SIGNIFICANCE OF SYNAPTIC PRUNING IN BRAIN HEALTH: A COMPUTATIONAL ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN ASD

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by

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I, **Anjali Sharma** (2K22/MSCBIO/66), hereby certify that the work which is being presented in the thesis entitled- **" SIGNIFICANCE OF SYNAPTIC PRUNING IN BRAIN HEALTH: A COMPUTATIONAL ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN ASD "** in partial fulfillment of the requirements for the award of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from May 2023 to May 2024 under the supervision of Prof. Yasha Hasija.

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Certified that ANJALI SHARMA (2K22/MSCBIO/66) has carried out their search work presented in this thesis entitled " **SIGNIFICANCE OF SYNAPTIC PRUNING IN BRAIN HEALTH: A COMPUTATIONAL ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN ASD** " for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Prof. Yasha Hasija,

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Place:

Date:

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ABSTRACT

Our brain is complex but is gifted by its internal processes that help in maintaining the balance between plasticity and homeostasis. One such method that is known to help refine our neural circuit is called Synaptic Pruning. This process ensures the removal of the excess synapses to ensure proper brain development. Apart from improving memory storage and learning capacity, pruning has also served as an inspiration for the algorithm to optimize neural networks. This thesis focuses on various roles of pruning with a primary stress on its significant role in neurodevelopmental disorders. The aberrations in pruning are one of the main reasons for neurodegeneration. In order to investigate further, we have taken a case of Autism Spectrum Disorder to perform a computational differential analysis using R to find plausible biomarkers that could be targeted to design therapeutics for controlling the impacts of pruning to prevent the onset of ASD. The effects of under-pruning and over-pruning are immense on the brain and are also known to influence the gut microbiota, as per studies. With advancements in studies around pruning, specific biomarkers could be found that correlate with cognitive resilience. We aim to provide a detailed review of how activity-dependent pruning has evolved over time and has guided the identification of new, therapeutically relevant mechanisms by which circuit development can go wrong in neurodevelopmental disorders

Keywords: *Synaptic Pruning, R programming, Differential gene expression, Autism, ASD.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION

ABBREVIATION	FULL FORM
ASD	Autism Spectrum Disorder
TREM2	Triggering receptor expressed on myeloid cells 2
FMR1	Fragile X Mental Retardation
CX3CR1	C-X3-Chemokine Receptor 1
CX3CL1	C-X3-Chemokine Fractalkine
MEF2	Myocyte Enhancer Factor 2
BDNF	Brain Derived Neurotrophic Factor
PTEN	Phosphatase and Tensin homolog
mTOR	Mammalian Target Of Rapamycin
FMRP	Fragile X Mental Retardation Protein

CHAPTER 1

INTRODUCTION

The human brain is a remarkably complex and intricate network comprising billions of neurons interconnected by trillions of synaptic connections. Synapses act as communication hubs that connect neurons, allowing them to form specialized circuits in the brain for various functions; these synaptic junctions serve as the fundamental communication hubs that facilitate the transmission of signals between neurons, enabling the formation of specialized neural circuits responsible for various cognitive functions, sensory processing, and behavioral outputs. Establishing, remodeling, and eliminating synaptic connections on dendritic spines play a crucial role in shaping these neural circuits during brain development. During the early stages of brain development, synapse initiation occurs when a neuronal axon contacts a target cell, followed by the assembly of essential synaptic components during synapse establishment. Synapses then undergo a process of maturation, becoming more robust and dynamic through activity-dependent changes(Huo et al., 2024a).

1.1 Synaptic Pruning: A Developmental Necessity

One of the fundamental phenomena in brain development is the reduction in the number of synapses that occur between early childhood and puberty. In recent years, many studies have investigated the temporal course of changes in synaptic density in primates, revealing the following picture. Beginning at the early stages of fetal development, synaptic density rises constantly until a peak level is attained (at 2–3 years of age in humans). Then, after a relatively short period of stable synaptic density (until the age of 5 in humans), an elimination process begins: synapses are constantly removed, yielding a marked decrease in synaptic density. This process proceeds until puberty, when synaptic density stabilizes at adult levels and is maintained until age. The peak level of synaptic density in childhood is 50% to 100% higher than adult levels, depending on the brain region(Chechik et al., 1998).

What advantage could such a seemingly wasteful developmental strategy offer? Some researchers have treated the phenomenon as an inevitable result of synaptic maturation lacking computational significance. Others have hypothesized that synapses that are strengthened at an early stage might be later revealed as harmful to overall memory function when additional memories are stored. Thus, synaptic elimination may reduce memory interference and yield better overall performance(Chechik et al., 1998). Synaptic pruning in postnatal cortical development can be divided into two phases. Phase 1, from birth to one year, involves rapid increases in synaptic density, dendritic growth, and cortical volume expansion. Phase 2, from one year to adolescence, is characterized by a gradual decline in synaptic and neuronal density, continued dendritic growth, and a decrease in synapse density along dendrites.

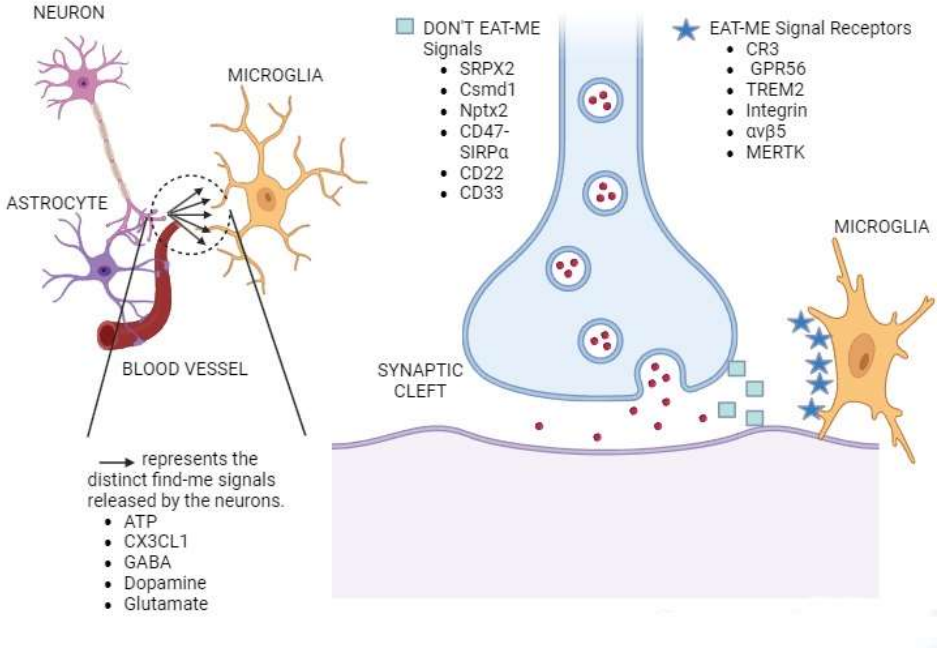
Synaptic pruning is believed to play a key role in the development of the brain and helps in efficient memory storage. Improving the pruning process can lead to better learning capacity of the human brain, thus ensuring proper synaptic plasticity without disturbing the brain's homeostasis. Synaptic densities in the human cerebral cortex are said to be at their peak during late infancy and childhood, which then keeps on declining as we grow older(Fernandes & Carvalho, 2016).

1.2 Microglial Role in Synaptic Pruning

The different molecules, like the complement system, glial cells like microglia and astrocytes, and signaling cascades like mTOR and MEF2 form the basis of the molecular mechanism that controls synaptic pruning. The complement system helps to tag the inactive synapses, and microglia further recognize and chop off these tagged synapses(Fernandes & Carvalho, 2016).

Microglia are the immune cells in the brain that play a vital role in cleaning the CNS, which is important for refining neural circuitry. Microglia works by executing three concurrent processes, i.e., recognition, engulfment, and elimination of the tagged synapses. Recognition is mediated through signals like phosphatidylserine (PS), CX3CL1, Glutamate, etc., and complement proteins binding to phagocytic receptors on microglia, initiating phagocytosis(Sierra et al., 2010). Complement proteins tags the inactive synapses and act as ‘eat-me’ signals(Chu et al., 2010; Stevens et al., 2007);

this signal is identified by one of the surface proteins of microglia CR3, and this signal transduction removes the tagged synapse (Stevens et al., 2007; Tian et al., 2024). Other molecular players involved includes TREM2 (The Triggering Receptor Expressed on Myeloid Cells 2) (Tian et al., 2024) and Progranulin (PGRN) (Lui et al., 2016).



(i) (ii)

Fig-1 (i) Find me signals are released to attract glial cells towards inactive synapses. These find-me signals include ATP, CX3CL1, GABA, DOPAMINE, and GLUTAMATE. (ii) Less active and immature synapses are labeled by eat-me signals like phosphatidylserine or complement proteins, which are identified by various receptors (CR3, GPR56, TREM2, integrin αvβ5, MERTK) on microglia. This marking facilitates synaptic pruning through direct microglial binding and engulfment of the tagged synapses. Additionally, microglial phagocytosis is modulated by don't eat-me signals, including inhibitors of the classical complement pathway (SRPX2, Csmd1, Nptx2), CD47-SIRPα interactions, and neuronal polysialylated protein interactions with CD22 and CD33, which prevent microglial activity.

Neuronal activity plays a central role in developmental synapse pruning, with active synapses being maintained and strengthened by the presence of "stabilization" signals, while weaker synapses are eliminated. This activity-dependent pruning occurs throughout the central nervous system (CNS) at different developmental periods, depending on the brain region and neuron subtype.

Neurons secrete and release multiple find-me signals that recruit microglia to nearby sites. Upon reaching these sites, microglia contact neurons through eat-me signals on the cell surface and initiate phagocytosis of tagged fragments, including pathogens, dying cells, cell debris, excess synapses, dendrites, axons, neurons, and protein aggregates. Microglia-mediated synapse pruning involves three main steps: recognition, engulfment, and digestion. Recognition is mediated via eat-me signals like phosphatidylserine (PS), calreticulin, Gas6, and complement proteins binding to phagocytic receptors on microglia. This binding initiates receptor-coupled signaling, remodeling the actin cytoskeleton to facilitate engulfment and digestion through phagosome-lysosome fusion. The best-studied eat-me signal is PS, which is exposed on dying neurons and less active synapses, facilitating their phagocytosis by microglial receptors and opsonins. For instance, PS binds to milk fat globule factor-E8, bridging the interaction with the microglial αvβ5 integrin receptor, thus potentiating phagocytosis of apoptotic cells. PS exposure also mediates selective synapse elimination, such as the microglial engulfment of developmental hippocampal and retinogeniculate synapses, depending on complement protein C1q and microglia-expressed PS receptor TREM2. Additionally, GPR56, an adhesion G protein-coupled receptor, binds to PS on the presynaptic compartment, controlling myelination and synapse elimination(Huo et al., 2024b).

1.3 Implications in Neurodevelopmental Disorders

Aberrant synaptic pruning has been implicated in the pathophysiology of various neurodevelopmental and neuropsychiatric disorders, such as autism spectrum disorder (ASD), schizophrenia, and intellectual disability. As a result of reduced synaptic pruning, there is an increase in synaptic density, which has been observed in autism(Tian et al., 2024). In contrast, increased synaptic pruning leads to cognitive

decline because of reduced synaptic density, as seen in the case of schizophrenia(Keshavan et al., 1994).

This thesis aims to understand the underlying mechanism of synaptic pruning and how improper pruning leads to neurodevelopmental disorders. By taking the case of Autism, we hope to decipher the gene expression profile, identify a few key genes that are differentially expressed, and finally identify potential biomarkers upon which further assay will lead towards therapeutic effects regarding Autism and Synaptic pruning.

Chapter 2

LITERATURE REVIEW

The human brain is characterized by a period of a process called synaptogenesis, in which new synapses between neurons form depending upon the experiences to which they are subjected. This process is followed by selective synaptic elimination, where the less frequently used connections are removed. This removal, occurring mainly during early childhood and adolescence, is called Synaptic Pruning and marks an important neurodevelopmental event (Huttenlocher & Dabholkar, 1997). Synaptic pruning is necessary for refining neural circuits, which includes improving memory, learning, and other behavioral adaptations(Selemon, 2013). Fine-tuning of the neural networks is achieved by activity-dependent mechanisms, which involve strengthening active neural connections and weakening inactive ones based on their experiences and environmental interactions(Kolb & Gibb, 2011). This refinement is crucial for developing sensory, motor, and cognitive abilities. Studies in animal models have shown the need for experience-based synaptic pruning, as visual experience is necessary for pruning synapses in the visual cortex, which is essential for developing normal visual acuity(Hooks & Chen, 2007). Any aberration in this pruning process can lead to either excessive synaptic connections, i.e., neural hyperconnectivity, or insufficient synaptic connections, i.e., neural hypoconnectivity, in both cases leading to various neurological disorders that hamper the proper functioning of the brain(Tang et al., 2014).

The risk of developing several social-emotional psychiatric disorders, as well as disorders such as schizophrenia, anxiety, and depression, often emerge during late adolescence and early adulthood because adolescence is often considered a second window of opportunity in brain development which includes heightened sensitivity and plasticity in behavior and cognition. This phase is marked by changes in brain structure and function, including synaptic pruning, which plays a crucial role in shaping adult behavior and is associated with vulnerability to psychiatric disorders(Rapee et al., 2019).

Throughout mammalian development, there are periods of increased changes in the brain that are restricted to definite developmental stages; these periods are referred to as sensitive and critical periods. Through complex series of events, including cell proliferation, differentiation, and network formation, normal brain functioning is shaped, thereby increasing brain sensitivity to external stimuli. The sensitive period allows gradual adaptability of functions by experience, while the critical periods require specific prior experience for fundamental changes in neural networks; these periods are key to achieving circuit refinement and plasticity. Studies have suggested a decline in synaptic density during adolescence, with the complement system and microglial signaling factors also playing a significant role during these critical periods, highlighting the importance of understanding neurobiological mechanisms underlying adolescent brain development and vulnerability to psychiatric risks(Westacott & Wilkinson, 2022).

During early postnatal years in mammals, there is an excess production of neurons, axons, and synapses, followed by activity-dependent pruning mechanisms that remove redundant synapses to enhance neural circuit efficiency and maturation. This synaptic pruning process begins in infancy, peaks during adolescence, and continues into the third decade of life, particularly in brain regions like the prefrontal cortex. Although the precise mechanisms of synapse disposal were previously unclear, recent research has highlighted the involvement of immune system proteins and cells in synaptic removal during critical developmental periods. Genetic models that lack proteins from the classical complement pathway and microglial signaling factors exhibit synaptic pruning loss, leading to immature synapse persistence beyond early critical periods(Huo et al., 2024).

2.1 Aberrant Synaptic Pruning

2.1.1 Mechanisms of Aberrant Synaptic Pruning

Aberrant synaptic pruning may result from genetic mutations, environmental factors, or disruptions in molecular pathways that regulate synapse elimination. That being said, many essential pathways and molecular players that play an integral part in synaptic pruning include the complement system. For elimination, synapses need to

be tagged to be identified by microglia; the complement system of our Immune system plays a pivotal role in this tagging process. Complement proteins, such as C1q and C3, mark synapses to be pruned, and microglia, the brain's resident immune cells, recognize the tags and prune the marked synapses by phagocytosis(Stevens et al., 2007). Any anomaly in the complement pathway can lead to improper synaptic pruning. For example, over-activation of the complement system can result in excessive synapse elimination, while under-activation can prevent the necessary pruning of weak synapses (Sekar et al., 2016). Genetic variations or mutations in complement genes also play a role in affecting the pruning process. For example, C4 has been associated with an increased risk of neurodevelopmental disorders like schizophrenia, potentially due to disrupted synaptic pruning. Microglia also play a crucial role in synaptic pruning by recognizing and engulfing the tagged synapses. Aberrant microglial function, such as impaired phagocytic activity or excessive inflammation, can contribute to abnormal synaptic pruning(Hong et al., 2016; Lui et al., 2016).

Other regulators of synaptic pruning include the mammalian target of rapamycin (mTOR); hyperactivation of mTOR has been associated with reduced autophagy, which results in the accumulation of excess synapses(Tang et al., 2014); in contrast, mTOR inhibition can impair synaptic pruning, contributing to cognitive loss(Huang et al., 2013). Another key factor is the Myocyte Enhancer Factor 2 (MEF2); it plays a vital role in activity-dependent pruning by regulating the expression of genes necessary for synaptic elimination, such as those genes which are coding for cell adhesion molecules or guidance cues(Flavell et al., 2006). Any deviation in MEF2 activity, either through genetic mutations or environmental factors, can disrupt normal synaptic pruning processes, contributing to the progression of neurodevelopmental disorders like autism and intellectual disability.(Harrington et al., 2016; Parikshak et al., 2013).

2.2 Effects and Impact of Aberrant Synaptic Pruning

Aberrant synaptic pruning can lead to increased or decreased pruning rates; while both exhibit distinct impacts, both lead to neural dysfunction.

2.2.1 Excessive synaptic pruning

Excessive synaptic pruning significantly reduces the number of synaptic connections, making it difficult for the neurons to communicate effectively. Excessive pruning can have far-reaching consequences on neural connectivity and cognitive function in the prefrontal cortex and other brain regions. The decline in the neural connection is said to be the reason behind cognitive loss and adverse symptoms associated with schizophrenia(Keshavan et al., 1994).

Additionally, excessive pruning in the prefrontal cortex and other brain regions involved in social cognition, such as the superior temporal sulcus and amygdala, may contribute to the negative symptoms (e.g., blunted affect, social withdrawal) and impaired social functioning observed in individuals(Adolphs, 2009; Glausier & Lewis, 2013).

2.2.2 Insufficient Synaptic Pruning

When insufficient pruning, the synaptic refinement process is disrupted, leading to the persistence of immature or inappropriate neural connections(Bagni & Greenough, 2005; Pfeiffer & Huber, 2009), and hence the neural circuit is disturbed. Such has been the case in the neurodevelopmental disorder Autism Spectrum Disorder. In ASD, the inability to adequately prune unnecessary synapses can result in an abundance of synaptic connections in some areas of the brain, resulting in hyperconnectivity(Belmonte et al., 2004; Zoghbi & Bear, 2012), which has been associated with cognitive insensitivity, sensory hypersensitivity and difficulties in integrating sensory information(Courchesne et al., 2007; Markram & Markram, 2010).

2.3 Autism Spectrum Disorder (ASD) and Aberrant Synaptic Pruning

2.3.1 Synaptic Pruning in ASD

ASD is a neurodevelopmental disorder characterized by repetitive behaviors, restricted interests, and difficulties in social communication. Synaptic pruning has its say in ASD as well. It can very easily be assumed that ASD leads to aberrant synaptic pruning, but it is wrong. Abnormal Synaptic Pruning is one of the contributing factors that result in ASD, i.e., Aberrant synaptic pruning results in ASD(Tang et al., 2014). Post-mortem reports of pateints have indicated higher spine densities on dendrites, implying either inadequate pruning or the creation of too many connections between nerve cells during early developmental stages(Hutsler & Zhang, 2010). This excessive growth of synapses in the brain is said to play a role in the atypical neural connections found in ASD patients, which could possibly explain the cognitive and behavioral changes experienced by individuals with the disorder(Pardo & Eberhart, 2007). The hyperconnectivity in sensory processing regions, such as auditory and visual cortices, is the reason behind the sensory overload experienced by the patients(Markram & Markram, 2010). Excess synaptic connections in these areas may lead to heightened and overwhelmed responses to sensory stimuli.

The altered synaptic pruning affects the functional connectivity between different brain regions, which is said to be the underlying mechanism contributing to social communication issues and integrating information across different domains(Supekar et al., 2013). Results suggest that the atypical connectivity patterns in ASD arise from deviations from this precise tuning in the pruning and refinement of large-scale, long-range neural networks.

2.3.2 Identification of Essential Genes involved in synaptic pruning and ASD

Six genes that play a crucial role in synaptic pruning and ASD were identified. All of these genes have a vital say in processes leading to synaptic pruning.

[1]. C4 Gene (Complement Component 4) :

The C4 gene is as essential in synaptic pruning as it is in synaptic pruning because of its ability to make the synapses for elimination and its ability for the complement proteins to enter the microglia. Increased C4 expression has been associated with overactive synaptic pruning and with greater schizophrenia risk.(Sekar et al., 2016).

[2]. MEF2C (Myocyte Enhancer Factor 2C) :

MEF2C is an important transcription factor that controls synaptic pruning by regulating synaptic pruning via activity-dependent transcriptional control of genes required for synaptic elimination. By controlling the number of excitatory synapses, MEF2C helps to guide the formation and refinement of neural circuits critical for cognitive function and behavior regulation. Due to dysregulation of the same 2 MEF2C target genes, abnormal synaptic pruning may underlie specific aspects of neurodevelopmental and psychiatric disease. The results of this study illustrate the important role of MEF2C in synaptic pruning, thus illuminating molecular mechanisms underlying brain development and function.(Flavell et al., 2006).

[3]. FMR1 (Fragile X Mental Retardation 1) :

Synaptic pruning is also regulated by the FMRP protein encoded from the FMR1 gene. By interaction with miRNAs and local translation regulation of synaptic proteins, FMRP ensures the correct elimination of wrong synapses. As a result, loss of FMR1 function is associated with abnormal synaptic pruning, which is thought to underlie some neurodevelopmental diseases like Fragile X Syndrome.(Edbauer et al., 2010).

[4]. BDNF (Brain-Derived Neurotrophic Factor) :

BDNF is crucial for the synapses' pruning owing to its activity-dependent release, TrkB receptors' interaction, and synaptic plasticity-modulating effect. It makes certain that neural circuits mature and become highly specialized

through the elimination of extra synapses and the preservation of those that are used(B. Lu et al., 2013).

[5]. PTEN (Phosphate and TENsin homolog) :

The importance of PTEN regarding synaptic pruning, neuronal arborization, and synaptic stability cannot be overemphasized. It ensures that proper synaptic pruning occurs, preventing the excess formation of synapses by controlling PI3K/Akt/mTOR pathways. The significance of this protein in performance-dependent synaptic pruning is essential for adaptable changes in neurons (or neural plasticity) and various aspects related to learning processes. Non-functioning PTEN may cause neurodevelopmental disorders(Kwon et al., 2006).

[6]. SYNPO :

It has been shown that SYNPO is a crucial gene for the formation and performance of the spine apparatus, which supports synaptic plasticity, keeps dendritic spine integrity, and manages calcium signaling while ensuring cytoskeletal dynamics are rightly controlled. This research has also shown that all these functions are important aspects of the synaptic pruning process, thereby indicating that SYNPO is essential for neurodevelopment and maintenance of synaptic homeostasis.(Deller et al., 2003).

Chapter 3

METHODOLOGY

3.1 Data Collection

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition with diverse presentations. To look at the underlying molecular mechanisms, researchers increasingly turn to transcriptomics, the study of gene expression levels. Hence, we were focused on investigating the transcriptomic profile associated with Autism Spectrum Disorder (ASD) using RNA sequencing data. RNA sequencing (RNA-Seq) is a powerful technique that offers a comprehensive snapshot of a cell's RNA transcripts (potential protein blueprints). This allows us to identify genes that might be abnormally expressed in ASD compared to healthy individuals. RNA-seq data is the large-scale data generated by high throughput sequencing technologies that measure the RNA transcript's expression levels in a biological sample.

This study used the Allen Atlas Brain data portal(https://portal.brain-map.org/), which provides high-quality RNA-Seq data from various brain regions, allowing us to focus on brain tissue relevant to ASD. We retrieved two datasets (H0351.2001 and H0351.2002) containing RNA-Seq data for all genes in a single brain. We carefully curated the downloaded data to ensure clarity and facilitate analysis, adding relevant details like sample information and gene annotations.

3.2 Data preprocessing

Preprocessing is an important step moving forward as it ensures that the data is clean, reliable, and ready for analysis. Properly processed data enables us to draw meaningful conclusions. Hence, before proceeding to further analysis it is crucial that we preprocess the retrieved data to maintain the level of quality and consistency.

RNA-Seq data was prepared using preprocessing tasks, including quality control assessment, data normalization and read alignment to the reference genome.

3.2.1 Quality Control Assessment

Certain unreliable data points, such as low-quality reads, adapter sequences, and potential contaminants, are present in the dataset that could introduce errors in our analysis. Such data points are removed during quality control assessment, making sure that only high-quality reads are retained in the dataset for further analysis and reducing the risk of misleading conclusions.

3.2.2 Read Alignment

Mapping the sequencing reads to their corresponding genomic locations helps us to identify transcripts and their expression levels. This mapping was done using Spliced Transcripts Alignment to a Reference (STAR) (Dobin et al., 2013), an ultrafast RNA-seq aligner. This allows us to identify which genes these reads originated from.

3.2.3 Quantification and Normalization

To measure the expression levels in the sample, a number of reads mapping to each gene was counted using a featureCounts tool (Liao et al., 2014). It provides us with the gene abundance in the sample.

During sequencing, technical variations pose a great threat to the inaccuracy of analysis and false results. Hence, the data must be normalized first, which involves removing these technical variations and the missing values from the dataset. A common normalization method, TMM(Robinson & Oshlack, 2010), was employed to do this task. It ensured the data was consistent, clean, and ready for analysis.

3.3 Differential Gene Expression

After preprocessing, we have completed an important step towards identifying the top significantly differentially expressed genes. First, we need to evaluate the dataset's expression levels and classify them into categories: upregulated and downregulated.

Expression analysis was carried out in an R environment using R programming, a widely used language for statistical analysis of datasets. Different libraries, such as DESeq2(Love et al., 2014), Tidyverse(Wickham et al., 2019), and Dplyr were installed and imported into the environment.

3.3.1 Data Import and Preprocessing

Before moving to any analysis, all the required files in the required dataset must be imported into the environment. This makes the files accessible by our program during the assay. After loading, to move forward, all the count data was rounded to the nearest integer values to avoid any conflict in our workflow.

3.3.2 DESeq2 Object Creation

Separate variables were created for both the datasets (dds_2001 and dds_2002) to store the RNA-seq counts data and the metadata regarding the sample. It is considered an optimal way to store data in a variable before performing Gene Expression analysis using DESeq2. A design formula was created, which served as the basis and based upon which the differentially expressed genes will be identified. This formula was based on the fact that ASD occurs dominantly in specific regions of the brain.

3.3.3 Differential Expression Analysis

On the variables created, one by one, the DESeq function was applied to perform the differential gene expression analysis by calculating the dispersion parameters and fitting the negative binomial model. After completion, the results were saved in two different variables (res_2001 and res_2002). Results can be visualized using display(res_2001) or saved in a file to be accessible anytime.

3.3.4 Result Extraction and Visualization

The result of DESeq contained various statistical metrics, which include log2 fold changes, p-values, p-adjusted values, base mean values, etc. For further analysis, the res_2001 and res_2002 were converted into data frames for easier data handling. A filter was applied over the whole data frame to keep the genes that have $p(adj) < 0.05$, which implies that such genes are significantly dysregulated. Since the metadata was stored in the R environment only, a source column was added to the data frame in order to identify which gene that specific metrics belonged to. The two data frames were combined to make one single data frame, which was then used to make a volcano plot.

Utilizing the functions provided by the ggplot2 library, a volcano plot was plotted, representing log2fc as a function of base mean.

This statistical framework provided by DESeq has made it quite easy to identify differentially expressed genes, and keeping them at the central focus of further analysis, we may get some potential biomarkers that can be targeted as drug targets for ASD.

3.4 Gene-Protein Mapping

Now that we have identified significant differentially expressed genes, we need to look into their functioning, and the best way to do so is to gather information regarding the protein they code. We employed the biomaRt(Durinck et al., 2005, 2009) package to create a map, associating each gene symbol with its corresponding protein accession ID. The column containing gene names in the combined data frame created a new one. The gene list was extracted. The getBM function retrieved the HGNC (HUGO Gene Nomenclature Committee) gene symbols and the corresponding UniProt/Swiss-Prot accession numbers from the Ensembl database. The filters argument specifies the type of identifier used for the input gene list (in this case, HGNC symbols), and the attributes argument specifies the desired output attributes (HGNC symbols and UniProt/Swiss-Prot accession numbers). The values argument provides the input gene list, and the mart argument specifies the Ensembl database to be queried. The resulting protein_mapping_combined data frame contained the gene symbols and their corresponding UniProt/Swiss-Prot protein accession numbers, facilitating the mapping of genes to their protein products.

This gene to protein mapping was an important preprocess step in order to introduce gene expression data into the next levels of the analysis that include functional annotations, protein protein interactions, and pathway memberships These protein pathways can help the researchers to understand the effects on a protein level, and their roles on the molecular mechanisms of Autism Spectrum Disorder (ASD).

Chapter 4

RESULTS

4.1 Differential Gene Expression

After data preprocessing, all the files were loaded into the R environment. There were 5 files for each sample:

1) RNAseqTPM.csv - Contains TPM values arranged by (row, column) = (genes, samples).
2) RNAseqCounts.csv - Contains fragment counts arranged by (row, column) = (genes, samples).
3) Genes.csv - Metadata for the genes in RNAseqTPM.csv, including information about gene identification, location, and size.
4) Ontology.csv - The ontology of brain structures used for sampling.
5) SampleAnnot.csv – All the annotations related to the sample.

For both the files, two variables were created, count_data and metadata. count_data variable stored RNA sequencing counts and had 22317 rows and 121 columns for both samples, whereas the metadata variable stored the sample annotations and had 121 rows and 21 columns for both samples.

A DESeq object was created, namely dds_2001 and dds_2002 for H0351.2001 and H0351.2002, respectively. The design for this object was based upon the main structure. Using the function Results(dds_2001) was stored in a variable res_2001, which was further stored and saved in a local csv file.

TABLE - 1 : TOP 10 UPREGULATED GENES FROM SAMPLE H0351.2001.

Gene ID	baseMean	**log2FoldChange**	lfcSE	stat	pvalue
CDH9	318.2377624	**11.19760998**	1.040435946	10.7624213	5.18E-27

ARX	365.4380552	**11.00380067**	1.02565585	10.72855059	7.48E-27
KIAA0748	2358.830056	**10.69541345**	0.625991258	17.0855636	1.90E-65
FOXG1	1759.235948	**10.60738049**	0.656466591	16.15829446	9.93E-59
RXFP1	345.9773089	**10.44472387**	1.057230079	9.879329082	5.12E-23
DDN	11027.75076	**10.41222255**	0.301201399	34.56897137	7.40E-262
GDA	4815.379114	**10.39527383**	0.460814327	22.55848663	1.11E-112
CARTPT	205.5255353	**10.39100707**	1.160157692	8.956547144	3.35E-19
FRMPD2P1	486.7582659	**10.27056488**	1.060308049	9.686397249	3.44E-22
SST	1478.506453	**10.23215278**	0.67848083	15.08097551	2.16E-51

TABLE-2 : TOP 10 DOWNREGULATED GENES FROM SAMPLE H0351.2001.

Gene ID	baseMean	**log2FoldChange**	lfcSE	stat	pvalue
ZP2	67.58953585	**-12.47650826**	0.565545171	-22.06102871	7.49E-108
BARHL1	18.66808668	**-11.33940914**	2.637113234	-4.299932591	1.71E-05
CCDC155	17.22168372	**-11.03681348**	0.558575007	-19.75887454	6.73E-87

TFAP2B	15.86131049	**-10.87909619**	2.024551138	-5.37358429	7.72E-08
EOMES	37.32017325	**-10.75154853**	0.88179085	-12.19285564	3.39E-34
PCP2	23.23227678	**-10.26196524**	0.54275076	-18.90732541	9.93E-80
OTX2	25.90644897	**-10.22523954**	1.4855076	-6.883330347	5.85E-12
CDH15	81.52465844	**-10.2129699**	0.522414232	-19.54956292	4.16E-85
UNCX	8.316915817	**-10.17011447**	3.283099459	-3.097717445	0.001950173
FAT2	1325.851923	**-10.16398555**	0.330502252	-30.75315069	1.11E-207

TABLE-3 : TOP 10 UPREGULATED GENES FROM SAMPLE H0351.2002.

Gene ID	baseMean	**log2FoldChange**	lfcSE	stat	pvalue
RXFP1	474.347186	**9.511278565**	0.846538646	11.23549245	2.73E-29
CIDEA	137.5126718	**9.399348275**	0.934825312	10.05465743	8.76E-24
CARTPT	247.3200486	**9.238512126**	1.059326542	8.721118336	2.75E-18
FOXG1	1698.794843	**9.219551378**	0.392795573	23.47162749	7.95E-122
TBR1	1835.522358	**9.014387042**	0.358299976	25.15877102	1.13E-139
TMEM155	2162.488758	**8.993560896**	0.329798439	27.26987103	9.66E-164

CDH9	354.4664815	**8.89544147**	0.74058719	12.01133585	3.10E-33
GDA	7023.602141	**8.85980681**	0.28762955 6	30.80283868	2.40E-208
CHRM1	3582.595983	**8.829078317**	0.24634547 2	35.84022982	2.61E-281
KCNV1	1117.770739	**8.766313017**	0.41078746 3	21.34026426	4.80E-101

TABLE-4 : TOP 10 DOWNREGULATED GENES FROM SAMPLE H0351.2002.

Gene ID	baseMean	**log2FoldChange**	lfcSE	stat	pvalue
ZP2	112.2831919	**-12.15212534**	0.82617256 3	-14.70894325	5.65E-49
BARHL1	33.92664468	**-11.85290885**	0.54754281	-21.64745593	6.42E-104
TFAP2B	32.11994909	**-11.39511377**	0.62066682	-18.35946986	2.77E-75
PCP2	30.30400823	**-10.75943342**	0.52649109 2	-20.43611675	7.98E-93
TLX3	14.3472333	**-10.61470558**	2.35841315 2	-4.500782898	6.77E-06

FGF3	11.50611069	**-10.47582499**	0.55171448	-18.98776518	2.15E-80
UNCX	11.51147791	**-10.47525993**	0.55341639 7	-18.92835124	6.66E-80
BARHL2	33.66384335	**-10.24251278**	0.46746745	-21.91064377	2.06E-106
CDH15	124.8092834	**-10.16372681**	0.26001361 2	-39.08921053	0
CCDC155	14.96754578	**-10.11686917**	0.54076122	-18.70857005	4.22E-78

In the res_2001 variable, we included the condition of the p-adj value being < 0.05 to filter out the significant genes from the rest of the genes. Using an R module ggplot, a Volcano plot was generated for these significant genes.

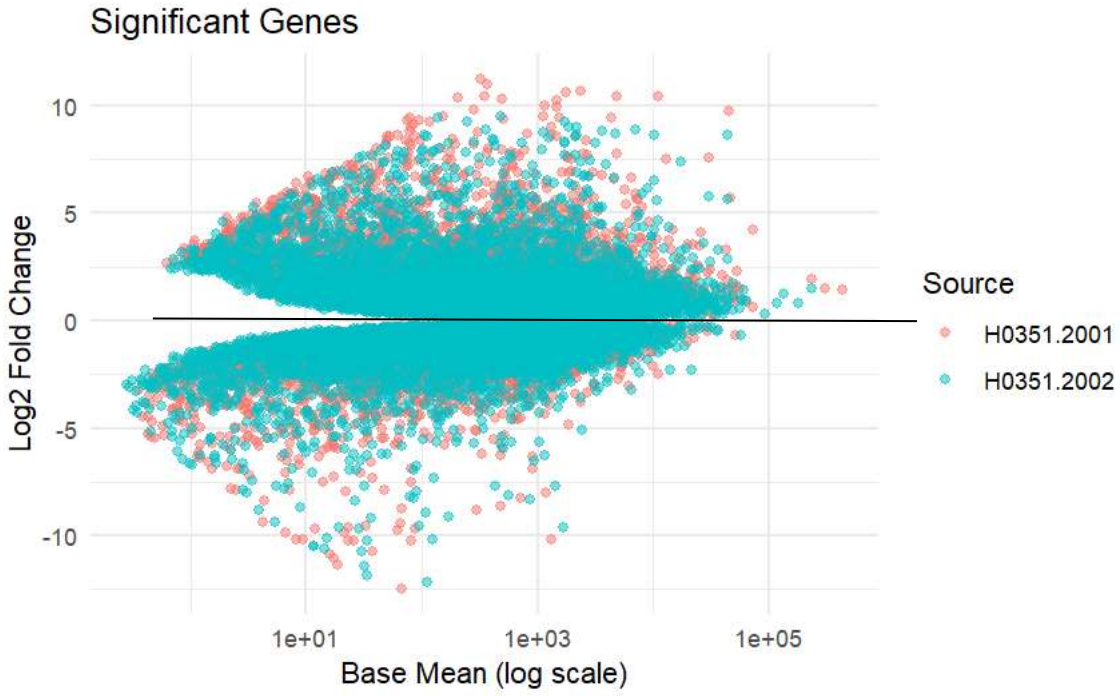


Fig-2 Volcano plot depicting significant upregulated and downregulated genes.

Volcano plot makes the representation of upregulated and downregulated genes much simpler; all the genes present above the horizontal line passing through the origin are upregulated, and all those below them are downregulated.

4.2 Gene Protein Mapping

After identifying the most impactful genes, the next step was to link them to their protein counterparts. Proteins are the cell's workhorses, and understanding which ones are affected in ASD can provide valuable clues about the underlying mechanisms. The next crucial step was to map these genes to their corresponding protein products. This mapping is essential for downstream analyses, such as pathway enrichment analysis and functional interpretation, as proteins are the functional units responsible for carrying out various biological processes within cells.

Recognizing the protein players behind the differentially expressed genes required a meticulous mapping process. With the list of genes prepared, we used the getBM function of biomaRt library of R to map the gene-protein.

TABLE 5 – GENE IDS MAPPED TO THEIR RESPECTIVE UNIPROT ACCESSION ID

Gene	Uniprot Accession ID
BCL2L14	Q9BZR8
BPY2	O14599
CDY2A	Q9Y6F7
BPY2C	O14599
DAZ4	Q86SG3
CDY2B	Q9Y6F7
DDX3Y	O15523
C1RL	Q9NZP8
CLSTN3	Q9BQT9
DAZ3	Q9NR90
DAZ1	Q9NQZ3
AMELY	Q99218
DAZ2	Q13117
CDY1B	Q9Y6F8
BPY2B	O14599
CDY1	Q9Y6F8
CXADR	P78310
ADAMTS13	Q76LX8
CLDN17	P56750
CYYR1	Q96J86

Of the 44,634 genes in the gene list, 33,168 were successfully mapped to their respective UniProt accession IDs, providing a comprehensive resource for further analysis and interpretation.

4.3 Identification of Potential Biomarker

Six genes identified as playing a crucial role in synaptic pruning during the literature review were suspected to play a role in ASD. These six genes, namely 'C4', 'MEF2C', 'FMR1', 'BDNF', 'Pten', and 'SYNPO', were searched across the significantly downregulated genes, as these six genes are known to promote synaptic pruning, so their upregulation may cure the condition and result in normal brain functioning.

TABLE 6 – SIGNIFICANT DOWNREGULATED GENES THAT ARE IMPORTANT FOR SYNAPTIC PRUNING AS WELL

Gene	log2FoldChange
BDNF	-0.40225
FMR1	-0.27782
FMR1	-0.49763

sThe gene search revealed that in both the samples, only one gene i.e., FMR1, has been significantly downregulated and targeted, which may further lead to any sort of improvement in the condition of ASD.

The FMR1 gene encodes the FMRP (Fragile x Mental Retardation Protein); it plays a crucial role in the local translation of synaptic proteins and ensures the correct elimination of wrong synapses. Loss of function is associated with wrong synaptic pruning.

Another set of genes was searched across the significantly upregulated genes; these genes are known to inhibit synaptic pruning, and overexpression of such genes may lead to abnormal synaptic density.

TABLE 7 – SIGNIFICANT UPREGULATED GENES THAT INHIBIT SYNAPTIC PRUNING

Gene	log2FoldChange
CX3CR1	1.793512061

TREM2	1.732042508
CX3CR1	0.666960224
TREM2	0.999986544

It was revealed that both samples possess a pair of significantly downregulated genes- CX3CR1 and TREM2- and the observed upregulation hints towards their involvement at molecular levels in ASD.

CX3CR1 (C-X-C3 motif Chemokine Receptor 1) encodes the CX3CR1 protein, which is a receptor protein present on the surface of microglial cells and it binds to the fractalkine CX3CL1 (a find-me signal) ligand which is expressed by the neurons. This protein-ligand binding is crucial for glial cells to identify the neurons to be pruned. The overexpression of this CX3CR1 gene may alter the microglial response to the fractalkine, resulting in decreased microglial activity at synapses. As a result synaptic pruning is decreased as microglia cannot recognize and remove the excess synapses.

The TREM2 gene encodes another receptor present on the glial surface that plays a key role in the phagocytic activity of microglia and response to neural signals. TREM2 activation is necessary for microglia to fully exhibit its phagocytotic nature as it influences the glial metabolism and aids in clearing the cell debris and synaptic material post-phagocytosis. Any irregularity in TREM2 expression can disturb the normal phagocytic activity of microglia. Overexpression may result in the failure of microglia to prune the synapses properly.

Identification of these two upregulated genes suggests that further investigation into the functional aspect of these genes could shed light upon the critical role they play in our body and potentially lead to the development of targeted therapeutic interventions.

4.4 Network Analysis

All three identified biomarkers were then subjected to network analysis to visualize the inter-genic connections they exhibit within our brain. Network maps were developed using STRING data resource.

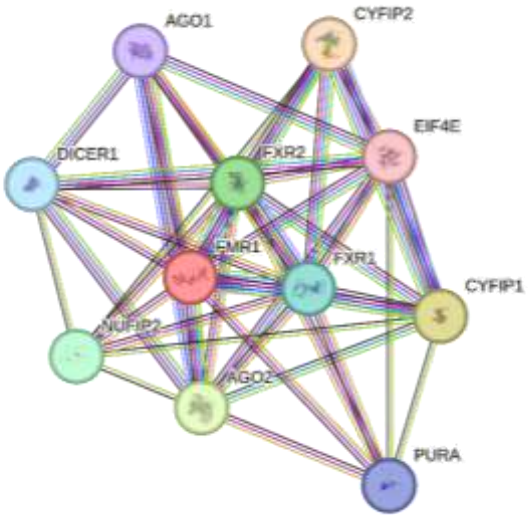


Fig-3 Network map of FMR1

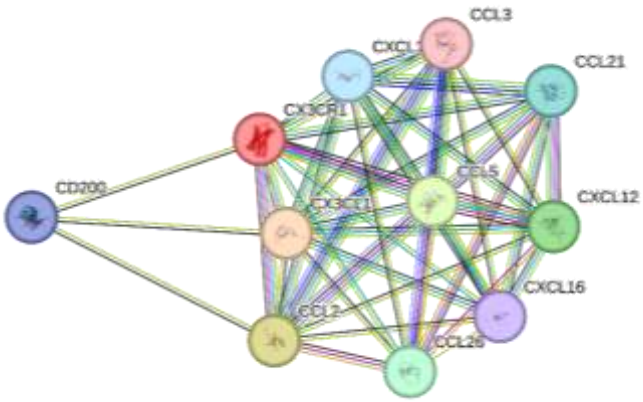


Fig-4 Network map of CX3CR1

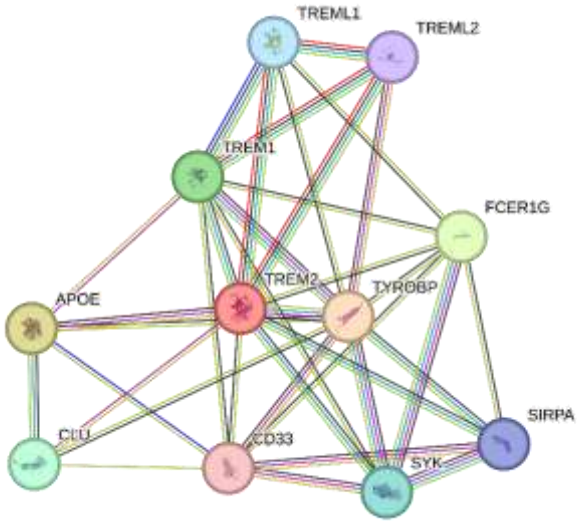


Fig-5 Network map of TREM2

In all the maps, the query is enclosed in a red circle, and the rest are the interactions that genes have. If the 3d structure of their protein is available, then it is present in the circle enclosing the gene.

4.5 3D-Structure Modelling

The 3D structure modeling of the potential biomarker proteins identified through the gene-protein mapping step provided valuable insights into their structural features and potential functional implications in the context of Autism Spectrum Disorder (ASD).

By accessing the Uniprot ID, it was found that the FMR1 gene codes for Fragile X Mental Retardation Protein, the CX3CR1 gene codes for the receptor protein CX3CR1, and the TREM2 protein codes for the membrane protein Triggering receptor expressed on myeloid cells 2. Sequences for the queries were retrieved in FASTA format. Each sequence was submitted as a query on the SWISS-MODEL workspace. Using the identified templates, the Swiss-Model server constructed 3D models of the

MEF2C and SYNPO proteins by aligning their sequences with the template sequences and modeling the conserved regions and variable regions accordingly.

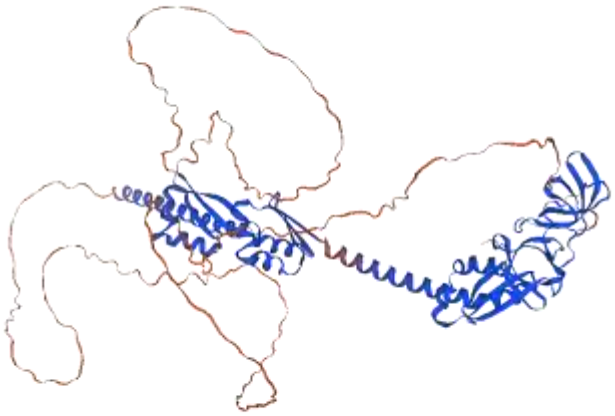


Fig-6 3D model of FMRP

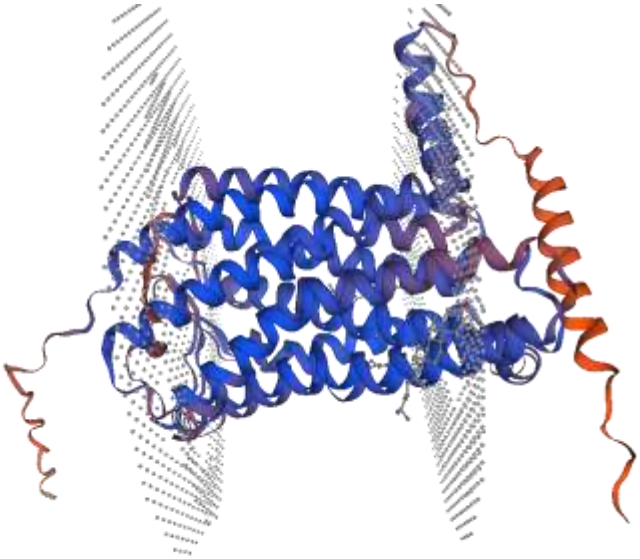


Fig-7 3D model of CX3CR1

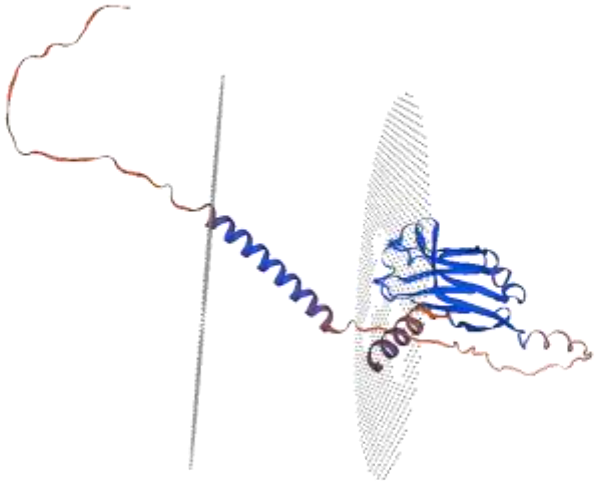


Fig 8 3D model of TREM2

The quality of the generated 3D models was evaluated using various statistical and structural analysis tools provided by the Swiss-Model server. These tools assessed stereochemical quality, energy minimization, and structural similarities to known protein folds.

Chapter 5

CONCLUSION AND FUTURE DIRECTION

5.1 Conclusion

This thesis has thoroughly reviewed the complex process of synaptic pruning and its crucial role in brain development. Synaptic pruning is a natural and necessary process aiming to refine the neural circuits and ensure they work effectively and efficiently. This removal of synapses is at its peak during childhood and early adolescence as the brain undergoes significant changes, allowing better neural communication. Brain's immune cells, the microglia, play a crucial part in this pruning process as they help identify and remove excess synapses through molecular mechanisms. However, when synaptic pruning does not occur properly, it is a problem because it may further lead to neurodevelopmental disorders such as ASD or schizophrenia. Since improper pruning can result in either too many synapses or too few, both disrupt neural connectivity and affect brain functioning. It is necessary to maintain accurate regulation of synaptic pruning for healthy brain development. Further, we emphasize ASD and how improper synaptic pruning is a contributing factor to the disease severity.

Using the RNA sequencing data, we moved to the gene expression profiling of two different brain samples. It was found that a total of 21,304 genes were upregulated, and 19,660 genes were downregulated in the combined dataset of the brain samples. 33,168 genes were successfully mapped to their respective Uniprot accession ID. Additional analysis revealed that the 'FMR1' gene is downregulated, FRM1, a crucial gene that ensures the correct elimination of synapses takes place, and two genes 'CX3CR1' and 'TREM2', were found overexpressed; both genes are responsible for inhibiting proper synaptic pruning upon their increased expression. To ensure the functional significance of these identified genes, network analysis was performed for all three genes, and the results revealed that all the genes play a central role in several pathways and processes in our brains. To further understand the structural implications of these findings, we analyzed the Ramachandran plots for the proteins encoded by the

identified genes. The Ramachandran plots provide insight into the conformational angles of amino acid residues in the protein structures, highlighting regions of energetically favorable conformations.

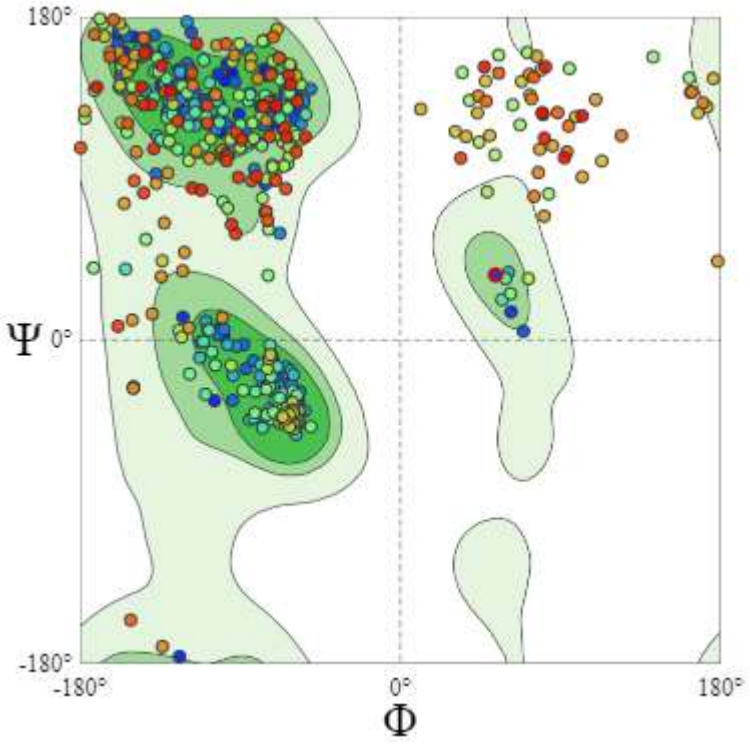


Fig-9 Ramachandran plot of FMR1 – showing 80.84% of the protein in the Ramachandran favored region.

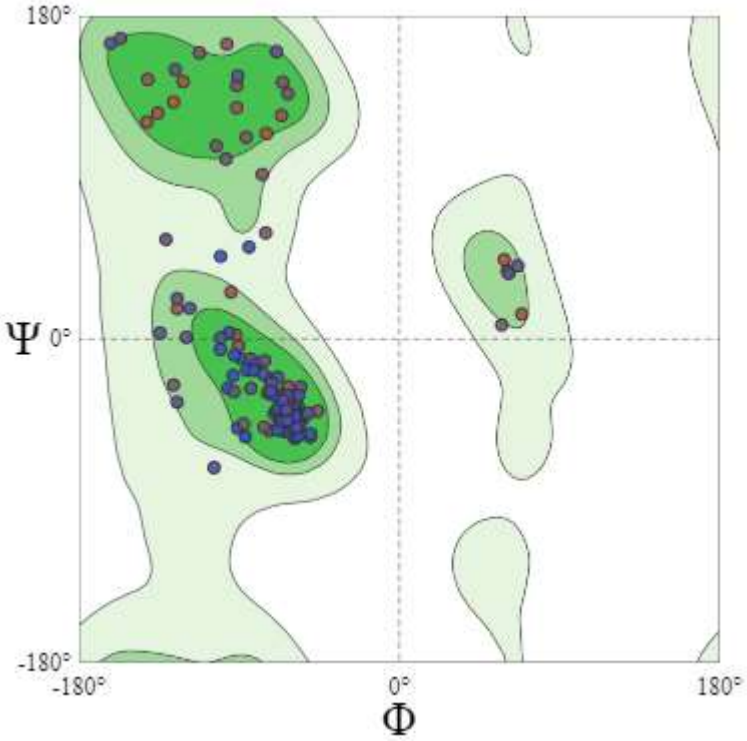


Fig-10 Ramachandran plot of CX3CR1- showing 97.2% of the protein in the Ramachandran favored region

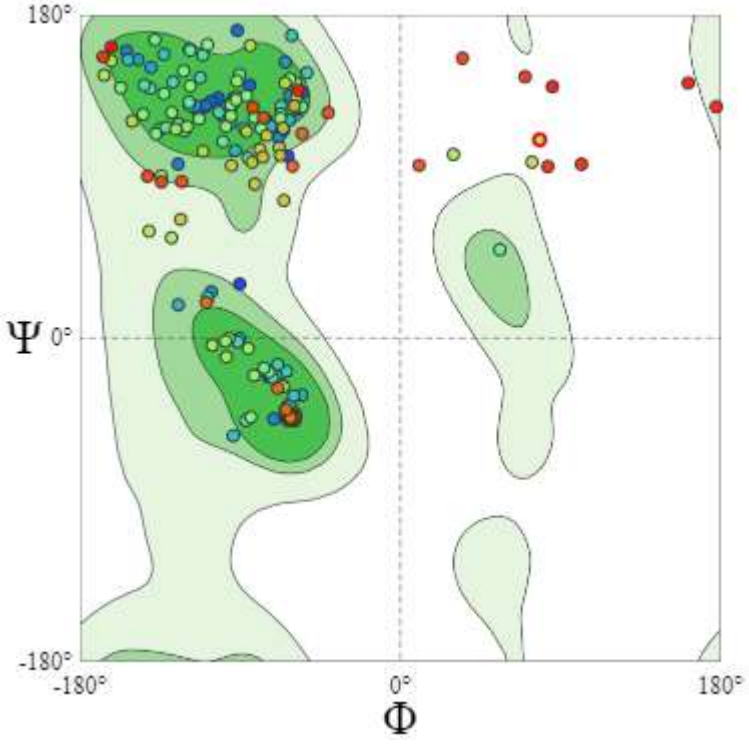


Fig-11 Ramachandran plot of TREM2- showing 66.84% of the protein in the Ramachandran favored region

All the plots show a high percentage of protein lying in the Ramachandran favored region, which accounts for the structural stability of the 3D structures. Although CX3CR1 shows the highest amount of protein in the favorable region (97.2%), it implies that CX3CR1 has the highest structural stability and can be used for further assay.

To conclude, this study has identified three potential biomarkers, viz C-X-C3 chemokine receptor protein, Fragile X Mental Retardation Protein, and triggering receptor expressed on myeloid cells 2. These biomarkers can be targeted for potential drug delivery mechanisms and mutational analysis to identify their role as target proteins further and evaluate their role in disease progression.

5.2 Future Directions

While this study lays a concrete groundwork of computational transcriptomic assay of ASD, it is necessary that the findings of this study are validated through wet lab experimentation.

Targeting the downregulated gene FMR1, which play a critical role in pruning process, can prove to be a avenue worth exploring as attempts to increase the expression levels of FMR1 in diseased cells can actually lead to the restoration of synaptic pruning back to normal levels and thus making an attempt towards curing autism. Meanwhile, upregulated genes that inhibit pruning can be targeted for Molecular Docking and Simulation analysis which will result in the identification of potential inhibiting perturbations that will hinder the functioning of these overexpressed genes.

Mutational analysis on the 3D structures of the protein can help us to gather information about significance of specific amino acid in the protein structure and whether or not any alteration to it will lead to some kind of structural change or will affect the stability of the protein.

In conclusion, in this study we have identified three key genes that have a say in autism as well as pruning. Further assays should be done on these genes as this proves to be a potential key to solve the problems regarding ASD and Synaptic pruning altogather.

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ACCEPTANCE NOTIFICATION

Submitted Paper:

NEUROBIOLOGICAL BASIS OF AUTISM SPECTRUM DISORDER: FOCUS ON SHANK2 GENE AND MOLECULAR DOCKING STUDIES

Kanchan Kumari, Anjali Sharma, Yasha Hasija*
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***Abstract*-Autism spectrum disorder (ASD) has been recognized as a complex chronic brain disorder. Evidence implicates the involvement of synaptic protein and many other genes in the emergence of neuropsychiatric disorder such as ASD. In this study we examined the SHANK2 gene, postsynaptic density scaffolding protein located at excitatory synapse and pivotal for proper function and development of synapse. Mutation in SHANK2 protein leads to synaptic dysfunction and associated with ASD related phenotype. By using the molecular docking, we identify the interactions between SHANK2 gene and candidate compounds. Moreover the advancement of genomics science and technology has the potential to revolutionize therapeutic approach and drug discovery process. Computational approach by using various bioinformatics tools shed some light on the potential treatment of ASD.**

Keywords- Autism spectrum disorder, SHANK2, Molecular Docking, Synaptic Protein, Synaptic Dysfunction, Mutation, Bioinformatics

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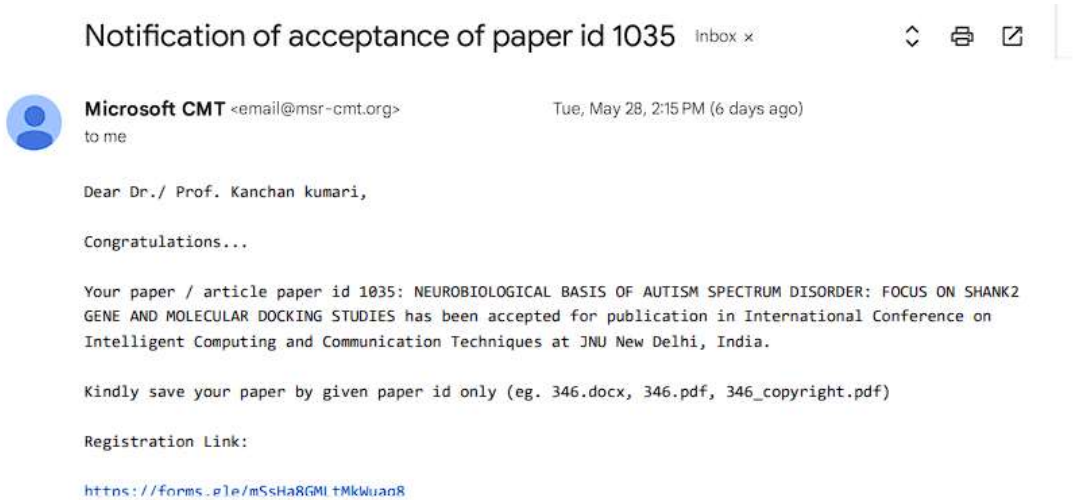

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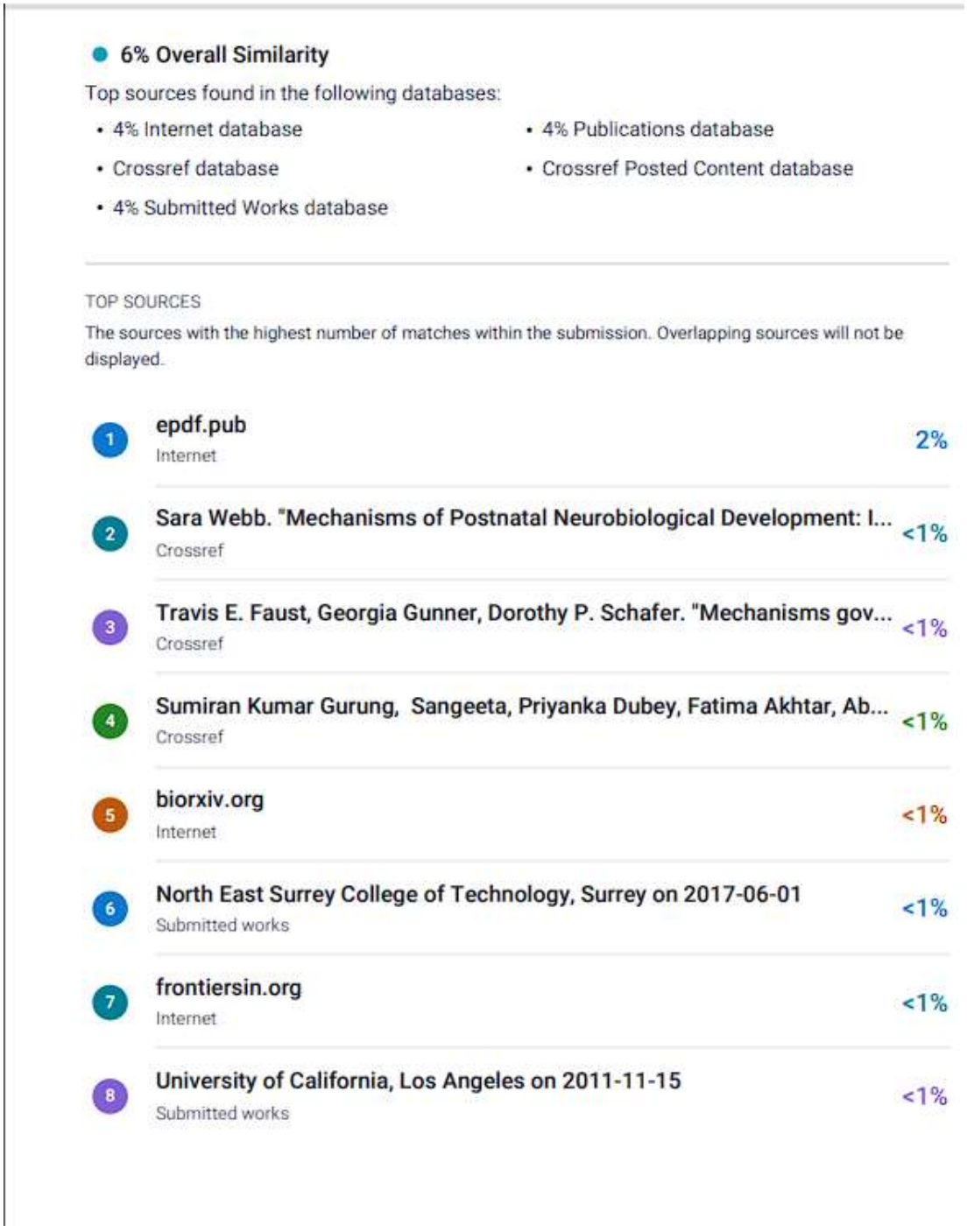

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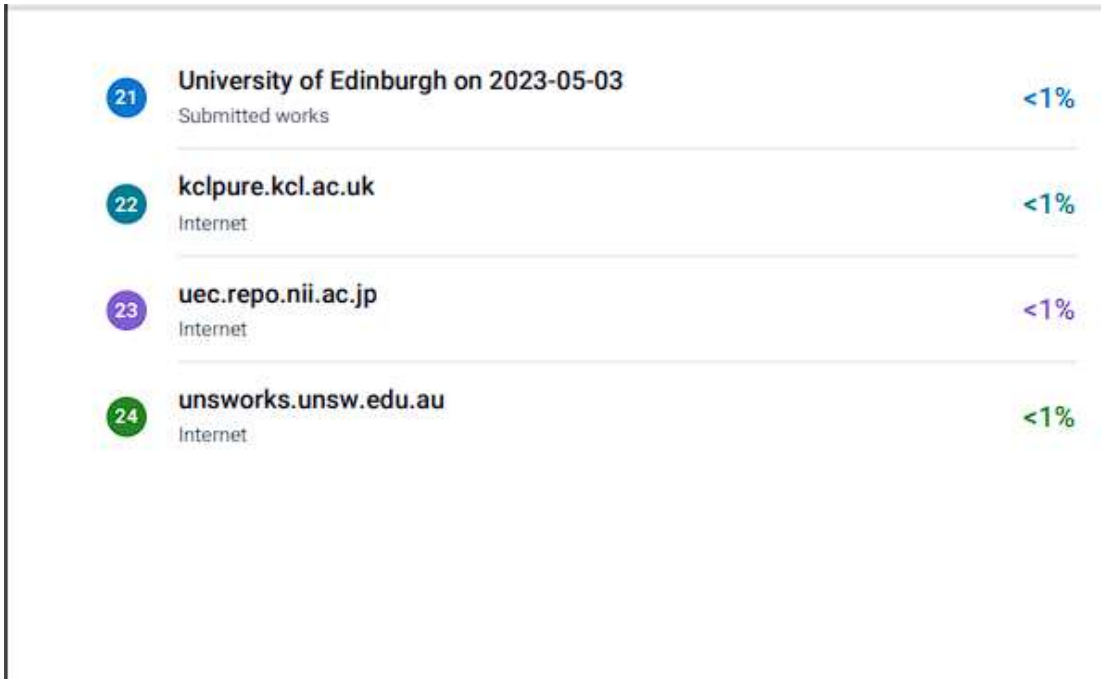
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CV

Anjali Sharma

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Education

Delhi Technological University — New Delhi, India
*Master of Science majoring in **Biotechnology** with **8.0/10 GPA*** — *September 2022 – Present*

Deshbandhu College, University of Delhi — New Delhi, India
*Bachelor of Science (Honors) majoring in **Biochemistry** with **8.77/10 GPA*** — *July 2019 – June 2022*

Experience

International Workshop - DecodeLife — 15th July – 11th August 23
Data Science with Python

- Acquired comprehensive skills in various domains, including utilizing Linux commands, Jupyter Notebook, Anaconda, file handling, String manipulation, Machine learning with Python, Chemoinformatics, Network bioinformatics with Python, Deep learning, and Transcriptomics.
- Practised in data analysis techniques, encompassing exploratory data analysis, statistical analysis, and data visualization to derive meaningful insights from diverse datasets.

One Week DST-STUTI Training Program — 30th Jan – 5th Feb 2023
Modelling for Molecular Biology of Human Diseases

- I was competitively accepted for the Department of Science & Technology hands-on training program on utilizing modern techniques in developing human disease models and alayzing the datasets based on human diseases at the Dept. of Toxicology, Jamia Hamdard – New Delhi

Virtual Research Fellowship: The Hackbio — July 2021 – October 2021
Proteomics and Computer Aided Drug Discovery

- Performed CADD studies in Python/Unix environment on Lujo Virus receptors FDA approved small molecule ligands, and employed proteomics tools for enumerating Prion-Like candidates in the Human proteome. Utilized data clustering techniques to identify distinct binding patterns between Lujo Virus receptors and FDA approved small molecule ligands, enabling a nuanced understanding of their interactions.

Summer School: International Youth Neuroscience Association (IYNA) — June 2020 – August 2020
Analysing the Role of Synaptic Pruning Processes in Neurodegenerative Disorders

- Worked on a systematic review on the role of synaptic pruning in neurodegeneration. Employed data preprocessing techniques to clean and organize raw data, ensuring its suitability for analysis.
- Applied statistical methods to analyze quantitative data, such as measuring synaptic density and assessing the significance of changes in pruning patterns.

Summer School: International Youth Neuroscience Association (IYNA) June 2020 – August 2020

Analysing the Role of Synaptic Pruning Processes in Neurodegenerative Disorders

- Worked on a systematic review on the role of synaptic pruning in neurodegeneration. Employed data preprocessing techniques to clean and organize raw data, ensuring its suitability for analysis.
- Applied statistical methods to analyze quantitative data, such as measuring synaptic density and assessing the significance of changes in pruning patterns.
- Developed graphical representations to convey complex relationships between synaptic pruning patterns and the progression of neurodegenerative disorders.

Skills

Analytical Skills
Tableau, Microsoft excel (using AI), Programming languages- Python/R to automate processes, Cloud Services to store and retrieve data, Basic ML (Scikit-Learn))

Computational Skills
Python/R/Unix, Latex PyMOL, Novice Genomics and Drug Discovery Tools, molecular docking, fundamental transcriptomics ,CADD methods, Multiple Sequence Alignment (CLUSTALW) , Basic computational Neuroscience (BRIAN neuron network Simulator)

Awards

SITARE BIIS June – July 2022
I was awarded a competitive acceptance into the Students Innovations for Translation & Advancement of Research Explorations (SITARE) – 12th Biotech Innovation Ignition School (BIIS) conducted Department of Biotechnology, India.

Conference Presentations

- **15th Conference of the Hellenic Society for Computational Biology & Bioinformatics (HSCBB21)**
  Sharma A *et al.*, "Pharmacoinformatics, and Molecular Dynamics Simulation towards identification of inhibitors against Human Pathogenic Lujo-virus 6GH8 target", 10 – 11th December 2021 (Flash Talk)
- **16th Annual Bioinformatics & Computational Biology Conference (BBCC 2021)**
  Pharmacoinformatics, Virtual Screening, and Molecular Dynamics Simulation towards identification of inhibitors against Human Pathogenic Lujo-virus 6GH8 Target", 1st – 3rd December 2021 (Poster Presentation)
  https://f1000research.com/posters/10-1202

- **16th Annual Bioinformatics & Computational Biology Conference (BBCC 2021)**
  Pharmacoinformatics, Virtual Screening, and Molecular Dynamics Simulation towards identification of inhibitors against Human Pathogenic Lujo-virus 6GH8 Target", 1st – 3rd December 2021 (Poster Presentation)
  https://f1000research.com/posters/10-1202
- **HEALTH 2021 International Conference on "Cancer Biology: Advances & Challenges"**
  Sharma A, "Designing a Multi Epitope Vaccine Against Non-Small Cell Lung Carcinoma Using Immunoinformatics", 11 – 13th November 2021. **Best Oral Presentation in Computational Oncology**
- **Horizons in Molecular Biology Symposium 2021**
  Sharma A & Shanahan M, "Reviewing The Various Roles Of Synaptic Pruning", 13 – 16th September 2021
- **BioEpistome 2021 at MITWPU, UIET**
  Sharma A, "Deleting Neurons: A Closer Look at Synaptic Pruning", 31st January 2021 (Poster Presentation) **1st Prize: Best Undergraduate Poster**
- **NeuroNovember Convention 2021**
  Sharma A & Shanahan M, "Deleting Neurons: A Closer Look at Synaptic Pruning", 28th November 2020 (Oral Presentation) **3rd Prize: Best Oral Presentation**
  https://app.oxfordabstracts.com/events/1839/program-app/submission/218782

Online Coursework

- **Introduction to Python.** Completed March 2022 at Coding Ninjas.
  Taught by Ankush Singla
- **Foundation of Modern Machine Learning Course**
  Completed September 2022 at International Institute of Information Technology, Hyderabad (IIITH).
- **SARS-CoV-2 Protein Modeling and Drug Docking.** Completed September 2021 at coursera.org.
  Taught by Bhagesh Hunakunti, University Of Manchester
- **Introduction to Genomic Technologies.** Completed October 2021 at coursera.org.
  Taught by Steven Salzberg and Jeff Leek, John Hopkins University
- **Fundamentals of Neuroscience for Neuroimaging.** Completed October 2020 at coursera.org.
  Taught by Arnold Bakker, John Hopkins University

Extra-Curricular Acitivities

- Head of Content at **BioSoc-DTU** (Biotechnological society of DTU) (2022-24)
- Actively participated in **Conference Presentations** and won prizes for best presentations. (2020-22)
- Won first prize in **Cine-Mania** (The Movies Quiz) conducted by Ecozest(Economics Society of Deshbandhu College) in its annual fest- Econfiesta'22.
- Participated in **Mimamsa** (National Science Level Quiz for Undergraduate students.) in 2018 and 2019