Quantitative studies on gene regulatory pathways for Alzheimer's disease

Report submitted in partial fulfilment of the degree of

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In

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Under the Supervision of

Dr. Tiratha Raj Singh

By

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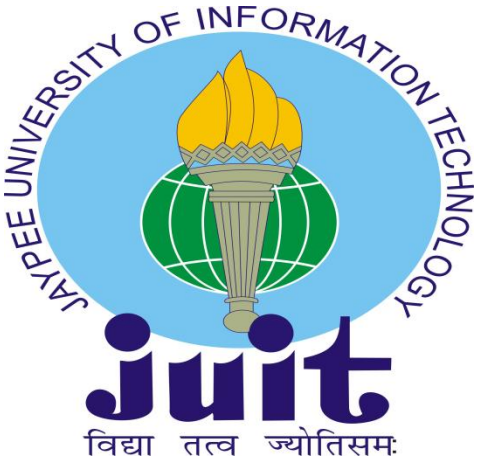
To



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DECLARATION

I hereby declare that the project titled "**Quantitative studies on gene regulatory pathways for Alzheimer's disease"** is submitted as a Project Work has been carried out by me at Jaypee University of Information Technology ,Solan under the guidance of Dr. Tiratha Raj Singh**.** Any further extension, continuation or use of this project has to be undertaken with prior express written consent from the Supervisor, Jaypee University of Information Technology, Solan-173234.

I further declare that the project work or any part thereof has not been previously submitted for any degree or diploma in any university.

Signature: Name:

Date:

ACKNOWLEDGEMENT

Though only my name appears on the cover of this dissertation, many people have contributed to its production. Every effort is motivated by an ambition and all ambition have an inspiration behind the height of reaching a milestone . First and above all I thank the almighty for bestowing upon me the courage to face the complexities of life, strength and patience to work through all these years so that today I can stand proud with my head held high.

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(GARIMA SANORIA)

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1. INTRODUCTION

Alzheimer's disease is a type of dementia an illness of the brain that affects a person's ability to carry out daily activities. Memory, emotions, mood, behaviour and language are all affected, and because the disease is progressive, the symptoms worsen over time. There are many forms of dementia, but Alzheimer's disease is the most common among older people. In the disease's early stages, people most often notice memory problems that can be severe enough to interfere with their ability to work or carry out everyday tasks. It's normal for people to forget some things as they get older mild forgetfulness or the occasional difficulty in finding a word is not necessarily cause for alarm. But in Alzheimer's disease, the change is more dramatic. People tend to forget things that they used to remember, like names, words, and where they've put everyday objects. More importantly, this difficulty is persistent, progressive, and severe, and there is usually a noticeable, rapid decline in cognitive skills. Alzheimer's disease is the most frequently seen form of dementia and it's frighteningly common in older people. In 2010, more than 500,000 Canadians were living with AD or a related dementia [1]. Of these, approximately 71,000 are under the age of 65. 1 in 11 Canadians over 65 has dementia . Women account for 72% of all Alzheimer cases, and 62% of all dementia cases [2, 3] . Today in Canada, over 450,000 people over the age of 65, and 1/3 of those over 85, have Alzheimer's disease or a related disease. What's more, as the country's 10 million baby boomers grow older, the number of people with the disease is expected to rise considerably [4].

Because the cause of Alzheimer's disease is still unknown, it's difficult to predict who will develop it in the course of their lifetime. Some risk factors, however, are known, the most important of which is age. In fact, after age 65 the frequency of all types of dementia just about doubles every five years [5-11]. By the time a person reaches age 85, they're at a 35% risk of dementia. People diagnosed with Alzheimer's disease may worry that they have passed the disease to their children, but this usually will not be the case. There are two types of Alzheimer's disease. One is a familial type. It is passed from one generation to another through a dominant gene. If one of your parents has this type of the disease, you always have a 50% chance of inheriting the gene and then developing the disease. The familial type of Alzheimer's disease is very rare and is seen in only 5-10% of cases. About 90% of cases

are the sporadic type of Alzheimer's disease [12]. You can develop this kind of Alzheimer's disease even if nobody in your family has had the disease, although having a family history still affects your chances of getting the disease, compared with someone with no Alzheimer's disease in their family the more family members who are affected, and the closer they are to you, the higher the risk for the disease. An important thing to note is that although dementia is different from "normal forgetfulness," there is a step before the disease occurs, a sort of in-between stage, called mild cognitive impairment. Alzheimer's disease (AD) is a devastating neurodegenerative disorder with a relentless progression. AD pathogenesis is believed to be triggered by the accumulation of the amyloid-β peptide (Aβ), which is due to overproduction of Aβ and/or the failure of clearance mechanisms. Aβ self-aggregates into oligomers, which can be of various sizes, and forms diffuse and neuritic plaques in the parenchyma and blood vessels. Aβ oligomers and plaques are potent synaptotoxins, block proteasome function, inhibit mitochondrial activity, alter intracellular Ca^{2+} levels and stimulate inflammatory processes. Loss of the normal physiological functions of Aβ is also thought to contribute to neuronal dysfunction [13,14]. Aβ interacts with the signalling pathways that regulate the phosphorylation of the microtubule-associated protein tau. Hyperphosphorylation of tau disrupts its normal function in regulating axonal transport and leads to the accumulation of neurofibrillary tangles and toxic species of soluble tau. Furthermore, degradation of hyperphosphorylated tau by the proteasome is inhibited by the actions of Aβ. These two proteins and their associated signalling pathways therefore represent important therapeutic targets for AD.

Microtubule-associated protein tau is abnormally hyper phosphorylated in the brain of patients with Alzheimer's disease, and is the major protein subunit of paired helical filaments. There is also a significant pool of non-paired helical filament abnormally phosphorylated tau in Alzheimer's disease brain. In the present study, the site-specific dephosphorylation of this Alzheimer's disease abnormally phosphorylated tau by protein phosphatase-2A was studied and compared with that by protein phosphatase-2B [15]. The dephosphorylation was detected by its interaction with several phosphorylation-dependent antibodies to various abnormal phosphorylation sites. Protein phosphatase-2A was able to dephosphorylate the abnormally phosphorylated tau at Ser-46, Ser-199, Ser-202, Ser-396 and Ser-404, but not at Ser-235 (the amino acids are numbered according to the largest isoform of human tau)[16,17]. Two major types of protein phosphatase-2A, protein phosphatase-$2A_1$ and -$2A_2$, dephosphorylated the abnormally phosphorylated tau at approximately the same rate. After the abnormally phosphorylated tau was

dephosphorylated by protein phosphatase-2A, its relative mobility on sodium dodecyl sulphate-polyacrylamide gel electrophoresis increased. The dephosphorylation of the abnormal tau by protein phosphatase-$2A_1$ and -$2A_2$ was markedly stimulated by Mn^{2+}.
These results suggest that tau dephosphorylation is catalysed by protein phosphatase-2A in addition to protein phosphatase-2B. A deficiency of either protein phosphatase-2A or -2B, or both, may be involved in abnormal phosphorylation of tau in Alzheimer's disease [18].

Because neurofibrillary degeneration plays a central role in the pathogenesis of AD, one of the most attractive therapeutic targets of AD is to inhibit neurofibrillary degeneration. the most promising approaches to achieve this goal are to inhibit the abnormal hyperphosphorylation of tau and to inhibit its sequestration of normal MAPs. The former approach is more effective since it should both rescue the disruption of microtubule and axoplasmic flow and prevent further deposition of NFTs. Several academic groups and pharmaceutical companies have been investigating this approach by restoring PP2A activity or inhibiting tau kinase activity in the brain. Memantine, a low-to-moderate-affinity antagonist of NMDA receptor, which improves mental function and the quality of daily life of individuals with moderate to severe AD , reverses the okadaic-acid-induced inhibition of PP2A activity and prevents tau hyperphosphorylation in hippocampal slice cultures from adult rats The restoration of PP2A activity to normal level by memantine also leads to restoration of the expression of MAP2 in the neuropil and a reversal of hyperphosphorylation and accumulation of neurofilaments [19-22]. Wang's group has demonstrated that treatment of brain slices and rats with melatonin can restore PP2A activity that is inhibited by okadaic acid or calyculin A and reverse hyperphosphorylation of tau and neurofilament proteins as well as cytotoxicities Melatonin also prevents tau hyperphosphorylation and aggregation induced by overactivation of GSK-3 or PKA. These are examples showing that inhibition of dysregulation of protein phosphorylation / dephosphorylation is a promising target to treat AD. Further investigation of new compounds that can inhibit abnormal hyperphosphorylation of tau will likely provide new treatments for AD.

2. REVIEW OF LITERATURE

More than 5 million Americans have Alzheimer's disease, which is the most common form of dementia accounting for 60 to 80 percent of all cases. That includes 11 percent of those age 65 and older and one-third of those 85 and older [22]. The disease also impacts more than 15 million family members, friends and caregivers.

2.1 Early History of dementia and alzheimers disease:

Refined definitions of psychiatric disorders, and particularly of dementia, were crucial for our understanding of different forms of dementias. Prior to the late 19th century, the definition of dementia was vague and the term usually referred to general mental deterioration associated with chronic brain disease.

By the end of that century dementia was largely synonymous with memory loss. Indeed, as early as 1838, Esquirol had defined several types of dementia, including a senile form, 'demence senile', which he said was associated with 'the progress of age and involved 'loss of memory especially recent memory'[23,24]. From the mid-19th century onwards, not only were efforts made to find pathological abnormalities associated with dementia (and other brain disorders), but also improved diagnostic techniques were available for both identifying and quantifying the severity of dementia . Until histological procedures were developed that allowed brain sections to be preserved and appropriately stained, attempts at discovering pathological changes in brain-associated diseases were based chiefly on gross anatomical changes, such as size, colour, mass, and relative amounts of fluid and solid material [25]. In 1822, for example reported changes in arachnoid in patients with motor and psychiatric symptoms, and numerous studies suggested that senile dementia was associated with cerebral cortical atrophy, enlarged ventricles and changes in the physical consistency of brain tissue.

With the advent of improved tissue storage, preservation and staining methods, combined with microscopy, scientists had at their disposal techniques for examining the brain with unprecedented detail, and many of them recognized that here was a chance to find pathological changes in mental disorders that may be too small to be detected using gross anatomical examination. During the last few decades of the 19th century, microscopic and

histological studies had allowed the visualization of necrotic brain cells, senile plaques and neurofibrillary tangles, even before Alzheimer described them in his first patient.

In 1882, Blocq and Marinesco described plaques (often called 'miliary foci' at the time) in the brains of an epileptic who had no dementia [26,27]. This is considered by many science historians to be the first ever description of neuritic plaques. Subsequently, similar plaques were found in the brains of patients with other diseases. For example, in 1898, Redlich observed plaques in brains of two senile patients with memory loss and confusion; both brains showed cerebral atrophy. In 1907, Fischer noted 'miliary necrosis' in the cerebral cortices of 12 out of 16 patients with senile dementia, but failed to find significant numbers of plaques in any of 45 patients diagnosed with progressive paralysis but having no dementia. Neurofibrillary tangles had also been detected in brain specimens before Alzheimer described them. Fragnito, in 1904, observed neurofibrillary degeneration and damage in cerebral cortical cells of brains from patients with senile dementia, and three years later Fuller also described neurofibrillary accumulations in senile dementia [28]. Alzheimer himself contributed significantly to the advances in preservation and staining of brain tissue and, by 1910, many methods were available for staining brain tissue; more than ten of them could detect neurofibrils. By the early 1900s, it was clear that at least some mental diseases were caused by pathological changes in the brain. Indeed, syphilis was one of the few mental diseases for which a specific physical cause was known [29,30]. Dementia due to cerebral infarcts caused by arteriosclerosis was also known to occur in elderly people, although other patients with dementia were found at autopsy to have general cerebral atrophy without arteriosclerotic lesions. It should be borne in mind that dementia was not as serious a public health problem in those days as it is nowadays: the life expectancy at the turn of the century was well below 65 years, so senile dementia was not so common [31]. However, it was known that dementia could occur at any age and 'presenile dementia' was a recognized disease entity. Freud's psychoanalysis movement was also powerful at the beginning of this century, and his ideas that mental illness was due mainly to disturbances of the subconscious mind were at odds with those who believed that such disorders were caused by specific pathological, cellular and chemical lesions. The great psychiatrist, Kraepelin, was one of the main proponents of the organic theory of mental illness and he was highly influential in the eventual definition of Alzheimer's disease.

2.2 Dementia:

Dementia is a general term for the loss of memory and other intellectual abilities serious enough to interfere with daily life.

2.3 Other types of dementia

2.3.1 Vascular dementia:

A decline in thinking skills caused by conditions that block or reduces blood flow to the brain, depriving brain cells of vital oxygen and nutrients [32]. These changes in thinking skills sometimes occur suddenly following strokes that block major brain blood vessels. It is widely considered the second most common cause of dementia after Alzheimer's disease.

2.3.2 Mixed dementia:

A condition in which abnormalities characteristic of more than one type of dementia occur simultaneously. Symptoms may vary, depending on the types of brain changes involved and the brain regions affected, and may be similar to or even indistinguishable from those of Alzheimer's or another dementia.

2.3.3 Parkinson's disease dementia

An Impairment in thinking and reasoning that eventually affects many people with Parkinson's disease. As brain changes gradually spread, they often begin to affect mental functions, including memory and the ability to pay attention, make sound judgments and plan the steps needed to complete a task.

2.3.4 Dementia with Lewy bodies

A type of progressive dementia that leads to a decline in thinking, reasoning and independent function due to abnormal microscopic deposits that damage brain cells [33,34].

2.3.5 Huntington's disease dementia

A progressive brain disorder caused by a defective gene. It causes changes in the central area of the brain, which affect movement, mood and thinking skills.

2.3.6 Creutzfeldt-Jakob disease

The most common human form of a group of rare, fatal brain disorders known as prion diseases. Misfolded prion protein destroys brain cells, resulting in damage that leads to rapid decline in thinking and reasoning as well as involuntary muscle movements, confusion, difficulty walking and mood changes.

2.3.7 Frontotemporal dementia

A group of disorders caused by progressive cell degeneration in the brain's frontal lobes (the areas behind the forehead) or its temporal lobes (the regions behind the ears) [35].

2.4 How alzheimer's affects the brain

The changes that take place in the brain begin at the microscopic level long before the first signs of memory loss. The brain has 100 billion nerve cells (neurons). Each nerve cell connects to many others to form communication networks. In addition to nerve cells, the brain includes cells specialized to support and nourish other cells. Groups of nerve cells have special jobs. Some are involved in thinking, learning and memory [36]. Others help us see, hear and smell. Still others tell our muscles when to move. Brain cells operate like tiny factories. They receive supplies, generate energy, construct equipment and get rid of waste. Cells also process and store information and communicate with other cells. Keeping everything running requires coordination as well as large amounts of fuel and oxygen. Scientists believe Alzheimer's disease prevents parts of a cell's factory from running well. They are not sure where the trouble starts. But just like a real factory, backups and breakdowns in one system cause problems in other areas. As damage spreads, cells lose their ability to do their jobs and, eventually, die.

2.5 The role of plaques and tangles:

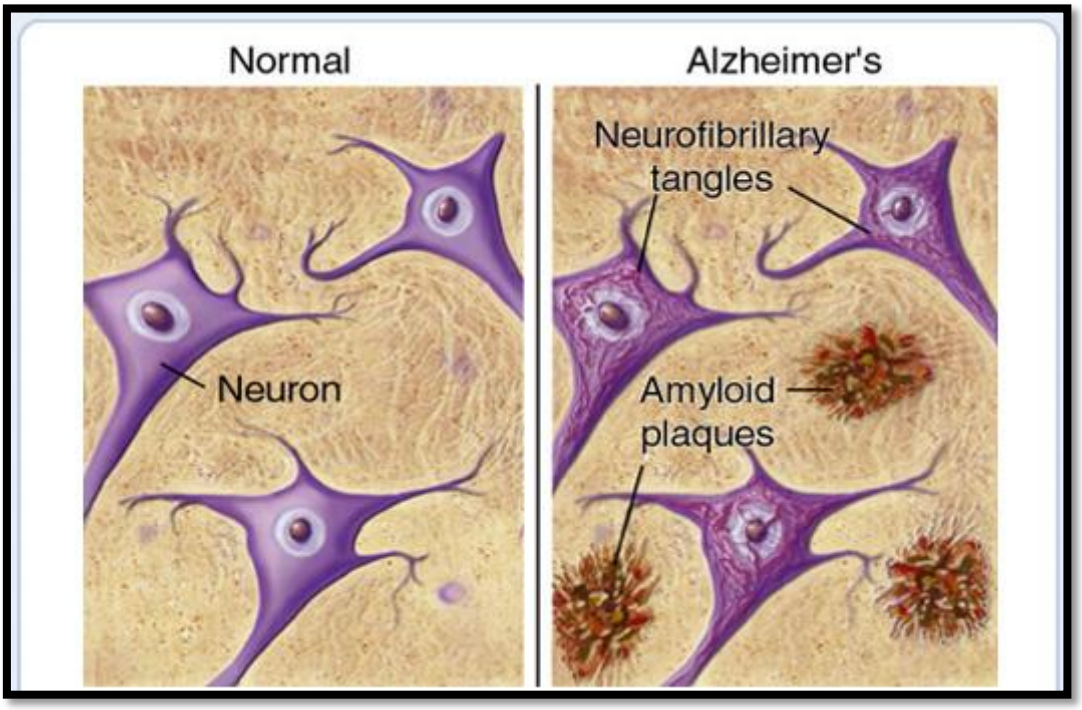


Fig 1: **How Alzheimers spreads in the brain.**

The brains of individuals with Alzheimer's have an abundance of plaques and tangles. Plaques are deposits of a protein fragment called beta amyloid that builds up in the spaces between nerve cells. Tangles are twisted fibres of another protein called tau that build up inside cells. Though autopsy studies show that most people develop some plaques and tangles as they age, those with Alzheimer's tend to develop far more. They also tend to develop them in a predictable pattern, beginning in the areas important for memory before spreading to other regions. Scientists do not know exactly what role plaques and tangles play in Alzheimer's disease. Most experts believe that they disable or block communication among nerve cells and disrupt processes the cells need to survive.

2.6 Phosphorylation of tau protein:

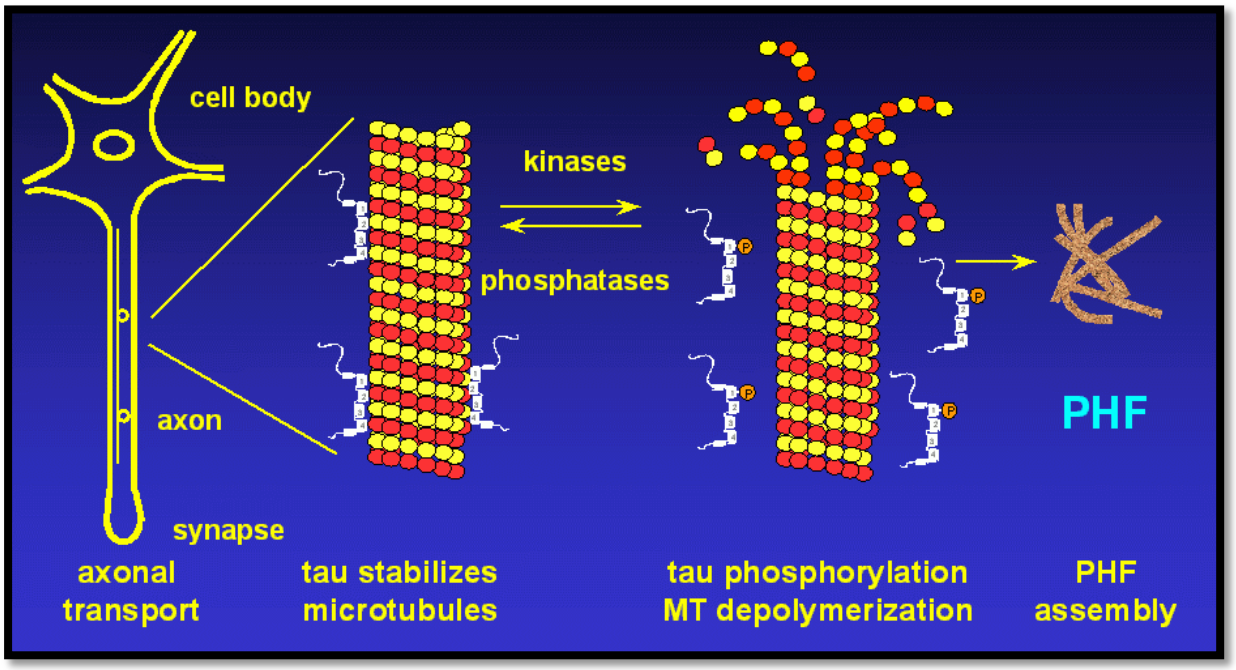


Fig:2 Phosphorylation of TAU protein in Alzheimer's disease.

Significant progress has been made in the elucidation of abnormal phosphorylation sites in tau in AD. Using site-directed mutagenesis and *in vitro* phosphorylation of mutant recombinant tau, were able to map the phosphorylation- dependent antibody mAb AT8. mAb AT8 recognizes A68-tau proteins before but not after dephosphorylation [37]. The antibody also fails to recognize the bulk of adult tau proteins that are isolated from normal brain tissues. The epitope of mAb AT8 is very close to, if not identical with, the Tau-1 epitope, around residues and is dependent on phosphorylation in the vicinity of Ser-202 mAb Tau-1 has a complementary property to mAb AT8 in that it requires dephosphorylation of Ser-199 and/or Ser-202 have used a series of phosphorylation-dependent neurofilament antibodies which also recognize A68-proteins, to show that tau is phosphorylated at Ser-235, Ser-396 and Ser-404, Ser-235 and Ser-396. Ser-46 in one of the N-terminal tau inserts has also been shown to be phosphorylated in the A68-tau proteins of 64 and 68 kDa mobility [38] . have shown variable phosphorylation at Thr-231 and Ser-262 by mass spectroscopy. The interest of Ser- 262 is that it is the only site of abnormal phosphorylation so far identified in the tandem repeat region. However, even this site is outside the minimal protease-resistant tau unit of the core PHF Most of the sites so far

identified are within Ser-Pro or Thr-Pro pairs, of which there are 17 distributed throughout the N- and C-terminal domains of tau.

Numerous enzymes have been found which phosphorylate tau *in vitro.* Mitogenactivated protein kinase (MAP kinase) phosphorylates most of the 17 Ser-Pro and Thr-Pro sites Glycogen-synthetase kinase-3 phosphorylates most of the Ser-Pro motifs .Both MAP kinase and GSK-3 3 are microtubule associated proteins as defined by the criterion of co purification with microtubules during cycles of assembly and disassembly Two further kinases, called tau protein kinase 1 and 2, have been purified from microtubule-associated proteins and found to phosphorylate tau Tau protein kinase 1 appears to be identical to GSK-3, and phosphorylates tau at Ser- 199, Thr-231, Ser-391 and Ser-413 [39]. Tau protein kinase 2 phosphorylates Ser- 202, Thr-205, Ser-235 and Ser-404, which is similar to MAP kinase. Rodder and In gram isolated two further kinases (PK36 and PK40), which phosphorylate tau at Ser-Pro and Thr-Pro sites, and also phosphorylate intermediate and heavy neurofilament subunits. Other kinases include cAMP-dependent kinase calcium-calmodulin-dependent kinase II casein kinase I casein kinase II proline-directed kinase cdc2 kinase and the cyclin-dependent cdk2 and cdk5 kinases have isolated a 35/41 kDa kinase from rat brain extract that phosphorylates Ser-262, which produces a substantial reduction in tau binding affinity for microtubules. It is not known which, if any, of these kinases are involved in the phosphorylation of tau *in vivo,* whether they are pathological and what their significance is regarding the development of neurofibrillary pathology in AD [40].

Since phosphorylation may also be caused by a loss of phosphatase activity, candidate phosphatases have also been examined. Protein phosphatase 2A dephosphorylates tau phosphorylated by p42 MAP kinase (also known as ERK2), whereas phosphatase 1 is ineffective Both phosphatises 2A and 2B (calcineurin) dephosphorylate phosphorylated Ser-Pro and Thr- Pro motifs as well as Ser-262 within the first repeat The significance of these observations is at present unknown. One argument that has been advanced in favour of attributing a pathogenic significance to states of abnormal phosphorylation of tau has been the observation that phosphorylated tau has a reduced binding affinity for microtubules have shown that tau protein isolated from the sarkosyl-insoluble fraction binds with less affinity to microtubules before dephosphorylation. Recombinant tau protein phosphorylatcd *in vitro* also has reduced binding affinity for microtubules , and phosphorylation at Sr appears to be particularly effective in this regard, although phosphorylation at Ser-Pro and Thr-Pro sites also contributes Similarly, MAP kinase- phosphorylated tau also has a marked reduction in binding affinity for microtubules. Two hypotheses regarding the pathogenesis of

ncurofibrillary degeneration in Alzheimer's disease have been advanced on the basis of this data. The first has been that abnormal phosphorylation of tau reduces binding affinity to microtubules and hence cause their destabilization, and so reduces fast axonal transport.

The second hypothesis is that abnormal phosphorylation of tau, by neutralizing positive charges in the basic residues just upstream from the repeat region, reduces electrostatic repulsion, and so favours the self-assembly of tau into PHFs [41].However, there is as yet no evidence that any abnormal state of phosphorylation of tau favours self-association. Indeed, the only study that has examined the effect of phosphorylation on the polymerization of tau *in vitro* failed to demonstrate any quantitative difference between phosphorylated and unphosphorylated tau.

2.7 Causes and risk factors

While scientists know that Alzheimer's disease involves the failure of nerve cells, why this happens is still unknown. However, they have identified certain risk factors that increase the likelihood of developing Alzheimer's.

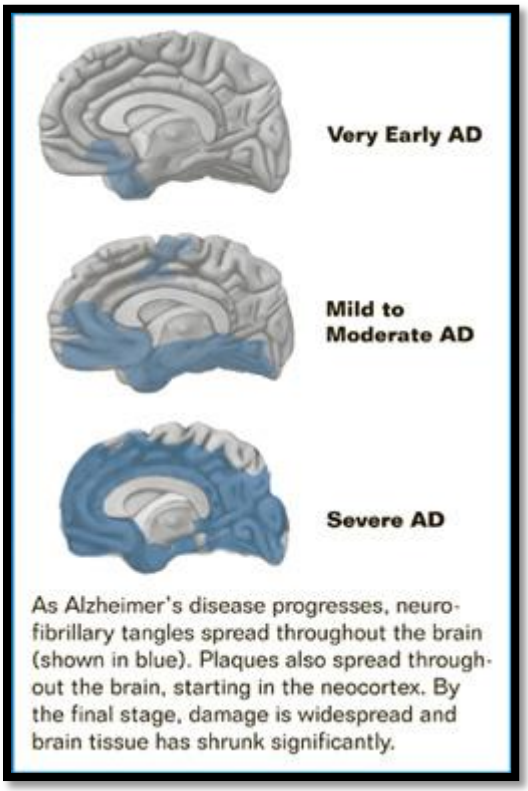


Fig:3 Stages of Alzheimer

2.7.1 Age :

The greatest known risk factor for Alzheimer's disease is increasing age. Most individuals with the illness are 65 and older. One in nine people in this age group has Alzheimer's. Nearly one-third of people age 85 and older have Alzheimer's.

2.7.2 Family history:

Another risk factor is family history. Research has shown that those who have a parent, brother or sister with Alzheimer's are more likely to develop the disease than individuals who do not. The risk increases if more than one family member has the illness.

2.7.3 Familial Alzheimer's and genetics :

Two categories of genes influence whether a person develops a disease: risk genes and deterministic genes. Risk genes increase the likelihood of developing a disease but do not guarantee it will happen. Deterministic genes directly cause a disease, guaranteeing that anyone who inherits one will develop a disorder. Researchers have found several genes that increase the risk of Alzheimer's. APOE-e4 is the first risk gene identified and remains the one with strongest impact. Other common forms of the APOE gene are APOE-e2 and APOE-e3. Everyone inherits a copy of some form of APOE from each parent. Those who inherit one copy of APOE-e4 have an increased risk of develop ping Alzheimer's; those who inherit two copies have an even higher risk but not a certainty [42]. Rare deterministic genes cause Alzheimer's in a few hundred extended families worldwide. These genes are estimated to account for less than 1 percent of cases. Individuals with these genes usually develop symptoms in their 40s or 50s.

2.7.4 Other risk factors:

Age, family history and genetics are all risk factors we can't change. Research is beginning to reveal clues about other risk factors that we may be able to influence. There appears to be a strong link between serious head injury and future risk of Alzheimer's. It's important to protect your head by buckling your seat belt, wearing a helmet when participating in sports and proofing your home to avoid falls. One promising line of research suggests that strategies for overall healthy aging may help keep the brain healthy and may even reduce the risk of developing Alzheimer's[43]. These measures include eating a healthy diet,

staying socially active, avoiding tobacco and excess alcohol, and exercising both the body and mind. Some of the strongest evidence links brain health to heart health. The risk of developing Alzheimer's or vascular dementia appears to be increased by many conditions that damage the heart and blood vessels. These include heart disease, diabetes, stroke, high blood pressure and high cholesterol. Work with your doctor to monitor your heart health and treat any problems that arise. Studies of donated brain tissue provide additional evidence for the heart-head connection. These studies suggest that plaques and tangles are more likely to cause Alzheimer's symptoms if strokes or damage to the brain's blood vessels are also present.

2.8 . How to find out if it's alzheimer's disease

Not everyone experiencing memory loss or other possible Alzheimer's warning signs recognizes that they have a problem. Signs of dementia are sometimes more obvious to family members or friends. The first step in following up on symptoms is finding a doctor with whom a person feels comfortable. There is no single type of doctor that specializes in diagnosing and treating memory symptoms or Alzheimer's disease. Many people contact their regular primary care physician about their concerns. Primary care doctors often oversee the diagnostic process themselves. In some cases, the doctor may refer the individual to a specialist such as a:

› Neurologist who specializes in diseases of the brain and nervous system.

› Psychiatrist who specializes in disorders that affect mood or the way the mind works.

› Psychologist with special training in testing memory and other mental function

There is no single test that proves a person has Alzheimer's. The workup is designed to evaluate overall health and identify any conditions that could affect how well the mind is working. When other conditions are ruled out, the doctor can then determine if it is Alzheimer's or another dementia. Experts estimate that a skilled physician can diagnose Alzheimer's with more than 90 percent accuracy. Physicians can almost always determine that a person has dementia, but it may sometimes be difficult to determine the exact cause[44,45].

2.9 Steps to diagnosis include:

Understanding the problem. Be prepared for the doctor to ask:

› What kind of symptoms have occurred.

› When they began.

› How often they happen.

› If they have gotten worse.

Reviewing medical history The doctor will interview the person being tested and others close to him or her to gather information about current and past mental and physical illnesses. It is helpful to bring a list of all the medications the person is taking. The doctor will also obtain a history of key medical conditions affecting other family members, especially whether they may have or had Alzheimer's disease or other dementias. Evaluating mood and mental status .Mental status testing evaluates memory, the ability to solve simple problems and other thinking skills. This testing gives an overall sense of whether a person:

› Is aware of symptoms.

› Knows the date, time and where he or she is.

› Can remember a short list of words, follow instructions and do simple calculations.

The doctor may ask the person his or her address, what year it is or who is serving as president. The individual may also be asked to spell a word backward, draw a clock or copy a design[46]. The doctor will also assess mood and sense of wellbeing to detect depression or other illnesses that can cause memory loss and confusion.

2.10 Physical exam and diagnostic tests

2.10.1 A physician will:

› Evaluate diet and nutrition.

› Check blood pressure, temperature and pulse.

› Listen to the heart and lungs.

› Perform other procedures to assess overall health.

The physician will collect blood and urine samples and may order other laboratory tests. Information from these tests can help identify disorders such as anaemia, infection, diabetes, kidney or liver disease, certain vitamin deficiencies, thyroid abnormalities, and problems with the heart, blood vessels or lungs. All of these conditions may cause confused thinking, trouble focusing attention, memory problems or other symptoms similar to dementia.

2.10.2 Neurological exam:

A doctor will closely evaluate the person for problems that may signal brain disorders other than Alzheimer's. The physician will also test:

› Reflexes.

› Coordination

. › Muscle tone and strength.

› Eye movement.

› Speech.

› Sensation.

The doctor is looking for signs of small or large strokes, Parkinson's disease, brain tumors, fluid accumulation on the brain and other illnesses that may impair memory or thinking.

The neurological exam may also include a brain imaging study. The most common types are magnetic resonance imaging (MRI) or computed tomography (CT)[47]. MRIs and CTs can reveal tumors, evidence of small or large strokes, damage from severe head trauma or a build up of fluid. Researchers are studying other imaging techniques so they can better diagnose and track the progress of Alzheimer's

2.11 When the diagnosis is alzheimer's:

Once testing is complete, the doctor will make an appointment to review results and share his or her conclusions. A diagnosis of Alzheimer's reflects a doctor's best judgment about the cause of a person's symptoms, based on the testing performed. You may want to ask the doctor:

› Why the diagnosis is Alzheimer's.

› Where the person may be in the course of the disease.

› What to expect in the future.

2.12 . Stages of the disease:

Alzheimer's disease gets worse over time. Experts have developed "stages" to describe how a person's abilities change from normal function through advanced Alzheimer's. It's important to keep in mind that stages are general guides, and symptoms vary greatly. Every person is unique, but there are some common patterns of the illness. Those with Alzheimer's live an average of four to eight years after diagnosis, but some live as long as 20 years[48].

Stage 1: No impairment

Normal function The person does not experience any memory problems. An interview with a medical professional does not show any evidence of symptoms.

Stage 2: Very mild decline

May be normal age-related changes or the earliest signs of Alzheimer's The individual may feel that he or she is having memory lapses — forgetting familiar words or the location of everyday objects. But no symptoms can be detected during a medical exam or by friends, family or co-workers.

Stage 3: Mild cognitive decline

Early-stage Alzheimer's may be diagnosed in some, but not all, individuals at this point Friends, family or co-workers begin to notice difficulties. During a detailed medical interview, doctors may be able to detect problems in memory or concentration. Common difficulties at this stage include:

› Noticeable problems coming up with the right word or name

. › Trouble remembering names when introduced to new people.

› Noticeably greater difficulty performing tasks in social or work settings.

› Forgetting material that one has just read.

› Losing or misplacing a valuable object.

› Increasing trouble with planning or organizing.

Stage 4: Moderate cognitive decline Mild or early-stage Alzheimer's

At this point, a careful medical interview should be able to detect clear-cut problems in several areas:

› Forgetfulness of recent events.

› Impaired ability to perform challenging mental arithmetic (e.g., counting backward from 100 by 7s).

› Greater difficulty performing complex tasks, such as planning dinner for guests, paying bills or managing finances.

› Forgetfulness about one's own personal history.

› Becoming moody or withdrawn, especially in socially or mentally challenging situations.

Stage 5: Moderately severe cognitive decline Moderate or mid-stage Alzheimer's

Gaps in memory and thinking are noticeable, and individuals begin to need help with day-to-day activities. At this stage, those with Alzheimer's may:

› Be unable to recall their own address or phone numberor the high school or college they attended.

› Become confused about where they are or what day it is.

› Have trouble with less challenging mental arithmetic (e.g., counting backward from 40 by subtracting 4s).

› Need help choosing proper clothing for the season.

› Still remember significant details about themselves and their family.

Stage 6: Severe cognitive decline Moderately severe or mid-stage Alzheimer's

Memory continues to worsen, personality changes may take place and individuals need significant help with daily activities. The person may:

› Lose awareness of recent experiences as well as their surroundings.

› Remember their own name but have difficulty with their personal history.

› Distinguish familiar and unfamiliar faces but have trouble remembering the name of a spouse or caregiver.

› Need help dressing properly and may, without supervision, make mistakes such as putting pajamas over daytime clothes or shoes on the wrong feet.

› Experience major changes in sleep patterns sleeping during the day and becoming restless at night.

› Need help handling details of the toilet.

› Have increasingly frequent trouble controlling their bladder or bowels.

› Experience major personality and behavioral changes,including suspiciousness and delusions (e.g., believing the caregiver is an impostor) or compulsive, repetitive behavior like hand-wringing or tissue shredding.

› Be at risk for wandering or becoming lost.

Stage 7: Very severe cognitive decline Severe or late-stage Alzheimer's

In the final stage of this disease, individuals lose the ability to respond to the environment, to carry on a conversation and, eventually, to control movement. They may still say words or phrases. At this stage, individuals need help with much of their daily personal care, including eating and using the toilet. They may also lose the ability to smile, to sit without support and hold their heads up. Reflexes become abnormal. Muscles grow rigid. Swallowing is impaired.

2.13 Treating the symptoms

Currently, there is no cure for Alzheimer's and no way to stop the underlying death of brain cells. But drugs and non-drug treatments may help with both cognitive and behavioural symptoms. A comprehensive care plan for Alzheimer's disease:

› Considers appropriate treatment options.

› Monitors treatment effectiveness as the disease progresses.

› Changes course and explores alternatives as necessary.

› Respects individual and family goals for treatment and tolerance for risk[49].

2.13.1 Cognitive symptoms

Two types of drugs are currently approved by the FDA to treat cognitive symptoms of Alzheimer's disease. The first type, cholinesterase inhibitors, prevents the breakdown of acetylcholine , a chemical messenger important for memory and learning. By keeping levels of acetylcholine high, these drugs support communication among nerve cells. Three cholinesterase inhibitors are commonly prescribed:

› Donepezil , approved in 1996 to treat mild-to moderate Alzheimer's and in 2006 for the severe stage.

› Rivastigmine ,approved in 2000 for mild-to-moderate Alzheimer's.

› Galantamine, approved in 2001 for mild-to moderate stages. The second type of drug works by regulating the activity of glutamate, a different messenger chemical involved in information processing:

› Memantine, approved in 2003 for moderate-to-severe stages, is the only currently available drug in this class. The effectiveness of both types of treatments varies from person to person. While they may temporarily help symptoms, they do not slow or stop the brain changes that cause Alzheimer's to become more severe over time[50].

2.13.2 Behavioral symptoms

Many find behavioral changes like anxiety, agitation, aggression and sleep disturbances to be the most challenging and distressing effect of Alzheimer's disease. These changes can greatly impact the quality of life for individuals living in both family situations and long-term residential care. As with cognitive symptoms of Alzheimer's, the chief underlying cause of behavioral and psychiatric symptoms is the progressive damage to brain cells.[50]

3. OBJECTIVE

Quantitative studies on gene regulatory pathways for Alzheimer's disease

- Researches ongoing globally are producing huge data related to genes with respect to Alzheimer's disease. Additionally role of TAU pathway also found critical for AD. In this Project, I aim to put my efforts to study the proteins involved in TAU pathway in AD.
- To perform quantitative simulation on various modules of TAU pathway independently as well as in group.

4. MATERIALS AND METHODS

4.1 DATA COLLECTION :

Search for various genes related to Alzheimer's disease from :

4.1.1 ALZGENE : (http://www.alzgene.org/)

The AlzGene database provides a comprehensive, unbiased and regularly updated field synopsis of genetic association studies performed in Alzheimer's disease. In addition, hundreds of meta analyses are available for all eligible polymorphisms with sufficient data.

4.1.2 PUBMED: (http://www.ncbi.nlm.nih.gov/pubmed)

PubMed is a free search engine accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics. The United States National Library of Medicine (NLM) at the National Institutes of Health maintains the database as part of the Entrez system of information retrieval.

From 1971 to 1997, MEDLINE online access to the MEDLARS Online computerized database had been primarily through institutional facilities, such as university libraries. PubMed, first released in January 1996, ushered in the era of private, free, home- and office-based MEDLINE searching. The PubMed system was offered free to the public in June 1997, when MEDLINE searches via the Web were demonstrated, in a ceremony, by Vice President Al Gore.

4.1.3 OMIM : (http://www.omim.org/)

Online Mendelian Inheritance in Man (OMIM) is a database that catalogues all the known diseases with a genetic component, and — when possible — links them to the

relevant genes in the human genome and provides references for further research and tools for genomic analysis of a catalogued gene.

4.1.4 NCBI : (http://www.ncbi.nlm.nih.gov/)

The NCBI houses a series of databases relevant to biotechnology and biomedicine. Major databases include GenBank for DNA sequences and PubMed, a bibliographic database for the biomedical literature. Other databases include the NCBI Epigenomics database. All these databases are available online through the Entrez search engine.

4.1.5 KEGG : (http://www.genome.jp/kegg/)

KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies.

4.2 Data Processing :

All the collected data was processes in order to generate a working framework so as to study the function of the collected data , using the tool :

4.2.1 Cell Designer : (http://www.celldesigner.org/)

CellDesigner is a modeling tool of biochemical networks with graphical user interface. It is designed to be SBW (Systems Biology Workbench) compliant, and support SBML (Systems Biology Markup Language) format.

Gene ontology in order to retrieve the molecular and biological function of the genes collected was performed using the tool :

4.2.2 Uniprot : (http://www.uniprot.org/)

UniProt is a comprehensive, high-quality and freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing

projects. It contains a large amount of information about the biological function of proteins derived from the research literature.

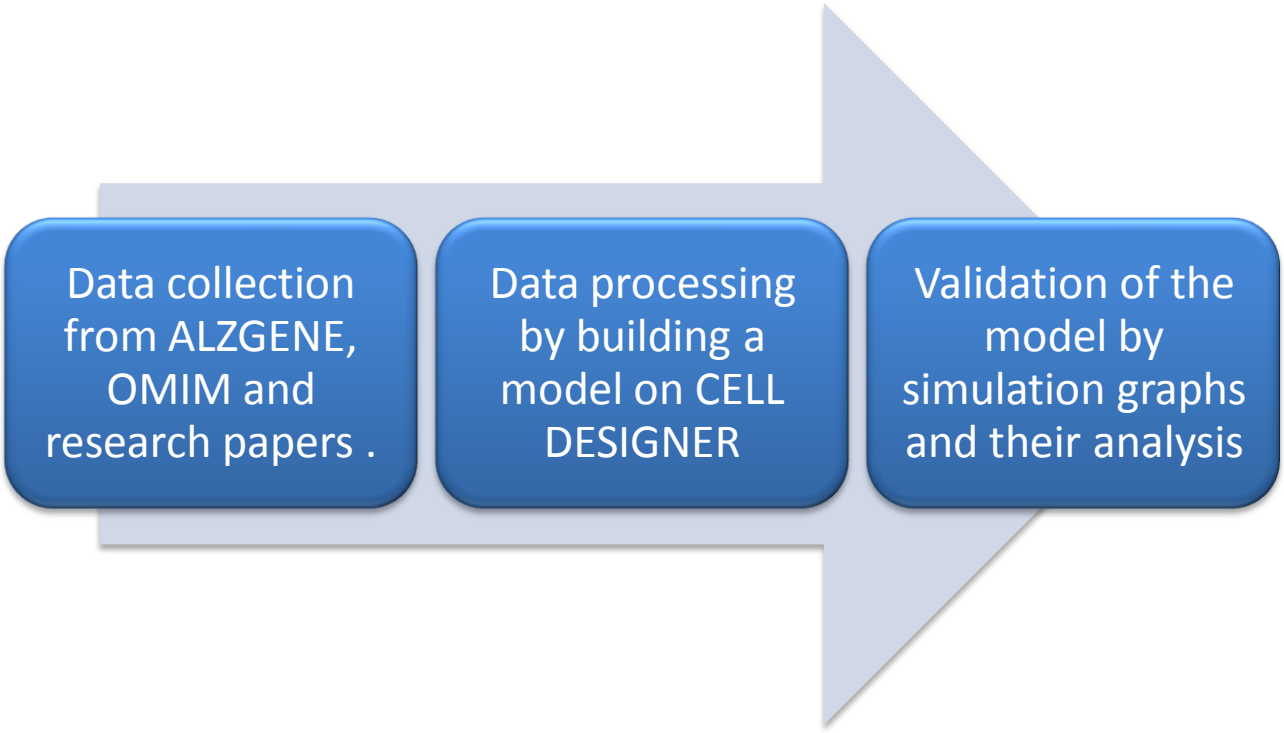


Fig: 4 Graphical representation of methodology adopted.

5. RESULTS

5.1 Retrieval of the genes related to TAU protein in Alzheimers disease .

Name of the genes with respect to their role in TAU pathway were collected from the database:

ALZgene (http://www.alzgene.org/) .

Table : 1 List of all the collected genes

S.No	ASTROCYTES	OLIGODENDROCYTES	FRONTAL AND TEMPORAL REGION
1	APQ4	CHGA	PHYHD1
2	GFAP	ENOS2	RELN
3	S100B	NEFL	MYO5C
4	EMR1	NEFH	COPS5
5	ALF1	SNAP25	DCHS2
6	LGALS3	SYT1	PTGS2
7	CD68	MAG	POT1
8		MBP	
9		MOG	
10		NSOX10	

5.2 Study the pathway of related genes in :

KEGG (http://www.genome.jp/kegg/) .

5.3 Construction of model with the collected genes in:

Cell Designer (http://www.celldesigner.org/).

On the basis of the genes collected and their functioning in alzheimers disease a refined model was created so as to show the TAU pathway and the relation between different genes.

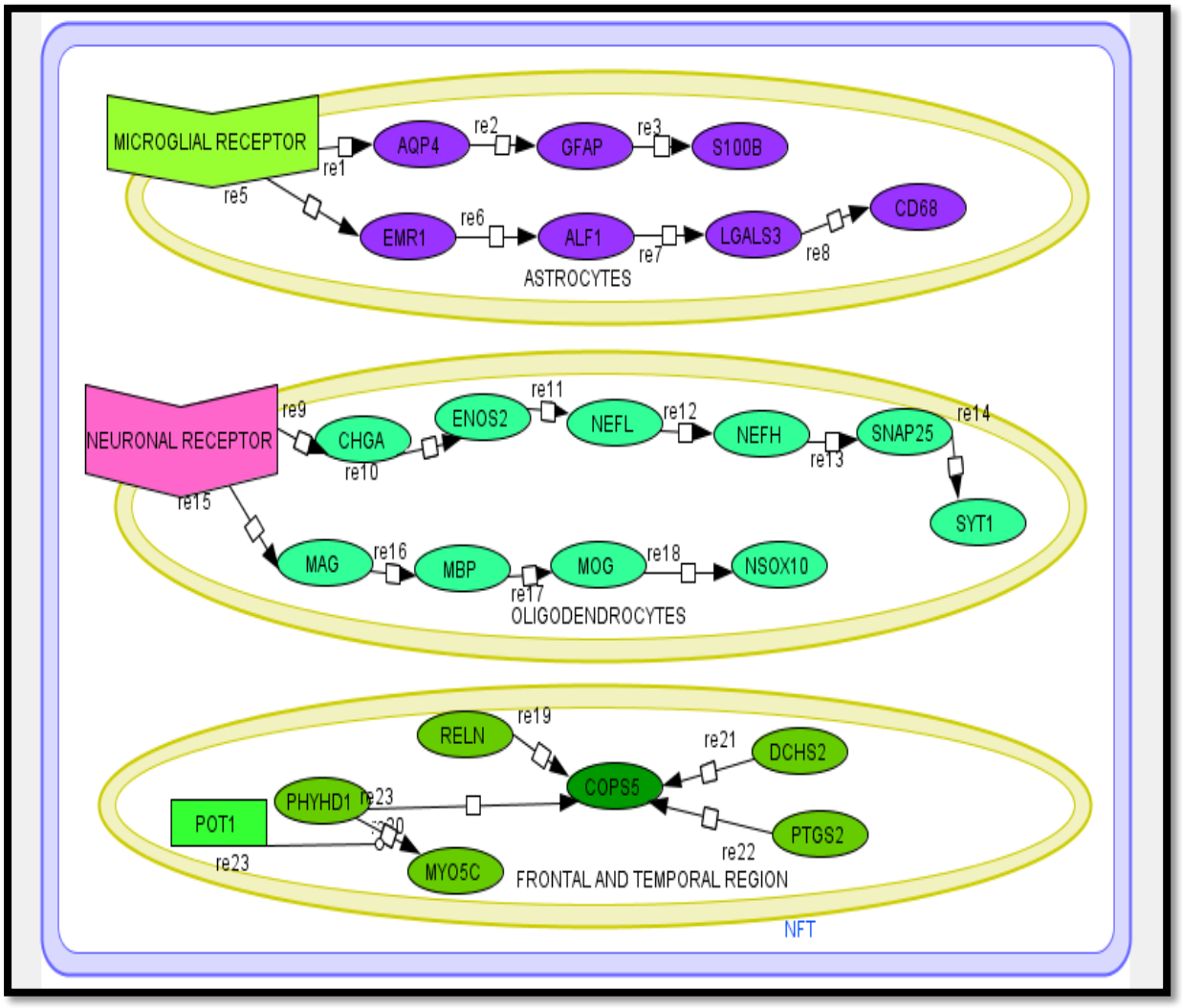


Fig: 5 Model of associated partner proteins linked to TAU

5.4 Study and analysis of the model with the help of graphs in:

Cell Designer (http://www.celldesigner.org/).

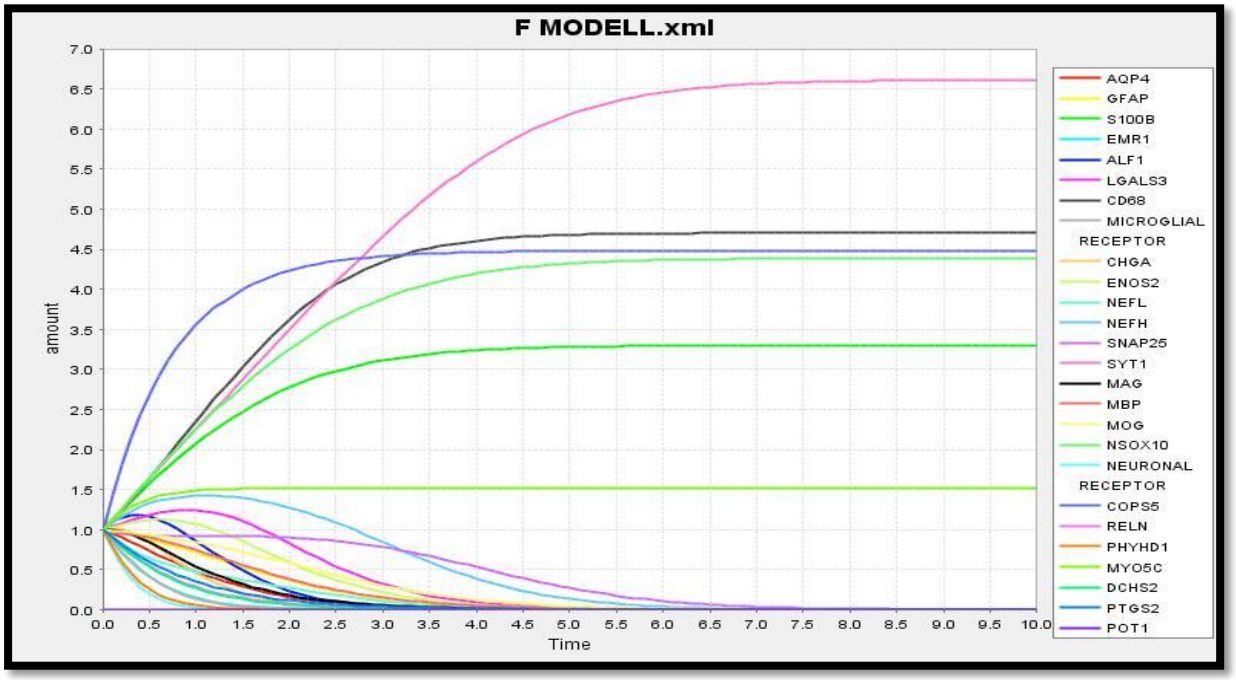


Fig:6 Dynamic behaviour analysis of all important genes at time 10msec and amount range from 0.0-7.0µmole.

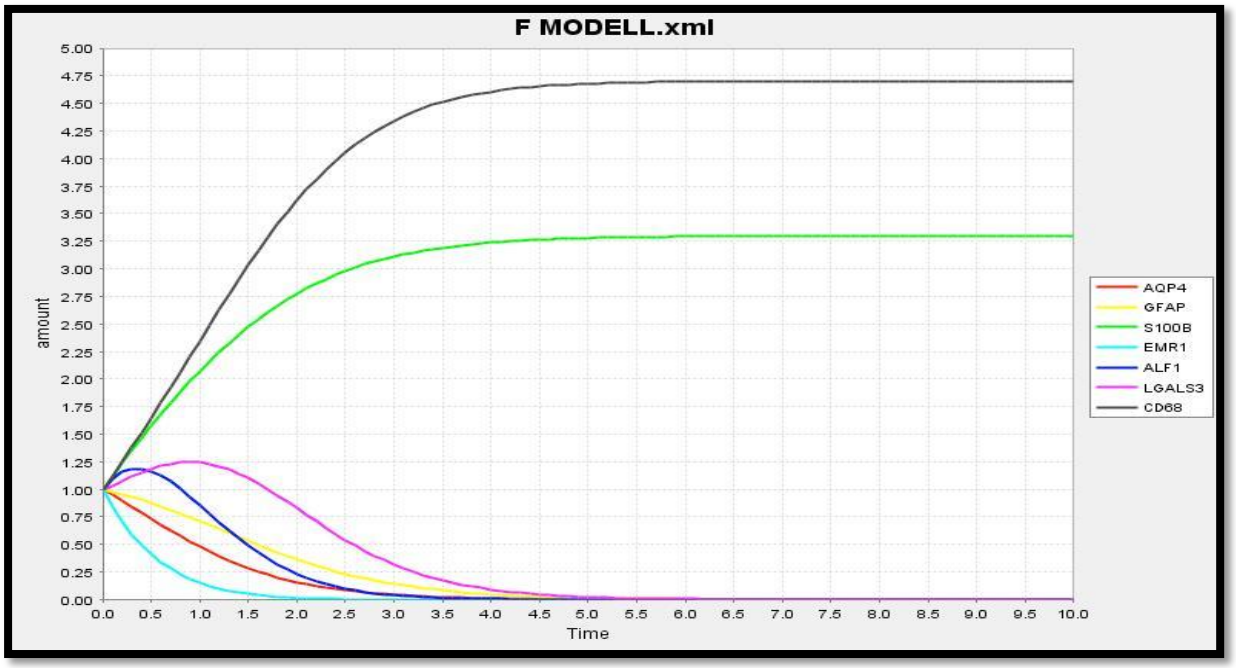


Fig:7 Dynamic behaviour analysis of important gene like APQ4, GFAP, S00B, EMR1,ALF1,LGAL53 and CD68etc. at time 10msec and amount range from 0.0-5.0µ mole.

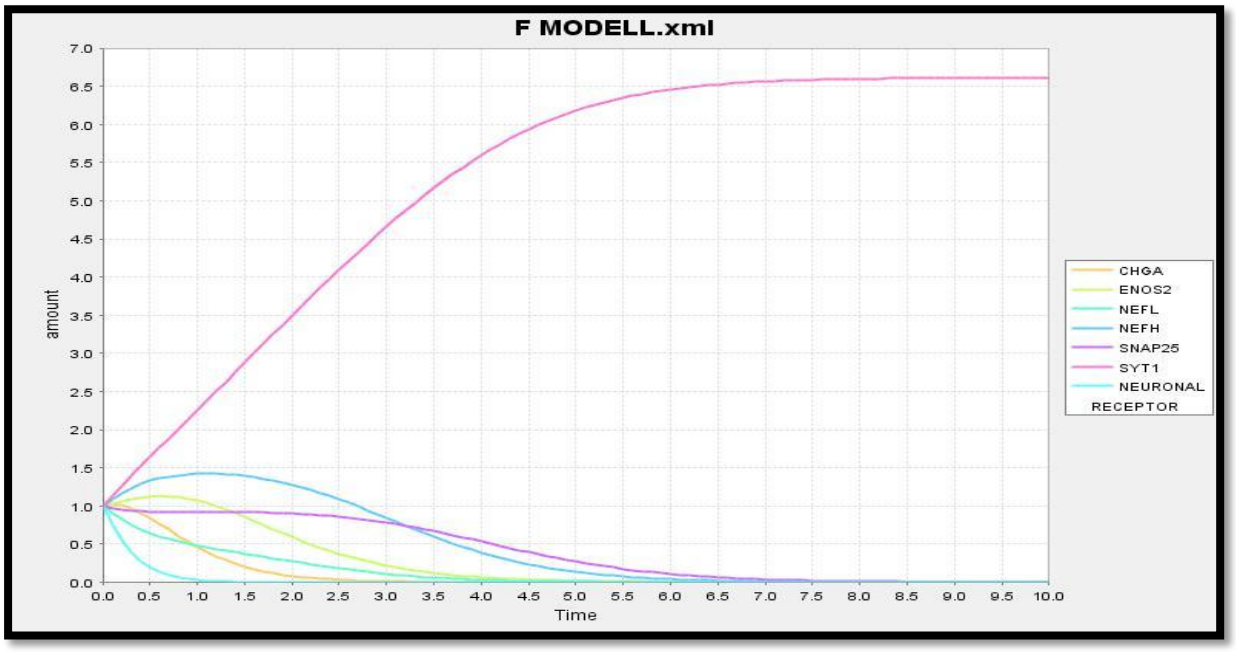
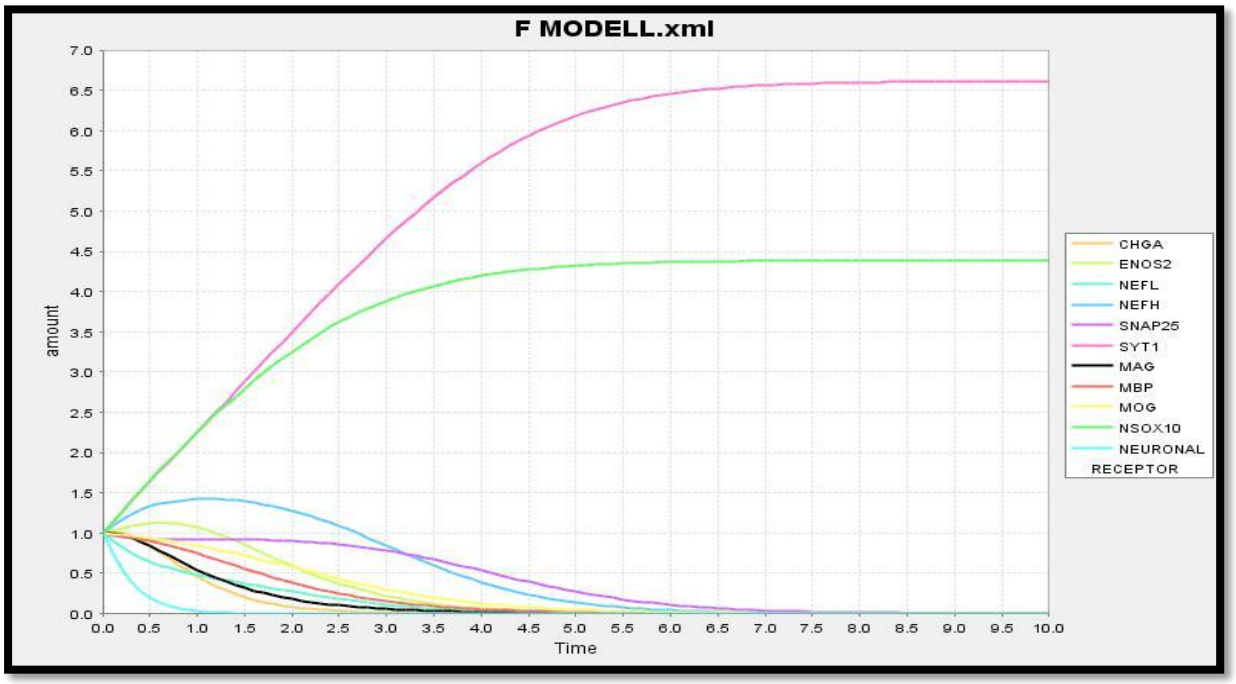


Fig: 8 Dynamic behaviour analysis of important gene like CHGA,ENOS2,NEFL ,NEFH, SNAP25, SYT1 and NEURONAL RECEPTOR. at time 10msec and amount range from 0.0-7.0µ mole.



.**Fig:9 Dynamic behaviour analysis of important gene like CHGA,ENOS2,NEFL ,NEFH, SNAP25, SYT1, MAG,MBP, MOG, NSOX10 and NEURONAL RECEPTOR. at time 10msec and amount range from 0.0-7.0µ mole.**

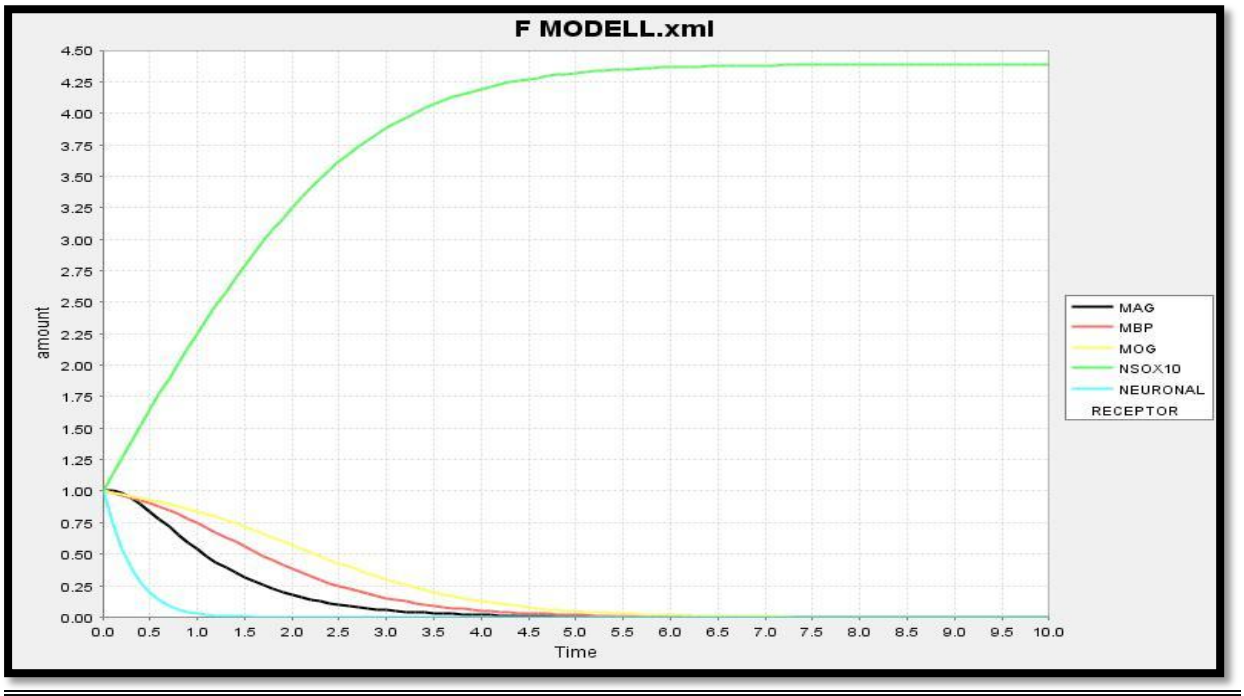


Fig:10 Dynamic behaviour analysis of important gene like MAG,MBP, MOG, NSOX10 and NEURONAL RECEPTOR. at time 10msec and amount range from 0.0-4.5µ mole.

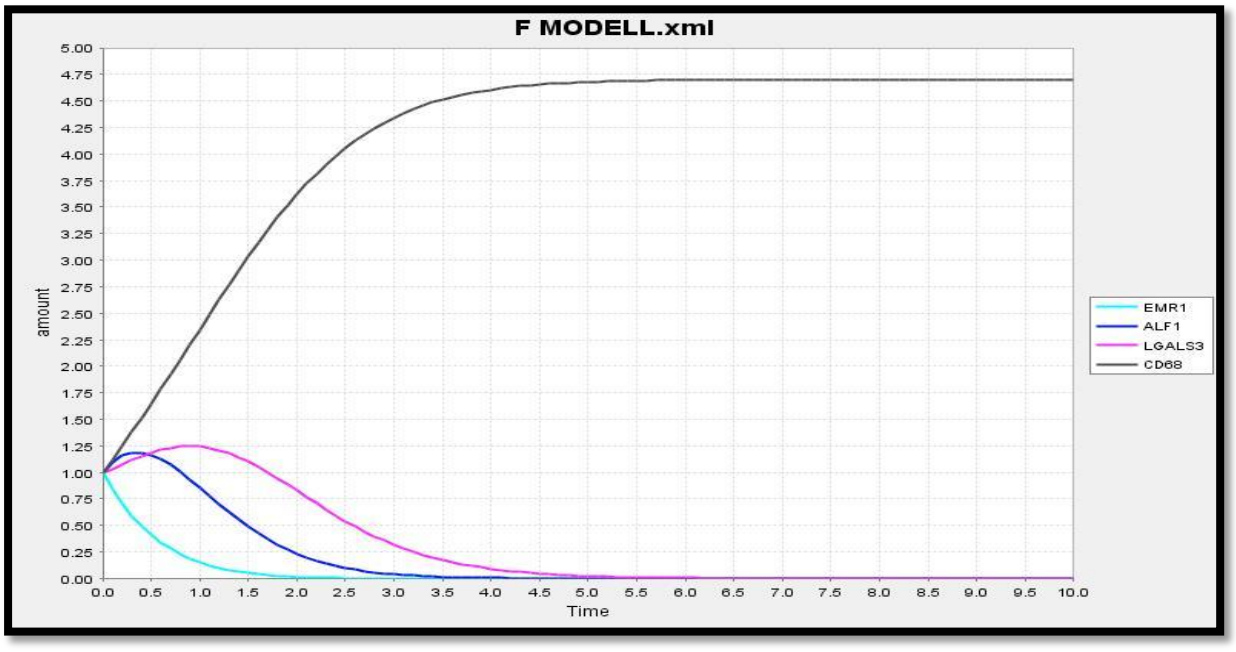


Fig:11 Dynamic behaviour analysis of important gene like EMR1,ALF1,LGALS3 and CD68. at time 10msec and amount range from 0.0-5.0µ mole.

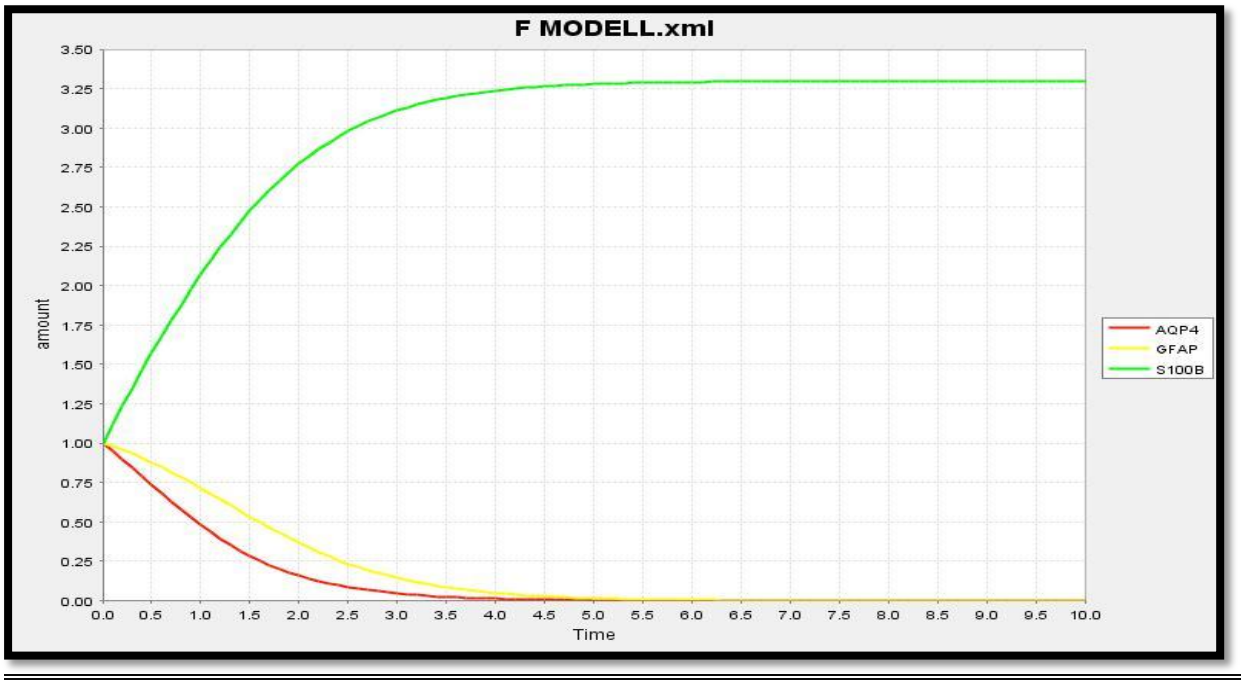


Fig:12 Dynamic behaviour analysis of important gene likeAPQ4,GFAP and S100B. at time 10msec and amount range from 0.0-3.5µ mole.

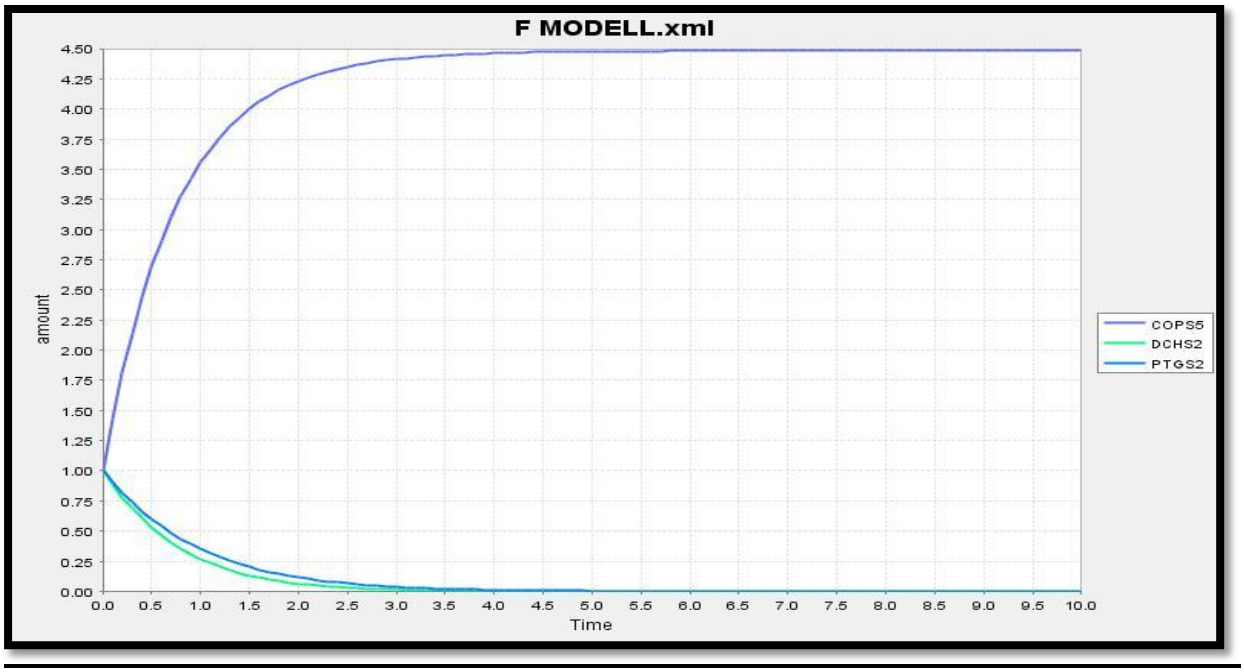


Fig:13 Dynamic behaviour analysis of important gene like COPS5,DCHS2 and PTGS2 . at time 10msec and amount range from 0.0-4.5µ mole.

5.4.1 Simulation Graph Summary :

Figure 6: It shows collective behaviour of all the genes collected in which different behaviour of each gene is seen but cannot be clearly distinguished from each other .

Figure 7: Gene CD68 gets stabilized at 4.6 μ mole and S100B at 3.15 μ mole at an end time of 4.3ms.

Figure 8: The behaviour of SYT1 is entirely diferrent from other involved genes in this graph. This SYT1 at initial concentration of 1 get progressed and stabilizes at 6.4 μ mole at an end time of 6 ms.

Figure 9: NSOX10 and SYT1 show a progressive behaviour where NSOX10 gets stabilized at 6.5 μ mole and SYT 1 at 4 μ mole with an end time of 6 ms and 4 ms .

Figure 10 : NSOX10 shows a progressive behaviour whereas rest of the genes show a retrogressive behaviour .If we add inhibitors for the genes MAG,MBP, MOG and Neuronal receptor then it promotes the gene expression of NSOX10.

Figure 11: CD68 shows a progressive behaviour and gets stabilized at 4.75 μ mole at an end time of 4.5ms which is different from the behaviour from the other genes EMR1,ALF1,LGALS3 as they show a retrogressive behaviour.

Figure 12:GFAP and S100B are antagonistic to each other where both of them attain a stable point at 0 μ mole and 3.25 μ mole at an end time of 4.5 ms .

Figure 13: Starting from an initial concentration of 1 μ mole COPS5 and PTGS2 show an antagonistic behaviour to each other .

5.5 Gene ontology was done to know the molecular function and biological process of genes in UNIPROT (http://www.uniprot.org/).

Table:2 Gene Ontology of all genes involved in Tau-pathogenesis of AD.

S.No	GENE NAME	Gene ontology (GO)	Gene ontology (biological process)	Gene ontology (molecular function)	Gene ontology (cellular component)
1	APQ4	Basolateral Plasma membrane, carbon dioxide transport	carbon dioxide transport	glycerol channel activity	basolateral plasma membrane
2	GFAP	astrocyte development	astrocyte development	structural constituent of cytoskeleton	astrocyte end-foot
3	S100B	astrocyte differentiation	astrocyte differentiation	calcium-dependent protein binding	cytoplasm ,extracellular region
4	CD68	cellular response to organic substance	cellular response to organic substance	endosome membrane	endosome membrane
5	NEFL	anterograde axon cargo transport	anterograde axon cargo transport	identical protein binding	axon development
6	NEFH	axon development	regulation of transport	axon development	neurofibrillary tangle
7	SNAP25	calcium-dependent protein binding	energy reserve metabolic process	calcium-dependent protein binding	cell junction [
8	SYT1	1-phosphatidylinositol binding	calcium ion-dependent exocytosis of neurotransmitter	1-phosphatidylinositol binding	cell junction
9	MAG	blood coagulation	blood	carbohydrate	integral

			coagulation	binding	component of membrane
10	MBP	aging	aging	structural constituent of myelin sheath	compact myelin internode region of axon
11	MOG	cell adhesion central nervous system development	positive regulation of MyD88-dependent toll-like receptor signaling pathway	integral component of membrane	integral component of membrane
12	COPS5	COP9 signalosome regulation of cell cycle	regulation of cell cycle	COP9 signalosome	COP9 signalosome
13	RELN	associative learning	associative learning axon guidance	lipoprotein particle receptor binding metal ion binding	cytoplasm dendrite
14	PHYHD1	dioxygenase activity	dioxygenase activity	dioxygenase activity	
15	MYO5C	ATP binding	motor activity	ATP binding	extracellular vesicular exosome
16	DCHS2	calcium ion binding	homophilic cell adhesion	calcium ion binding	integral component of membrane
17	PTS2	angiogenesis	arachidonate 15-lipoxygenase activity	arachidonate 15-lipoxygenase activity	caveola

6. Discussion

The model presented here is based on a simplified scheme of the Tau-signalling pathway. The scheme is still incomplete in the sense that there are other components of Tausignalling, Increasing body of evidence supports the complex genetic model of AD, which suggests that polygenic network of susceptibility genes may underlie the disease. Since the predisposing gene variants confer only fractional risk, genetic interactions may have a major role in contributing to neurodegeneration in AD.The interactions were investigated in pairs of polymorphisms, and the pairs were selected on the basis of the same chromosomal localization and/or on the involvement of the same pathogenic mechanism in AD. Logistic regression analysis revealed an strong interaction between the APQ4 and the GFPA.

According to the logistic regression model there is no interaction between the BDNF and APOE polymorphisms. Our study demonstrates an interaction between genotypes of Tau or PTGS2 that seems to increase the risk for AD.

In summary, our results support the involvement of the following genes in AD Tau aetiology: CD68, GFAP, S100B, APQ4 and PTS2.0

7. Conclusion

Modeling and simulation techniques has applications in medical, pharmaceutical studies, and it is being used for epidemiological, agricultural and educational purposes also. Modeling and Simulation techniques are important tools to conduct researches for biologists. It makes biologists understand more complex processes in biological system which were not possible to explain with mathematical biological system. Modeling and Simulation techniques help us to understand biological system, to answer biological queries, to develop new medicine, to develop more strong and more productive species and to improve the environment. It is anticipated that the information generated in this work will be useful to the scientific community and will help in the designing of better therapeutic options for AD.

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