Investigations on microglial genes for their assertive role in Alzheimer's Disease

Project report submitted in partial fulfillment of the requirement for the degree of

Master of Science

in

Biotechnology

By

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Under the supervision of

Dr. Tiratha Raj Singh

to

Department of Biotechnology & Bioinformatics

Jaypee University of Information Technology Waknaghat, Solan-173234, Himachal Pradesh

DECLARATION

I hereby declare that the work presented in this project entitled "**Investigations on microglial genes for their assertive role in Alzheimer's Disease**" in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology submitted in the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology Waknaghat is an authentic record of my own work carried out over a period from January 2023 to May 2023 under the supervision of **Dr. Tiratha Raj Singh** Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Solan, Himachal Pradesh.

I also authenticate that I have carried out the above-mentioned project work under the proficiency stream.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Ankita Singh, 217804

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

Dr. Tiratha Raj Singh

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Dated:

CERTIFICATE

This is to certify that the project report was carried out by **Ankita Singh (217804)** student of M.Sc. Biotechnology, IVth Semester, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Solan, Himachal Pradesh, during January to May 2023 as presented in the report was under the guidance and supervision of **Dr. Tiratha Raj Singh** Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Solan, Himachal Pradesh. The project entitled "**Investigations on microglial genes for their assertive role in Alzheimer's Disease**" is therefore being forwarded for acceptance in partial fulfillment of the requirements for the award of M.Sc. Biotechnology of Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Solan, Himachal Pradesh.

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Ankita Singh (217804)

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
BBB	Blood Brain Barrier
CNS	Central nervous system
FANMOD	Fast Network Motif Detection
GWAS	Genome wide association study
HDL	high-density lipoprotein
IL- β	Interleukin-1β
KEGG	Kyoto Encyclopedia of Genes and Genomes
NFTs	Neurofibrillary tangles
SNPs	Single nucleotide polymorphisms
SIFT	Sorting Intolerant From Tolerant
SORL1	Sortilin-related receptor 1
TGN	Trans-Golgi network
TF	Transcription Factor
WHO	World Health Organization

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ABSTRACT

The most prevalent type of dementia is Alzheimer's disease (AD), which damages the CNS by forming beta-amyloid plaque and neurofibrillary tangles (NFTs). By the time AD is clinically diagnosed, neuronal death has already occurred in numerous brain and retinal locations. There is now no effective treatment for AD, which is an incredibly complex neurological condition that is spreading quickly around the globe. The search for a treatment for AD has recently been the focus of several initiatives. One of the most prominent indications of Alzheimer's disease is the activation of microglia, which are seen near amyloid plaques and NFTs. Research on human genetics reveals that microglia are important in the aetiology of AD. The microglia in the brain significantly express the bulk of AD-risk genes, and many of them are only expressed in certain circumstances. A growing body of research demonstrates that decreased microglial activation and impaired microglial responsiveness to beta-amyloid increase the likelihood of developing AD despite the microglia's protective role. The function of microglial genes has been demonstrated in this report, and co regulated genes have been identified for SORL1 by using iRegulon. By using FANMOD network motifs were detected. Deleterious SNPs have been identified using structural and sequence-based analyses. It is anticipated that this study will provide an insights for the management of AD and its associated regulatory processes.

Keywords: Alzheimer’s disease, Microglia, SORL1, Amyloid plaques, FANMOD, iRegulon.

CHAPTER-1 INTRODUCTION

Alzheimer's disease is an untreatable neurological condition. It is characterised by the gradual deterioration of brain regions needed for learning and memory. Over the course of months or years, the illness progressively gets worse, impairing a person's memory, logic, judgement, communication skills, and even capacity to carry out ordinary tasks [1]. AD, the most common type of dementia, usually has an impact on people over 65. In AD, extracellular and intracellular protein aggregations build up. The main component of amyloid plaques is the 39–42 amino acid peptide known as amyloid beta. Synaptic dysfunction causes PHF and NFT to accumulate, which ultimately causes neuronal death. Our communities and wellness financial systems are being increasingly burdened by dementia and other diseases of cognitive decline. In the most recent Global Burden of Disease Report from the World Health Organization (WHO) [2]. When German physician Alois Alzheimer conducted an autopsy on a lady who had memory and language problems, it is believed that he made the first official discovery of AD. AD originally manifested in the patient's cerebral cortex as abnormal neurofibrillary tangles and senile plaques [3]. Cognitive impairment is brought on by these clinical symptoms of Alzheimer's disease, which result in neuronal dysfunction, neurotoxicity, and inflammation. Several AD diseases, including the emergence of tau-containing intra cellular neurofibrillary tangles and the production of amyloid-beta plaques within neurons, begin to develop decades before symptoms show up [4]. The amount of neuropathology increases as Alzheimer's disease worsens, causing ventricular enlargement and cortical shrinkage, which reduces the overall mass of the brain by 35%. The para-hippocampal regions are crucial for creating new memories early in the disease, but as it progresses, they lose neurons and synapses [3]. An immune reaction in the spinal cord or brain is referred to as "neuroinflammation." These messengers are produced by local CNS glia, endothelial cells, and peripherally derived immune cells. These responses have biochemical, psychological, and physiological immune effects. The context, progression, and duration of the initial stimulus or insult determine the degree of neuroinflammatory responses [2]. The primary purpose of neuroinflammation, which is the mind's triggering of the innate immunity system, is to defend the CNS from contagious slurs, harm, or disorder. It is well known that neurodegenerative diseases and ailments like Alzheimer's disease are actively influenced by neuroinflammation [5]. These include the activation of peripheral immune cells as well as the proinflammatory cytokines

produced in the brain. Each of these elements, alone or in combination, has the potential to cause neurons in the brain to deteriorate and eventually lead to neurodegeneration [6]. The primary purpose of neuroinflammation, which is the brain's triggering of the body's immune response, is to defend the CNS from infectious insults, harm, or illness [7]. A number of molecular and cellular changes, the stimulation of peripheral lymphocytes, the activation of specific intracellular signaling pathways, and the production of inflammatory mediators into the brains are all components of this complex process. Each of these variables, whether acting separately or in combination, can cause neuronal dysfunction and fatality in AD [8]. Chronic aseptic low-grade inflammatory response, also known as inflammation, is a feature of aging. Inflammation is characterized by immune senescence, cellular senescence, and mitochondrial dysfunction. Meta inflammation can be caused by chronic overnutrition or obesity [9]. Over the years of research, many therapeutic objectives have been employed to either cure Alzheimer's disease or alleviate its symptoms. One of the most researched methods of treating AD is beta-amyloid clearance through passive or active immunization, but so far, this approach has proven to be ineffective and even harmful [10]. Numerous brand-new medications under development aim to alter the course of the disease by affecting one or several of the numerous, extensive brain changes brought on by AD. These alterations present possible candidates for new medications that aim to halt or slow the progression of the disease. The fact that AD is a multisystem condition is now widely acknowledged [8]. The neuroinflammation method is not caused solely by innate immunity; rather, it is also triggered through other CNS resident molecules, collectively called microglia, neurons, and endothelial cell [11]. Inflammation, or the body's response to injury, is one of the key mechanisms in the development and aggravation of Alzheimer's disease (AD). Inflammation works to eliminate the original injury's cause as well as the soft tissue infections and cell debris that resulted from it [12]. An estimated 4 million people in India have dementia of some kind, and by 2050 it's anticipated that this number will have tripled. AD accounts for 60-70 percent of all dementia cases worldwide [13]. AD is presently the 7th main cause of death in the US [14]. One person worldwide develops dementia every three seconds, a surge in relative risk that reflects the disease's effects. Finding a therapy and a cure for this illness is urgently needed [15], [16]. A variety of factors contribute to the disease, including oxidative stress, amyloid beta accumulation, inflammation, tau phosphorylation, lipid dysregulation, and mitochondrial dysfunction. In the case of amyloid beta accumulation, the beta-amyloid proteins bind to neuronal cell receptors and are

internalized, resulting in the formation of senile plaques [17]. Researchers have attempted to develop a cure for Alzheimer's using amyloid-based medicines, such as medication detection and immune regulation. Researchers also considered tauopathies, which are caused by excessive production of the microtubule-stabilizing protein tau inside nerve cells, as a potential treatment [18], [19]. The main events may or may not involve molecular processes. Surgical or traumatic events may also set off the inflammatory apparatus. According to the microglial priming model, the presymptomatic AD pathology, which is located in small levels of proinflammatory mediators, may have long-term effects on microglia in AD pathology, which is connected with low levels of proinflammatory mediators, may have long-term effects on microglia. Additionally, inflammation, infection, and stress may act as secondary stimuli that alter these primed cells, causing them to become activated and start an inflammatory response that aids in the pathogenesis of AD [20].

OBJECTIVES:

1. Computational investigations to identify master regulators and other regulatory targets for SORL1.

2. Identification and analysis of network motifs for AD pathway.

3. To identify the SNPs using structure and sequence based tools of SORL1 gene and carry out its analysis in order to determine its crucial role in AD

CHAPTER-2 REVIEW OF LITERATURE

2.1 Microglia

Any discussion of neuroinflammation should center on the microglia. These cells are resident CNS cells and can be identified in the white and grey matter in the spinal cord and brain. The major immune monitoring of the CNS is carried out by these innate immune cells, which also perform macrophage-like tasks such as generating cytokines and chemokines. 10% of Central Nervous System's population are microglia [21]. Actually, macrophages, the body's other long-lived tissues, and microglia share the same origin. This hypothesis is supported by the observation that, over the course of a person's lifetime, myeloid cells in the bone marrow differentiate into long-lived cells called microglia with a low rate of turnover [22]. Microglia that are concentrated all around amyloid plaques in the brain are numerous and stimulated, which is one of the most obvious signs of AD. Research on genetic recombination reveals that microglia are crucial in the emergence of AD. The brain's microglia express the greatest risk genetic variants for AD, many of which are only expressed under specific conditions. Since altered microglial responses to beta-amyloid and decreased microglial activity are associated with a higher risk of acquiring AD [5]. The unpredictable morphological characteristics and microglia and astrocyte proliferation are reflected in the reactive gliosis of AD histopathology. Astrogliosis and microgliosis are frequently seen in a variety of neurodegenerative illnesses with different etiologies. However, it is uncertain whether these histopathological changes signify glial cells' helpful, detrimental, or minimal role in the neurodegenerative process. The embryonic developing embryo is where erythromyeloid neural stem cells mature into innate immune cells known as microglia, which are present in the CNS [21].

2.2 Indication from human biology implicating microglia in late-onset AD

The production of amyloid beta, its collection, and the development of amyloid plaques are considered to be to some of the major pathogenic factors for Alzheimer's disease. Ten or two years before AD symptoms appear [23]. According to dominant inheritance, the amyloid precursor protein or its production enzyme mutations are the genetic causes of autosomal AD with early onset [21]. However, inherited genetic AD is extremely uncommon; most AD cases are "sporadic" and manifest later in life. Aging and a combination of environmental and genetic factors, including those that affect people's capacity to clear amyloid beta, appear to be the main causes of the late

onset of AD. In the past ten years, human genetic studies, notably GWASs employing SNPs, have discovered more than 20 genetic regions that are highly related to the risk of AD. APOE is a gene, that generates the apolipoprotein E variations apoE3, apoE4, and apoE2, and has three major alleles, that are responsible for the genetic risk for AD. It is possible to explain a significant percentage of the genetic risk for sporadic AD using the APOE gene, which has 3 main alleles and encodes the apolipoprotein E subtypes apoE3, apoE4, and apoE2. A unique apoE4 or apoE2 allele bestow a roughly 3-fold enhanced or roughly doubling decreased chance of developing AD relative to the most prevalent apoE3 version, in both. Amyloid beta plaques contain ApoE, the main protein in lipoprotein particles that resemble high-density transport lipids and lipoprotein HDL, cholesterol, and hydrophobic particles in the nervous system. In comparison it seems that apoE4 and apoE3 boost plaque formation and decrease approval of amyloid beta [24], [25].

The maintenance glial cells play a key role in brain development and control. Stress has the capacity to open up the BBB, enabling the recruitment of T-cells and other specialised immune cells from the periphery, which is an effective immune interventions therapy for dementia [26]. Understanding the interaction with glial and peripheral immune cells is necessary for effective immune intervention therapy for neurodegeneration [27]. Table: 1. shows both Immune responses, innate and adaptive, that have been connected to AD's damage and repair mechanisms [28].

Table : 1. Adaptive and Innate immunity in AD.

Disease	Innate immunity	Adaptive immunity
Alzheimer's Disease	Amyloid plaques express cytokines, complements, and chemokines. Amyloid beta stimulates innate immune expression in culture. Microglia contribute to the elimination of amyloid beta.	Activating the adaptive system to attack the amyloid beta peptide will help with clearance as a treatment strategy.

2.3 Glial cells interactions in AD

Microglia release a wide range of activated innate related genes in the early stages of the disease. Different levels of microglial activation's effects on the development of neurological diseases has identified [29]. Although astrocytes display a variety of functioning changes when the illness worsens, the effect of astrocytic and astrogliosis problems on the condition of neurons has long been limited. Since astrocytes have been found to express a wide variety of immune-related genes, this suggests that they may be a part of a deliberate immune-activated response to a neurodegenerative disorder. In Prion diseases, the mechanism by which this glial communication occurs has been uncovered [30]. When an infection is just getting started, activated microglia secrete a number of cytokines that cause astrogliosis. Astrocytes consequently promote chemokine expression, which results in local microglial proliferation and increased microglial activity [31]. More cytokines are released into the environment by reactive microglia, which leads to progressive astrogliosis. In disease, the glial trigger does not appear to gradually decline with time, it tends steadily grow over time, indicating that this feedback cycle is a permanent cycle specific to the glial [26]. Most recent research suggests that cytokines like IL-1β trigger astrogliosis syndrome, which results in AD neurodegeneration. Studies that have discovered evidence of microglial-mediated astrogliosis resolution emphasise the possible therapeutic value of focusing on the interaction among glial cell in Alzheimer's Disease [30], [32]. In contrast to normal cultures, a prion-infected nerve cell culture showed chemotactic properties when administered to mice,

according to a previous study [33]. This discovery demonstrates that microglia were attracted to the injected location by either neurons and astrocytes. Another investigation discovered that pro-inflammatory cytokine IL-1β is essential in the activation of astrogliosis, or the populations of microglia produce a remarkable amount of IL-1β when they are unwell [34], [35]. Different genes are expressed by microglia to develop the innate immune system. Due to the numerous immune-related genes they produce, astrocytes take part in the immune-triggered neurological diseases AD neurodegeneration results from the astrogliosis illness caused by IL-1β. The recruitment of microglial cells at the injection site may be mediated by neurons, astrocytes, or both. Because it is required for the activation of astrogliosis, IL-1β production is significantly increased during illness [24].

Table: 2. Microglial functions of Alzheimer's disease risk genes have been discovered through genome-wide associations.

Gene	**Function**	**Microglial activity and Alzheimer's Disease**
SORL1	Various receptors, including those for vesicular lipoprotein sorting.	Amyloid beta is directed to the lysosome by binding to it. Familial Alzheimer's disease is a rare variant in this field. high human affirmation of microglia.
TREM2	Ties ligands that are anionic or lipophilic.	involved in chemotaxis, phagocytosis, and cell viability. Illness changes affect how apoE and apoJ communicate.
ABCA7	Lipids are transported by an ATP-binding cassette transporter and a multipass transmembrane protein.	Resides in the phagocytic cup and is thought to be involved in membrane remodeling. Amyloid beta phagocytosis is

		impaired in mice lacking the Abca7 gene.
MS4A6A	Unknown function of a four-pass transmembrane protein from the MS4A family.	Like MS4A1 in MS4A2 and B cells in the microglial receptor complex, likely involved.
CD33	Sialylated ligands are bound, and phosphorylated ITIM draws in the phosphatase SHP-1.	The protective allele increases Amyloid beta absorption while lowering surface CD33 stages. The meta-analysis failed to replicate the Alzheimer's Disease association.
CR1	C1q and C3b/C4b are bound by complement receptor 1.	Recognizes targets for opsonization. disables C3b and C4b. Alzheimer's disease risk is increased by a modified version with additional C3b/C4b-binding domains.
HLA-DRB1/5	Class II protein of the major histocompatibility complex for extracellular antigen presentation	The host immune system may use intracellular adaptors.

2.4 Biological Proof for SORL1's Implication in AD

SORL1 is a gene that codes for a receptor on the membrane that has several domains that are engaged in sorting proteins among endosomes, the plasma membrane, and the trans-Golgi network. The most prevalent type of dementia, AD, is genetically linked to it. It has great associations with both the uncommon, typical, early-onset, and late-onset sporadic forms of AD hereditary variety. In 2007, the first genetic characterization of the SORL1 variant in AD was made using a specific gene method. In 6 cohorts with hereditary and random LOAD from different

ethnic origins, 29 SNPs at the SORL1 gene were associated with Alzheimer's disease. The odds ratios (ORs) for each of these unique SNPs ranged from 1.4 to 2.6. (especially in comparison to 14.9 for homozygosity for the APOE 4 allele) [36]. These connections were confirmed by a haplotype study using a moving window of three SNPs, which found independent associations between AD and the SORL1 5' and 3' clusters of SNPs. The 5' cluster in introns 6 of SORL1 is composed of SNPs 8, 9, and 10 (Figure- 1).

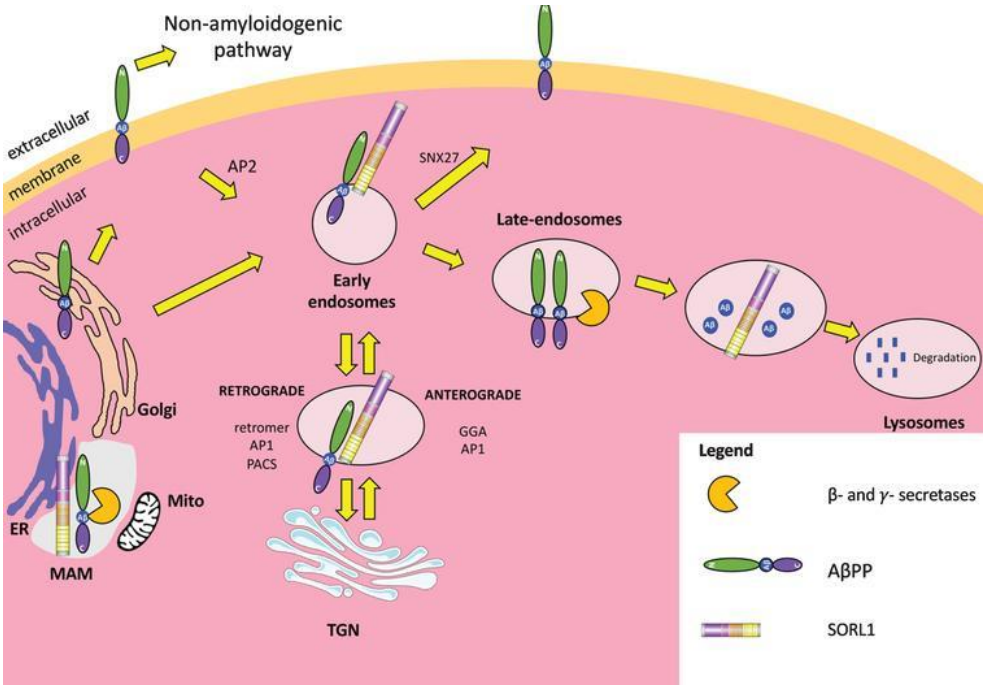


Figure-1. The secretory pathway for SORL1 begins in the endoplasmic reticulum (ER), wherein cleavage by the furin enzyme eliminates the pro-peptide. The receptor can then follow a signalling cascade or a trafficking route as it moves towards to the plasma membrane. The intracellular domain of the receptors has the capacity to enter the nucleus and regulate the transcription of unknown genes.

CHAPTER-3 MATERIAL AND METHODS

3.1 Data Collection

The coding region of the SORL1 gene was collected from the NCBI (https://www.ncbi.nlm.nih.gov/gene/6653). The transcription factor of SORL1 was obtained from Literature Review [37]. The KEGG pathway of Alzheimer's Disease was downloaded from KEGG server. The website dbSNP (https://www.ncbi.nlm.nih.gov/snp) provided information about SNPs. The dbSNP database was developed by the National Centre of Biotechnology Information to solve the issue of large-scale sampling design for gene mapping and evolutionary biology [38]. To screen for such mutations, SNP data were obtained from dbSNP. A total of 65423 SNPs were identified for the SORL1 gene, of which 1802 missense SNPs were selected for further analysis. The UniProt ID Q92673 was used to retrieve the gene's sequence [39]. Protein Data Bank (PDB) was used to retrieve the structure.

3.2 Regulatory Network Identification By Using iRegulon: A regulon is made up of cis-regulatory control elements with shared TF binding sites and includes its direct transcriptional targets and transcription factor (TF). By using patterns found in a group of co-regulated gene, the iRegulon plugin aids in the identification of regulons. Cytoscape plugins run as an internet-connected, the server-side daemon was accessed by a Java client. MySQL is used to collect and access the motif based whole-genome rankings via the Python-based iRegulon server-side daemon. The client receives the outcomes following the submission of a gene set or network to the service. The user can review the motif identification findings, choose a TF from the prioritized collection of TFs, and direct regulator-target 'edges' and add upstream regulators to the input gene set or network under investigation. [40].

3.3 Network Motifs Detection By Using FANMOD: FANMOD is a tool for discovering what is known as network motifs in a network, which are small vertex-induced subgraphs that happen noticeably more frequently than in random networks, for an introduction to network motifs in general. FANMOD can look for network motifs with three to eight vertices in size. Additionally, it can analyze colored networks, enabling the user to add more data to the network than just connectivities. FANMOD is faster than similar programmes based on other algorithms in detecting motifs because it uses the so-called rand-esu method, especially for larger patterns. FANMOD is

a graphical interface that makes setting up the algorithm settings simple; the output can be converted to HTML [41].

3.4 Sequence-Based Tools for the Identification of deleterious SNPs

Various Sequence-based tools such as PANTHER, SIFT, Meta-SNP, Predict-SNP, SNAP2, PhD-SNP, SNP&GO.

1. PANTHER (http://www.pantherdb.org/tools)

PANTHER typically operates under the premise of how long an amino acid has been kept in a lineage that results in protein formation. More preservation time increases the possibility that the protein may change in function. PANTHER-PSEP (position-specific evolutionary preservation) is the name of this method of determination. Identification of SNPs involved in genetic variation that can cause human disease is a critical function of PANTHER-PSEP [42]. PANTHER determines the purpose of the proteins, a gene's product, through curated datasets of proteins from various families [43]. PANTHER frequently evaluates protein function using an evolutionary model that anticipates the effects of genetic variety. Currently, PANTHER consists of 5000 protein family trees, which are further divided into 30000 [39].

2. Meta-SNP (http://snps.biofold.org/meta-snp)

Meta-predictor of Variants Causing Disease. Protein input for Meta-SNP is needed, and it can either be in FASTA format or as the protein sequence. Additionally, a list of mutations with commas separating them is needed. The types of scoring systems include:

• For PANTHER, a mutation is anticipated to be harmful if the score is higher than 0.5.

• For PhD-SNP, a mutation is determined to be harmful if the score is higher than 0.5.

• For SIFT, a positive number indicates that the mutation is neutral if the score is more than 0.05.

• For SNAP, a score of more than 0.5 is considered to have detrimental effects and to be a sign of sickness.

• Meta-SNP: If the score is larger than 0.5, a negative effect is anticipated [44].

3. SNAP2 (https://rostlab.org/owiki/index.php/Snap2)

Multiple sequence alignment's extractions of evolutionary data provide a significant input signal for the predictions. Different sequences and their properties are used to differentiate between non-synonymous and synonymous. Scores range from -100 to +100, with -100 denoting strong neutrality and +100 denoting sick state [45].

4. PhD-SNP (http://snps.biofold.org/phd-snp/)

PhD-SNP is designed to determine whether a certain single point protein mutation is a neutral polymorphism or associated with a disease. The following inputs are needed: Protein Sequence, Position, New Residue, and Prediction [46].

5. SIFT (http://sift-dna.org)

A method called Sorting Intolerant From Tolerant (SIFT), which is based on sequence homology, can assess if altering an amino acid would have an effect on how a protein behaves [47].

6. Predict SNP1 (http://loschmidt.chemi.muni.cz/predictsnp)

To determine the impact of a mutation on a protein's function, Predict SNP performs a number of programs. A combination of MAPP, Predict SNP, Polypehn-1, PANTHER, and PhD-SNP results are examined. To determine whether a mutation is harmful or neutral, a scoring system is applied. If the value, is within the range of (-1,0) then the consequences of mutations are considered to be neutral. It is determined that mutations are harmful if the value is between 0 and 1 [48].

7. SNPs & GO (https://snps.biofold.org/snps-and-go/snps-and-go.html)

SNPs & GO is a precise method that uses protein sequences, evolutionary data, and Gene Ontology words to determine if a variant is associated with a disease. It beats other predictive techniques because it gathers in-depth framework data from protein sequences, evolutionary data, and function. To find the damaging SNPs, an SVM-based classifier is applied [49].

3.5 Structure-based tools to identify deleterious SNPs

The deleterious missense SNPs were identified using five structure-based methods.

1. I-Mutant

I-Mutant 2.0 is a tool that uses support vector machines (SVMs) to automatically forecast how single point mutations would affect protein stability.

Either the protein structure or—more crucially—the protein sequence are used as starting points for the predictions.

I-Mutant2.0 can be applied as a regression estimator to foretell the corresponding Delta Delta G values as well as a classification tool to foretell the direction of the protein stability change following mutation.

The accessibility of the protein structure and sequence determines which predictive mode can be chosen using the Web interface.

Even in situations where the atomic resolution of the protein structure is unknown, I-Mutant2.0 is a special and useful tool for protein structure [50].

2. CUPSAT

A technique to forecast changes in protein stability following point mutations is called CUPSAT. The prediction model analyses the amino acid environment surrounding the mutation site using the torsion angle distribution and amino acid-atom potentials. Additionally, different amino acid environments can be distinguished using the secondary structure and prediction model's solvent accessibility specificity [51].

3. DynaMut

To examine and visualise measuring the impact of mutations on protein dynamics and stability by sampling conformations caused by changes in vibrational entropy, researchers use a web service called DynaMut [52].

4. Align-GVGD

A-GVGD combines MSA of orthologous sequences with the Grantham distance to classify missense variations or to identify human disease susceptibility missense alterations from modifications of low clinical importance. A Grantham Difference score (GD) is derived from the differences between these characteristics and those of the variant amino acid under consideration, and a Grantham Variation score (GV) is derived from the biochemical variation for every alignment site. Depending on how likely it is to result in a function-interfering modification, the expected consequence is categorized as C0, C15, C25, C35, C45, C55, or C65 [53].

5. MUpro

The purpose of the machine learning tool MUpro is to forecast how single-site amino acid changes would affect the stability of proteins. Support Vector Machines and Neural Networks, two machine learning approaches, were trained on a sizable mutation dataset and had an accuracy of 84%. This method outperformed previous approaches in the literature, as it can forecast changes in protein stability without the need for tertiary structures [54].

3.6 Conserve regions analysis of SORL1: Based on the evolutionary relationships between homologous sequences, a bioinformatics tool called ConSurf manages the process of detecting conserved portions in nucleic acids or amino acids. As a result, regions that are crucial to the makeup and functionality of proteins are discovered. For the query sequence, a multiple sequence alignment is done, and a phylogenetic tree is made to show an evolutionary relationship. Then, using this knowledge, the rate of evolution at each site within the sequence is calculated [55].

3.7 Interaction analysis of SORL1: The STRING database, a substantial collection of known and anticipated protein-protein signalling links, will be used for interaction analysis. This strategy is appropriate for the inquiry at hand because gene changes surely affect how the network's supporting interactions and related functions function. The STRING db was used to perform SORL1 interaction analysis. Homo *sapiens* was chosen as an organism and SORL1 was entered as the input name. The result was a node and edge representation of proteins and interactions [56].

3.8 Evaluating the structure effect of mutations: The S383F, A338V, Y258C, R176W, and V116M alterations were visualized using the HOPE (Have Your Protein Explained) server65, and some understanding of the mutations' structural effects was also gained [57].

CHAPTER-4 RESULTS AND DISCUSSION

4.1 Regulatory Network Identification

Gene Regulatory networks have been detected. HIF1A is a Transcription Factor of the SORL1 gene and master regulators. Arrows are regulatory networks the of SORL1 gene shown in figure-2. In Table – 3 on the basis of rank it has been mentioned AUC, Motif ID, CLUSTER CODE, TF, NES, and Target genes.

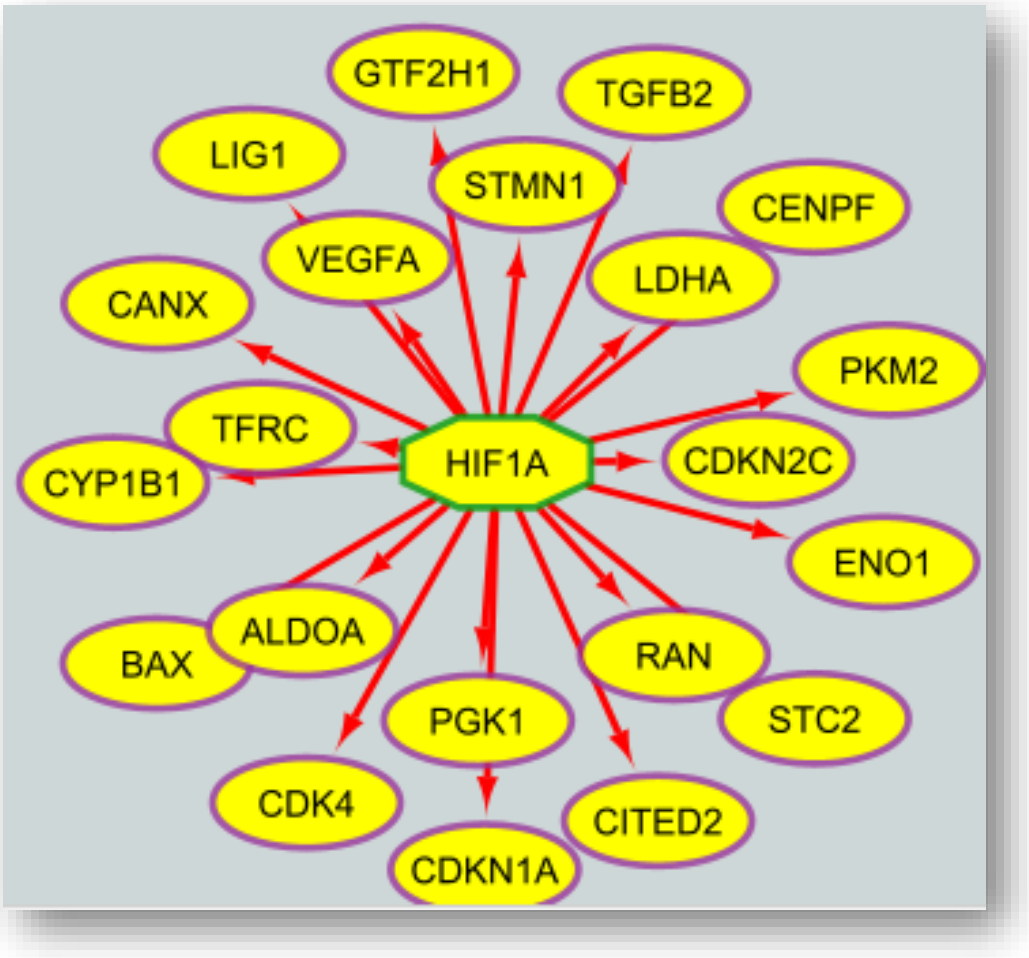


Figure -2. Regulatory Networks of SORL1

Table:3. Representation of Regulatory networks on the basis of rank

Rank	Motif ID	AUC	NES	CLUSTER CODE	Transcription factor	Target genes
1	homer-M00083	0.383476	9.28113	M1	HIF1A	LIG1,CITED2,CDKN2C,BAX,ENO1,CDK4,PKM2,TFRC,GTF2H1,LDHA,STMN1,PGK1,STC2,TGFB2,CDKN1A,HIF1A
2	transfac_pro-M02012	0.367917	8.86589	M1	HIF1A ,ARNT	LIG1,LDHA,CITED2,STC2,TGFB2,BAX,CDK4,GTF2H1,STMN1,ENO1
3	transfac_pro-M00797	0.36452	8.77523	M1	HIF1A, ARNT,EPAS1	ENO1,LIG1,LDHA,CITED2,VEGFA,TGFB2,CANX,TFRC,STMN1,CENPF,HIF1A,RAN,CDKN2C,STC2
4	transfac_pro-M00976	0.304117	7.16321	M3	AHRR, ARNT2, HIF1A, RNT, AHR	LIG1,STC2,ENO1,CITED2,TGFB2,BAX,CYP1B1,TFRC,CDK4,CDKN1A,LDHA,VEGFA,HIF1A
5	transfac_pro-M00466	0.298342	7.00908	M1	HIF1A, ARNT	ENO1,LDHA,VEGFA,LIG1,TFRC,CITED2,STC2,PKM2,TGFB2,CENPF,HIF1A,CANX,CDKN1A,CDKN2C

6	transfac_pro-M02378	0.290053	6.78786	M4	ARNT,HIF1A, EPAS1	LIG1,CITED2,LDHA,STC2,ENO1,TFRC,TGFB2,STMN1,PGK1,HIF1A,GTF2H1,CDK4,CDKN1A,CDKN2C,ALDOA,PKM2,CENPF

4.2 Network Motifs Detection:

Formulae of significance profile (SP_i)-

$$SP_i = \frac{Z_i}{\sqrt{\Sigma_i Z_i^2}}$$

By using FANMOD it has been identified that the majority are three nodes motifs that is seven, and four node motifs are three, six nodes are two and seven nodes are one as shown in table- 4. In figure 3 significance profiles of different nodes has been identified. In Figure- 4 the nodes are genes and edges are directions. It shows activation and inhibition of different genes from Hub nodes.

Table: 4. shows the Different nodes and their Values.

Nodes	Motif ID	Z Score	P Value	Significance Profile
3	6	-1.2419	0.85	-0.3935
3	6	-1.2419	0.85	-0.3935
3	6	-1.2419	0.85	-0.3935
3	6	-1.2419	0.85	-0.3935
3	6	-1.2419	0.85	-0.3935
3	36	-1.4798	0.846	0.4689
3	12	0.23547	0.679	0.0746
4	**2116**	**1.9707**	**0.019**	**0.479**
4	**14**	**3.4897**	**0.002**	**0.848**
4	28	0.927	0.169	0.2253
6	**266352**	**3.0605**	**0.009**	**0.5394**
6	**62**	**4.7773**	**0.001**	**0.842**
7	**126**	**5.1386**	**0.001**	**1**

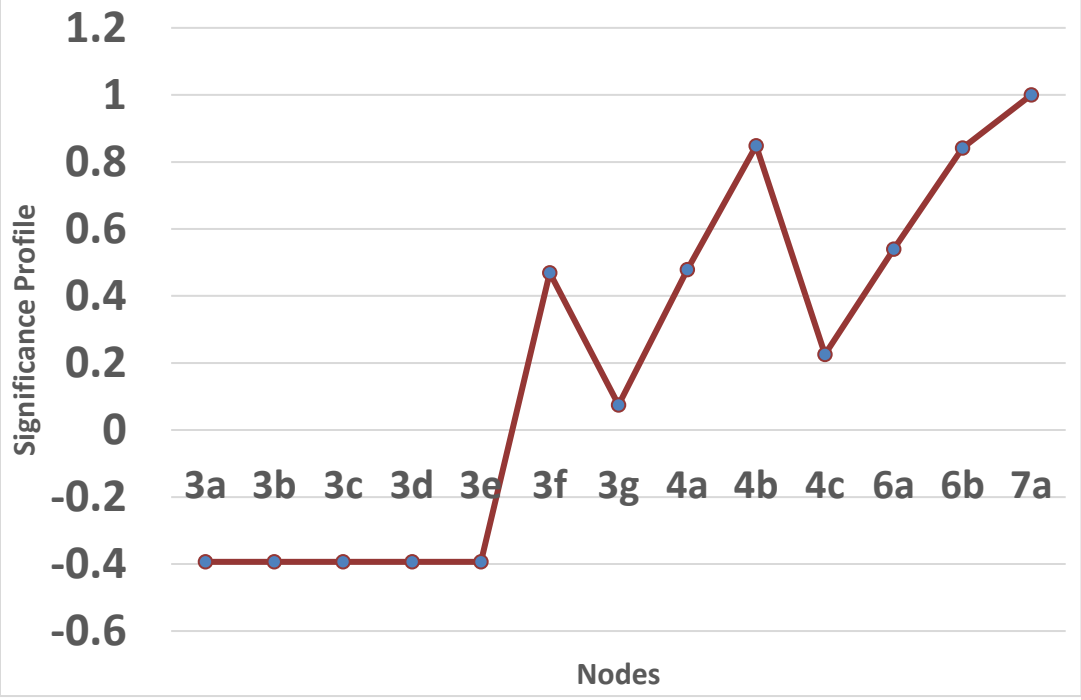


Figure - 3. Representation of the significance profile of different nodes

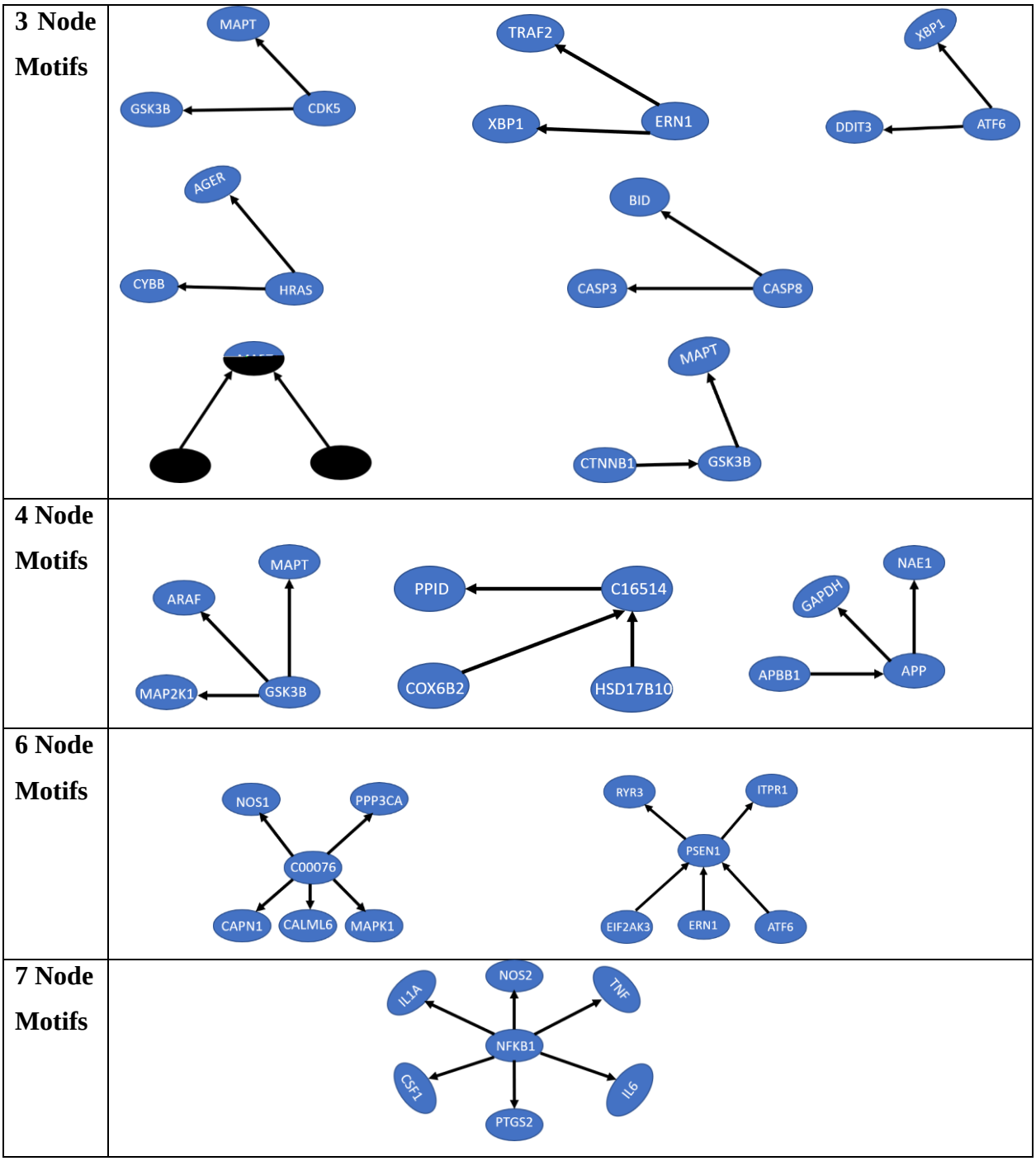


Figure- 4. shows the activation and inhibition of different nodes from Hub nodes.

Table: 5. Representation of Genes and its functions which are obtained in FANMOD

Gene	Chromosome Location	Protein	Function
MAPT	17q21	Tau	The MAPT gene directs the production of tau, which is present in nervous system and neurons [58].
GSK3B	3q.13.33	Glycogen synthase kinase-3 beta	Negative regulation of glucose homeostasis affects metabolic processes, mitochondrial malfunction, apoptotic pathways, and energy inflammation [59].
CDK5	7q36.1	kinase CK1	Neuronal cell death is caused by neurofibrillary tangles, synaptic malfunction, mitochondrial dysfunction, and cell cycle reactivation [60].
XBP1	22Q12.1	X-box-binding protein 1	XBP1 regulates memory function, improving AD-like pathology in the hippocampus [61].
TRAF2	9q34.3	TNF-alpha	Regulating physiological functions such as T and B cell signaling, organogenesis cell survival and inflammatory reactions [62].
ERN1	17q23.3	Serine/threonine-protein kinase/endoribonuclease IRE1	Contributes to multivesicular body information during ER stress, whileautophagy and unfolded protein response are also activated [63].
DDIT3	12q13.3	DNA damage-inducible transcript 3 protein	Protein acts as dominant-negative inhibitor by inhibiting other C/EBP members [64].

ATF6	1q23.3	ATF6	Cleaved by proteases due to the buildup of misfolded proteins in the ER [65].
AGER	21	Advanced glycosylation end-product specific receptor	AGER molecules involved in inflammation, homeostasis, and Alzheimer's [66].
HRAS	11	H-Ras	HRAS is responsible for controlling cell division by transmitting messages to the nucleus through signal transduction [67].
CYBB	Xp21.1-p11.4	cytochrome b-245	The CYBB gene provides instructions for the production of a protein beta chain, which is essential for the immune system [68].
BID	22q11.21	Bax	The protein encoded by this gene forms heterodimers with BAX or BCL2 to control apoptosis, and is a mediator of caspase-8's mitochondrial damage [69].
CASP3	N/A	Caspase-3	Involved in AD brain plaque development, neuronal cell death, and functional impairment [70].
CASP8	2q33–2q34	Caspase-8	Connected to a variety of mechanisms, including memory, learning, microglia pro-inflammatory activity and synaptic plasticity [71].
TUBB3	16	beta-tubulin	The beta-tubulin protein is made using instructions from the TUBB3 gene, which creates and arranges microtubules in cells [72].

TUBA1B	12q13.12	Tubulin alpha-1B	Microtubules expand by adding GTP-tubulin dimers to a stabilizing cap, which is GDP-bound [73].
CTNNB1	3p22.1	beta-catenin	Beta-catenin is a protein made by the CTNNB1 gene, found in many cell and tissue types, and is found at junctions that link nearby cells [74].
MAP2K1	15q22.31	MEK1 protein kinase	Protein is a component connecting the nucleus to chemical signals from the outside [75].
ARAF	Xp11.3	Raf protein	Activates cell proliferation and cell cycle progression [76].
PPID	4	tau and alpha-synuclein	Alzheimer's disease, miRNA impacts, and apoptosis in synovial fibroblasts are all linked to PPID [77].
APBB1	11p15.4	Thymidylate synthase	The thymidylate synthase gene is believed to control transcription and stop the progression of the cell cycle in Alzheimer's disease [78].
APP	21q11	Amyloid precursor protein	The APP gene produces the amyloid precursor protein, which is found in the CNS [79].
GAPDH	12p13.31	Sirtuin-1	GAPDH interacts with proteins linked to neurodegenerative diseases, leading to decreased activity in Alzheimer's disease [80].
NAE1	16q22.1	NEDD8-activating enzyme E1	Beta-amyloid precursor protein interacts with NAE1, allowing it to transmit signals and play a role in the aetiology of AD [81].

NOS1	12q24.22	Nitric oxide	Nitric oxide increases oxidative and nitro-oxidative stress, leading to mitochondrial dysfunction and apoptosis, leading to cognitive and functional decline in AD [82].
PPP3CA	4q24	A-kinase anchoring protein	The serine/threonine protein phosphatase is involved in the recycling of synaptic vesicles and is dependent on calcium and calmodulin [83].
CAPN1	11q13.1	Calpain-1 catalytic subunit	The brain controls a larger range of genes than the muscle, which are involved in AD and protein quality regulation [84].
CALML6	1p36.33	Calmodulin Like 6	Neurofibrillary tangle development involves three calmodulin-binding proteins, but only a small number have been experimentally confirmed to do so [85].
MAPK1	22q11.22	scaffold protein	Dephosphorylation of MAPKs at their tyrosine and threonine residues is a key regulator of MAPK signalling [86].
RYR3	15q13.3-q14	Ryanodine receptor 3	Intracellular Ca2+ regulates autophagy, a cellular process that causes cytosolic proteins and organelles to degrade in the lysosomes [87].
PSEN1	14q24.3	presenilin 1	PSEN1 is a component of gamma-secretase affects APP cleavage, Notch

			signalling, beta-cadherin processing [88].
ITPR1	3p26.1	Inositol 1,4,5-Trisphosphate Receptor Type 1	ITPR1, most prevalent isoform of ITPR in the brain, is linked to neurological illnesses with the highest number of human mutations [89].
EIF2AK3	2p11.2	PERK	The endoplasmic reticulum regulates proinsulin trafficking and quality control by acting as a metabolic sensor in beta-cells [90].
NFKB1	4q24	NF-kappa-B p105	Noncanonical and canonical pathways trigger NF-κB signalling, with the canonical route being important for inflammatory responses [91].
NOS2	17p13. 1	lipopolysaccharide	Nitric oxide is a biological mediator that is produced by nitric oxide synthase, which is activated by cytokines and lipopolysaccharide [92].
IL1A	2q14.1	Interleukin-1 alpha	IL-1 overexpression is linked to the development of beta-amyloid plaque in the Alzheimer's brain, and it interacts with other genetic risk factors [93].
CSF1	1p13.3	colony stimulating factor 1 receptor (CSF-1 receptor)	CSF1 mutations cause adult-onset leukoencephalopathy with axonal spheroids and pigmented glia and other neurodegenerative diseases [94].

PTGS2	1q31.1	prostanoids	PTGS2 is a key factor in AD progression, mimicking lowered neurite outgrowth and improved cell apoptosis in a PC12 cellular AD model [95].
IL6	7p15.3	Interleukin-6	IL-6 stimulates microglia and astrocytes, leading to the release of proinflammatory cytokines and C-reactive protein in the AD brain, which is responsible for development of AD .
TNF	6p21.33	Transmembrane TNF-α	TNF-alpha levels are linked to cognitive decline in AD, promoting amyloid beta synthesis, neuronal loss, and cell death [96].

4.1) SNPs for SORL1 GENE in dbSNP

Missense SNPs 1802 were chosen for screening out of a total of 65423 SNPs that were collected from dbSNP in order to find the harmful SNPs linked to disease. Missense SNPs are SNPs found in coding areas that have an impact on the protein's function and structure.

4.3 Analysis using sequence-based tools

An 1802 missense SNPs were taken and screened using sequence-based tools: PANTHER, SIFT Meta-SNP, PhD-SNP, PredictSNP1, SNAP2, and SNPs&GO. The SNPs were screened in order to identify the deleterious nature of SNPs.

Table : 6. Total number of deleterious SNPs analyzed in various sequence-based tools

TOOLS	Total Number of Deleterious SNP
PANTHER	1227
Ma-SNP	420
SNAP	337
PhD-SNP	315
SIFT	139
PredictSNP1	116
SNPs&GO	26

Table:7. List of ns SNPs predicted to be deleterious using seven sequence-based techniques.

Variant ID	Mutations	PANTHER	Ma-SNP	SNAP	PhD-SNP	SIFT	Predict SNP1	SNPs&GO
rs79037187	R1936C	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs143536682	S2175R	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs199717181	G1536S	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs372116149	D1267E	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs372549539	D1449E	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs564006388	V116M	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs752726649	T588I	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs753082593	S383F	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease

rs767645899	C1471Y	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs946548088	E1990G	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs1379473559	D2207G	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs1483653291	A338V	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs2134858130	S474P	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs2134948229	L1996M	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs140814048	R1684C	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs144806633	D2117Y	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs201415809	G1524R	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs201465902	Y258C	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs367544750	T1814I	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs375087515	L2119P	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs376229351	C1471R	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs376573235	C1296Y	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease

rs546537917	R176W	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs745596761	G1440R	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs745596761	G1440W	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease
rs745984652	E1903G	probably damaging	Disease	Effect	Disease	Deleterious	Deleterious	Disease

RESULT: Out of 1802 nsSNPs, only 26 were found to be deleterious in all seven-sequence-based tools. These nsSNPs were then further screened using structure-based tools.

4.4 Screening using structure-based tools

The 26 nsSNPs were further screened using structure-based tools. Prediction was carried out using 5 structure based tools i.e. I-Mutant, DynaMut, Align-GVGD, MUpro, CUPSAT. Out of 26 missense SNPs only 5 missense SNPs were obtained to be deleterious in all five tools.

Table: 8. List of missense SNPs expected to be deleterious by using 5 Structure based tools.

Variant ID	Mutation	I-Mutant	CUPSAT	Dynamut	Align GVGD	MUpro		
						SVM[a]	SVM[b]	Neural Networks
rs201465902	Y258C	Decrease	Destabilising	Destabilising	Most Likely	Decrease	Decrease	Decrease
rs546537917	R176W	Decrease	Destabilising	Stabilising	Most Likely	Decrease	Decrease	Decrease
rs564006388	V116M	Decrease	Stabilising	Destabilising	Less likely	Decrease	Decrease	Increase
rs753082593	S383F	Increase	Destabilising	Stabilising	Most Likely	Decrease	Decrease	Decrease
rs1483653291	A338V	Increase	Destabilising	Stabilising	Most Likely	Decrease	Decrease	Increase

4.5 Conserve regions analysis of SORL1:

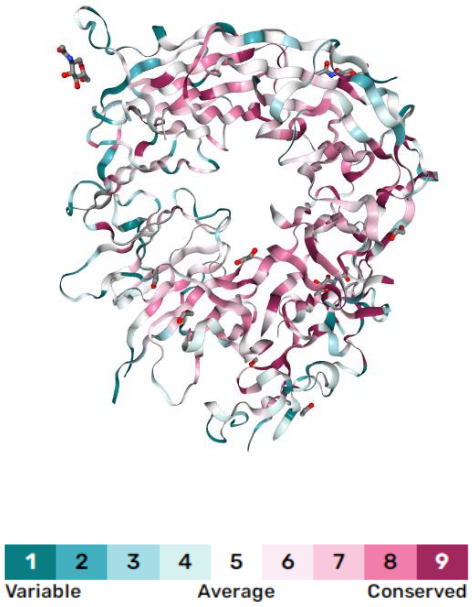


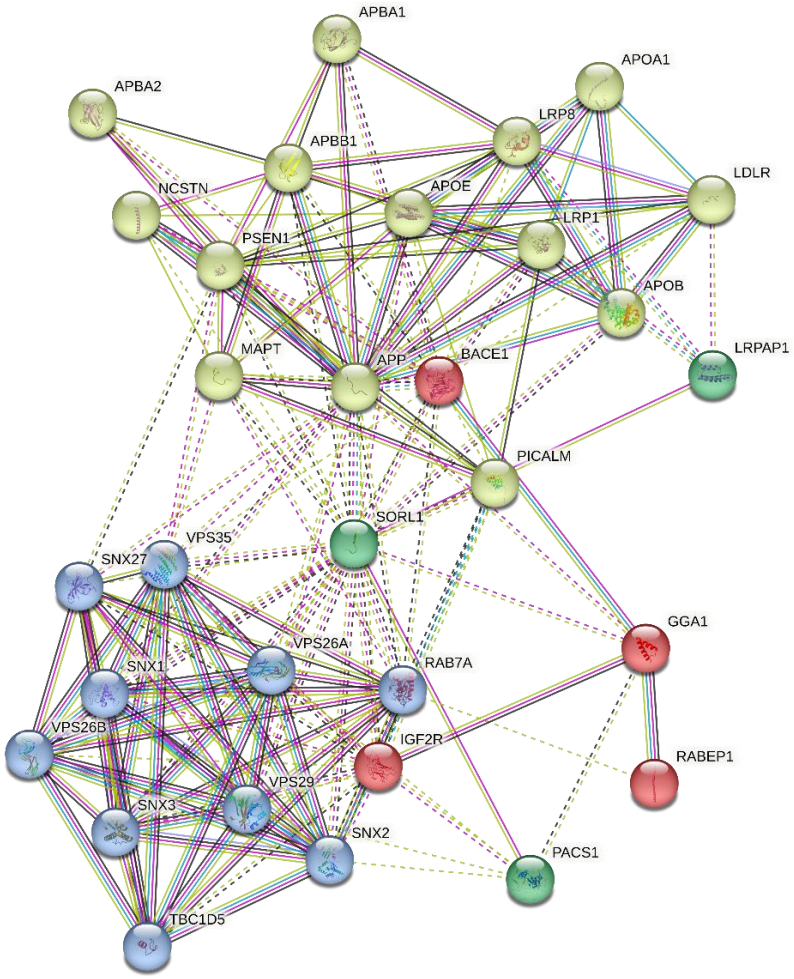
Figure-5 shows conservation regions of SORL1

The least conserved residue in this diagram has a value of 1, while the most conserved residue has a score of 9. Protein structure 3WSX was provided as an input to ConSurf. for analysis of conservation regions. ConSurf created a scoring table for the five SNPs that were chosen using structure-based methods, and it was discovered that four SNP was conserved throughout evolutionary time. There was conservation at these sites.

Table: 9 Conservation regions and score of SORL1

Position	Residue	Conservation Score
383	S	8
338	A	8
176	R	8
116	V	9

4.6 Interaction analysis of SORL1:

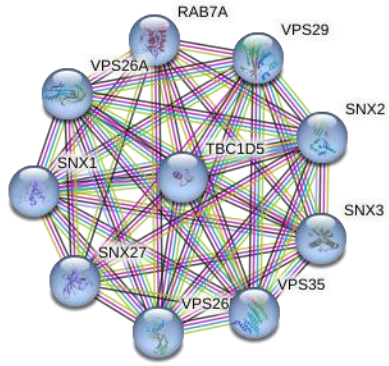


Nodes Number : 31

Edges Number : 169

Avg. Node degree: 10.9

Average local clustering coefficient : 0.686



Nodes Number : 10

Edges Number: 45

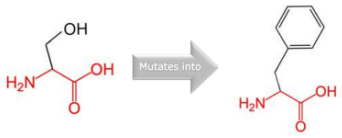
Avg. Node degree: 9

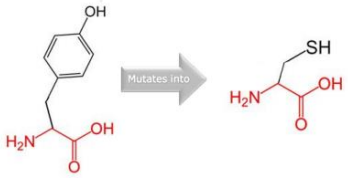
Average local clustering coefficient: 1

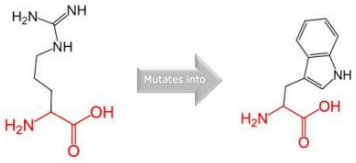
Figure-6 shows the interaction analysis of SORL1

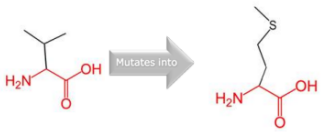
4.6 Evaluating the structure effect of mutations:

Table : 10. Effects of Structural Changes Obtained through the HOPE Server on SORL1

Mutation	Structure Alteration	Properties of Amino acids	Domain
S383F		Sizes of the wild-type and mutant amino acids vary. The mutant residue is larger than the residue of the natural type. The residue is found on the protein's surface, and its mutation may interfere with how the protein interacts with other molecules or with other regions of the protein. The hydrophobicity of the mutant and wild-type residues is different.	A domain with an unidentified function's surface contains the mutant residue. In the employed structure, the residue was not discovered to be in touch with any other domains whose function is known. Even so, this mutation may have an impact on interactions with other molecules or domains.
A338V		Sizes of the wild-type and mutant amino acids vary. The mutant residue is larger than the residue of the natural type. The	The core of a domain contains the residue. The essential structural integrity of this domain may be disturbed by the

		protein's central region was where the wild-type residue was hidden. Since the mutant residue is larger, it probably won't fit.	variations between the wild-type and mutant residue.
Y258C		The sizes of the mutant and wild-type amino acids are different. More compact than the wild-type residue is the mutant residue. This could result in a lack of interactions with the outside world. The wild-type and mutant residues have different hydrophobicities.	The altered residue is found in a region of the protein that is crucial for the binding of other components. Contact exists between the altered residue and residues from a different domain. The mutation might interfere with these interactions. The mutant residue is situated in a region crucial for other molecules to bind to it, and it is in touch with residues in another region equally crucial for binding. The mutation might

			impede the communication between these two domains, which would impact the protein's functionality.
R176W		Wild type amino acid and the mutant amino acid have different charges. This mutation eliminates the charge from the wild-type residue. Interactions between molecules may be lost as a result of this. The sizes of the wild-type and mutant amino acids vary. The mutant residue is larger than the residue of the natural type. The residue is found on the protein's surface, and its mutation may interfere with how the protein interacts with other molecules	The altered residue is found in a region of the protein that is crucial for the binding of other components. Contact exists between the altered residue and residues from a different domain. The mutation might interfere with these interactions. The mutant residue is situated in a region crucial for other molecules to bind to it, and it is in touch with residues in another region equally crucial for binding. The mutation might

		or with other regions of the protein. The hydrophobicity of the mutant and wild-type residues is different.	impede the communication between these two domains, which would impact the protein's functionality.
V116M		The sizes of the mutant and wild-type amino acids are different. Greater in size than the wild-type residue is the mutant residue. The protein's centre concealed the wild-type residue. Because it is larger, the mutant residue probably won't fit.	The mutated residue is found in a region of the protein that is crucial for the binding of other components. Contact exists between the altered residue and residues from a different domain. There's a chance that the mutation messes up these contacts.

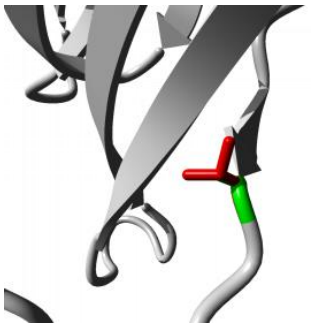
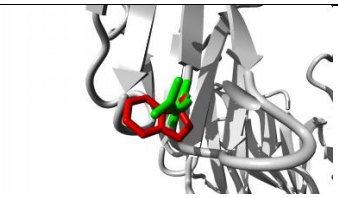
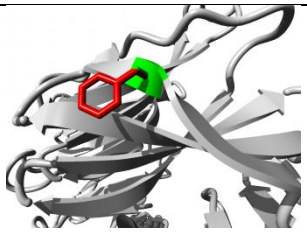
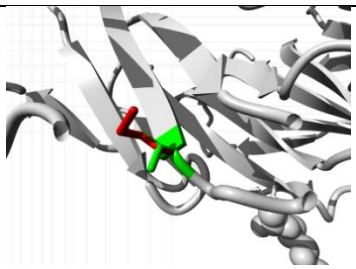
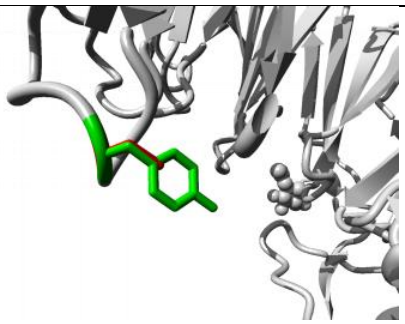
A338V	
R176W	
S383F	
V116M	
Y258C	

Figure-7. shows mutated amino acid residues (red) and wild-type residues(green).

DISCUSSION

By using iRegulon Gene Regulatory networks have been detected. HIF1A is a Transcription Factor of the SORL1 gene and master regulators. Arrows are regulatory networks of the SORL1 gene shown in figure-1. In Table – 3 on the basis of rank, it has been mentioned AUC, CLUSTER CODE, Motif ID, TF, NES, and Target genes. By using FANMOD it has been identified that majority are three nodes motifs that is seven, and four node motifs are three, six nodes are two and seven nodes are one as shown table- 4. In figure -3 significance profiles of different nodes has been identified. Less than 0.05 that is 4a, 4b, 6a, 6b, and 7 have proper Significance profile. In figure- 4 the nodes are genes and edges are directions. It shows activation and inhibition of different genes from Hub nodes. XBP1 gene is similar in iRegulon as well as FANMOD. Missense SNPs 1802 were chosen for screening out of a total of 65423 SNPs that were collected from dbSNP in order to find the harmful SNPs linked to disease. Missense SNPs are SNPs that are located in coding regions and have an effect on the structure and function of the protein. An 1802 missense SNPs were taken and screened using sequence-based tools: SNAP2, PANTHER, PhD-SNP, Meta-SNP, PredictSNP1, SNPs&GO and SIFT. To determine the SNPs' harmful characteristics, the SNPs were tested. Out of 1802 nsSNPs, only 26 were found to be deleterious in all seven-sequence-based tools. These nsSNPs were then further screened using structure-based tools. The 26 nsSNPs were further screened using structure-based tools. Prediction was carried out using 5 structure based tools i.e. I-Mutant, DynaMut, Align-GVGD, MUpro, CUPSAT. Out of 26 missense SNPs only 5 missense SNPs were obtained to be deleterious in all five tools. The least conserved residue has a value of 1, while the most conserved residue has a score of 9 in ConSurf. By using ConSurf protein structure 3WSX was given as an input for analysis of conservation regions. ConSurf created a scoring table for the five SNPs that were chosen using structure-based methods, and it was discovered that four SNPs were conserved throughout evolutionary time. There was conservation at these sites. The interactions of SORL1 was identified by STRING db which shows in Fig- 6. SORL1 was the input name, and Homo *sapiens* was chosen as an organism. The results were nodes and edges, which stand for proteins and interactions. With the aid of HOPE Server from table-10, Only Y258C exhibits mutant residue that is smaller than wild-type residue, the other mutant residues being R176W, V116M, S383F, and A338V are larger than the wild type residue.

The mutant residue Cysteine will not be stable in the protein's core, according to Y258C, because it is bigger than the wild-type residue Tyrosine.

CHPTER-5 CONCLUSION

In this research work, it can be concluded that by using iRegulon various target genes are obtained and HIF1A is the Transcription factor of the SORL1 gene. It can also be concluded that by using FANMOD three nodes have seven, four nodes have three, six nodes have two, and seven nodes have only one regulatory motifs which are statistically significant. It can also be concluded that out of 1802 missense SNPs, only 26 were found to be deleterious by using sequence based tools, and by using structure based tools only 5 SNPs were found deleterious. By using a conservation analysis server that is ConSurf, we identified that only four SNPs were found conserved, which can affect the function and structure of the protein. According to the HOPE Server, all mutant residues R176W, V116M, S383F, and A338V displayed mutant residues were larger than wild-type residues, while only Y258C shown mutant residues that were smaller. Since mutant residue Cysteine is larger than wild-type residue Tyrosine, it can be inferred from Y258C that it will not be stable in the protein's core. It is believed that the presented piece of information will be useful for the scientists working in this domain and it will also help to design new experiments for these biological entities such as gene, transcription factor, network motifs and SNPs, associated with AD.

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