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Bacterial Succession as a Forensic Chronometer: Bioinformatics Analysis of Cadaver Decomposition

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Abstract: ***Decomposition is a complex biological process influenced by both biotic and abiotic factors. Microorganisms play a major role in carrion decomposition by colonizing and proliferating within tissues after death. Post-mortem decomposition is characterized by the dynamic succession of bacterial communities that develop in predictable stages over time. These microbial changes provide measurable biological indicators that can be correlated with the post-mortem interval (PMI).***
The present study focuses on the bioinformatics analysis of bacterial succession in postmortem human brain tissues using sequencing data retrieved from the Sequence Read Archive (SRA) database. Comparative analysis of different brain regions, including the dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, thalamus, putamen, and caudate nucleus, was performed to evaluate microbial prevalence, evolutionary significance, and tissue-specific bacterial diversity. The study also examined the predominance of bacterial groups such as Proteobacteria, Firmicutes, and Actinomycetota during various stages of decomposition.
In addition to forensic microbiology, the study examined the neurological and evolutionary significance of postmortem brain tissues. Comparative analysis of eukaryotic prevalence and evolutionary similarity with primate groups such as Simiiformes and Catarrhini revealed notable differences between cortical regions. The dorsolateral prefrontal cortex demonstrated greater similarity with Catarrhini, whereas the ventrolateral prefrontal cortex exhibited stronger association with Simiiformes, reflecting evolutionary specialization and cortical lateralization. Furthermore, the analysis of thalamic and putamen asymmetry provided insights into neurological development, neurobehavioral disorders, and functional specialization within the human brain.
The findings suggest that bacterial community succession can serve as a reliable forensic chronometer for estimating the age of cadavers. Furthermore, the integration of forensic microbiology, neuroscience, and bioinformatics provides new insights into postmortem tissue analysis and microbial evolution.
The integration of forensic microbiology, neuroscience, evolutionary biology, and bioinformatics in this study highlights the interdisciplinary potential of microbial analysis in postmortem investigations. By utilizing publicly available sequencing datasets and advanced computational approaches, the research contributes toward the development of standardized microbial biomarkers for PMI estimation. The findings support the concept that bacterial succession patterns within cadaveric tissues can serve as dependable forensic chronometers and provide valuable information regarding decomposition stages, tissue specialization, and evolutionary relationships. Overall, this study emphasizes the growing importance of microbial ecology and bioinformatics in modern forensic science and demonstrates their application in improving the scientific accuracy of postmortem interval estimation.
Keywords: Postmortem, Brain tissues, Homo sapiens, Bacterial communities, Firmicutes, Forensic microbiology, Bioinformatics, Post-mortem interval (PMI).

I. INTRODUCTION

A. Cadavers and Postmortem

Cadavers play a critical role in the study of the human brain, especially in the fields of Neuroscience and Neuropathology. The brain is a highly complex organ, and many of its diseases—particularly neurological disorders—can only be fully understood through post- mortem examination of brain tissues.

Cadaver-based brain studies provide direct insight into structural abnormalities, cellular damage, and biochemical changes that are often not completely detectable in living patients.

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B. *Role of Cadavers in Brain Tissue Study*

1) Anatomical Study of the Brain

- Cadavers are used to study the gross anatomy of the brain
- Helps identify:
  - Cerebral hemispheres o
    Brainstem and cerebellum o
    Lobes and functional regions
- Provides a realistic understanding of spatial relationships

This is essential for medical students and neurosurgeons.

2) Neuropathological Examination

Cadaver brain tissues are examined to detect disease:

- Gross examination → identifies visible abnormalities
- Microscopic examination → reveals cellular-level changes

Used to diagnose:

- Alzheimer's disease
- Parkinson's disease
- Multiple sclerosis

3) Histological and Molecular Studies

- Brain tissues are stained and analyzed under microscopes
- Detects:
  - Protein deposits (amyloid, tau) o Neuronal degeneration o
    Inflammation

These studies help explain disease mechanisms.

4) Research and Brain Banks

- Cadaver brains are stored in brain banks for research
- Support long-term studies on neurological disorders
- Aid in development of drugs and therapies

Organizations like the National Institute of Neurological Disorders and Stroke promote such research.

5) Forensic Applications

In Forensic Medicine, cadaver brain tissues help:

- Determine cause of death
- Identify trauma (head injury, haemorrhage)
- Detect poisoning or hypoxia

C. *Neurological Conditions Studied Using Cadavers*

Neurodegenerative Disorders

- Alzheimer's disease → plaques and tangles
- Parkinson's disease → neuron loss

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Demyelinating Disorders

- Multiple sclerosis → myelin damage

Traumatic Brain Injuries

- Diffuse axonal injury
- Brain edema and haemorrhage
- Infectious Disorders
- Encephalitis
- Meningitis

D. Significance of Studying Brain Tissue Using Cadavers

1) Accurate understanding of brain anatomy
- Provides real, three-dimensional knowledge of brain structures essential for Neuroscience.

2) Definitive diagnosis of neurological disorders
- Confirms diseases like Alzheimer's disease and Parkinson's disease through direct tissue examination.

3) Study of disease mechanisms
- Reveals cellular and molecular changes (e.g., protein deposits, neuron loss).

4) Validation of clinical findings
- Helps compare symptoms observed during life with actual brain pathology (clinicopathological correlation).

5) Improvement of diagnostic tools o Enhances accuracy of imaging techniques like MRI and CT scans.

6) Advancement of medical research o Supports development of new drugs and therapies for neurological diseases.

7) Forensic investigation support
- Assists in determining cause of death in brain-related cases within Forensic Medicine.

8) Medical education and training
- Provides hands-on learning for students and surgeons in neuroanatomy.

9) Understanding rare and complex disorders
- Enables study of conditions that are difficult to diagnose in living patients.

10) Contribution to public health and society
- Improves healthcare outcomes through knowledge gained from cadaver stu Postmortem:

A post-mortem examination of brain tissue is a specialized part of autopsy focused on identifying **structural, microscopic, and biochemical changes in the brain after death**. It is a core practice in Neuropathology and widely used in Forensic Medicine.

This examination helps determine:

- Cause of death
- Presence of neurological disorders
- Extent of brain injury or disease

E. Steps in Brain Post-Mortem Examination

1) Removal of the Brain

- The skull is opened using a craniotomy
- The brain is carefully removed to avoid damage
- Spinal cord may also be examined if needed

2) Gross Examination
- The brain is inspected for:
  - Size and weight o Shape and symmetry o Lesions, hemorrhage, or tumors

Examples:

- Atrophy in Alzheimer's disease
- Pigment loss in Parkinson's disease

3) Fixation and Sectioning
- Brain is preserved in formalin (10%)
- Fixation improves tissue quality
- The brain is cut into sections (usually coronal slices)

4) Microscopic (Histopathological) Examination
- Tissue is examined under a microscope
- Special stains are used (H&E, silver stain, etc.)

Findings include:
- Neuronal loss
- Protein deposits
- Inflammation or infection

F. Conditions Identified Through Brain Post-Mortem

Neurodegenerative Disorders
- Alzheimer's disease
- Parkinson's disease

Demyelinating Disorders
- Multiple sclerosis

Cerebrovascular Disorders
- Stroke (infarction or hemorrhage)

Traumatic Brain Injury
- Diffuse axonal injury
- Brain swelling (edema)

Infectious and Inflammatory Disorders
- Encephalitis
- Meningitis

G. Significance of Post-Mortem Study of Brain Tissue

1) Accurate determination of cause of death
- Identifies brain-related causes such as hemorrhage, stroke, or trauma within Forensic Medicine.

2) Definitive diagnosis of neurological disorders
- Confirms diseases like Alzheimer's disease and Parkinson's disease that may be uncertain during life.

3) Understanding disease pathology o Reveals structural and cellular changes in the brain studied under Neuropathology.

4) Clinicopathological correlation o Links symptoms observed before death with actual brain findings after death.

5) Advancement of medical research
- Helps in discovering mechanisms of neurological diseases and developing treatments.

6) Validation of diagnostic techniques o Confirms and improves imaging methods like MRI and CT scans.

7) Detection of hidden or silent conditions o Identifies diseases that showed no clear symptoms during life.

8) Forensic and legal importance o Provides scientific evidence in criminal investigations and court cases.

9) Medical education and training
- Enhances learning for students and professionals studying brain anatomy and pathology.

10) Public health and epidemiological value
- Provides data on disease prevalence and helps improve healthcare planning.

H. Possible markers which can be sued to find the state of the person.

Markers to Determine the State of a Dead Person & Neurological Disorders

In Forensic Medicine and Neuropathology, various morphological, biochemical, and molecular markers from brain tissue are used to determine:
- State of death (time since death, cause)
- Presence of neurological disorders

1. Markers to Determine the State of the Dead Person

a) Physical & Structural Brain Markers

- Brain edema (swelling) → indicates trauma, hypoxia, or infection
- Hemorrhage → suggests injury or stroke
- Infarction (dead tissue) → indicates ischemic stroke
- Skull/brain injury patterns → help determine cause of death

These markers help identify whether death was due to natural disease, trauma, or external causes.

b) Post-Mortem Biochemical Markers

- Neurotransmitter levels (dopamine, serotonin)
- Enzyme degradation (e.g., cholinesterase activity)
- Electrolyte imbalance in brain tissue

These help estimate time since death and metabolic disturbances.

c) Cellular & Molecular Markers

- RNA degradation patterns
- Protein breakdown rates
- Apoptosis (cell death markers)

Used in advanced forensic studies to estimate post-mortem interval (PMI).

d) Hypoxia/Ischemia Markers

- Neuronal shrinkage
- Eosinophilic neurons (“red neurons”)

Indicate lack of oxygen before death (e.g., drowning, suffocation).

2. Markers for Detecting Neurological Disorders

a) Neurodegenerative Disease Markers

Alzheimer’s disease

- Amyloid plaques
- Neurofibrillary tangles (tau protein)

Parkinson’s disease

- Lewy bodies
- Loss of dopamine neurons

b) Demyelination Markers

- Multiple sclerosis o Loss of myelin sheath o Plaque formation in white matter

c) Inflammatory Markers

- Microglial activation
- Cytokine presence

Seen in encephalitis and autoimmune disorders

d) Vascular Markers

- Infarcts and hemorrhages
- Vessel damage

Indicate stroke or vascular dementia

e) Traumatic Brain Injury Markers

- Diffuse axonal injury
- Brain contusions
- Hematomas

Important in forensic cases

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I. Significance of Brain Tissue Markers in Post-Mortem Analysis

1) Accurate determination of cause of death
- Brain markers (e.g., hemorrhage, infarction) help identify whether death was due to trauma, stroke, or disease in Forensic Medicine.

2) Estimation of time since death (PMI)
- Biochemical and cellular changes in brain tissue assist in determining the post- mortem interval.

3) Detection of neurological disorders o Confirms diseases like Alzheimer's disease and Parkinson's disease.

4) Identification of hidden or undiagnosed conditions o Reveals diseases that were not detected during life.

5) Understanding disease mechanisms o Helps study cellular and molecular changes in Neuropathology.

6) Support in forensic investigations o Provides scientific evidence in cases of suspicious or unnatural death.

7) Correlation with clinical symptoms
- Links observed symptoms before death with actual brain pathology (clinicopathological correlation).

8) Advancement of medical research
- Contributes to development of new treatments and therapies for neurological disorders.

9) Improvement of diagnostic techniques o Validates and refines imaging methods like MRI and CT scans.

10) Public health and legal importance
- Helps in maintaining accurate death records and supports legal decision-making.

II. REVIEW OF LITERATURE

The study of post-mortem brain tissue and microbial succession has emerged as a rapidly expanding interdisciplinary field that combines Neuroscience, Neuropathology, Forensic Medicine, Molecular Biology, and Bioinformatics. Traditional forensic investigations have long relied on physical and morphological indicators such as rigor mortis, livor mortis, algor mortis, and insect colonization to estimate the post-mortem interval (PMI). However, these methods are often influenced by environmental conditions, body mass, temperature, humidity, and external contamination, which can reduce the accuracy of PMI estimation. In recent years, researchers have increasingly focused on microbial communities associated with decomposing human tissues as biological indicators capable of providing more reliable and objective forensic evidence.

Human decomposition is a biologically dynamic process involving the interaction of endogenous microbes, environmental microorganisms, and host tissues. Following death, immune regulation ceases, allowing bacterial populations to proliferate and migrate through tissues in a predictable pattern. This phenomenon, commonly referred to as microbial succession, has been proposed as a “microbial clock” that can assist in estimating the time elapsed since death. Simultaneously, post-mortem brain tissue studies continue to provide essential information regarding neurological disorders, structural abnormalities, molecular pathology, and evolutionary development of the human brain.

The present literature review discusses previous studies related to cadaveric brain tissue analysis, microbial succession during decomposition, forensic microbiology, brain evolution, and bioinformatics approaches used in post-mortem studies. The review also highlights current limitations and identifies gaps in existing research that justify the need for the present investigation.

A. Cadaveric Brain Tissue Studies in Neuroscience

Cadaver-based research has played a central role in the advancement of modern Neuroscience. Historically, Andreas Vesalius revolutionized anatomical science through direct human dissection, challenging earlier misconceptions regarding the structure of the human body. Since then, post-mortem brain examination has become an indispensable method for understanding the anatomy and pathology of the nervous system.

According to Greenfield’s Neuropathology, post-mortem examination remains the gold standard for confirming many neurological disorders. Brain tissues obtained from cadavers allow researchers to study structural organization, neuronal architecture, neurochemical pathways, and disease-associated abnormalities that cannot be fully observed in living individuals. Gross examination of cadaveric brains helps identify atrophy, hemorrhage, infarction, tumors, and traumatic injuries, while histopathological analysis reveals cellular- level changes such as neuronal degeneration, inflammation, demyelination, and protein aggregation.

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Several neurodegenerative disorders have been characterized primarily through post-mortem studies. Alzheimer's disease is associated with amyloid-beta plaques and neurofibrillary tangles composed of tau protein. Parkinson's disease is characterized by degeneration of dopaminergic neurons in the substantia nigra along with the presence of Lewy bodies. Multiple sclerosis demonstrates extensive demyelination within white matter tracts, while Amyotrophic Lateral Sclerosis (ALS) involves progressive degeneration of motor neurons. These pathological features are often difficult to confirm clinically and therefore require direct tissue examination.

Adams and Victor's Principles of Neurology emphasizes the importance of clinicopathological correlation in neurological research. This concept involves comparing symptoms observed during life with pathological findings identified after death. Such comparisons have improved the classification and diagnosis of several neurological conditions. In many cases, post-mortem examinations have revealed discrepancies between clinical diagnosis and actual pathology, leading to revisions in disease classification systems.

Post-mortem brain tissue is also important for molecular and biochemical studies. Hynd et al. (2003) reported that human autopsy tissue is critical for neurochemical research because it enables direct measurement of neurotransmitters, gene expression patterns, and abnormal proteins associated with disease progression. Immunohistochemistry, molecular genetics, and advanced sequencing technologies have further expanded the scope of cadaveric research by allowing investigators to detect biomarkers associated with neurodegenerative disorders.

B. Brain Banks and Ethical Considerations in Post-Mortem Research

The establishment of brain banks has significantly improved neurological research by providing standardized and well-preserved tissue samples for scientific investigation. Brain banks collect, preserve, catalogue, and distribute post-mortem brain tissues obtained from donors with informed consent. Organizations such as the National Institute of Neurological Disorders and Stroke (NINDS) have promoted brain banking as an essential resource for studying neurological diseases.

Brain banks support long-term studies involving Alzheimer's disease, Parkinson's disease, Huntington's disease, autism spectrum disorders, and psychiatric illnesses. The availability of high-quality tissue samples allows researchers to conduct reproducible studies and compare pathological findings across populations. Furthermore, the integration of brain tissue studies with neuroimaging techniques such as MRI and PET scans has improved diagnostic accuracy and understanding of disease progression.

Despite their advantages, post-mortem studies face several limitations. One major concern is the post-mortem interval (PMI), which influences tissue integrity. Delays in preservation can result in autolysis, RNA degradation, protein breakdown, and microbial contamination. Rapid fixation using formalin or freezing techniques is therefore essential to maintain tissue quality.

Ethical considerations also remain central to cadaver-based research. The World Health Organization and various medical regulatory bodies emphasize the importance of informed consent, confidentiality, and respectful handling of human remains. Ethical frameworks ensure that research involving cadaveric tissues is conducted responsibly while maintaining public trust in scientific practices.

C. Brain Regions and Functional Significance in Post-Mortem Studies

Different regions of the brain exhibit unique structural and functional characteristics, making them valuable targets for post-mortem analysis. Among these, the dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC), thalamus, putamen, and caudate nucleus have received considerable attention in neuroscience research.

D. Dorsolateral and Ventrolateral Prefrontal Cortex

The dorsolateral prefrontal cortex is associated with executive functions such as working memory, planning, decision-making, and cognitive flexibility. Plakke and Romanski (2014) reported that the DLPFC represents one of the most recently evolved regions of the human brain and demonstrates advanced cortical organization compared to other prefrontal regions. The DLPFC possesses a highly developed granular layer IV, which is particularly prominent in primates and humans.

The ventrolateral prefrontal cortex, on the other hand, is associated with emotional regulation, language processing, and social cognition. Comparative studies suggest that the VLPFC shares more conserved evolutionary characteristics with simian primates, whereas the DLPFC has undergone substantial expansion during human evolution.

Research involving post-mortem tissue analysis has demonstrated regional variation in neuronal composition, molecular expression, and evolutionary significance between these cortical regions. Such studies contribute to understanding both cognitive specialization and evolutionary adaptation.

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E. Thalamus and Brain Lateralization

The thalamus functions as a major relay center responsible for transmitting sensory and motor information to the cerebral cortex. Functional imaging studies by Llano (2013) highlighted the role of the thalamus in language processing and cortical integration. Research by Müller et al. (1998) demonstrated increased left thalamic activation during language tasks, suggesting hemispheric specialization.

Post-mortem studies of thalamic tissues have been useful in understanding aphasia, cognitive disorders, and neural connectivity. Structural asymmetry between the left and right thalamus has also been associated with functional lateralization and cognitive dominance.

F. Putamen and Neurobehavioral Disorders

The putamen forms part of the basal ganglia and plays an essential role in movement regulation, motor learning, and speech articulation. Fazl and Fleisher (2019) described the putamen as a critical component of neural circuits controlling voluntary movement.

Studies have shown that asymmetry in putamen development may be associated with neurobehavioral disorders. Qu et al. (2023) demonstrated alterations in putamen structure among Parkinson's disease patients, while research involving bipolar disorder and ADHD indicated abnormal lateralization patterns. These findings suggest that post-mortem analysis of the putamen can provide important insights into neurological and psychiatric disorders.

G. Caudate Nucleus and Addiction Research

The caudate nucleus has been extensively studied in relation to addiction, schizophrenia, and neurodegenerative diseases. Korpi et al. (1987) reported that the caudate contains high concentrations of dopamine receptors, making it essential for studying neurotransmitter imbalance.

Post-mortem examinations have revealed structural and biochemical alterations within the caudate nucleus among individuals with cocaine addiction and psychiatric disorders. Such findings support the use of caudate tissue as a marker for investigating substance abuse and neuropsychiatric conditions.

H. Evolutionary Perspectives of the Human Brain

The evolution of the human brain has attracted significant scientific interest due to its relationship with cognition, language, and behavior. Comparative studies involving Simiiformes and Catarrhini have shown that human brain development represents an extension of evolutionary trends observed among primates.

Zhuang et al. (2023) explained that the human brain underwent accelerated neuronal proliferation, increased cortical expansion, and prolonged developmental timing compared to non-human primates. Molecular and genetic studies have identified rapidly evolving regulatory sequences associated with primate brain evolution.

The DLPFC has been identified as one of the most expanded cortical regions in humans, contributing to advanced cognitive abilities. White et al. (2023) noted that the left DLPFC demonstrates greater specialization for verbal processing and executive control, whereas the right DLPFC retains functional characteristics more closely related to ancestral primate patterns.

Preuss (2022) proposed that the VLPFC retains more conserved features shared with simian primates, including structural organization and connectivity patterns. Comparative analysis of cortical regions therefore provides insight into both evolutionary conservation and human- specific adaptations.

These evolutionary perspectives are important in forensic and neuropathological studies because they help explain regional specialization, neuronal complexity, and susceptibility to disease.

I. Forensic Microbiology and Microbial Succession

Forensic microbiology is an emerging discipline that investigates microbial communities associated with decomposition and their applications in forensic science. After death, the cessation of immune function allows bacteria to proliferate throughout the body, resulting in predictable microbial succession.

The microbial populations associated with decomposition are collectively referred to as the thanatomicrobiome when present within internal organs and the epinecrotic microbiome when present on body surfaces. These microbial communities undergo sequential changes influenced by oxygen availability, tissue breakdown, environmental temperature, moisture, and insect activity.

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Metcalf et al. (2013) demonstrated that microbial succession follows reproducible patterns during decomposition, supporting the concept of a microbial clock for PMI estimation. Early decomposition stages are typically dominated by aerobic bacteria, while later stages involve anaerobic and spore-forming organisms.

Proteobacteria and Firmicutes are among the most extensively studied bacterial phyla in forensic microbiology. Dong et al. (2019) reported that Gammaproteobacteria become increasingly dominant during the bloat and active decay stages. Their abundance correlates strongly with decomposition progression, making them useful biomarkers for PMI estimation.

Firmicutes and Actinomycetota have also been associated with advanced stages of decomposition. Moitas et al. (2024) observed that Actinomycetota abundance increases during advanced decay and dry remains stages due to their ability to survive in nutrient-depleted environments.

Research by Huang et al. (2026) indicated that low abundances of Pseudomonadota and Firmicutes are characteristic of the fresh stage of decomposition. Such microbial profiles can therefore provide information regarding the approximate time since death.

The use of microbial communities for forensic investigation offers several advantages over traditional methods. Microbial succession patterns are less subjective than morphological indicators and may remain informative even when environmental conditions alter physical decomposition. Additionally, modern sequencing technologies allow researchers to identify bacterial communities with high sensitivity and precision.

J. Bioinformatics and SRA-Based Studies in Forensic Research

The development of high-throughput sequencing technologies and bioinformatics tools has transformed forensic microbiology and post-mortem research. Public databases such as the Sequence Read Archive (SRA) provide access to large volumes of genomic and metagenomic data generated from human tissues and microbial communities.

Bioinformatics enables researchers to analyze sequencing reads, determine taxonomic composition, identify evolutionary relationships, and compare microbial prevalence across tissue types. SRA-based studies allow investigators to conduct large-scale analyses without the need for extensive laboratory experimentation. Several studies have used metagenomic approaches to characterize bacterial populations associated with decomposition. These investigations commonly involve taxonomic profiling, comparative genomics, phylogenetic analysis, and microbial abundance estimation. The integration of bioinformatics with forensic science has improved the ability to identify microbial biomarkers associated with specific decomposition stages. Furthermore, comparative genomic analysis has provided insights into evolutionary similarities between human brain tissues and primate lineages. Despite these advancements, challenges remain regarding standardization of sequencing protocols, contamination control, database quality, and reproducibility. Variability in sample collection methods and environmental conditions can influence microbial profiles, making it necessary to establish standardized analytical frameworks.

K. Research Gap

Although considerable progress has been made in post-mortem neuroscience and forensic microbiology, several important gaps remain in the existing literature.

1) Most forensic studies focus primarily on external decomposition indicators such as insects, body temperature, and physical tissue changes, while relatively fewer investigations examine bacterial succession within brain tissues.
2) Existing research on microbial succession has mainly concentrated on gut microbiota and surface microbial communities, with limited emphasis on microbial dynamics within specific regions of the human brain.
3) Comparative analysis between different brain regions such as the dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, thalamus, and putamen remains insufficiently explored.
4) Very few studies integrate forensic microbiology with evolutionary neuroscience to evaluate similarities between human brain tissues and primate groups such as Simiiformes and Catarrhini.
5) Although high-throughput sequencing data are widely available in repositories such as the SRA database, limited studies have utilized these datasets for comprehensive bioinformatics-based forensic analysis.
6) Current literature lacks standardized microbial biomarkers that can accurately estimate post-mortem interval across different tissue types and environmental conditions.
7) There is inadequate understanding regarding the relationship between bacterial prevalence and neurological tissue specialization during decomposition.
8) Most available studies are experimental and laboratory-based, whereas large-scale computational mining of publicly available sequencing datasets remains underutilized.

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9) Limited research has investigated whether bacterial community composition can provide information regarding neurological state, cognitive specialization, or neurobehavioral disorders.

10) The application of bacterial succession as a reliable forensic chronometer still requires validation through integrated molecular, bioinformatics, and comparative tissue studies.

The present study attempts to address these gaps by integrating bioinformatics analysis of SRA- derived sequencing data with forensic microbiology and brain tissue analysis. The investigation explores bacterial succession patterns across different post-mortem brain tissues while also examining evolutionary similarities with primate lineages. By combining microbial prevalence analysis with comparative neurological evaluation, the study aims to contribute toward the development of more accurate microbial markers for post-mortem interval estimation.

III. AIM AND OBJECTIVES

A. Objective 1

Data mining of the brain tissues from postmortem tissues.

To study the significance of the brain tissues in neuronal behavioral or state of the victim, and the tissues significance in evolutionary behaviour, we extracted percent data of the brain tissues of postmortem people. Data was collected from at least 10 submitted sequences and the average was represented in the study.

Analysed data was retrieved from the SRA data base experiments. The data was collected in percentages and used for analysis. For easy comparison, the different parts of the tissue are arranged in the order of the tissues.

B. Objective 2

Data mining of the bacterial community prevalence data and determining the age of the cadaver or tissue.

Bacterial community's percent data was extracted from the SRA data base of the different brain tissues of the human postmortems and used for analysis of the prevalence of the bacterial communities.

C. Objective 3

BACTERIAL COMMUNITY GROWTH AND AGE OF CADAVER

Bacterial Markers for postmortem interval (PMI) estimation.

Bacterial markers for cadaver decomposition, often referred to as the thanatomicrobiome (internal organs) and epinecrotic community (body surface), provide a "microbial clock" that acts as a valuable tool for estimating the postmortem interval (PMI). As a body decomposes, the microbial community shifts from aerobic bacteria to anaerobic bacteria, with specific phyla and genera dominating different stages

IV. METHODS

SRA NUMBERS	TISSUE NAMES
SRR34682015	Brain tissue from left frontal cortex
SRR34682059	Brain tissue from the secondary visual cortex (BA18)
SRR34682005	Brain tissue from right cerebral cortex
SRR38115900	Left hemisphere
SRR28209966	Right Thalamus
SRR28155764	Left Thalamus
SRR32638529	Ventrolateral prefrontal cortex
SRR37507557	Dorsolateral prefrontal cortex
SRR32883898	Caudate Nucleus
SRR28249887	Right Putamen

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SRR28093939	Left Putamen
SRR37405783	Right cerebral cortex
SRR37511977	Left cerebral cortex
SRR34681983	Mesial temporal lobe
SRR34682032	Anterior temporal cortex
SRR28075249	Right Frontal Cortex
SRR37855543	Motor cortex
SRR28093931	Left Globus Pallidus
SRR28228311	Right Globus Pallidus
SRR37539857	Middle Frontal Gyrus
SRR28172436	Left Occipital Cortex
SRR28075221	Right Occipital Cortex
SRR28075265	Left Temporal Cortex
SRR28272645	Right Temporal Cortex
SRR34681961	Brain tissue from right parieto-occipital temporal cortex

This study investigates the dynamics of bacterial communities during decomposition and explores their evolutionary parallels with primate lineages, specifically *Simiiformes and Catarrhini.*

In this study, we analyze the progression of bacterial community structures and their metabolic activity to establish reliable indicators for determining the age of a post-mortem body. High- throughput sequencing data was mined from SRA database to estimate the percent of predominance of several bacterial communities. A total of 25 sequencing data was retrieved from SRA database and estimated for their percent similarity to the eukaryotic groups especially *Catarrhini* and *Simiiformes.*

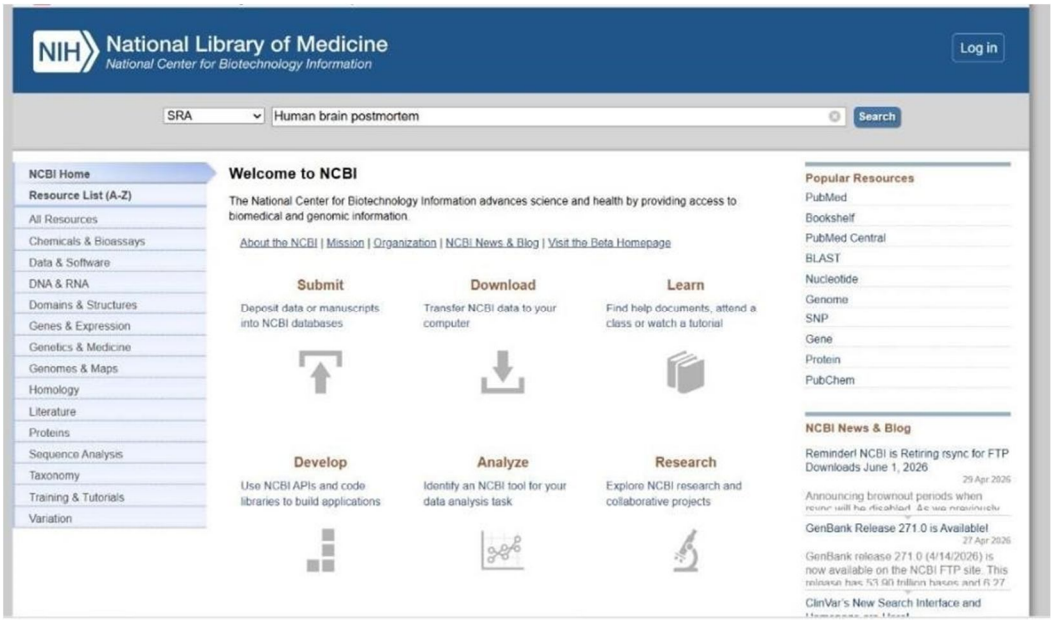


Fig 1.1 Representation of bacterial succession patterns in postmortem brain tissues.

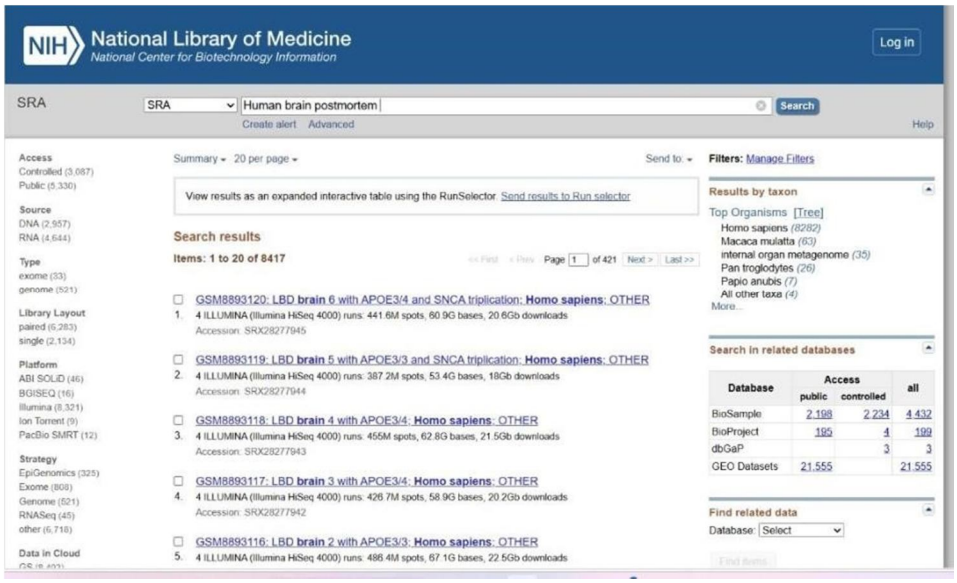


Fig 1.2 Bioinformatics workflow used for SRA data extraction and analysis.



Fig 1.3 Distribution of bacterial communities in postmortem human brain samples.

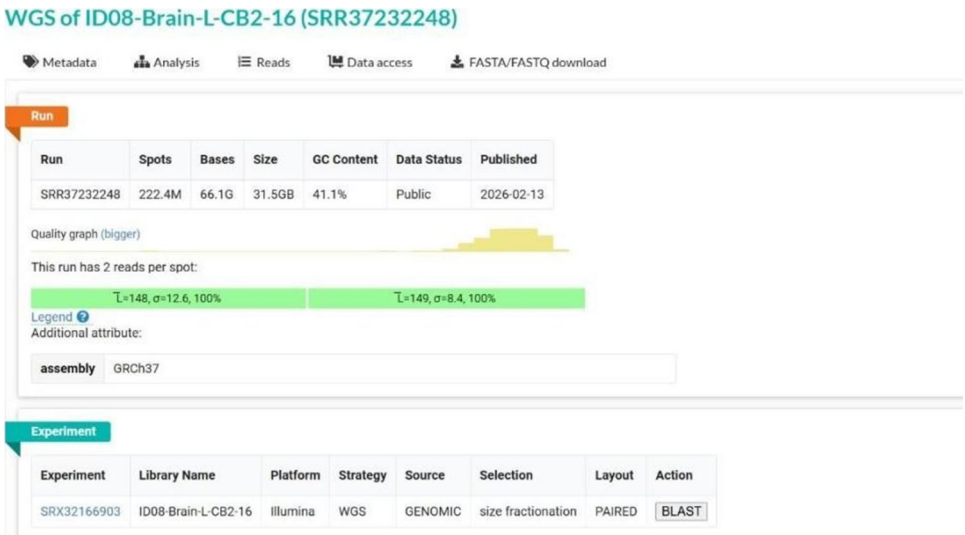


Fig 1.4 Visualization of microbial diversity associated with cadaver decomposition.

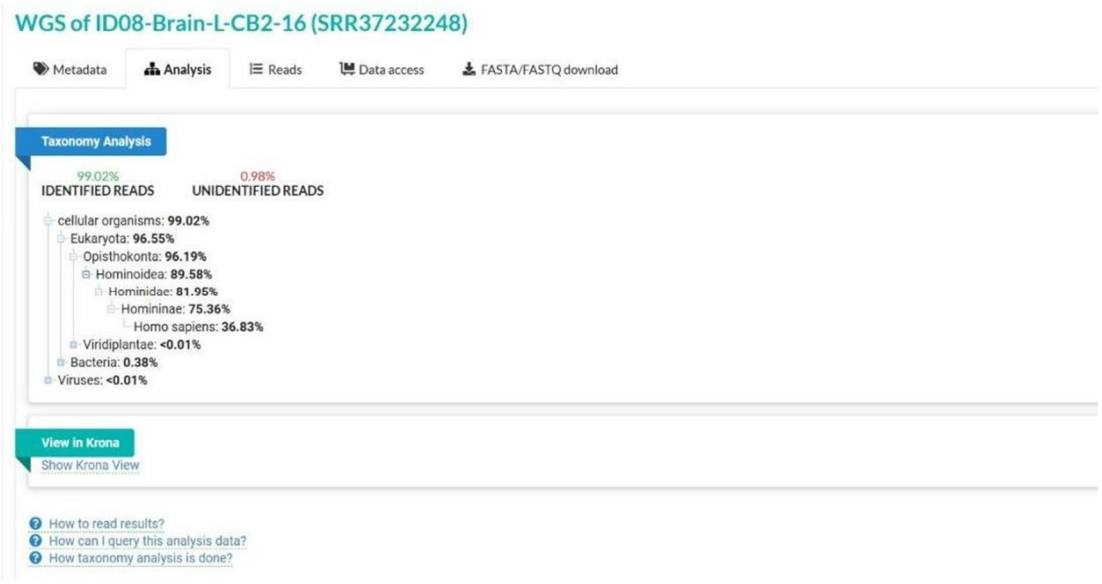


Fig 1.5 Comparative evolutionary similarity of brain tissues with primate groups.

V. RESULTS AND DISCUSSION

A. Objective 1

Results:

1) Brain Tissue and Development

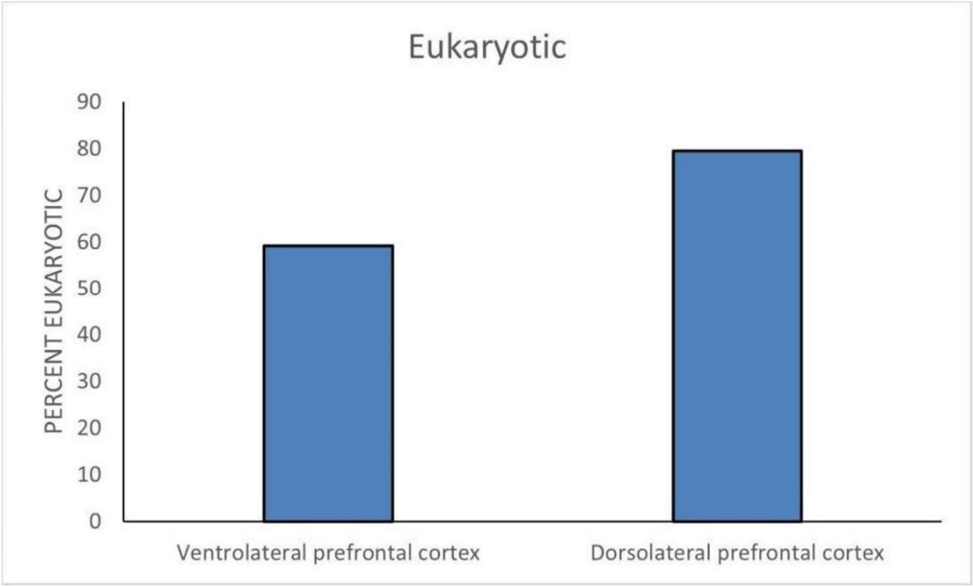
Dorso lateral pre frontal cortex and ventro lateral prefrontal cortex

Hypothesis 1

Null hypothesis: Dorso lateral pre frontal cortex and ventro lateral prefrontal cortex are developed equally and has no significance in the brain development.

Alternate hypothesis: Dorso lateral pre frontal cortex is more developed than the ventro lateral prefrontal cortex and has significance in the brain development.

From the data analysed we found about 79.55% was eukaryotic in dorsolateral prefrontal cortex and on the other hand, 59.23% was eukaryotic in ventro lateral prefrontal cortex.



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Figure 2: Histogram showing the percent eukaryotic in dorsolateral prefrontal cortex and ventro lateral prefrontal cortex tissues of post mortem human brain.

The dorsolateral prefrontal cortex (dlPFC) is generally considered to be a more recently evolved structure in human evolution compared to other parts of the prefrontal cortex, including the ventral and orbital areas. [Plakke, B., 2014].

DlPFC is more highly developed, recently derived, or structurally complex is supported by evolutionary, developmental, and cognitive research.

The dlPFC is considered one of the most recently derived parts of the human brain being why the tissue is more eukaryotic in nature. It is essential for complex cognitive functions such as working memory, planning, cognitive flexibility, and executive attention.

Moreover, the dlPFC is distinguished by having a highly developed granular layer IV, a feature that is more pronounced in primates and humans than in other species.

2) Left thalamus and right thalamus

Hypothesis 2:

Null hypothesis: Left thalamus and right thalamus are developed equally and has no significance in the brain development.

Alternate hypothesis: Left thalamus is more developed than the right thalamus and has significance in the brain development.

From the data analysed we found about 99.27% was eukaryotic in left thalamus and on the other hand, 95.32% was eukaryotic in right thalamus.

In most cases, there was left greater than right thalamic activation and this was associated with widespread activation of areas of the inferior frontal and superior temporal cortex. Several of these studies showed greater thalamic activation to word generation than with repetition (Müller et al., 1998), which would be consistent with the clinical literature that shows preserved repetition and impaired naming in thalamic aphasic patients (De Witte et al., 2011).

A highly developed left thalamus, especially when compared to the right, often correlates with specialized, enhanced functionality in language processing, verbal memory, and analytical thought. The thalamus functions as the brain's central "relay station," filtering sensory and motor information before it reaches the cerebral cortex. Because the left hemisphere of the brain is dominant for language in most people, the left thalamus plays a more active role in managing language-related inputs than the right. [Llano D. A. 2013].

a) From the cadaver post mortem report, though brain tissues are the first to be decomposed, brain tissues especially hypothalamus regions states the victims activeness of the brain. Whether the victim is left brainy or right brainy.

b) Right brainy has left thalamus highly developed while, left brainy has right thalamus more developed.

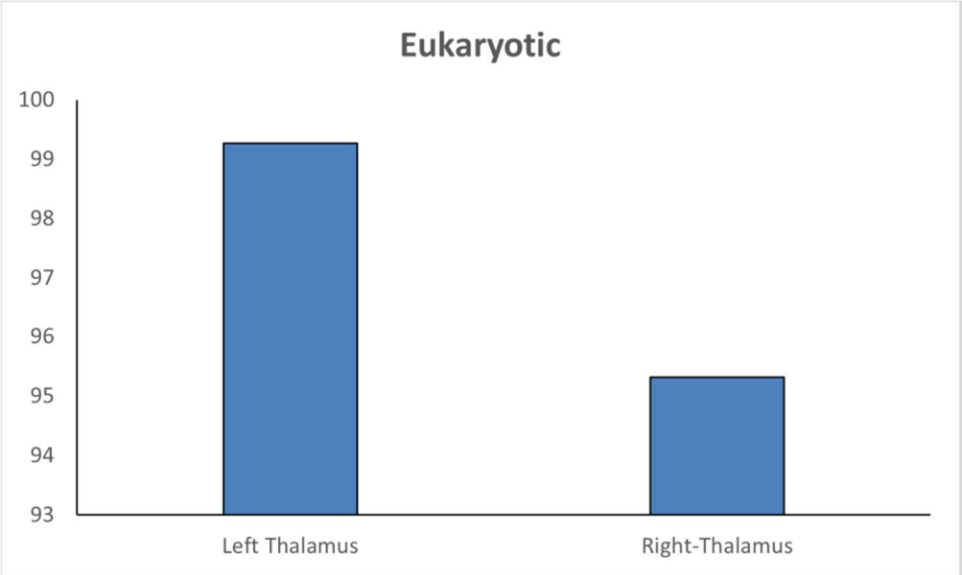


Figure 3: Histogram showing the percent eukaryotic in thalamus regions of post mortem human brain.

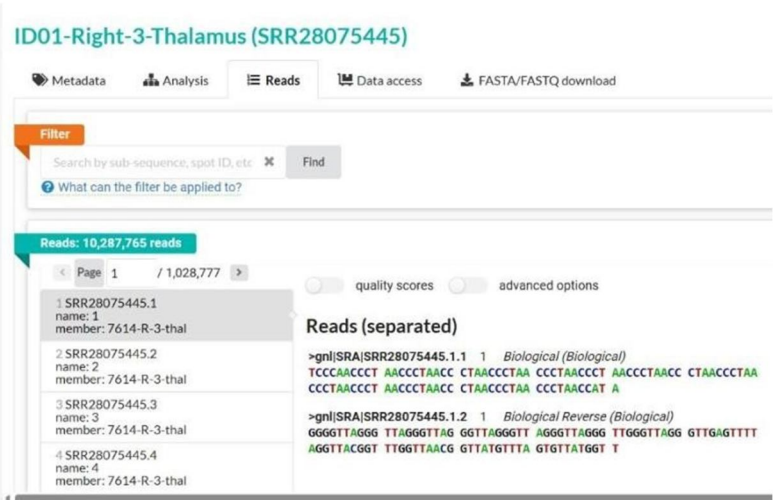


Fig 3.1 Thalamic tissue comparison in postmortem brains.

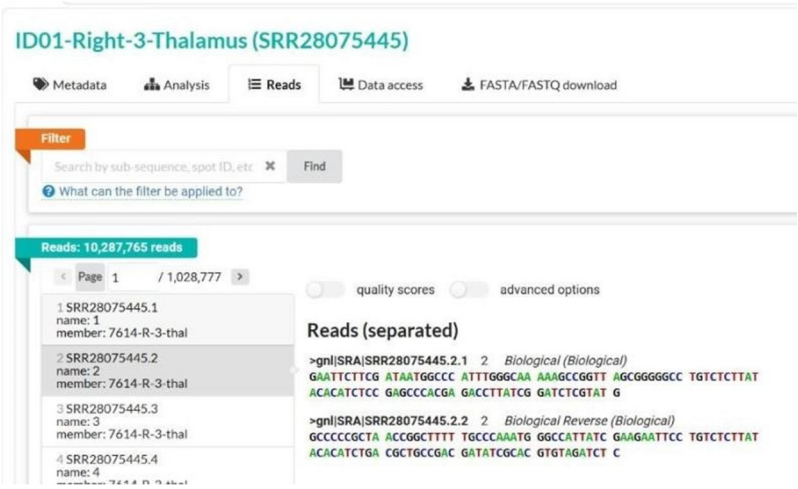


Fig 3.2 Visualization of developmental variation between left and right thalamus

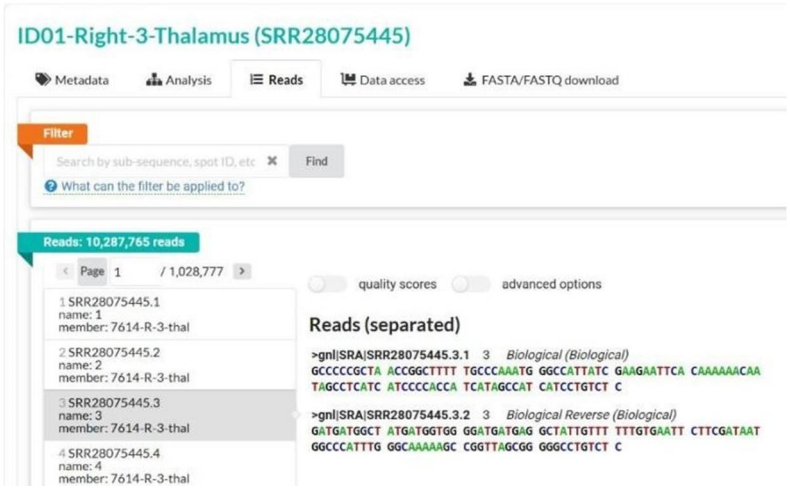


Fig 3.3 Comparative analysis of eukaryotic prevalence in thalamic regions.

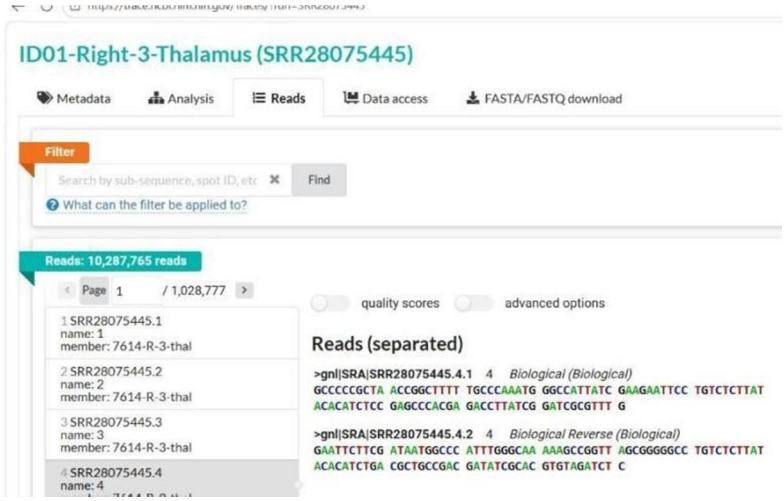


Fig 3.4 Comparative analysis of eukaryotic prevalence in thalamic regions.

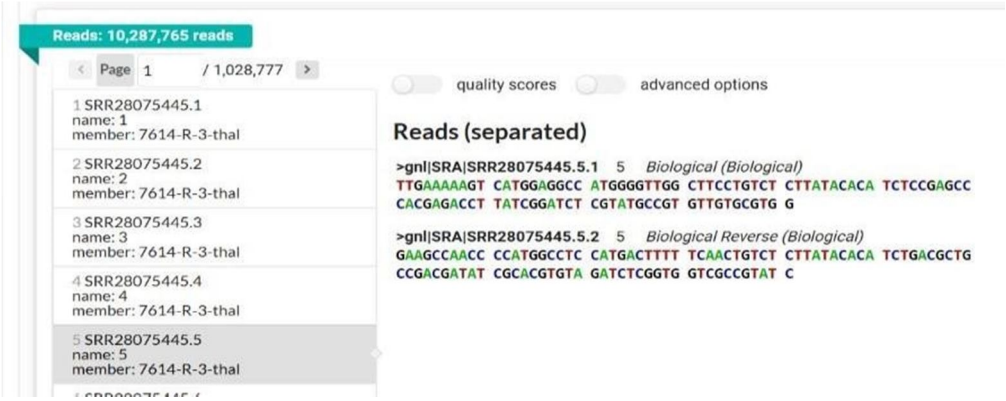


Fig 3.5 Illustration of thalamic asymmetry in brain tissue development.

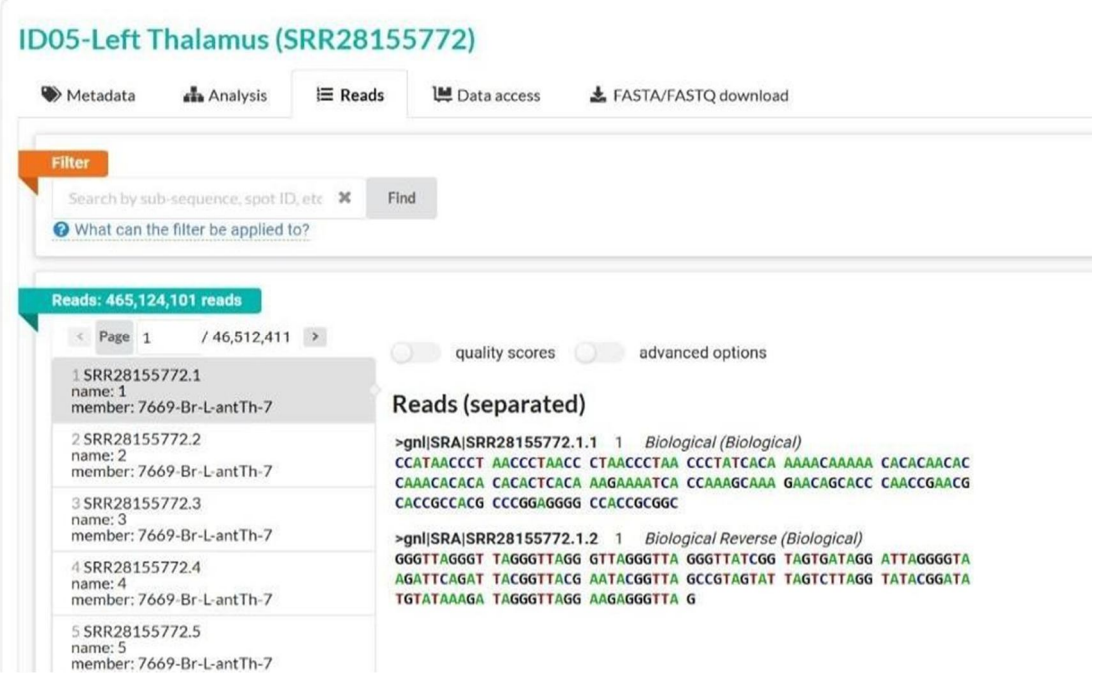


Fig 3.6 Analysis of thalamic tissue evolution and functional specialization.

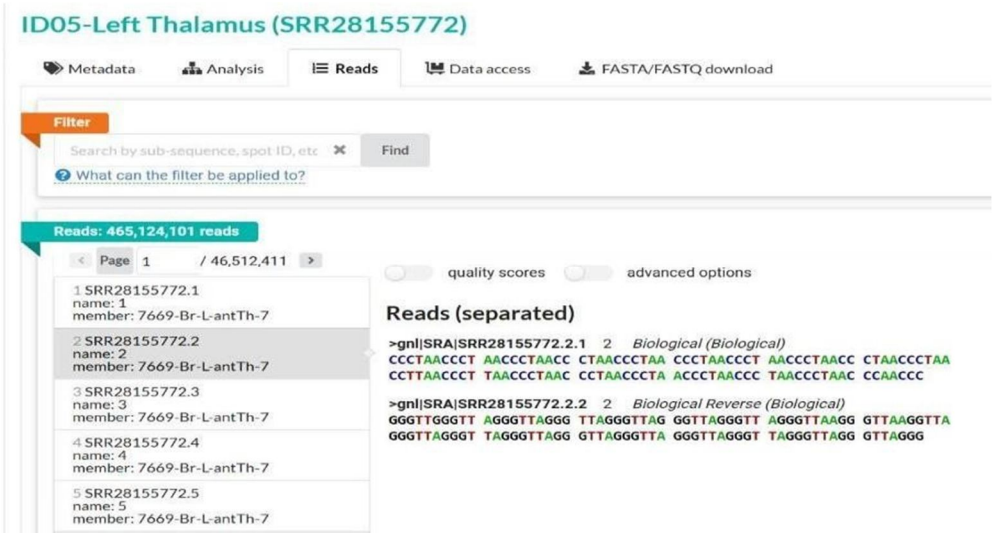


Fig 3.7 Representation of left-right thalamic activity and brain lateralization.

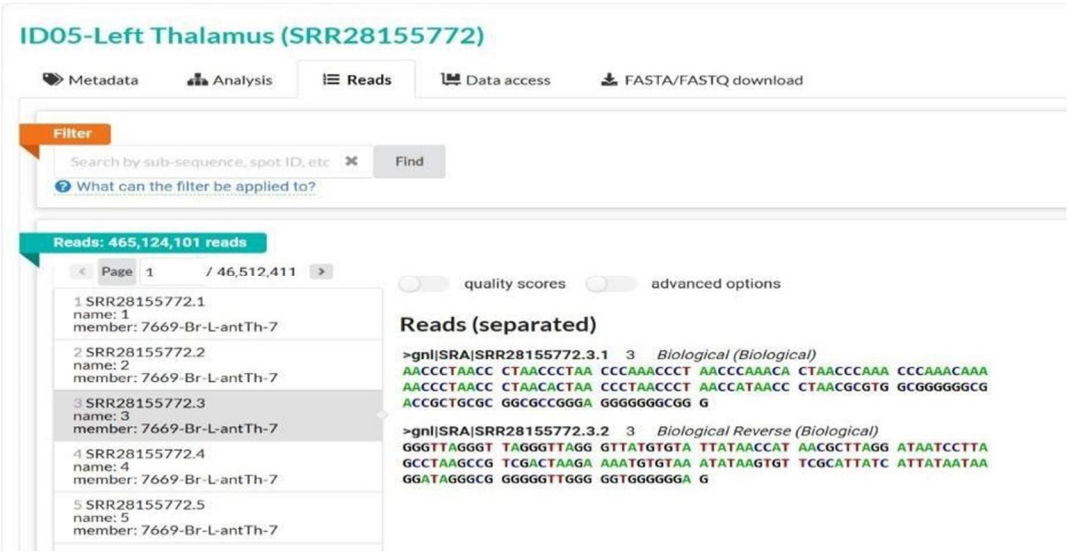


Fig 3.8 Comparative histogram of postmortem thalamus tissue characteristics.

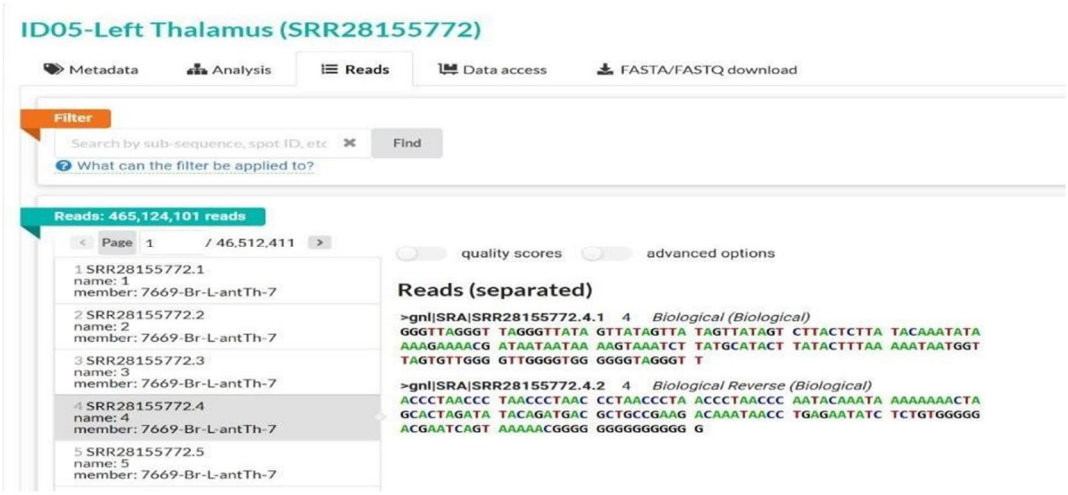


Fig 3.9 Graphical summary of thalamic tissue findings from SRA analysis.

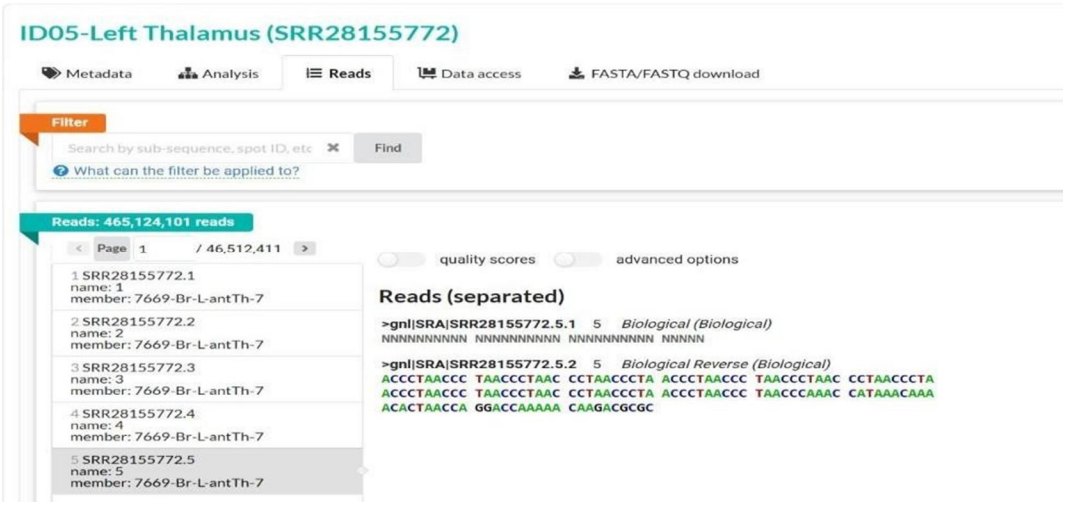


Fig 3.10 Visualization of brain lateralization associated with thalamus development.

Left putamen and right putamen

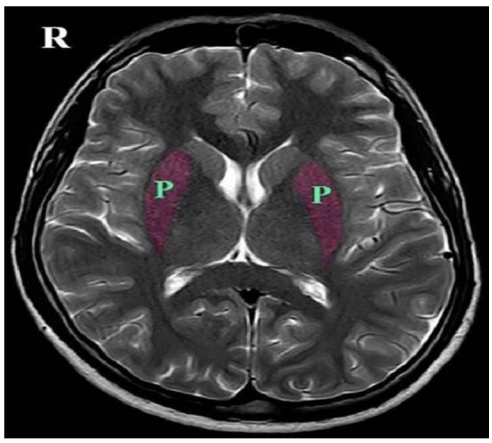


Figure 4: MRI Image of the Bilateral putamen

Hypothesis 3:
Null hypothesis: Left and right putamen are developed equally and has no significance in the brain development.
Alternate hypothesis: Left putamen is more developed than the right putamen and has significance in the brain development.
From the data analysed we found about 99.27% was eukaryotic in left putamen and on the other hand, 95.32% was eukaryotic in right putamen.

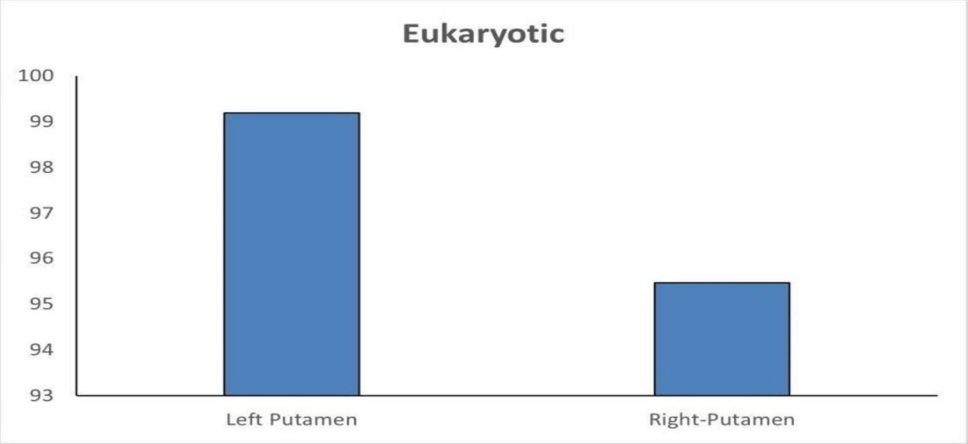


Figure 5: Histogram showing the percent eukaryotic in putamen regions of post mortem human brain.

The left putamen is generally considered more highly developed or larger in right-handed individuals due to its specialized role in language, speech, and complex motor control. The putamen acts as part of the basal ganglia, which is involved in movement regulation, reinforcement learning, and habit formation [Fazl, Arash; 2018]. The left putamen is strongly associated with motor functions related to speech articulation. Research shows that in children and adults, there is often a "leftward lateralization" (larger left side) in the anatomy of the putamen, which can be further enhanced by learning activities like inhibitory control training.

The right putamen is more involved in spatial awareness, non-verbal emotional recognition, and non-literal language processing. The left putamen specifically contributes to articulatory processes when speaking a second language, particularly when the speaker is not highly proficient, indicating its high development in complex, learned language motor tasks [Alexander GE; 1990].

Scientific research states that not all but people with neurobehavioral disorders have a more developed right putamen than a left putamen [Hernán MA, 2002]. In case of Bipolar Disorder (BD), studies have shown that individuals at risk and patients with BD often exhibit larger volumes in the right putamen compared to healthy controls, suggesting this could be a potential marker of vulnerability [Dal Ben M, 2019]. In case of ADHD, some studies suggested that children with ADHD often exhibit a smaller left putamen compared to the right, which is a reversal of the typical asymmetry found in control groups. In case of Parkinson's Disease (PD), this condition typically involves more severe dopamine depletion in one side, leading to an asymmetrical reduction in putamen function (often left putamen in some studies) rather than increased development [Qu, B., 2023].

1) From the cadaver postmortem report, though brain tissues are the first to be decomposed, brain tissues especially Putamen regions states the victims neuro-physical disorders in the brain. Whether the victim is having a bipolar or autism or Parkinson's disorder.

2) Left putamen is highly developed in control groups, and these hyper development in the right putamen may indicate the victim be a person with neuronal disorder (Autism, Bipolar disorder).

3) From our analysis, we found this region of brain (putamen) can be used as a non invasive marker to diagnose children with neuronal problems at a young age.

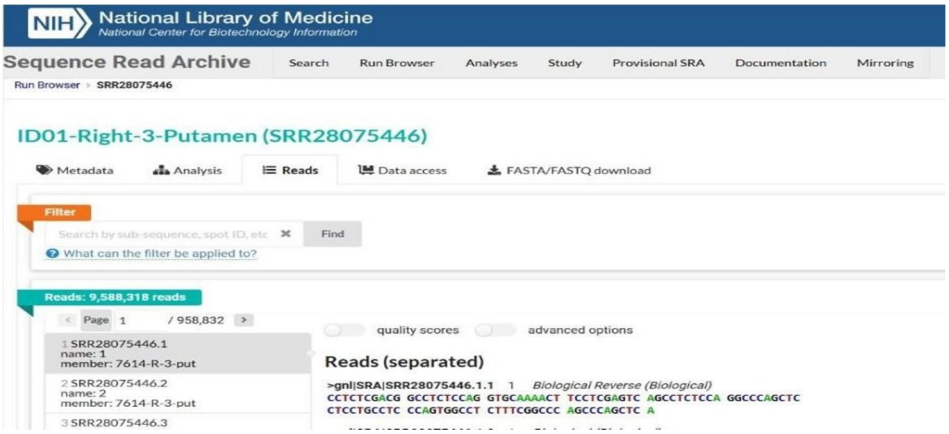


Fig 5.1 Representation of structural variation in putamen regions.

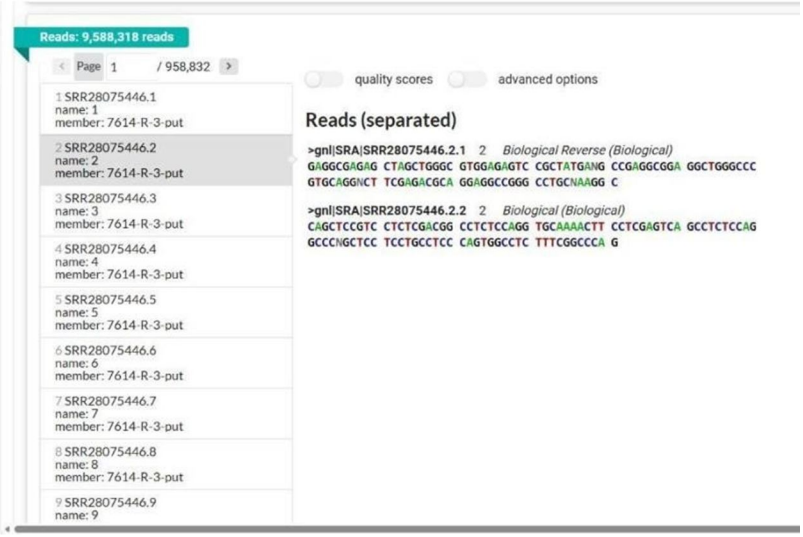


Fig 5.2 Comparative analysis of left and right putamen development.

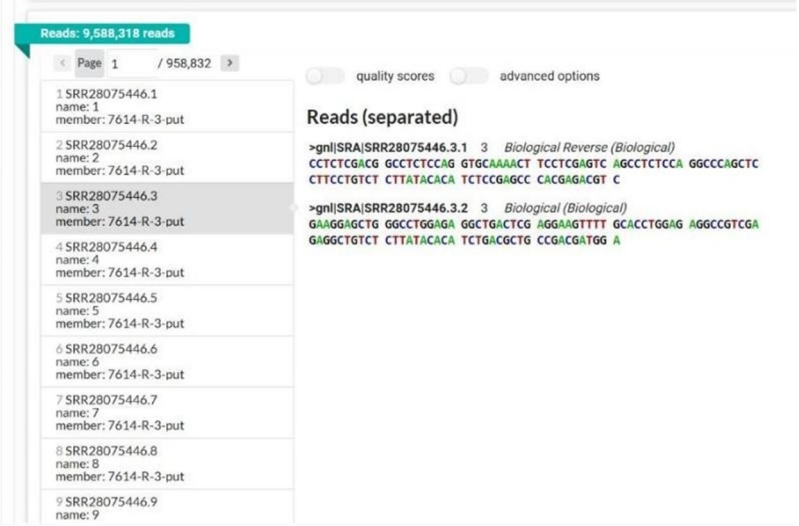


Fig 5.3 Visualization of putamen-related neuronal activity and asymmetry.

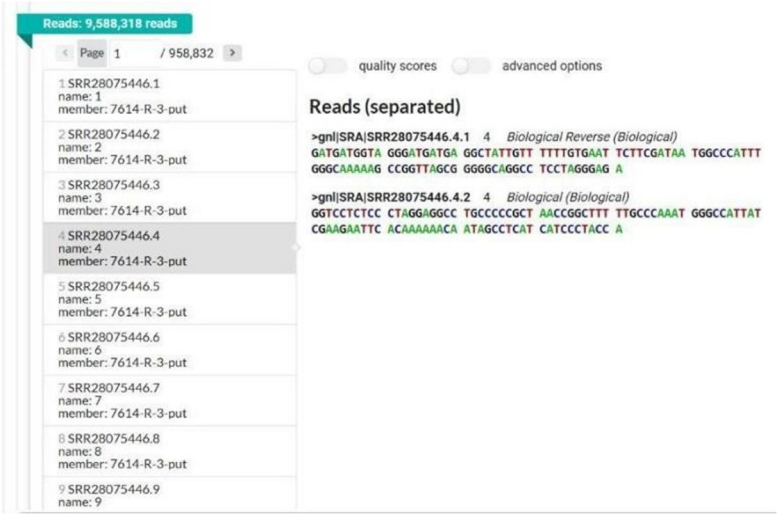


Fig 5.4 Putamen involvement in motor regulation.

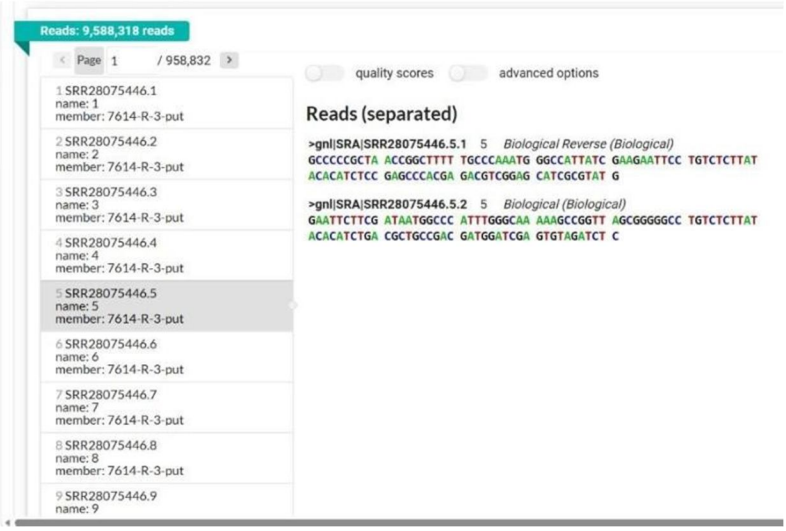


Fig 5.5 Putamen involvement in motor regulation.

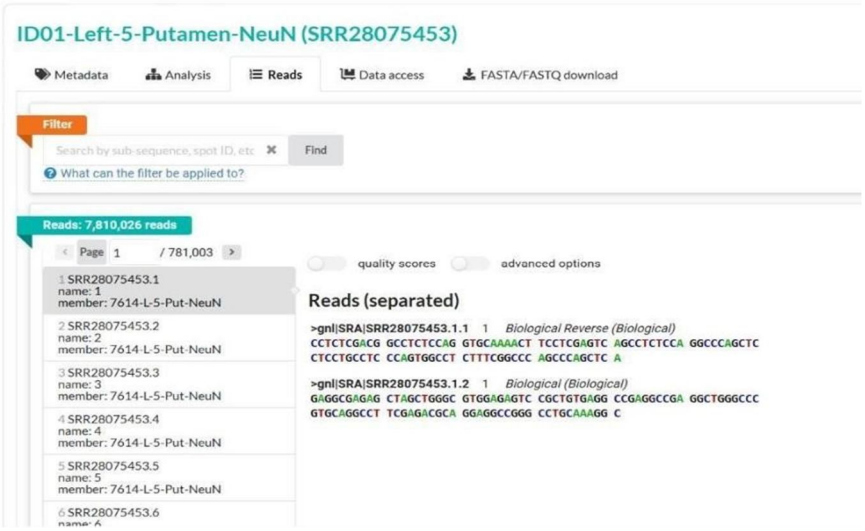


Fig 5.6 Illustration of leftward lateralization in the putamen region.

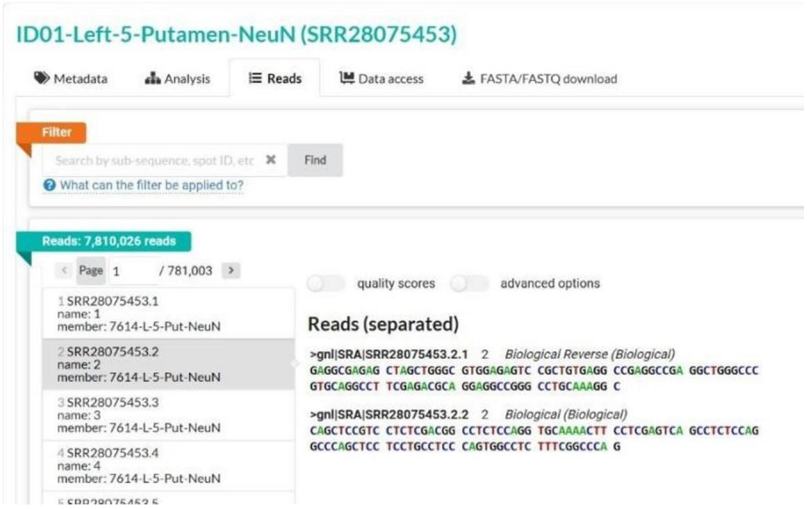


Fig 5.7 Visualization of putamen changes associated with neurobehavioral disorders.

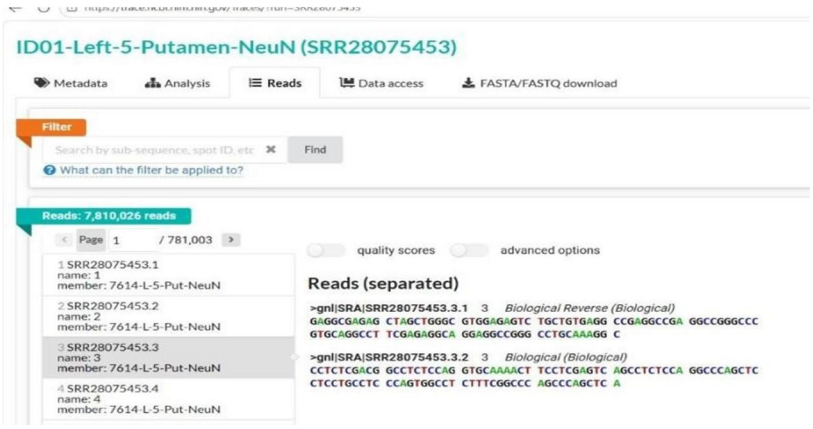


Fig 5.8 Comparative representation of putamen tissue analysis in postmortem brains.



Fig 5.9 Putamen findings from evolutionary and forensic analysis.

3) Caudate Nuclei in Postmortem

Hypothesis 4:

Null hypothesis: Caudate nuclei cannot be used as marker in diagnosing drug or addicted brains.

Alternate hypothesis: Caudate nuclei can be used as marker in diagnosing drug or addicted brains.

The caudate nucleus is a key brain region for understanding, diagnosing, and researching the physical brain changes associated with both neurodegenerative diseases and severe psychiatric conditions during post-mortem examination. Studies suggest changes in caudate volume are associated with cocaine addiction, where it may appear larger, alongside increased neural activity, aiding in the investigation of drug-related deaths.

Post-mortem brain tissue from the caudate is essential for analyzing neurotransmitter changes. High concentrations of dopamine and dopamine receptors are located there, making it crucial for studying schizophrenia and stimulant addiction [Korpi ER, 1987].

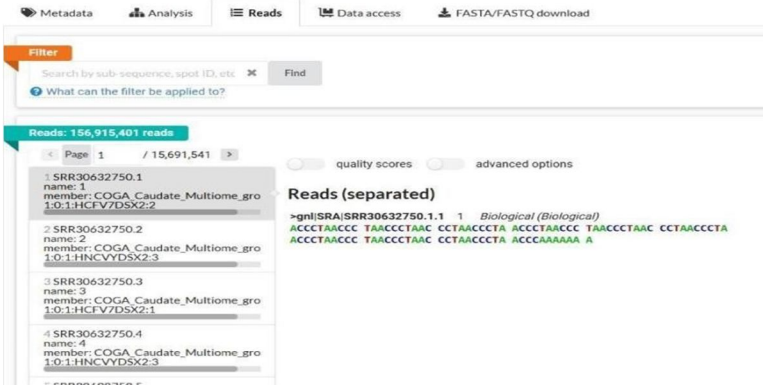


Fig 6.1 Representation of caudate nucleus involvement in addiction-related studies.

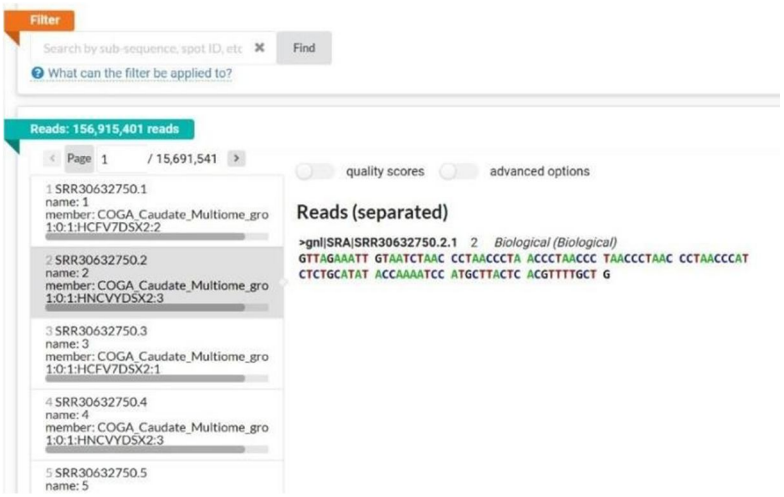


Fig 6.2 Visualization of neurotransmitter changes in caudate nucleus tissues.

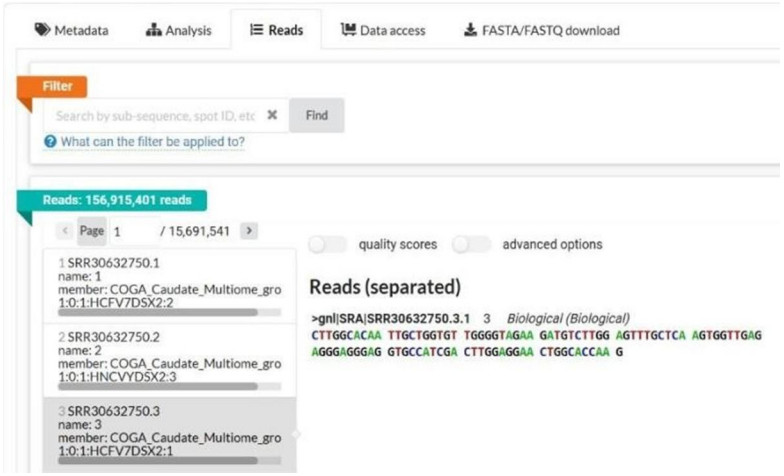


Fig 6.3 Comparative analysis of caudate nucleus structure in postmortem brain.

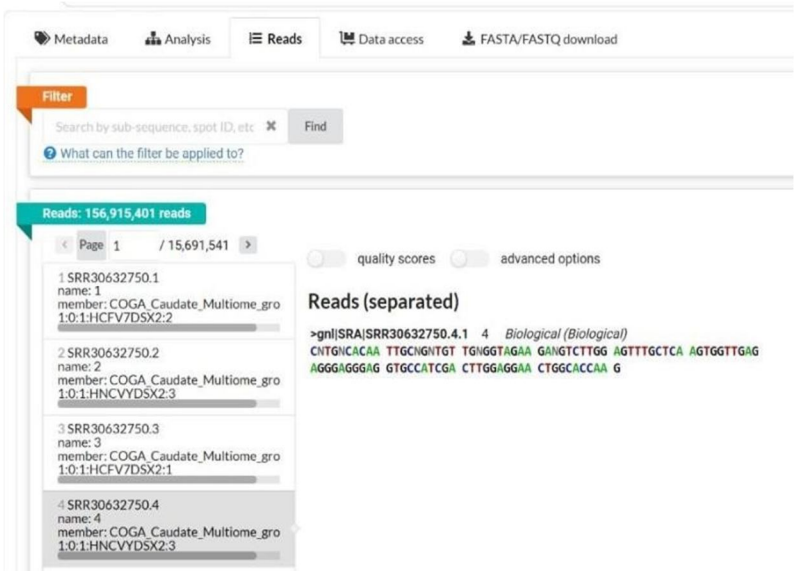


Fig 6.4 Dopamine-related activity in the caudate nucleus.



Fig 6.5 Illustration of caudate nucleus alterations in psychiatric disorders.

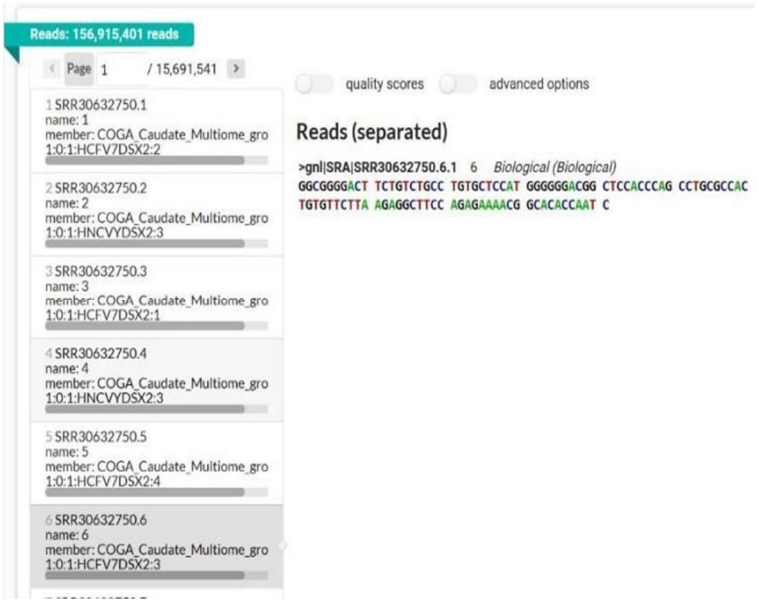


Fig 6.6 Visualization of addiction-related structural changes in the caudate region.



Fig 6.7 Comparative representation of caudate nucleus abnormalities in forensic studies.



Fig 6.8 Analysis of caudate nucleus involvement in neurodegenerative conditions.

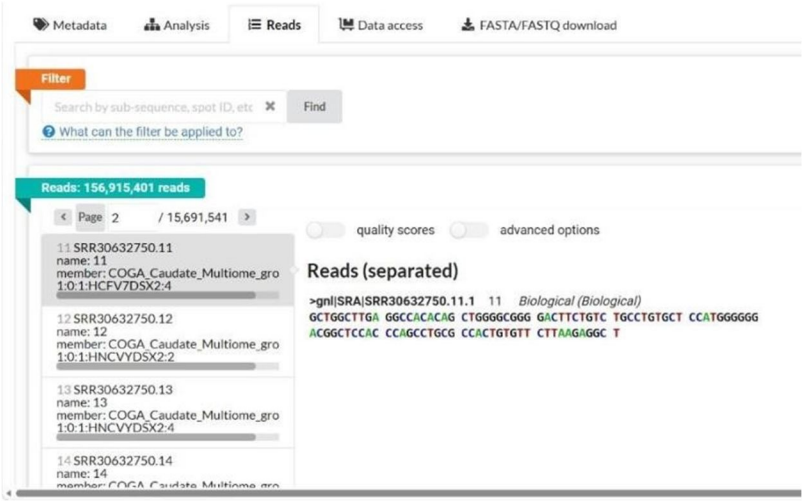


Fig 6.9 Postmortem caudate nucleus observations.

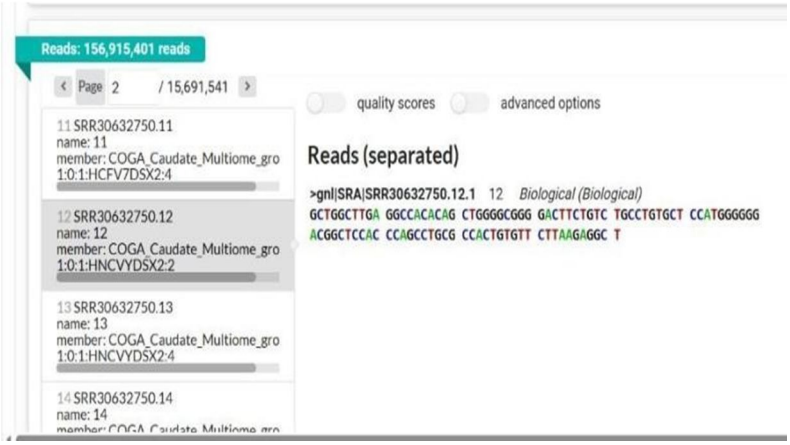


Fig 6.10 Visualization of caudate nucleus significance in forensic neuropathology.

4) Evolutionary Context

The evolution of the human brain is a specialized extension of trends observed within **Simiiformes** (simians) and **Catarrhini** (Old World monkeys and apes), marked by accelerated neuronal proliferation, increased cortical volume, and prolonged developmental timing. While human brains are roughly three times larger than those of African apes, they share a common ancestor and fundamental, albeit accelerated, developmental trajectories. [Zhuang, X. L., 2023].

5) Evolutionary Significance With Catarrhini Group

Based on the SRA read data, we found dorsolateral prefrontal cortex (DLPFC) is more similar to catarrhini than the ventro lateral prefrontal cortex.

DLPFC is 53.56% more similar to Catarrhini group than the VLPFC (24.12%).

Based on research into the molecular and structural evolution of the primate brain, evidence suggests that the right dorsolateral prefrontal cortex (DLPFC) in humans exhibits some characteristics more similar to our catarrhine relatives (such as macaques and apes) compared to the more highly evolved left DLPFC (Shaojie Ma et al., 2022). The left DLPFC is strongly associated with verbal processing and planning, which are highly expanded in humans, whereas the right DLPFC is more involved in spatial and non-verbal tasks. Some studies have shown that in primates, including humans, the left-right asymmetry of the DLPFC is linked to structural and functional variations, with the left often showing greater, or more specialized, development. Therefore, in studies investigating brain lateralization and evolution, the right DLPFC is sometimes described as having functional or structural patterns that resemble the less lateralized, more "traditional" primate model, while the left DLPFC shows a more human- specific, or more highly "lateralized," divergence [White, L. K., 2023].

6) Evolutionary Significance With Simiiformes Group

Based on the SRA read data, we found dorsolateral prefrontal cortex (DLPFC) is more similar to simiffformes than the ventro lateral prefrontal cortex.

VLPFC is 65.79% more similar to Simiiformes group than the DLPFC (32.12%).

Based on comparative studies, the ventrolateral prefrontal cortex (VLPFC)—often considered part of the ventral prefrontal system—generally shows more fundamental, shared, and conserved features with Simiiformes (monkeys and apes) than the human dorsolateral prefrontal cortex (DLPFC) [Preuss, T. M., 2022].

While the DLPFC is highly developed and specialized in humans, both the VLPFC and DLPFC emerged as distinct granular structures in the ancestor of simian primates. However, in terms of evolutionary similarity in organization, connectivity, and function, the ventral areas are considered to have more direct, ancestral counterparts, while the human DLPFC represents a massive expansion and reconfiguration. Both the dorsal and ventral regions are characterized by a "granular isocortex" that is shared among simians (catarrhines/platyrrhines) but absent or much less developed in non-primate mammals and strepsirrhines [Preuss, T. M., 2022].

	Eukaryota	Simiiformes	Catarrhini
Ventrolateral prefrontal cortex	59.23	65.79	24.12
Dorsolateral prefrontal cortex	79.55	32.19	53.56

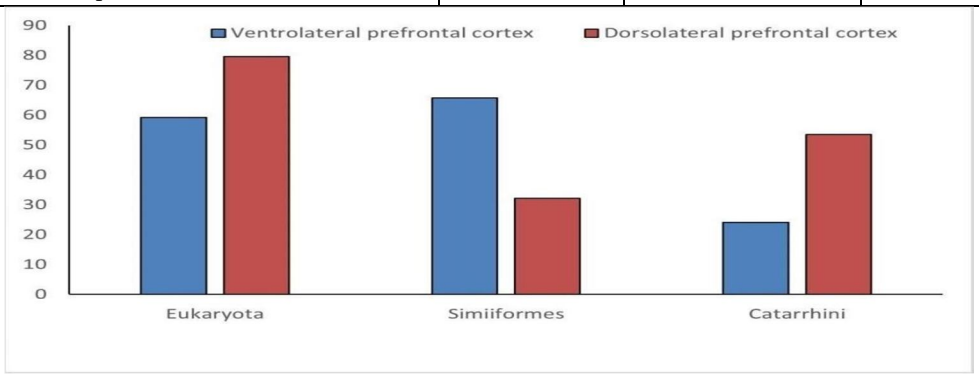


Figure 7: Histogram showing the percent similarity of the brain tissues during the development with the major groups Simiiformes and Catarrhine.

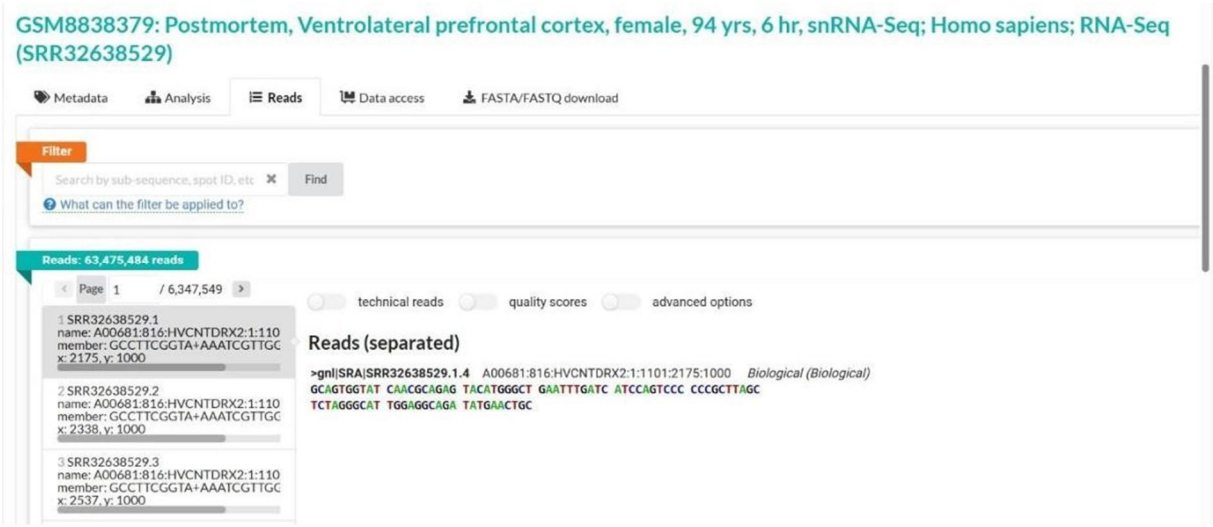


Fig 7.1 Comparative evolutionary analysis of DLPFC and VLPFC tissues.

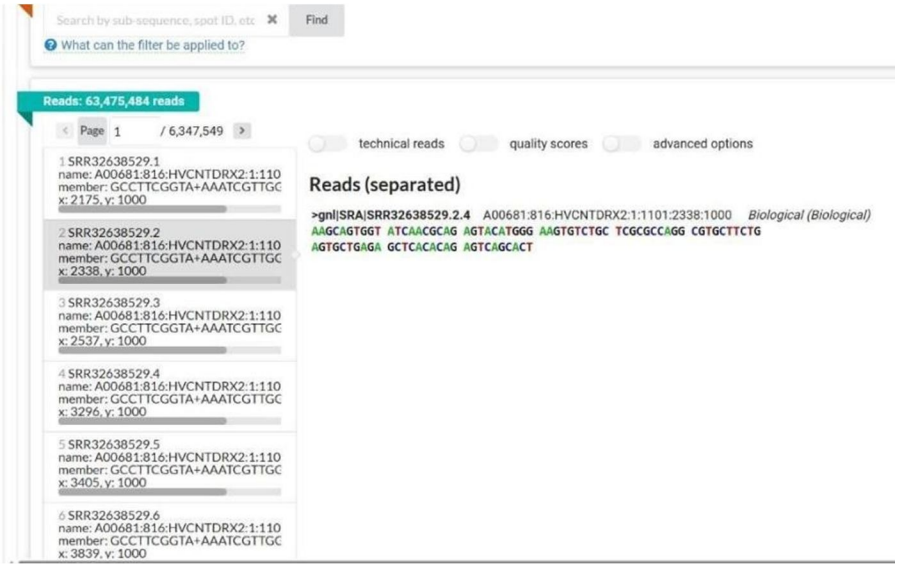


Fig 7.2 Visualization of cortical similarity with Catarrhini lineage.

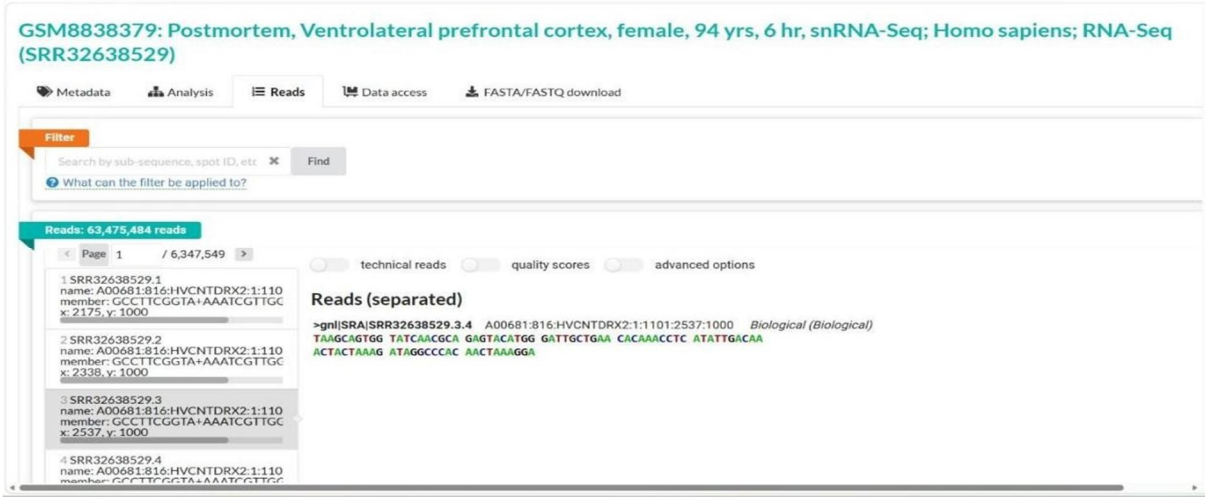


Fig 7.3 Primate evolutionary trends in brain tissues.



Fig 7.4 Comparative study of DLPFC evolution and cortical specialization.

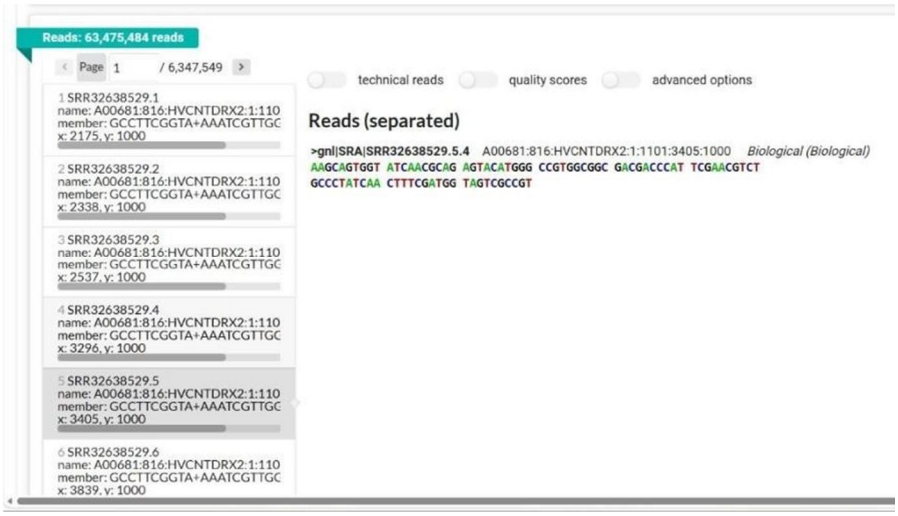


Fig 7.5 Visualization of VLPFC similarity with Simiiformes groups.

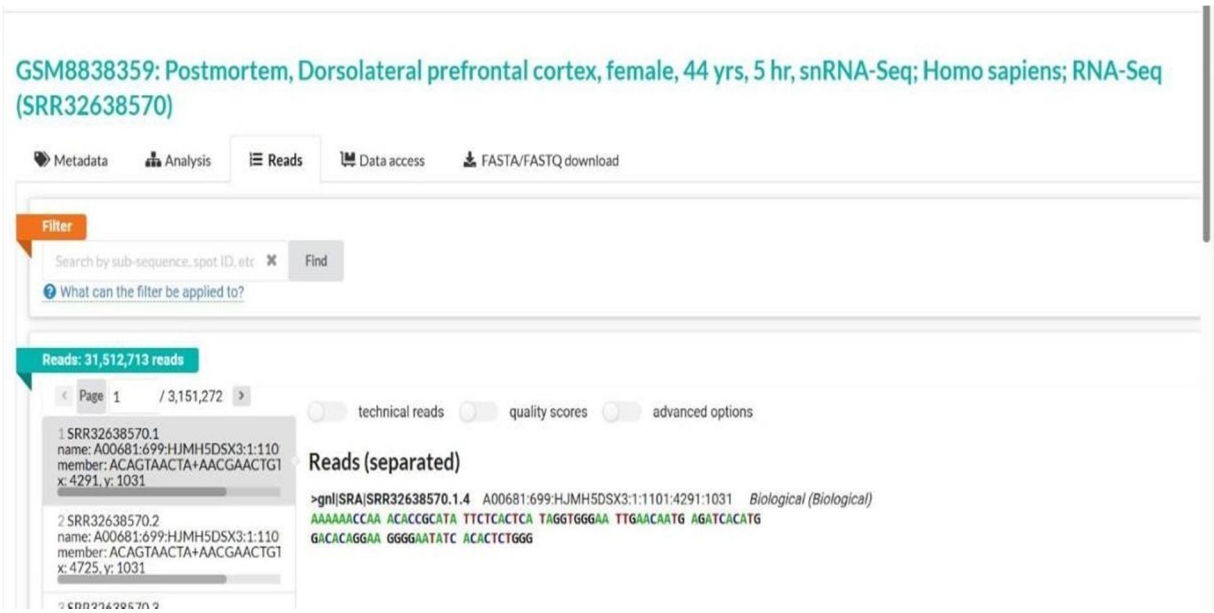


Fig 7.6 Representation of evolutionary divergence between cortical regions.

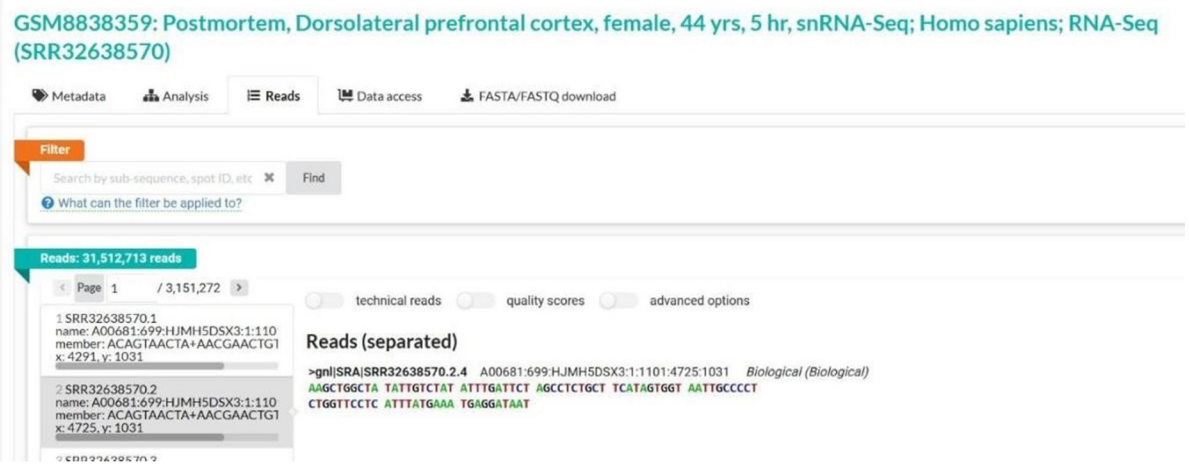


Fig 7.7 Primate-related brain tissue development.

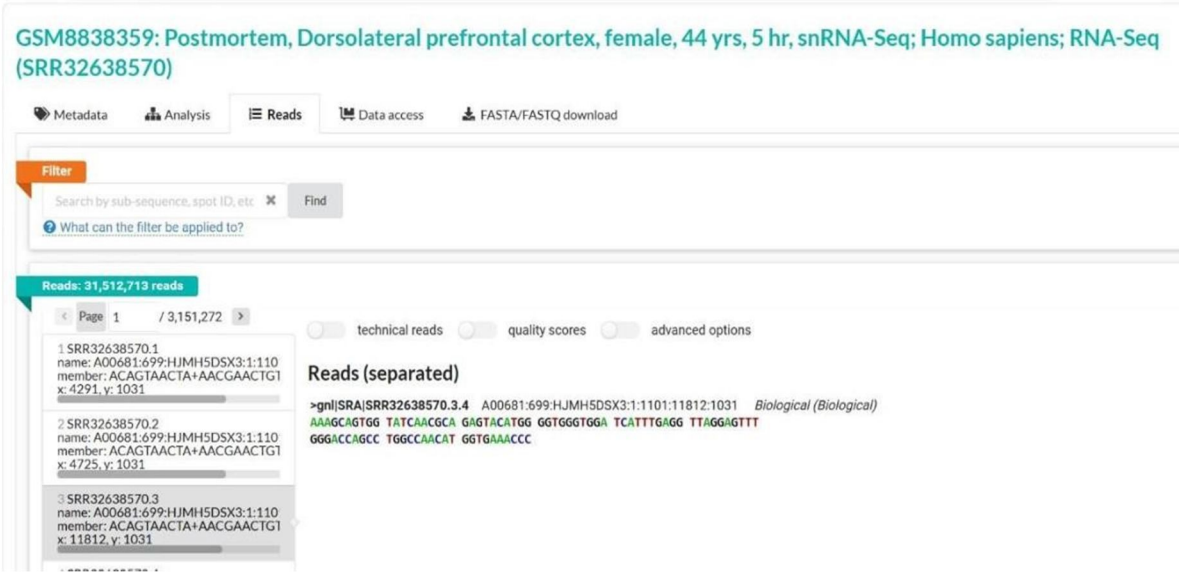


Fig 7.8 Visualization of evolutionary lateralization in prefrontal cortex tissues.

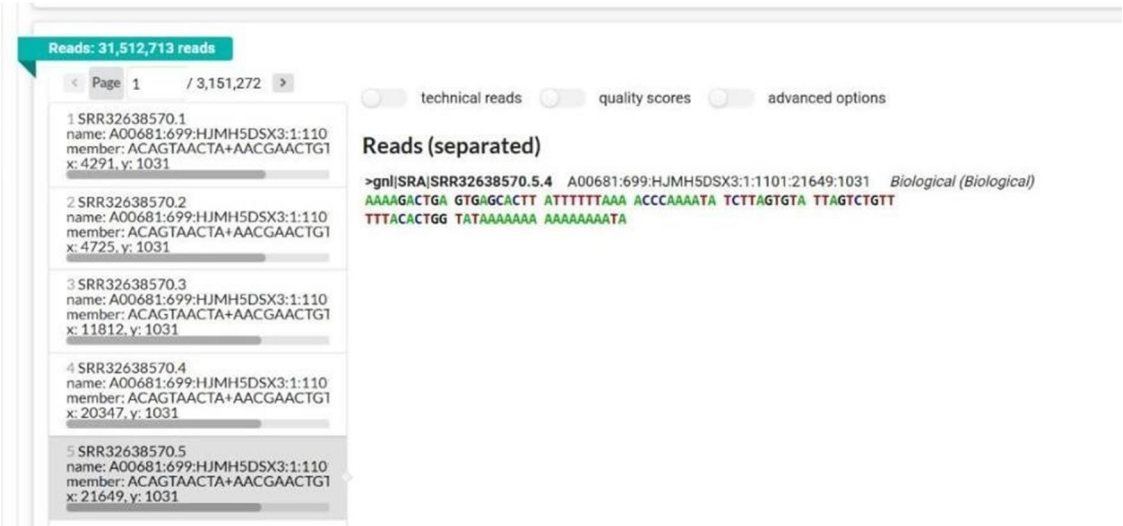


Fig 7.9 Evolutionary similarity findings from SRA data.

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7) Bacterial Community Growth And Age Of Cadaver

Bacterial Markers for postmortem interval (PMI) estimation.

Bacterial markers for cadaver decomposition, often referred to as the thanatomicrobiome (internal organs) and epinecrotic community (body surface), provide a "microbial clock" that acts as a valuable tool for estimating the postmortem interval (PMI). As a body decomposes, the microbial community shifts from aerobic bacteria to anaerobic bacteria, with specific phyla and genera dominating different stages.

From our study, we found the bacterial communities to be in mixed proportions and not in definite proportions. The community numbers depicted in % are given below in the tables.

SRX29888414	Gut microbiome: post mortem: female	Bacteria: 93.20%
		Proteobacteria: 24.39%
		Gammaproteobacteria: 8.07%
		Enterobacterales: 0.15%
		Acidiferrobacterales: <0.01%
		Alphaproteobacteria: 0.04%
		Betaproteobacteria: 0.01%
		delta/epsilon subdivisions: <0.01%
		Terrabacteria group: 15.70%
		Firmicutes: 15.67%
		Actinobacteria: 0.03%
		FCB group: 0.07%

>gnl|SRA|SRR34861541.2.4A00257:587:H73FFDRXY:1:2101:3839:1031 *Biological (Biological)*
ACTCCAGTTGACACAAAATAGACTACGAAAGTGGCTTTAACATATCTGAACACACAATAGCTAAG
ACCCAAACTGGGATTAGATACCCCA

>gnl|SRA|SRR34861541.5.4A00257:587:H73FFDRXY:1:2101:4562:1031 *Biological (Biological)*
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GACTGGTGACACGCTTCTGAGCATG

>gnl|SRA|SRR34861541.8.4A00257:587:H73FFDRXY:1:2101:5050:1031 *Biological (Biological)*
AAGCAGTGGTATCAACGCAGAGTACATGGGTAACTGAGAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAA
>gnl|SRA|SRR34861541.10.4A00257:587:H73FFDRXY:1:2101:5195:1031 *Biological (Biological)*
GTGGTATCAACGCAGAGTACATGGGGCTTTTAAGCTGCAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAA

SRX29888414	Gut microbiome: post mortem: female	Bacteria: **93.20%**
		Proteobacteria: **24.39%**
		Gammaproteobacteria: **8.07%**
		Enterobacterales: **0.15%**
		Acidiferrobacterales: **<0.01%**
		Alphaproteobacteria: **0.04%**
		Betaproteobacteria: **0.01%**
		delta/epsilon subdivisions: **<0.01%**
		Terrabacteria group: **15.70%**
		Firmicutes: **15.67%**
		Actinobacteria: **0.03%**
		FCB group: **0.07%**

SRR38153523	Ventrolateral human brain male	Bacteria: **3.05%**
		Pseudomonadati: **0.66%**
		Pseudomonadota: **0.29%**
		Gammaproteobacteria: **0.18%**
		Enterobacterales: **0.06%**
		Enterobacteriaceae: **0.05%**
		Escherichia: **0.02%**
		Escherichia coli: **0.02%**
		Klebsiella/Raoultella group: **0.02%**
		Klebsiella: **0.02%**
		Klebsiella pneumoniae: **0.02%**

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SRR34682058	Secondary visual cortex	Bacteria: **0.50%**
		Pseudomonadota: **0.04%**
		Gammaproteobacteria: **0.04%**
		Enterobacterales: **0.01%**
		Enterobacteriaceae: **0.01%**
		Escherichia: **0.01%**
		Escherichia coli: **0.01%**
		Klebsiella/Raoultella group: **<0.01%**
		Klebsiella: **<0.01%**
SRR25368388	Heart; Homo sapiens	Bacteria: **11.20%**
		Terrabacteria group: **0.09%**
		Actinomycetota: **0.09%**
		Actinomycetes: **0.09%**
		Streptosporangiales: **0.08%**
		Actinomadura cremea: **0.08%**

SRR27345611	Human liver tissue	Bacteria: **3.49%**
		Terrabacteria group: **0.03%**
		Bacillota: **0.01%**
		Bacilli: **0.01%**
		Bacillales: **0.01%**

SRR34861541	Muscle tissue Homo sapiens	Bacteria: **10.16%**
		Firmicutes: 25.87
		Actinomycetota: 13.45
		Actinomycetes: 10.23
		Mycobacteriales: **0.02%**
		Mycobacteriaceae: **0.02%**
		Mycolicibacterium mageritense: **0.02%**
		Nocardiaceae: **<0.01%**
		Nocardia: **<0.01%**

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SRR29633722	DNA extracted from adult brain tissue	Bacteria: **73.44%**
		Pseudomonadota: **1.27%**
		Terrabacteria group: **0.87%**
		FCB group: **0.03%**
		Campylobacterota: **0.03%**

Basing on the prevalence and predominance of the bacterial communities thriving within the cadaver, we could analyse the age of the cadaver or post mortem interval.

1) Analysis 1

Proteobacteria, particularly within the class Gammaproteobacteria, play a dominant role in post-mortem decomposition, often becoming the most prevalent phylum as decomposition
progresses. They are essential in early to mid-stage decay, especially after the rupture of the body, as they thrive in the fluctuating aerobic and anaerobic conditions
While Firmicutes may dominate earlier stages, Proteobacteria increase significantly during the bloat and early decay stages.
Specific Gammaproteobacteria are used as biomarkers to estimate the post-mortem interval (PMI) because their increasing abundance strongly correlates with the time since death. [Dong, K., 2019].
Based on research regarding microbial community succession during decomposition, a 15.8% relative abundance of Firmicutes at the phylum level, often observed alongside shifts in Bacteroidetes and Proteobacteria, typically indicates the Bloat Stage or early stages of Active Decay. [Metcalf, J. L., 2013].

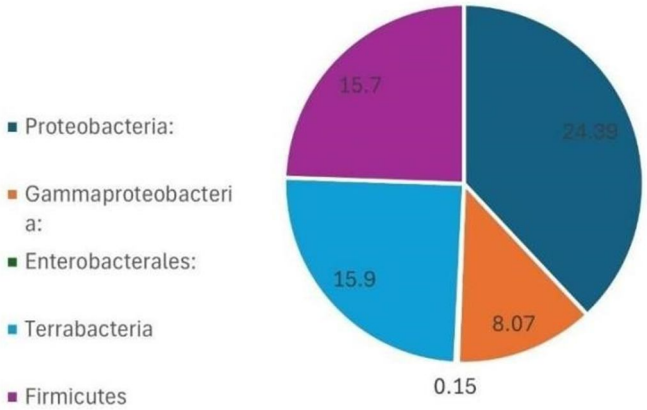


Figure 8: Gut microbiome: post mortem: Female

2) Analysis 2:

Based on the provided percentages (Pseudomonadota 0.7% and Firmicutes 0.2%), this microbial profile most closely suggests the Fresh Stage of post-mortem decomposition, or potentially a very early, cool-environment state. [Huang, X., 2026]
As decomposition progresses, Pseudomonadota typically increase and become highly dominant (often 25–88%) during active putrefaction. The very low numbers provided indicate that the massive, accelerating anaerobic bacterial growth characteristic of bloat and active decay has not yet started.
Therefore, this profile reflects a very early time point, likely shortly after death.

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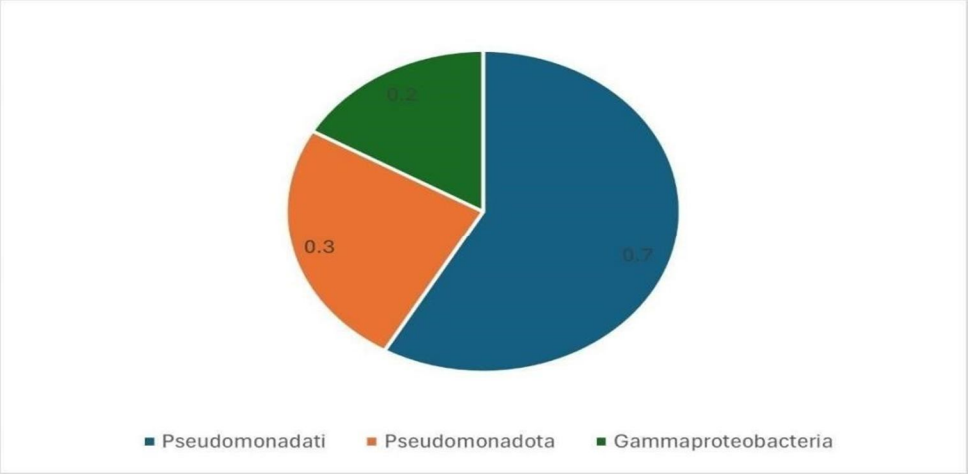


Figure 9: Ventrolateral human brain male

3) Analysis 3:

A post-mortem decomposition sample showing very low levels (e.g., 0.7%) of *Actinomycetota* (formerly Actinobacteria) along with *Terrabacteria* likely corresponds to the Early Stage or the transition from the Fresh to Bloat stage. [Moitas, B., 2024].

- Low percentages indicate the carcass has not yet reached the advanced decomposition or dry stage, where these bacteria thrive.
- The low presence of these microorganisms suggests of transition phase where the body is likely in the early phase of putrefaction (0 to 5 days post-mortem), where soft tissue breakdown is just beginning.

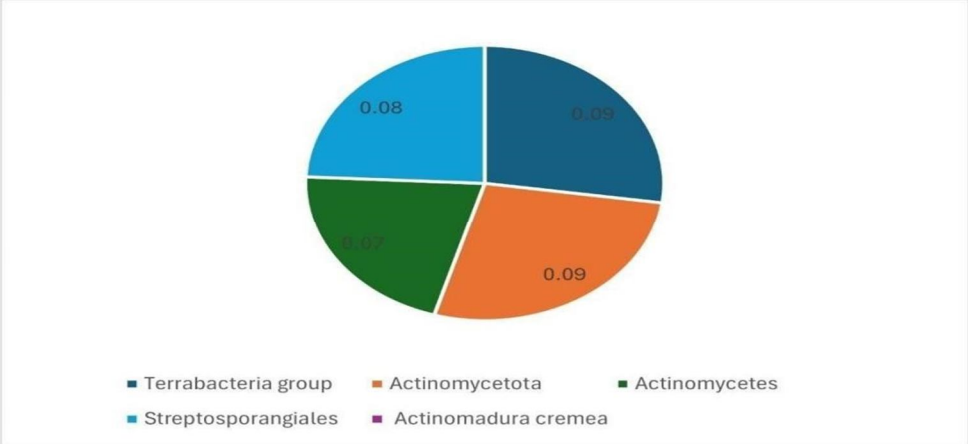


Figure 10: Heart, human Male

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>gnl|SRA|SRR25368388.4.1NS500731:94:HWMFGBGXC:1:11101:4445:1046 *Biological (Biological)*
TTGATNGTGCCATCAAAAGACTCTTTGCCAAAGAAGAGGCCAATTTATGAAGAAAAAAAAAAAAAA
AAAAGGAGCGG

>gnl|SRA|SRR25368388.6.1NS500731:94:HWMFGBGXC:1:11101:7080:1048 *Biological (Biological)*
TTCCANCCACTGCACTCCAGCCTGGCTGACAGAGCAAGACTGCATCTCAAAAAAAAAAAAAAAAAA
AGGGGCGGGGG

4) Analysis 4:

We found 25.47% to be found predominant followed by Actinomycetota which suggests of the advanced decay stage.

During the advanced decay stage post-mortem, the bacterial community shifts significantly from anaerobic bacteria that dominate during the bloat stage to a diverse community dominated by Bacillota (formerly Firmicutes) and Actinomycetota (formerly Actinobacteria). As soft tissue is removed and the body dries out, these bacteria, along with fungi, dominate the remaining tissues and soil interface. [Moitas, B., 2024].

The dorsolateral prefrontal cortex (DLPFC) contained a higher proportion of eukaryotic reads (79.55%) compared to the ventrolateral prefrontal cortex (VLPFC) (59.23%). The left thalamus showed a greater eukaryotic proportion (99.27%) than the right thalamus (95.32%). Overall, the data indicate regional variation in eukaryotic representation, with the thalamus showing consistently higher levels than the prefrontal cortex. Analysis of SRA read data revealed distinct differences in similarity between brain regions and primate groups. The dorsolateral prefrontal cortex (DLPFC) showed higher similarity (53.56%) compared to the ventrolateral prefrontal cortex (VLPFC) (24.12%). The ventrolateral prefrontal cortex (VLPFC) exhibited greater similarity (65.79%) than the DLPFC (32.12%). These results suggest that the evolutionary relationship of bacterial community patterns varies depending on the cortical region examined, with DLPFC aligning more closely with Catarrhini, while VLPFC shows stronger similarity to Simiiformes. The Early Stage or the change from the Fresh to Bloat stage is most likely represented by a post-mortem decomposition sample with extremely low levels (e.g., 0.7%) of Actinomycetota (previously Actinobacteria) and Terrabacteria. Actinomycetota was discovered to be prevalent at 25.47%, indicating an advanced degradation stage.

VI. CONCLUSION

These microbial signatures, when mapped against decomposition timelines, enable forensic investigators to refine post-mortem interval estimation with greater accuracy than traditional morphological methods. The findings underscore the potential of microbial ecology as a forensic tool, highlighting how bacterial growth trajectories serve as biological clocks that define the age of a body after death. Although this research indicates that bacterial communities can be used as a “microbial clock” for the estimation of post-mortem interval, further work is required to better understand this concept. Bacteria act like tiny timekeepers after death. As a body decomposes, different groups of bacteria grow and take over in a predictable order. Early bacteria appear first, followed by others that thrive as the environment changes. Because these changes happen in a regular sequence, scientists can use the growth of bacterial communities as a kind of “biological clock” to estimate how long a body has been decomposing. This makes bacteria an important tool in forensic science, helping investigators determine the post-mortem timeline more accurately.

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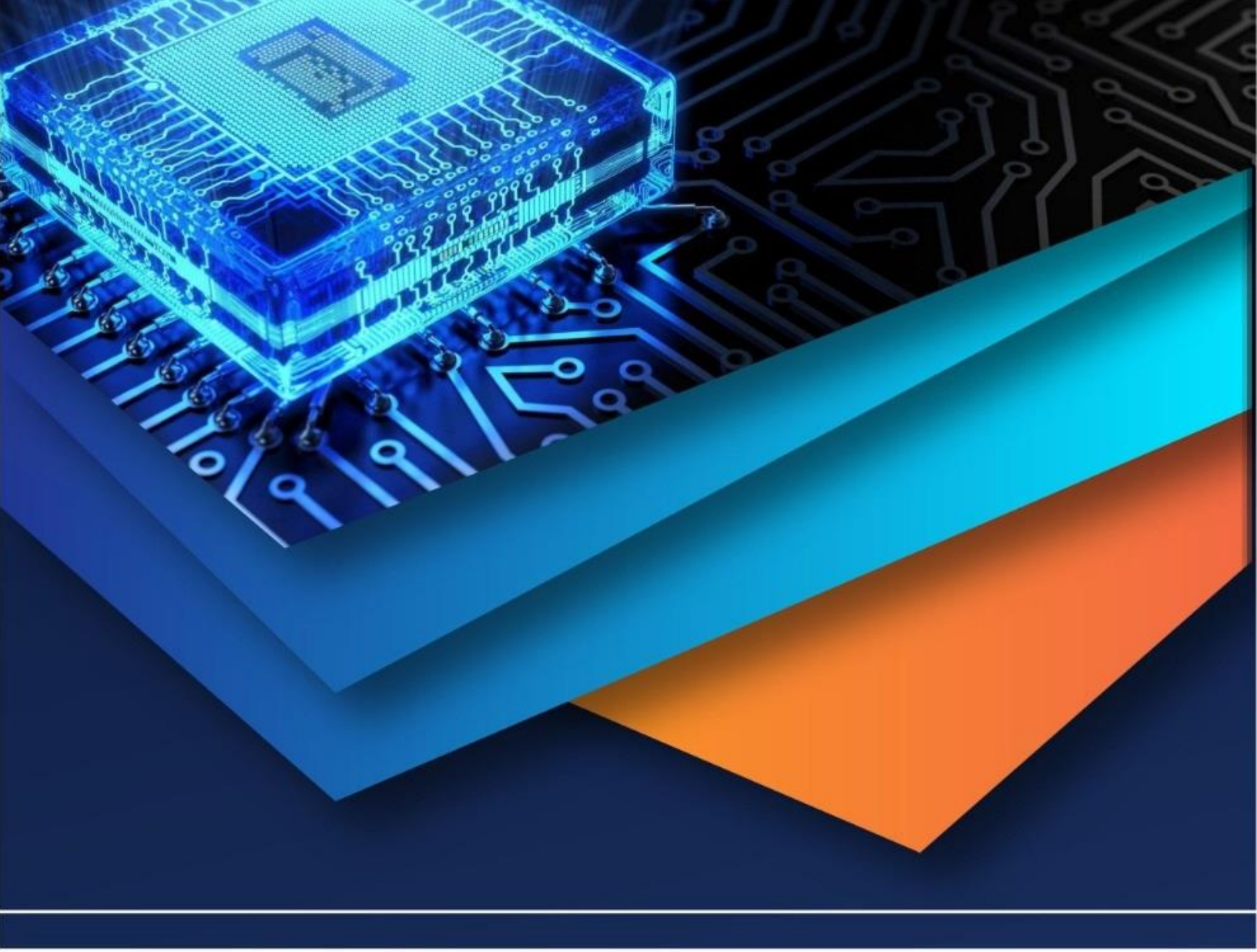
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