people across various countries worldwide, providing a comparative snapshot of this critical public health issue. The countries represented include a mix of high, medium, and low suicide rate regions. Notably, Lesotho stands out with the highest suicide rate, approximately 87.5 per 100,000 people, followed by Guyana, South Korea, and Lithuania, all exceeding 25 per 100,000. These elevated rates suggest serious underlying mental health challenges and potential gaps in mental health care and support systems. In contrast, countries like Syria and Lebanon show extremely low reported suicide rates—below 1 per 100,000—which may reflect cultural stigmas, underreporting, or protective social factors. Middle-range countries such as India, the USA, and Japan report suicide rates between 12 to 15 per 100,000, indicating ongoing concerns that warrant focused mental health interventions. The graph, rendered in a calm light blue tone, visually emphasizes the disparities in suicide rates globally and

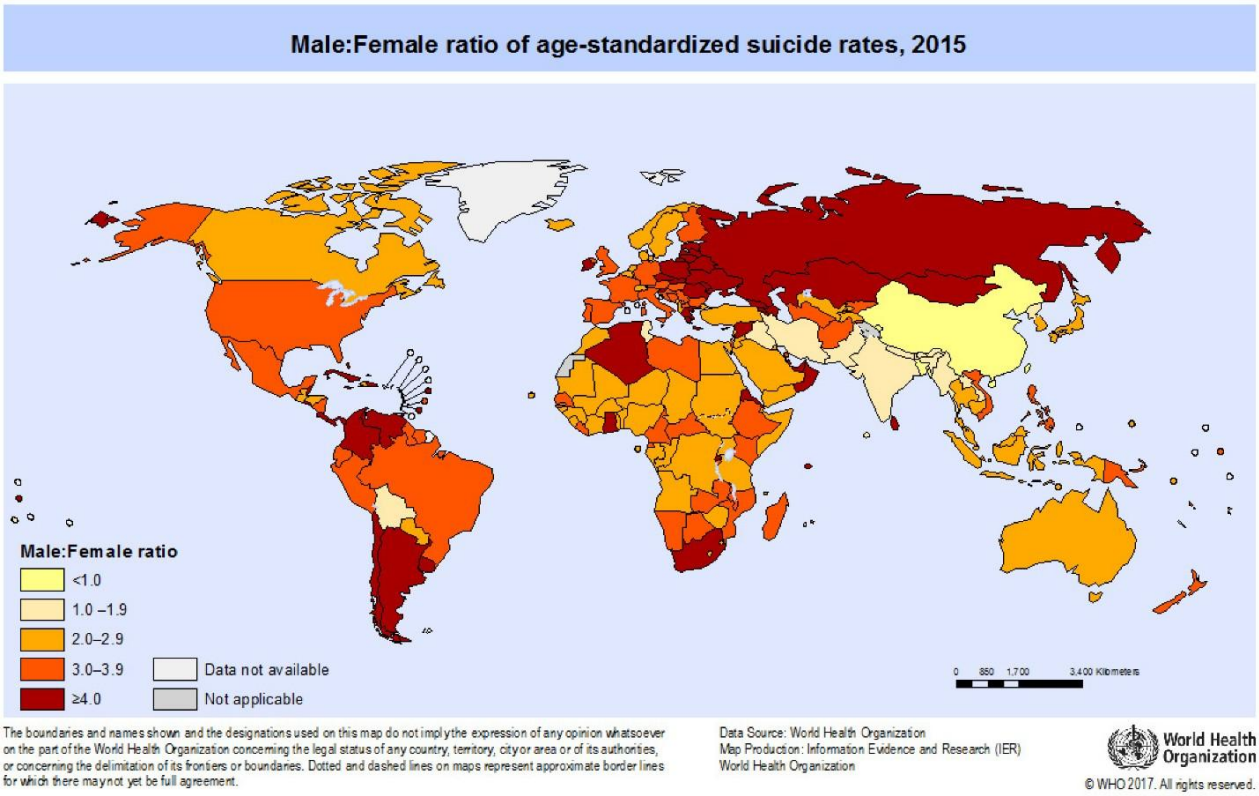

Fig. 1. Global Male-to-Female Ratio of Age-Standardized Suicide Rates in 2015.

underscores the urgent need for improved mental health awareness, support, and suicide prevention strategies tailored to each country's unique social and cultural context.

Suicidal tendencies can be detected using several methods, such as psychological assessments, machine learning, and physiological measurements [4]. Psychological assessments use questionnaires to evaluate the risk [5]. Then, trained mental health professionals conduct clinical interviews, and machine learning models analyze text data from social media and health records to identify those at risk [6]. Physiological measures such as sleep patterns, heart rate, and cortisol levels can provide more insight into the mental state (Mishica et al., 2021). Combining all these methods can improve the accuracy and result in early detection, leading to timely intervention and support [7]. However, to date, there is no clinical test that can be used to detect suicidal tendencies or assess the extent of such tendencies.Docking is a technique for determining how two molecules fit and interact [8–11]. It involves placing one molecule into the binding site of another to determine how well they interact [9]. Docking is essential in drug discovery and medicine because it helps scientists understand how well a potential drug binds with the target [9, 12]. This process uses computer algorithms and scoring systems to find the best binding positions [12].

Recently, in 2024, Claudin-5 relation with suicide has been reported. In addition, the relation of Claudin-5 has also been linked with ADHD and child depression [2]. In these neurological diseases, the Claudin 5 protein becomes disrupted, leading to its presence in the blood. We hypothesize that Claudin-5 has a binding site to which specific antibodies can bind and be used as a biomarker for these diseases. In the current work, we have computationally selected antibodies that could effectively bind and detect these claudin proteins. We have screened nine antibodies, and the best antibody has been selected. Antibody IX were bound to the druggable site of these nine antibodies. The antibody selected in this research has a higher tendency to bind the claudin-5 protein and could be used for its detection in patient blood.

2. RESULTS

This paper used computational simulations to study how well nine antibodies bind to the Claudin-5 protein. After evaluating the strength of their interactions, we selected Antibody IX as the best candidate for binding to Claudin-5. This finding could help develop diagnostics tools to detect Claudin-5 as a potential biomarker for suicide risk. Since Claudin 5 was obtained using AlphaFold 3, understanding its surface properties is crucial for this research. Therefore, we first tried to understand the binding properties of this protein. We used GrASP web server, which uses a graph neural network to find the binding region on the protein's surface. GrASP shows the surface of the proteins and maps out their features, helping users find possible binding sites and understand how the proteins interact. The best binding site in the Claudin-5 protein is shown in green in Figure 2a. The electrostatic surface potential showed the positive and negative charges on a molecule's surface, with red for negative charges, blue for positive charges, and white for neutral charges. The binding site of Claudin-5 had a negative charge, Figure 2b.

Molecular docking simulations were conducted to investigate the interactions between Claudin-5 and antibodies, resulting in the formation of a Claudin-5-antibody complex. Using HDOCK2.0 software, we identified the specific Claudin-5 antibody complex with the binding site shown in Figure 3. To select the best binding antibody, we focused on three key pa-

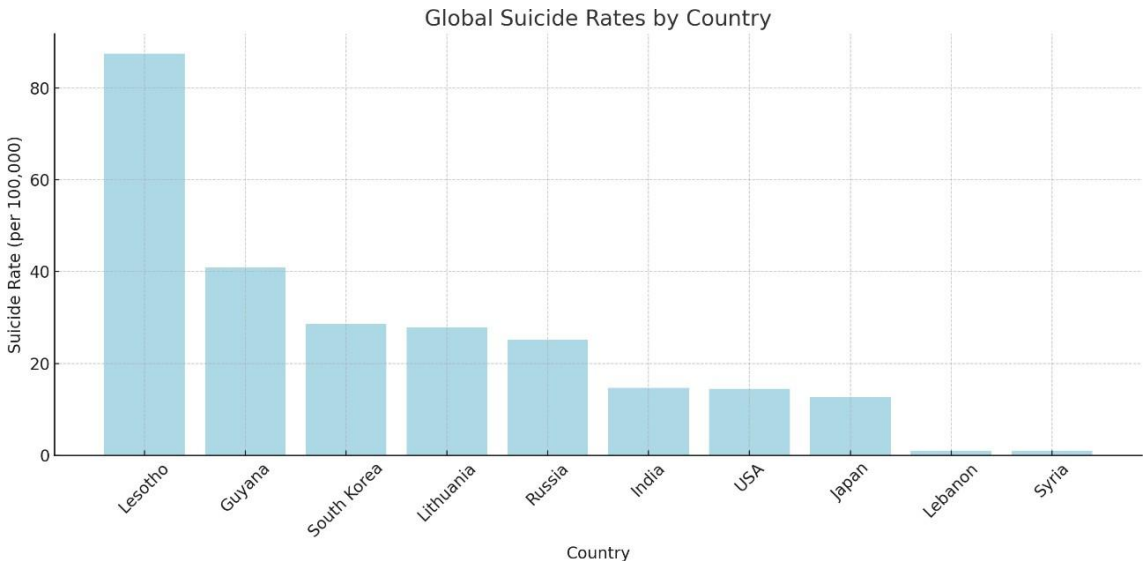

Fig. 2. This bar graph displays suicide rates per 100,000 people in a selection of countries around the world.

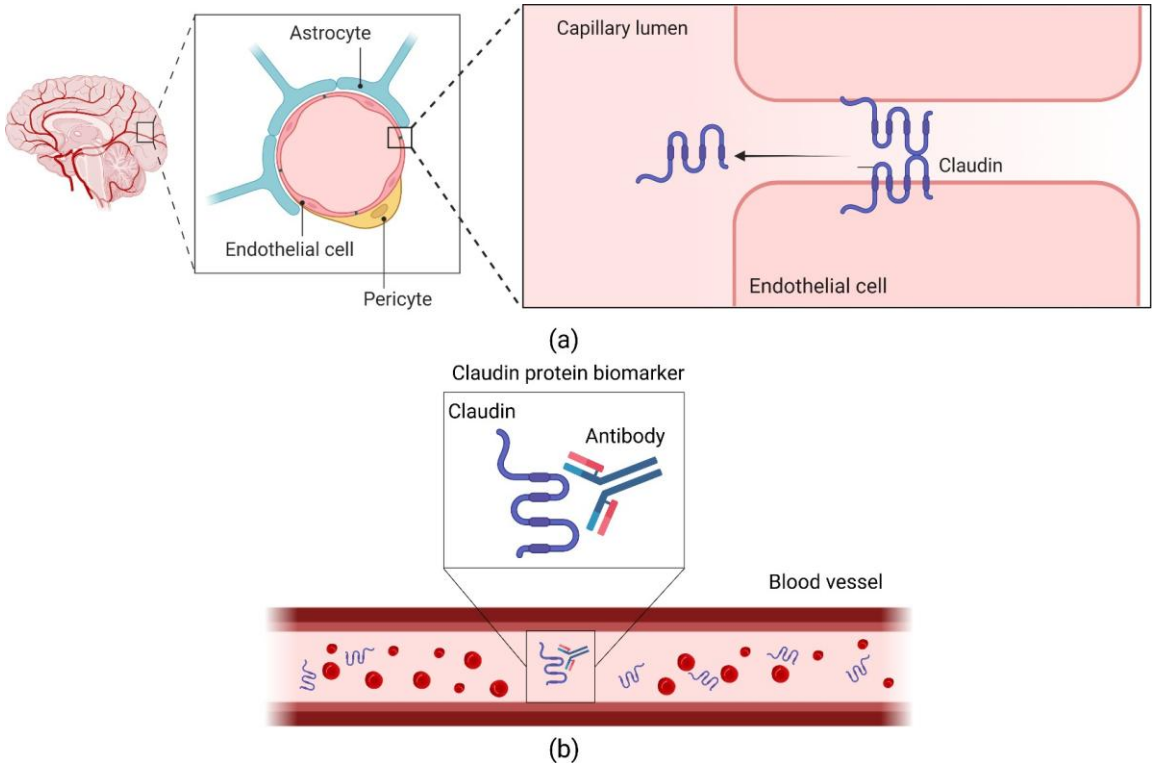

Fig. 3. Claudin-antibody interaction. (a) Claudin protein could potentially serve as a biomarker for suicide. The figure was generated using Biorender.

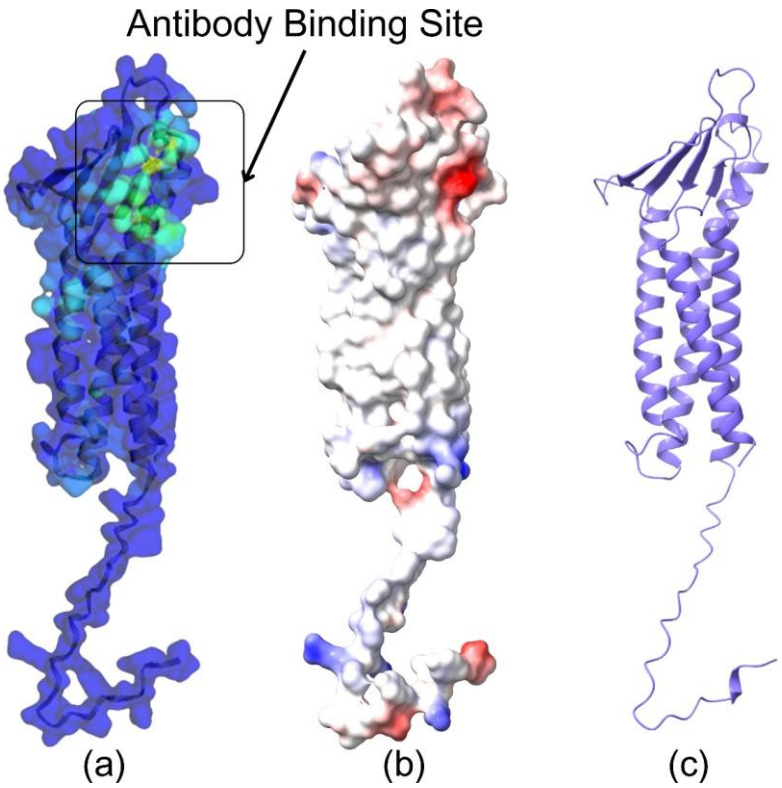

Fig. 4. Structure of claudin protein. (a) The yellow-green shot shows the antibody binding site predicted by the graph neural network; (b) the binding site is negative (red) as indicated by electrostatic surface potential (ESP); and (c) the claudin structure for reference.

rameters: the ability to bind at the site predicted by the GrASP web server, the number of hydrogen bonds, and the binding energy. Visual inspection with ChimeraX revealed that only antibodies IV, VIII, and IX bound properly to the predicted site. Hydrogen bonds are attractions between a hydrogen atom and another molecule with a negative charge. We have arranged the antibodies in decreasing order of the number of hydrogen bonds. From hydrogen bond analysis only antibodies VIII, and IX were selected. They can form between an antibody and Claudin-5, helping hold them together. We used the PRODIGY web server to confirm these findings and calculate the binding energy for each antibody-claudin-5 complex, as shown in Table 1. Binding energy measures the strength of the interaction between two molecules; lower energy means a stronger bond. Binding energy helps predict the strength and stability of the antibody binding to the clavalin-5 protein. This analysis confirms that Antibodies IX had the strongest binding affinity and effectively targeted the druggable site of the protein, making them the best candidate when they're for claudin-5 targeting. This thorough approach helped us identify antibodies with the best binding characteristics.

Overview of various suicide risk assessment methods, including their examples, classification types, and brief descriptions of how eachtechnique contributes to identifying or monitoring suicide risk.

3. DISCUSSION

Claudin-5 is a crucial protein that holds the epithelial cells of the blood-brain barrier (BBB) [2]. It is crucial to prevent harmful substances from entering the brain [2]. Reduced levels of Claudin-5 are linked to conditions like Alzheimer's, multiple sclerosis, stroke, epilepsy, and certain mental health disorders that can weaken the BBB [13]. A compromised BBB can allow harmful substances and immune cells to enter the brain and worsen these conditions [14]. Researchers are exploring ways to target Claudin-5 to either temporarily open the BBB for better drug delivery or to strengthen it to protect the brain [15]. This approach could offer new treatment options for neurological diseases.

Although Computational docking is crucial in drug discovery and molecular biology, it has many drawbacks. Some include inaccurate scoring functions, poor protein and ligand flexibility handling, and oversimplified solvent models, which lead to incorrect binding predictions. It also has high computational costs, challenges in identifying accurate binding sites, and results that depend heavily on parameters. It also struggles to verify the results of experimental data, often giving wrong positives and negatives. Nonetheless, it remains a vital resource when used with experimental methods for studying molecular interactions.

Claudin-5 plays a crucial role in maintaining the blood-brain barrier (BBB) and its connections to suicidal behavior. When Claudin-5 is disrupted, the BBB can be compromised, allowing for harmful substances to impact the decision-making and behavior control areas, increasing the risk of suicide. Using advanced computational docking and structural predictions, the study provides valuable insights into Claudin-5's interactions, despite challenges with docking accuracy. The findings emphasize Claudin-5's potential as a biomarker for early detection of any suicidal tendencies. By combining psychological assessments, machining learning, and physiological measures, one can

Table 1. Overview of modern suicide risk assessment methods across clinical, biological, technological, and AI-based domains.

Method	Examples	Type	Explanation
Psychological Screening Tools	PHQ-9, Columbia-Suicide Severity Rating Scale (C-SSRS)	Clinical	Standardized questionnaires to assess mental health status and suicide risk.
Behavioral Analysis	Monitoring social media, behavioral patterns	Observational	Observes changes in daily behaviors and online activity for warning signs.
Biomarker Analysis	Claudin-5, Cortisol levels, Serotonin	Biological	Measures biological markers linked to stress, mood, or neuroinflammation.
Neuroimaging	fMRI, PET scans	Imaging	Visualizes brain structure and activity to identify neurological abnormalities.
Genetic Testing	SNP analysis related to psychiatric conditions	Genomic	Identifies genetic variants associated with mental health disorders.
Machine Learning Algorithms	Predictive models using EHR, social media, speech	AI-Based	Uses data patterns to predict risk of suicide or mental health deterioration.
Wearable Devices	Heart rate, sleep patterns, physiological sensors	Technological	Continuously tracks physiological signals to detect stress or mood shifts.
Natural Language Processing	Text analysis from chat logs, social media posts	AI-Based	Analyzes language for signs of distress, ideation, or emotional changes.
Electrophysiological Tests	EEG pattern recognition	Clinical/Biological	Detects abnormal brain wave activity linked to psychiatric conditions.
Voice Analysis	Tone, pitch, and speech rate analysis	AI-Based	Evaluates vocal characteristics for emotional or cognitive health insights.

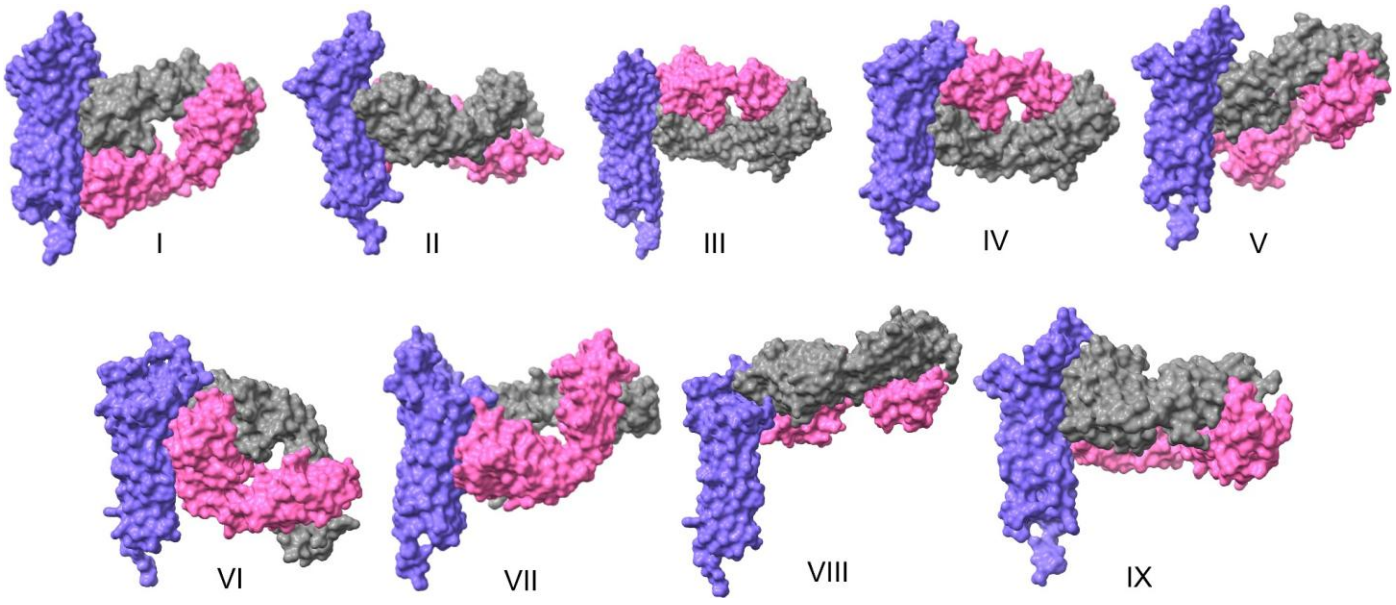

Fig. 5. Claudin-antibody complex obtained from molecular docking simulations. Almost all the antibodies bind to the binding site predicted by the graph neural network.

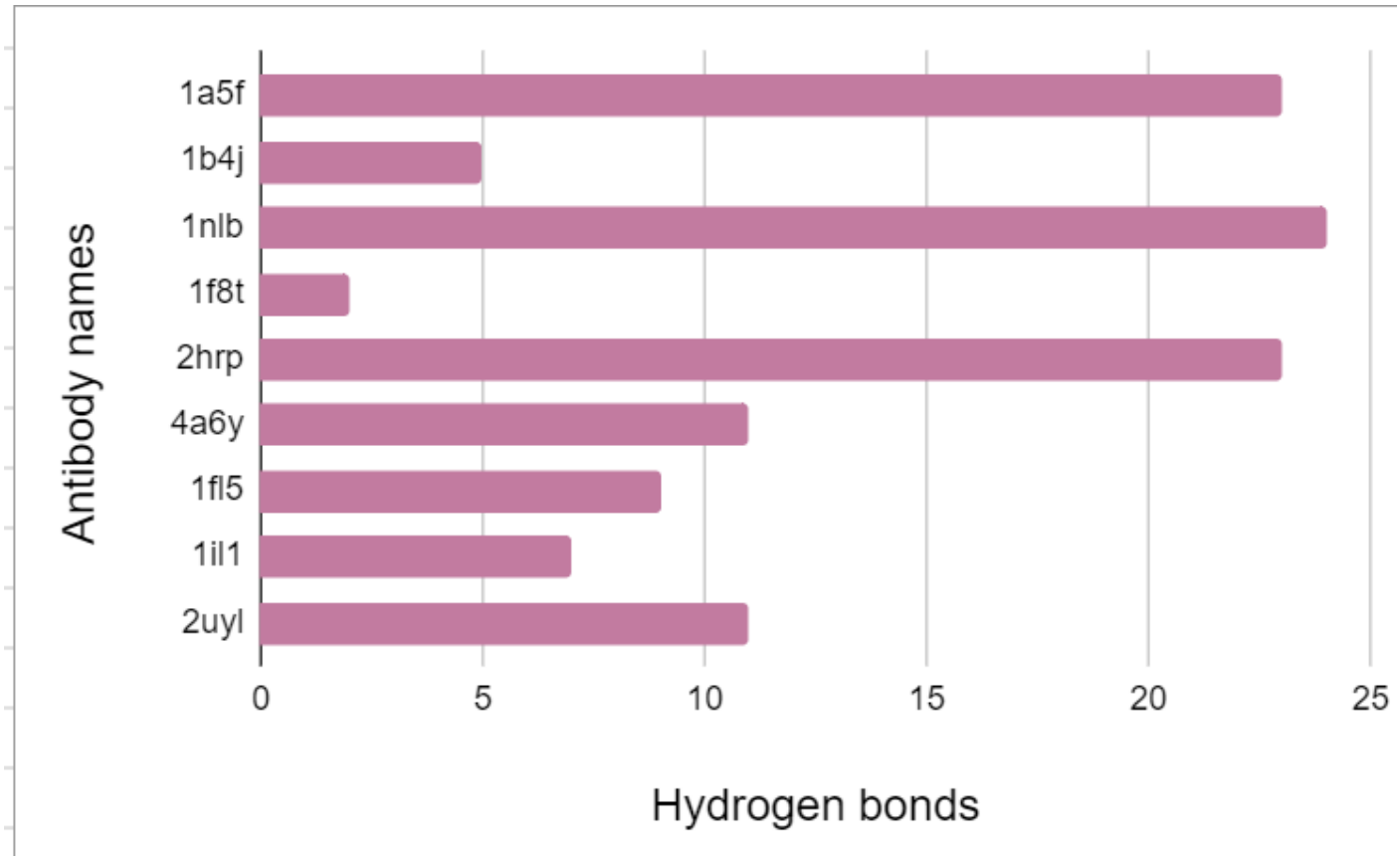

Fig. 6. Claudin 5-antibody interactions: The number of hydrogen bonds between Claudin 5 and antibodies formed. Only Anti- body VIII and IX were selected from the hydrogen bond analysis.

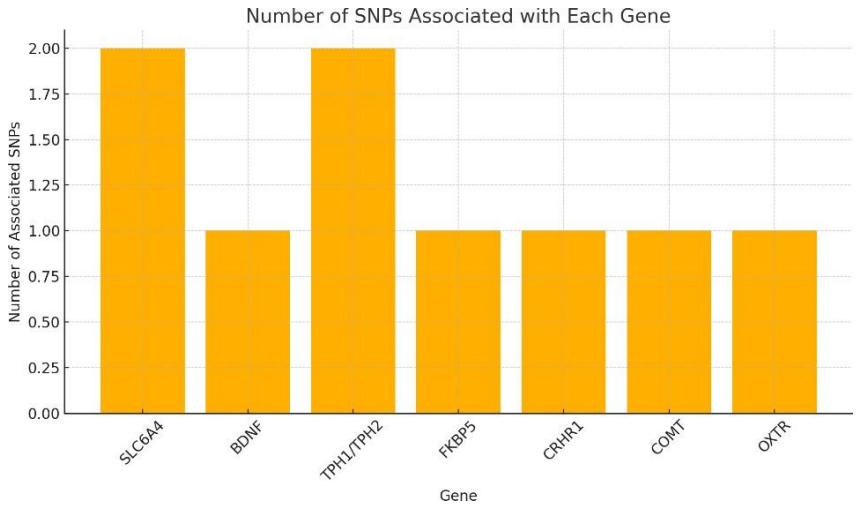

Fig. 7. Distribution of SNPs Across Key Genes Associated with Suicidal Behavior.This bar graph illustrates the number of single nucleotide polymorphisms (SNPs) identified for each gene implicated in emotional regulation, stress response, and neurotrans- mission. Notably, SLC6A4 and TPH1/TPH2 exhibit multiple SNP associations, suggesting a more complex genetic influence on serotonergic signaling pathways.

improve early detection and intervention, allowing an approach to reduce suicide risks.

The bar chart shown in Figure 5, titled "Number of SNPs Associated with Each Gene," illustrates the distribution of single nucleotide polymorphisms (SNPs) linked to various genes commonly studied in the context of neurological and psychological disorders. Among the genes shown, SLC6A4 and TPH1/TPH2 have the highest number of associated SNPs, with two SNPs each. These genes are known for their roles in serotonin transport and synthesis, respectively, which may explain their higher relevance in genetic studies of mood regulation and mental health. The remaining genes—BDNF, FKBP5, CRHR1, COMT, and OXTR—each have one associated SNP. These genes are also significant as they are involved in processes like brain-derived neurotrophic signaling, stress response, and neurotransmitter regulation. The uniform use of orange bars highlights the differences in SNP counts without introducing visual bias. Overall, this figure provides a clear comparison of SNP associations across key genes and may help prioritize genes for further functional or clinical research.

4. METHOD

First, the amino acid sequence of the Claudin 5 protein was obtained using the UniProt web server [16]. Using the UniProt web server, we obtained the amino acid sequence for the Claudin-5 protein in humans, designated as CLD5_HUMAN. We utilized AlphaFold 3 to generate the 3D structure of the Claudin 5 protein [17]. AlphaFold 3 is an AI tool developed by DeepMind that predicts the 3D structure of proteins and their interactions with other substances, improving drug discovery [17]. Using HDOCK2.0 software, we performed antibody docking with the Claudin protein. We prepared and uploaded input files, and the docking process started with default parameters to predict how the peptides bind to the protein. Molecular docking is a computational method that helps predict how molecules will interact with each other [12]. After docking, we analyzed various structures to identify the optimal binding based on interaction analysis and docking scores. The software used is the Prodigy web server, which predicts the binding energies of Claudin-5 and the antibodies, estimating the strength of their bond [18]. We used ChimeraX, a tool for visualizing and analyzing 3D structures of biomolecules [18]. It enables users to visualize molecular surfaces and analyze interactions. We checked identified sites against known binding areas using GrASP, a graph neural network-based software, to validate our results [19]. GrASP is a tool for visualizing protein surfaces and their properties, including binding sites. A binding site is a specific location on a protein where another molecule can attach. The more hydrogen bonds formed at this site, the stronger the interactions are.

3: Binding energy of claudin 5-antibodies. Binding energy is an energy requited to separate two molecules. Hence, lower binding (more negative) energy indicates a stronger and more stable interaction. Among the selected Antibody VIII and IX, Antibody IX showed the highest binding energy and was selected.

5. REFERENCES

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Table 2. Types of SNPs related to depression.

Gene	SNP ID	Function / Association
SLC6A4 (serotonin transporter)	rs25531, 5-HTTLPR	Polymorphisms in the promoter region associated with serotonin dysregulation and higher suicide risk, especially under stress.
BDNF (brain-derived neurotrophic factor)	rs6265 (Val66Met)	Affects neuronal plasticity. The Met allele is linked to increased risk of depression and suicidal behavior.
TPH1 / TPH2 (tryptophan hydroxylase)	rs1800532, rs7305115	Involved in serotonin synthesis; associated with suicidality and aggressive behavior.
FKBP5	rs1360780	Involved in HPA axis regulation; linked to childhood trauma and suicidal behavior.
CRHR1 (corticotropin-releasing hormone receptor 1)	rs110402	Affects stress response and is associated with depression and suicidality.
COMT (catechol-O-methyltransferase)	rs4680 (Val158Met)	Influences dopamine levels and is linked with suicidal risk, especially in schizophrenia and mood disorders.
OXTR (oxytocin receptor gene)	rs53576	Involved in social behavior; some variants linked with emotion regulation deficits and suicidality.

Table 3. Key Genetic Variants (SNPs) Associated with Suicidal Behavior and Psychiatric Conditions. This table summarizes specific single nucleotide polymorphisms (SNPs) across various genes that are implicated in neurotransmission, stress response, and emotional regulation—factors associated with an increased risk of suicidality and related mental health disorders.

Antibody ID	I	II	III	IV	V	VI	VII	VIII	IX
Binding Energy (kcal/mol)	-16.8	-13.5	-11.6	-13.8	-14.2	-6.0	-16.7	-10.8	-13.7