# Trisha Gaur MSc Thesis

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## ABSTRACT

PKD and TSC, characterized by different genetic disorders converge at a molecular level with the help of some common pathways in case of contiguous gene syndrome. TSC related brain abnormalities have been widely studied however, the contribution of PKD-associated pathways to abnormalities in the cerebral cortex remains obscure. In this study, a protein–protein interaction (PPI) network was created to investigate and analyze the shared molecular mechanisms and signaling pathways between PKD and TSC, putting a major focus on their potential roles in cortical development as well as the neurological function.

Network analysis displayed a highly interconnected network centered on the hub proteins MYC, EIF4EBP1, and NOS3, indicating convergence of proliferative, translational, and neurovascular signaling pathways. Abnormal MYC activity was linked with changes in neural progenitor cell growth and improper neuronal differentiation. At the same time, continuous activation of EIF4EBP1 indicated disturbed mTOR-dependent protein synthesis, which may affect normal synaptic development and neuronal communication. The NOS3 identified as an important hub gene in the PPI network specifically indicated that neurovascular dysfunction and metabolic imbalance might play a major key role in the PKD–TSC-associated cortical abnormalities, although this aspect has not been studied extensively before.

All in all, these findings suggest that cortical abnormalities, increased neuronal excitability, and metabolic they all work together in causing neurological problems such as epilepsy and cognitive impairment. The present study conducted provides a broader understanding of how PKD and TSC are connected to each other at the molecular level and also identifies some important genes and pathways that could be explored further for future therapeutic and experimental studies.



**KEYWORDS**: mTOR Signaling Pathway; MYC; EIF4EBP1; NOS3; Protein–Protein Interaction Network

# CHAPTER 1

## 1. Introduction

### 1.1 Overview of PKD–TSC Overlap Syndrome

Polycystic Kidney Disease and Tuberous Sclerosis Complex are inherited genetic disorders that are known to affect multiple organs of the body simultaneously, mainly through dysregulation of important cellular signaling pathways that are involved in the disease and further promotes the progression of the disease [2,3]. These disorders are the product of mutations in different genes and give rise to distinct clinical manifestations, still many reliable scientific evidences strongly suggest that they have several shared molecular mechanisms that are involved in abnormal cellular growth, metabolism, proliferation, and tissue organization. The coexistence of these diseases in contiguous gene syndrome has generated significant interest in understanding how overlapping molecular pathways contribute to neurological dysfunction and cortical abnormalities.

PKD is can be characterized by the formation of fluid- filled cysts within the kidneys, which can be attributed to cause problems like renal enlargement and chronic kidney disease if the gene is found to be mutated. This condition arises following the harmful mutations happening in the genes such as PKD1 or PKD2, which encode the proteins polycystin-1 and polycystin-2 respectively [1,3]. These are the proteins that are believed to be concentrated in the primary cilia of epithelial cells and play an important part in calcium signaling, mechanosensation, cell polarity, and regulation of cellular architecture. If polycystic gets disrupted, the signaling disturbs the entire intracellular calcium homeostasis and activates proliferative signaling pathways that promote cystogenesis and abnormal tissue remodeling.

Although PKD has traditionally been considered a renal disorder, recent studies have suggested that its effects are beyond the kidneys which includes vascular complications, hepatic cyst formation, hypertension, aneurysms, and neurological abnormalities and have increasingly been associated with PKD. These findings indicate that PKD involves systemic dysregulation affecting multiple biological pathways and organ systems.

TSC is also a disorder that is inherited and is caused by the mutations in the TSC1 or TSC2 genes, which are known for encoding hamartin and tuberin proteins, respectively and they together form a regulatory complex which functions to inhibit the mammalian target of rapamycin (mTOR) signaling pathway inside the body. The mTOR pathway mentioned is responsible for regulating the cellular growth, metabolism, protein synthesis, and autophagy, but under normal physiological conditions, the TSC1–TSC2 complex suppresses excessive mTOR activity and acts to maintain cellular homeostasis [1,10].

Mutations that happens either in TSC1 or TSC2 gene tend to disrupt the formed regulatory mechanism and result in constitutive activation of mTOR signaling. The persistent and prolonged activation of mTOR pathway causes uncontrolled cellular proliferation and formation of benign tumors usually known as hamartomas in many of the organs including the brain, kidneys, lungs, skin, and heart [4]. Neurological manifestations have represented some of the most severe complications associated with TSC and significantly affect the patient's life.

One of the most significant of all molecular links between PKD and TSC is the chromosomal localization of the PKD1 and TSC2 genes on chromosome 16p13.3 [7,12]. Huge deletions in

thechromosome named 16p13.3 which may involve both genes can lead to PKD–TSC contiguous gene syndrome, a rare but clinically severe condition determined by the combined manifestations of both disorders. Patients affected by this syndrome often develop severe polycystic kidney disease during early childhood along with prominent neurological abnormalities such as epilepsy, developmental delay, intellectual disability, autism spectrum-related symptoms, and behavioral dysfunction [8].

Many clinical studies have shown that patients with PKD–TSC contiguous gene syndrome tend to show more aggressive renal disease compared with isolated PKD and more severe neurological symptoms compared with classical TSC disorder. These observations suggest that the molecular interactions occurring between PKD-associated and TSC-associated pathways will only synergistically intensify disease progression or its development [5,6].

Neurological abnormalities are the most observable features in the PKD–TSC overlap syndrome which includes common brain abnormalities such as cortical tubers, subependymal nodules, abnormal neuronal migration, cortical dysplasia, and disrupted cortical organization, all of which are have known to be associated with major problems like seizures, cognitive impairment, autism spectrum disorders, and learning disabilities.

The cerebral cortex is a highly organized part of our brain which is known for being responsible for performing higher cognitive functions such as memory, language, sensory processing, and motor coordination. We also know that for the normal cortical development brain requires proper regulation of biological processes such as neural progenitor proliferation, neuronal migration, differentiation, axonal guidance, synaptic maturation, and neurovascular organization, and disruption in any of these processes can lead to abnormal cortical architecture and impaired neuronal connectivity[4].

Many of the evidences have suggested that molecular interactions between PKD-associated pathways and TSC-mediated mTOR signaling may largely contribute to cortical abnormalities due to the dysregulated proliferative signaling, impaired translational control, oxidative stress, and neurovascular dysfunction may collectively disrupt cortical development and increase neuronal hyperexcitability.

Recent advances and studies in the transcriptomics, network biology, and bioinformatics have enabled researchers to investigate complex molecular interactions associated with neurological disorders whereas systems biology approaches have become particularly important in understanding diseases involving multiple interconnected pathways because they provide insight into molecular convergence rather than isolated gene defects.

In the context of PKD–TSC overlap syndrome, identification of common molecular regulators may help explain how distinct genetic disorders converge to produce similar neurological phenotypes. Such understanding is important not only for improving knowledge of disease pathogenesis but also for identifying potential biomarkers and therapeutic targets associated with cortical dysfunction.

## 1.2 Molecular Mechanisms Involved in PKD–TSC Overlap Syndrome

### 1.2.1 mTOR Signaling Pathway

mTOR is a serine/ threonine is a protein kinase which is a member of PI3K- related kinase (PIKK) family comprising of two complexes mTOR complex 1(mTOR1) and mTOR complex 2 (mTOR2) [5]. The mammalian target of rapamycin (mTOR) signaling pathway is considered to be one of the most important intracellular pathways involved in cellular growth, metabolism, autophagy, and protein synthesis and plays a crucial role in all these processes. This pathway has been majorly known for integrating signals from nutrients, growth factors, cellular energy status, and environmental stress to regulate cellular homeostasis [5,7,12].

Under normal circumstances, the TSC1–TSC2 complex functions negatively to suppress the mTOR activity by inhibiting the small GTPase Rheb protein, thereby preventing excessive cellular growth within the specified region which otherwise is seen normal [9]. When mutation occurs in TSC1 or TSC2 it generally impairs this inhibitory mechanism and leads to the constitutive activation of mTOR signaling by activating this GTPase Rheb protein.

Prolonged activation of mTOR has shown major effects on the neuronal development and brain organization which are not normal because the hyperactivation of this pathway disrupts neuronal migration, dendritic branching, synapse formation, and axonal development. Such abnormalities usually interfere with the normal cortical organization and have a great influence on the neuronal hyperexcitability [8,9].

mTOR dysregulation is associated with epilepsy, cortical dysplasia, autism spectrum disorders, and cognitive dysfunction as several studies have been conducted so far have also suggested that increased and abnormal mTOR signaling usually promotes abnormal neuronal connectivity and excessive excitatory signaling within cortical networks.

In PKD, defective polycystin proteins affects calcium signaling pathways which in turn indirectly influences mTOR activity, where reduced intracellular calcium levels can activate proliferative and metabolic signaling pathways, further contributing to mTOR dysregulation. Therefore, abnormal mTOR activation represents a major molecular convergence point between PKD and TSC [2,3].

### 1.2.2. Interaction between PKD1 and mTORC1

In normal physiological conditions PC-1 inhibits mTORC1 activity which in return suppresses PC-1 expression at controlled level. This reciprocal inhibition creates a homeostatic balance, ensuring controlled cell size and proliferation as well as the prevention of abnormal cyst formation. When mTORC1 is hyperactivated, it negatively regulates the expression of polycystin-1 (PC-1), reduced PC-1 fails to restrain MtorC1 forming a negative feedback loop. As a result, abnormal cell growth, proliferation and cystogenesis (usually normal than ADPKD) occurs [5].

### 1.2.3. Cellular Proliferation and Neural Development

For normal tissue growth and organ development to happen in a systematic manner it is essential and crucial at the same time to undergo controlled and well-regulated cellular proliferation. During cortical development, neural progenitor cells undergo carefully regulated proliferation and differentiation to generate mature neurons and glial cells.

In both PKD and TSC, dysregulated signaling pathways usually known to promote excessive cellular proliferation and impairs normal differentiation processes which can easily disrupt the cortical layering and neuronal migration and synaptic organization as well. The transcription factor MYC has a central role in regulating genes associated with cell cycle progression, metabolism, apoptosis, and differentiation as it is highly active as observed during embryonic development and is particularly important in rapidly dividing tissues such as present in the nervous system [10].

Overactivation of MYC signaling can lead to excessive neural progenitor expansion and impaired neuronal maturation. Such dysregulation may contribute to cortical disorganization and abnormal neuronal circuitry. MYC also regulates metabolic pathways including glycolysis and mitochondrial function, both of which are essential for neuronal survival and development.

Studies has also demonstrated close interaction between MYC and mTOR signaling pathways since hyperactivation of mTOR may enhance MYC-associated proliferative signaling, thereby promoting abnormal tissue growth and cortical abnormalities.

**1.2.4. Protein Translation and Synaptic Function**

Protein synthesis is a critical process involved in neuronal development because various activities such as synapse formation, dendritic growth, and synaptic plasticity all of them greatly depends on properly regulated translational activity.

The mTOR pathway controls protein translation through downstream regulators like EIF4EBP1 which regulates translation initiation by tightly binding to eukaryotic initiation factor 4E (eIF4E) and controlling the formation of the translation initiation complexes.

As seen in the normal physiological conditions, phosphorylation of EIF4EBP1 by mTOR pathway generally releases eIF4E and it allows controlled protein synthesis. However, if persistent mTOR activation occurs continuously, it disrupts this regulatory process and results in excessive translational activity [4]. Abnormal protein synthesis can cause impaired synaptic maturation and altered neuronal communication because excessive production and translation of synaptic proteins can act to disrupt the balance between excitatory and inhibitory signaling within cortical networks, which increases susceptibility to abnormal conditions such as seizures and cognitive dysfunction.

Several neurodevelopmental disorders characterized by mTOR dysregulation shows up abnormalities in translational control. Therefore, altered EIF4EBP1 signaling is considered an important contributor to cortical abnormalities and neurological manifestations in PKD–TSC overlap syndrome.

**1.2.5. Neurovascular Dysfunction and Oxidative Stress**

Normal brain development depends not only on neuronal signaling but also on proper neurovascular organization and for this purpose, blood vessels provide oxygen and nutrients for developing neural tissues and actively participate in signaling interactions that regulate proper well- coordinated neuronal migration and differentiation.

NOS3 has been known for encoding endothelial nitric oxide synthase (eNOS) which is an enzyme that is responsible for nitric oxide production in vascular endothelial cells and has an

essential role in vascular tone regulation, cerebral blood flow maintenance, oxidative balance, and neuronal survival [8].

Altered NOS3 signaling can affect neurovascular homeostasis and can also increase the oxidative stress within the developing brain and if nitric oxide availability has been reduced it can disrupt cerebral blood flow to the brain and impair metabolic support for neurons [8]. Oxidative stress is often considered to be the detrimental effect during the cortical development because neurons are highly sensitive to metabolic imbalance which can damage cellular proteins, lipids, and DNA, ultimately affecting neuronal survival and synaptic stability.

Recent studies indicate that vascular dysfunction and metabolic imbalance can contribute significantly to epilepsy and cortical dysplasia. In PKD, endothelial dysfunction and vascular abnormalities are frequently observed due to altered calcium signaling associated with defective polycystin proteins.

The identification of NOS3 as a highly connected molecular regulator suggests that neurovascular dysfunction may represent an important but underrecognized mechanism contributing to PKD–TSC-associated cortical pathology.

### 1.3 Importance of Systems Biology and Network Analysis

Complex disorders such as PKD–TSC overlap syndrome involve interactions between multiple genes, proteins, and signaling pathways but traditional approaches focuses on individual genes are often insufficient to explain the broader biological mechanisms underlying disease progression. Systems biology has emerged as a powerful approach for having a better understanding of complex diseases because it integrates computational, molecular, and biological data to analyze interactions within entire biological networks.

One important systems biology method is protein–protein interaction (PPI) network analysis that we have mentioned in our study. Proteins involved rarely function independently within the cells instead, they interact with other proteins present to regulate biological processes. PPI network as already discussed multiple times help identify functional relationships among proteins involved in disease mechanisms.

Databases such as STRING performs to integrate experimentally validated and predicted protein interactions from multiple sources including experimental evidence, computational prediction, co-expression analysis, and literature mining. Visualization of these interactions enables identification of highly connected proteins known as hub genes.

Hub genes are biologically so important because they have the potential of regulating multiple pathways simultaneously and often play central roles in disease development or progression. Their identification can therefore provide insights into the key molecular drivers associated with neurological dysfunction.

Functional enrichment analysis done further improves network interpretation by identifying pathways significantly associated with selected genes. Pathways related to mTOR signaling, oxidative stress, synaptic regulation, cellular proliferation, and neurovascular signaling are particularly relevant in PKD–TSC overlap syndrome.

Integrating gene association data with PPI network analysis provides a systems-level understanding of molecular convergence between PKD and TSC. Such approaches may help

identify novel therapeutic targets and improve understanding of cortical abnormalities associated with neurological disease.

### 1.4 Research Gap

Although enough progress so far has been made to understanding the individual biology of Tuberous Sclerosis Complex and Polycystic Kidney Disease, the molecular basis of PKD–TSC overlap syndrome now remains insufficiently understood, especially with respect to cortical abnormalities and neurological dysfunction. Existing research that has been conducted so far and largely examined these disorders independently, leaving several interconnected mechanisms poorly explored.

Most of neurological studies related to TSC that have been examined have mainly put an emphasis on the hyperactivation of the mTOR signaling pathway and its association with cortical tubers, epilepsy, and neurodevelopmental impairment. On one hand, mTOR dysregulation is widely accepted as a central pathogenic mechanism, while on the other hand, less attention has always been given to how other biological processes such as abnormal cellular proliferation, neurovascular imbalance, and dysregulated protein translation interact with mTOR signaling to influence cortical pathology.

Similarly, investigations involving PKD have traditionally focused on renal cyst formation and kidney-related complications. The potential involvement of PKD-associated signaling pathways in brain development and cortical organization has received comparatively little attention. Although defective polycystin signaling is known to alter calcium homeostasis and cellular growth pathways, its contribution to neuronal dysfunction and cortical abnormalities in the context of PKD–TSC overlap syndrome is still not clearly understood.

One important limitation in the current literature is the lack of systems-level analysis of PKD–TSC contiguous gene syndrome. Because maximum of the available studies are either clinically descriptive or mutation-oriented, concentrating primarily on phenotypic presentation rather than integrated molecular interactions. Consequently, the functional relationships between shared disease-associated genes and their interacting protein networks remain inadequately characterized.

In addition, only a limited number of studies have combined gene association analysis with protein–protein interaction (PPI) network analysis to identify key molecular regulators involved in PKD–TSC-associated neurological pathology. Because of this, several biologically important hub genes that may coordinate multiple signaling pathways could remain unidentified.

The neurovascular dysfunction in PKD–TSC overlap syndrome still represents a poorly explored area of research, while vascular abnormalities are well studied in PKD alone, however, their influence on cortical organization, neuronal excitability, and cognitive dysfunction has not been investigated till now. To be specific, the involvement of NOS3 and related neurovascular signaling pathways has received relatively less attention in studies of cortical pathology.

Likewise, translational dysregulation facilitated by EIF4EBP1 again remains poorly studied in PKD–TSC overlap syndrome, despite the established role of mTOR-dependent protein synthesis in neuronal development, synaptic maturation, and epilepsy. Abnormal translational

control may significantly contribute to cortical dysfunction, still, this aspect has not been explored extensively using integrated network-based approaches.

In addition, although abnormal cell growth is considered a common feature of both PKD and TSC, the specific role of MYC in cortical abnormalities and neuronal dysfunction has not been explored in detail. It is still not fully understood how MYC interacts with pathways related to protein translation and neurovascular function in the brain. Studying these interactions may help in better understanding the progression of the disease and the neurological complications associated with PKD–TSC overlap syndrome.

Therefore, the present study attempts to address these gaps by integrating disease-associated gene analysis with protein–protein interaction network analysis to identify shared molecular regulators involved in PKD–TSC-associated cortical pathology. By focusing on hub genes and interconnected pathways related to proliferation, translational regulation, and neurovascular dysfunction, this study provides a broader systems-level perspective on the molecular mechanisms underlying epilepsy, cognitive impairment, and cortical abnormalities in PKD–TSC overlap syndrome.

## 1.5 Objectives of the Study

### 1.5.1 Primary Objective

To investigate shared molecular mechanisms involved in PKD–TSC overlap syndrome using systems biology and protein–protein interaction network analysis.

Specific Objectives

1. To identify disease-associated genes related to PKD and TSC using bioinformatics databases.
2. To identify common genes shared between PKD and TSC.
3. To construct protein–protein interaction networks associated with overlapping genes.
4. To identify key hub genes involved in cortical abnormalities and neurological dysfunction.
5. To analyze molecular pathways associated with proliferative signaling, translational regulation, and neurovascular dysfunction.
6. To propose a systems-level molecular model explaining epilepsy and cognitive impairment associated with PKD–TSC overlap syndrome.

# CHAPTER 2

## 2. Literature Review

### 2.1 PKD–TSC Contiguous Gene Syndrome

Polycystic Kidney Disease and Tuberous Sclerosis Complex as discussed are inherited disorders that are caused by mutations affecting important cellular signaling pathways, despite them being clinically distinct, several studies have shown a clear molecular overlap between them, especially in cases that generally involves contiguous gene syndrome. The coexistence of PKD and TSC can be attributed to the large chromosomal deletions involving adjacent PKD1 and TSC2 genes located on the chromosome number 16p13.3.

PKD is primarily associated with the mutations that take place in the PKD1 and PKD2 genes, which encode for the proteins called polycystin-1 and polycystin-2 respectively which are localized in the primary cilia and are involved in mechanosensation, intracellular calcium regulation, and maintenance of epithelial architecture. Loss of normal polycystin function disrupts calcium signaling and activates proliferative pathways that contribute to cyst formation and tissue remodeling and to no surprise may cause problems associated with the disorder.

Whereas, TSC on the other hand, is caused by mutations taking place in the TSC1 or TSC2 genes, encoding hamartin and tuberin proteins, respectively. These proteins form a complex that negatively regulates the mammalian target of rapamycin (mTOR) signaling pathway. Dysfunction of the TSC1–TSC2 complex results in persistent activation of mTOR signaling, leading to uncontrolled cellular growth and formation of hamartomas in multiple organs.

PKD–TSC contiguous gene syndrome represents a severe clinical phenotype because deletion of both PKD1 and TSC2 combines the pathological effects of both individual disorders. Patients affected by this PKD–TSC contiguous gene syndrome often develop rapidly progressive renal cystic disease during early life along with severe neurological manifestations such as epilepsy, developmental delay, intellectual disability, and autism spectrum disorders.

Previous experimental and clinical studies many a times have demonstrated that the renal disease progression is more aggressive in PKD–TSC contiguous gene syndrome than compared with isolated PKD. Similarly, neurological symptoms tend to be more severe than those observed in classical TSC. These observations suggest that shared molecular mechanisms between PKD and TSC might synergistically contribute to the disease progression eventually.

At the molecular level, both of the disorders PKD and TSC involve dysregulation of pathways associated with the following processes such as cellular growth, metabolism, and signal transduction. Defective polycystin signaling has been shown to influence mTOR activity indirectly through altered calcium signaling and metabolic imbalance. As a result, mTOR dysregulation may serve as a major molecular convergence point between the two disorders.

Recent advances in systems biology have enabled researchers to investigate the interaction between PKD-associated and TSC-associated pathways more comprehensively. However, despite increasing evidence regarding molecular overlap, the precise mechanisms through which PKD-related signaling contributes to cortical abnormalities and neurological dysfunction remain insufficiently understood.

**2.2 Cortical Dysplasia and Neurodevelopment**

The cerebral cortex as we know is a highly organized structure responsible for higher cognitive functions including learning, memory, language, sensory integration, and motor coordination, therefore, studies have often been found saying that the cortical development occurs through closely monitored biological processes involving neural stem cell proliferation, neuronal

migration, differentiation, synaptic maturation, and organization of neuronal circuits. Any disturbances if at all occurs in these developmental processes can result in cortical dysplasia, a condition often determined by certain abnormal cortical changes and defective neuronal organization. Cortical dysplasia, hence, is one of the major pathological features associated with epilepsy and neurodevelopmental disorders.

On the other hand, in TSC brain abnormalities usually arise due to the development of cortical tubers which contain dysmorphic neurons, huge cells, abnormal astrocytes and disrupted cortical layering. These lesions are closely linked with seizures and cognitive dysfunctions. During the study we found that many studies have shown that abnormal neuronal connectivity and increased excitatory signaling within cortical tubers contribute to neuronal hyperexcitability.

Abnormal activation of the mTOR pathway has come out to be a major mechanism involved in cortical dysplasia, since its hyperactivation disrupts neuronal migration, axonal guidance, dendritic development, and synaptic plasticity which cause huge interference with formation of functional neuronal circuits and increase susceptibility to epilepsy.

Along with mTOR dysregulation, oxidative and metabolic imbalance have also proved to be the major contributors to the cortical pathology. Neurons as we know are the structural units of the brain and they are highly dependent on tightly regulated metabolic activity and oxygen supply during development. So, any kind of disturbances if, however are reported in the mitochondrial function, energy metabolism, or vascular support can greatly affect the neuronal survival and synaptic maturation.

Studies conducted in recent times have suggested that the neurovascular signaling appears to be a crucial pathway and plays an important role in the cortical development. In the cortical brain, cerebral blood vessels not only provided us nutrients and oxygen but also regulate neuronal differentiation and migration through signaling interactions. Altered vascular organization is therefore expected to contribute to the abnormal cortical structure and neuronal dysfunction.

In PKD–TSC overlap syndrome, cortical abnormalities are likely to be arised due to the combined effects of excessive proliferative signaling, impaired translational regulation, metabolic stress, and neurovascular dysfunction. However, it has been found that the role of PKD-associated pathways in influencing cortical dysplasia remains poorly explored as compared with classical TSC-related mechanisms.

Understanding the molecular basis of cortical abnormalities is important because neurological manifestations such as epilepsy, cognitive impairment, and developmental delay significantly affect quality of life in patients with PKD–TSC overlap syndrome.

### 2.3 MYC in Neural Pathophysiology

MYC is a transcription factor that regulates all the genes involved in cellular proliferation, differentiation, apoptosis, metabolism, and growth and also known for playing a critical role during embryonic development while it is highly important in tissues such as nervous system that have high proliferative activity.

Not only this, but during the development of the brain, MYC acts to regulate neural progenitor cell proliferation and controls the balance between self-renewal and neuronal differentiation as well. Proper MYC expression is required for maintaining the normal cortical organization and neuronal maturation, however, dysregulation of MYC signaling can disturb these developmental processes and contribute to neurological abnormalities.

Exaggerated MYC activation promotes uncontrolled cellular proliferation and may likely the impair neuronal differentiation process as well. Studies have repeatedly demonstrated that abnormal MYC expression could also be the reason for cortical disorganization, defective neuronal migration, and altered synaptic development. MYC dysregulation has also been seen to be effectively associated with neurodevelopmental disorders specifically determined by epilepsy and cognitive dysfunction.

In addition to its role in proliferation, MYC also regulates metabolic pathways including glycolysis, mitochondrial function, and oxidative stress responses. As it is a known fact that neurons are extremely sensitive to metabolic imbalance, and therefore abnormal MYC associated signaling might increase their susceptibility to damaged state during cortical development.

MYC also interacts tightly with the mTOR signaling pathway but the hyperactivation of the mTOR can enhance MYC-associated proliferative signaling, leading to excessive neural progenitor expansion and abnormal tissue growth. Because both PKD and TSC involve abnormal proliferative pathways, MYC has emerged as an important molecular regulator potentially contributing to PKD–TSC-associated cortical pathology.

Systems biology studies frequently identify MYC as a hub gene due to its extensive interactions with proteins involved in cell cycle regulation, signal transduction, and neuronal function. Highly connected hub genes to many other genes are seemingly appears to be biologically important because they influence multiple pathways simultaneously and can significantly affect disease progression and can lead to neurological manifestations.

The identification of MYC as a central node in molecular interaction networks suggests that proliferative dysregulation may play a major role in cortical abnormalities associated with PKD–TSC overlap syndrome.

**2.4 EIF4EBP1 and mTOR Regulation**

EIF4EBP1 is an important downstream regulator of the mTOR signaling pathway and plays a major role in controlling protein synthesis. EIF4EBP1 regulates translation initiation by binding to eukaryotic initiation factor 4E (eIF4E), thereby preventing formation of translation initiation complexes.

Under normal conditions, mTOR-mediated phosphorylation of EIF4EBP1 releases the eIF4E and allows the controlled protein synthesis which is importantly required for cellular growth and neuronal function. However, if, persistent activation of mTOR signaling continues for the longer time, it may disrupt this regulatory mechanism and results in excessive translational activity.

Protein synthesis is specifically more important in the nervous system because neuronal differentiation, dendritic growth, synapse formation, and synaptic plasticity all of these

processesdepend on tightly regulated translational processes. Abnormal protein translation can impair neuronal communication and disrupt formation of functional neural networks.

Several studies have even proved that dysregulated EIF4EBP1 signaling contributes to problems like epilepsy, autism spectrum disorders, intellectual disability, and cortical dysplasia. In TSC inherited disorder, hyperactivation of the mTOR leads to persistent phosphorylation of EIF4EBP1 and increased synthesis of proteins associated with abnormal cellular growth and neuronal hyperexcitability.

Too much of the protein synthesis can also likely to affect the composition of synaptic proteins and disturb the normal synaptic development in the brain which may disrupt the balance between excitatory and inhibitory signals in neuronal circuits, making neurons more prone to hyperexcitability and seizures.

Recent studies based on the network biology approaches have determined EIF4EBP1 as an important connecting bridging molecule between mTOR signaling, synaptic dysfunction, and neurodevelopmental abnormalities. Its interaction with proteins involved in metabolism, translation, and neuronal signaling further highlights and confirm its role in the cortical pathology.

EIF4EBP1 is a promising therapeutic target in neurological disorders related to mTOR pathway dysregulation because according to the study conducted, it shows that abnormal protein translation is a major outcome of exaggerated mTOR activation due the mutation in some genes related to the disorders.

**2.5 NOS3 in Vascular and Cortical Development**

NOS3 encodes endothelial nitric oxide synthase (eNOS), it is an enzyme that is responsible for production of nitric oxide within vascular endothelial cells. Nitric oxide is an essential and required signaling molecule which is involved in the processes like vascular tone regulation, cerebral blood flow maintenance, oxidative balance, and cellular metabolism.

During brain development, neurovascular signaling is very essential for supplying oxygen and nutrients to rapidly growing neural tissues and it is also common known fact that proper vascular organization supports neuronal survival, migration, differentiation, and synaptic maturation. Therefore, disturbances in vascular homeostasis can significantly affect cortical development.

Nitric oxide production has an important role in maintaining cerebral blood flow and protecting neural tissues from oxidative stress. It's production is mediated through NOS3 gene but if nitric oxide production is reduced it might impair the endothelial function and may increase neurons susceptibility to the injury. On the other hand, it has been observed that the excessive oxidative stress can further disrupt neuronal signaling and synaptic stability.

Recent studies have highlighted how neurovascular dysfunction in epilepsy and neurodevelopmental disorders are closely related to each other. Because abnormal vascular signaling is known to contribute to impaired oxygen delivery, metabolic imbalance, and neuronal hyperexcitability. In PKD, vascular abnormalities are commonly observed due to altered calcium signaling and endothelial dysfunction associated with defective polycystin

proteins which goes on to interact with TSC-associated mTOR dysregulation and intensify cortical pathology.

NOS3 is closely associated with oxidative stress pathways and inflammatory signaling, both of which greatly influence the neuronal survival and cortical organization but systems biology analyses frequently identify NOS3 as a key network component involved in neurovascular regulation and metabolic homeostasis.The identification of NOS3 as a hub gene in PKD–TSC interaction networks suggests that neurovascular dysfunction may represent an important but previously underrecognized mechanism contributing to cortical abnormalities and neurological manifestations.

By and large, the involvement of NOS3 in vascular regulation, oxidative balance, and cortical development highlights the importance of neurovascular signaling in understanding the molecular basis of PKD–TSC-associated neurological dysfunction.

12

# CHAPTER 3

## 3. Methodology

### 3.1 Study Design

The study was carried out to understand and investigate the molecular mechanisms that are responsible for cortical abnormalities usually associated with PKD-TSC contiguous gene syndrome. A bioinformatics approach was followed where different computational and network analyses tools were utilized to important genes, protein interactions, and biological pathways that may contribute to disease progression. The methodology also involved protein-protein interaction (PPI) network construction, gene expression and pathway enrichment analysis since both disorders interconnected through multiple signaling pathways.

The emphasis was put on identifying shared genes associated with PKD and TSC, constructing a protein–protein interaction (PPI) network, analysis of the network and finally analyzing the functional significance of highly connected genes within the network. The entire methodology was divided into sequential stages including gene selection, identification of overlapping genes, network construction, hub gene analysis, and biological interpretation of results.

The overall workflow of the study included:

1. Selection of the top 100 genes associated with PKD and TSC using the CTD database
2. Identification of common genes between the two diseases using Google Colab
3. Construction of the protein–protein interaction network using STRING database
4. Identification of common hub genes based on network connectivity
5. Functional interpretation of the identified hub genes and associated pathways

This approach enabled the identification of central molecular mechanisms potentially involved in PKD–TSC-associated cortical pathology.

**3.2 Selection of Disease-Associated Genes**

**3.2.1 Comparative Toxicogenomics Database (CTD)**

The first and the foremost step of the study was gene selection in which genes associated with both the diseases Polycystic Kidney Disease (PKD) and Tuberous Sclerosis (TSC) were identified using publicly accessible database called Comparative Toxicogenomics Database (CTD) which contains almost all information from gene- disease relationships to protein interactions, pathways and toxicogenomic data.

For conducting the study CTD databse was chosen because it is not only a integration of experimentally validated associations and literature-based evidences, but also helps researchers in understanding the relationship between genes, chemicals, associated diseases and molecular pathways that may contribute to the progression of the disease. It is a manual curation model, where trained biomedical scientists assemble molecular interaction data from primary published sources and manually annotate genes, disease and chemicals with standardized terms.

CTD also calculates an inference score for each gene- disease pair, statistical measure based on co- occurrence frequencies in the curated literature, allowing evidence-weighted gene prioritization rather than simple frequency-based ranking.

Separate searches for each disease was performed to collect disease specific gene information.

- Polycystic Kidney Disease (PKD)

- Tuberous Sclerosis Complex (TSC)

For each disease, the top 100 associated genes were selected from the CTD database on the based on their inference scores and their reported biological relevance. It is an important parameter provided by the database that reflects the strength of the relationship between a particular gene and a disease and is generally calculated using evidence collected from previously published scientific studies and curated research data. Higher the inference score, stronger and more reliable is the association between the gene and the disease.

During the gene selection process, apart from the inference score, the biological relevance of each gene was also taken into the consideration in which genes having important cellular functions, signaling pathways, disease mechanism or tissue specific abnormalities were given more importance. This helped the study in selecting the most relevant and scientifically supported genes.

The selected genes were considered important for understanding the molecular basis of PKD and TSC because they are more likely to participate in disease progression and pathological changes. These genes were further used for downstream bioinformatics analyses such as identification of common genes, protein–protein interaction network construction, hub gene analysis, and pathway enrichment studies. This approach helped in focusing the study on the most meaningful genetic factors associated with the disease conditions.

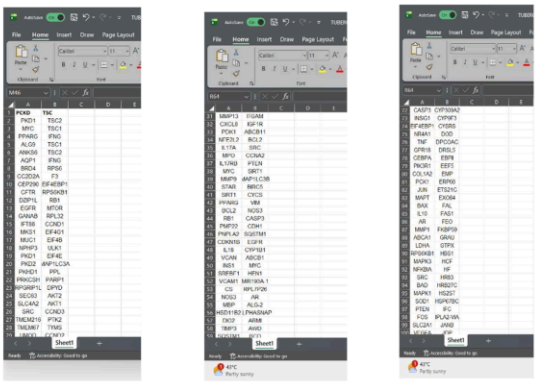
The selected genes included regulators involved in the following mechanisms:

- Cellular proliferation
- Signal transduction
- mTOR signaling
- Calcium homeostasis
- Protein synthesis
- Neurodevelopment
- Oxidative stress
- Vascular signaling

The gene associated with PKD and TSC were downloaded from the CTD database and were arranged separately in spreadsheet format for easier organization and further computational analysis, which made it much easier in comparing the genes of both the diseases and performing different bioinformatics analyses more efficiently.

As already mentioned above, top 100 genes were selected to put more emphasis on the genes that had the strongest association with the diseases. These genes were considered more biologically important and more likely to play a role in disease development and progression and at the same time, limiting the dataset to the top 100 genes helped in keeping the analysis more organized and manageable for network construction and downstream bioinformatics studies.

Figure1: List of genes retrieved from Comparative Toxicogenomics Database (CTD), associated with Polycystic kidney disease (PKD) and Tuberous sclerosis (TSC).



### 3.3 Identification of Common Genes Using Google Colab

Next step in the study was to identify the molecular overlap between both the diseases PKD and TSC, so the gene lists of both diseases were compared using Google Colab, which is an online platform used for Python programming and data analysis. The list of genes retrieved from the Comparative Toxicogenomics Database were uploaded on google colab to find out the common genes to both the diseases.

For analyzing the data it uses Python libraries such as Pandas and NumPy which potentially filter the data by removing duplicate gene entries in the gene list to make the data look more well- organized and accurate.

After the data was filtered and organized into a proper format, next task for the google colab was to compare the genes of both the diseases to make a separate list of common overlapping genes. The comparison was made using certain set of operations in the python which made it much easier for us to take a look at all of the overlapping genes.

To carry the study forward, it was important to identify the common genes to both the disorders because through such overlapping genes could likely be involved in the similar biological functions and disease related processes. The common gene obtained from the list of genes carrying 100 genes linked to both conditions were used for the construction of PPI network which was later analyzed to get a better understanding of the common link between PKD and TSC contiguous gene syndrome.

Google Colab is a cloud-based computational platform that supports Python programming and data analysis. Google Colab used in the study is a freely accessible online platform developed by the Google that allows users to write and run Python code through a web browser and is widely used for data analysis, machine learning, and bioinformatics research because it

provides simple, easy and accessible user-friendly coding environment without the need to install any software on a computer. It mainly works through online notebooks where researchers can write code, upload datasets, perform analysis, and visualize results in a step-by-step manner. It also supports many useful Python libraries such as Pandas, NumPy, and Matplotlib, which are commonly used for the analyzing biological data.

The unique and one the advantages of google colab is that it offers computational resources that are free of cost and automatically saves the work without any manual interruption. This feature allows researchers and students to perform computational analysis from any location around the world with just an internet connection. Along with this unique feature, it offers other advantages such as it requires no extra efforts for installing additional software or using high-end computer systems, therefore has made the process much simple and accessible.

Google colab is efficient in handling large datasets and organizing it in a more structured way. The platform uses different Python libraries for cleaning the data, for conducting comparison and analysis which proved to be of great help in managing the datasets in a more systematic way. Furthermore, it repeats and modifies the analyses whenever it is required since the complete workflow could be saved online in a simple format to maintain transparency and accuracy throughout the study.

In addition, it supported the efficient implementation of Python-based bioinformatics workflows. It allowed smooth execution of data processing, intersection analysis, and downstream computational analysis in a simple and user-friendly environment. These are some of the features that make Google Colab a useful and reliable platform for carrying out the present bioinformatics study. The overlapping genes obtained from this analysis formed the foundation for subsequent protein interaction studies.

Table 1: The table below shows overlapping genes in PKD-TSC contiguous gene syndrome

| S.No. | COMMON GENE IDENTIFIED | GENE NAME |
|---|---|---|
| 1. | SIRT1 | Sirtuin 1 |
| 2. | BCL2 | B-cell lymphoma 2 |
| 3. | RPS6KB1 | Ribosomal Protein S6 Kinase B1 |
| 4. | RB1 | RB Transcriptional Corepressor 1 |
| 5. | EGFR | Epidermal Growth Factor Receptor |
| 6. | EIF4EBP1 | Eukaryotic Translation Initiation Factor 4E Binding Protein 1 |
| 7. | SRC | SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase |
| 8. | CASP3 | Caspase 3 |
| 9. | SQSTM1 | Sequestosome 1 |
| 10. | AR | Androgen Receptor |
| 11. | MYC | MYC Proto-Oncogene, BHLH Transcription Factor |
| 12. | CCND2 | Cyclin D2 |
| 13. | NOS3 | Nitric Oxide Synthase 3 |
| 14. | PTEN | Phosphatase and Tensin Homolog |
| 15. | AKT1 | AKT Serine/Threonine Kinase 1 |

### 3.4 Construction of Protein–Protein Interaction (PPI) Network

Protein–protein interaction (PPI) analysis was performed to study and understand the functional relationships between the overlapping genes identified in PKD and TSC disorder separately. The analysis was required to get a snapshot of how proteins encoded by the common genes interact with one another and how they are possibly involved in similar biological processes and disease mechanisms.

The common genes obtained from the Google Colab were imported into STRING for the construction of the PPI network to understand the interconnected molecular mechanisms. STRING is a widely used bioinformatics database by the students and researchers that provides information about known and predicted interactions between proteins. The interaction data provided by the STRING is believed to be collected from different sources that includes experimental studies by the researchers, computational prediction methods, co-expression analysis, text mining, and information from other biological databases.

STRING's detailed mapping of the interactions occurring among different genes and its easy-to-understand network image, clearly showing proteins as nodes represented by different colours really pushed the study forward in interpreting the results more effectively. Therefore, it was preferred over other databases available.

Within the PPI network, each protein encoded by the gene was demonstrated as node- hub proteins with red, bridging proteins as orange nodes and yellow nodes represented other proteins. Interactions between proteins were represented by straight lines known as edges making the students understand which protein is interacting with another protein. A confidence score threshold was applied during the analysis to increase the reliability of the interactions and reduce the possibility of false-positive results.

The generated PPI network helped in visualizing the molecular connections among proteins associated with PKD–TSC overlap syndrome. Special emphasis was given to highly interconnected regions within the network because such regions often represent important biological pathways or signaling clusters that may play a significant role in disease development and progression.

The constructed network was analyzed to understand how shared genes interact at the molecular level and contribute collectively to:

- mTOR signaling
- Cellular proliferation
- Synaptic regulation
- Neurovascular signaling
- Oxidative stress response

PPI network analysis is particularly important in complex disorders like TSC and PKD because proteins tend to function in co-ordination with other biological systems rather than acting independently.

### 3.5 Identification of Hub Genes

To proceed with the study, PPI network analysis was carried out to identify the genes that seem to be playing important roles within the network in the progression of the disease. The analyses made us understand the strong influence of each gene on multiple molecular mechanisms that were involved in the PKD-TSC contiguous gene syndrome as it is clearly visible within the network that there are some genes that are interacting with many other several genes. Such genes are called hub genes that are the key drivers in the progression of the disease.

Hub genes are biologically significant because them being the main genes can potentially influence multiple biological processes simultaneously, because if any abnormal changes are reported in these genes, it may affect several pathways at the same time, which can contribute to disease development, progression and sometimes even worsen the disease. In many diseases, hub genes are closely associated with major pathological changes and are therefore considered potential biomarkers or therapeutic targets.

The identification of the hub genes helped us understand the important regulators among the common genes shared between PKD and TSC, which on further investigation provided the better understanding of their possible role in causing cortical abnormalities and neurological dysfunction linked with PKD-TSC overlap syndrome.

The PPI network generated from STRING was analyzed based on the following reliable parameters: -

- Degree centrality
- Number of interactions
- Connectivity patterns

Genes showing the highest interaction with other genes were identified as hub genes. Among the identified hub genes, special attention was given to the genes mentioned below: -

- MYC
- EIF4EBP1
- NOS3

These genes had shown the strong interaction connectivity among various genes or proteins and were closely associated with pathways involved in cellular proliferation, translational regulation, mTOR signaling, neurovascular homeostasis and cortical development.

MYC emerged out to be the major proliferative regulator involved in neural progenitor expansion and neuronal differentiation, while EIF4EBP1 was associated with mTOR-mediated protein translation and synaptic regulation and NOS3 was linked to vascular signaling, oxidative balance, and cerebral blood flow regulation.

Identification of these hub genes provided insight into the key molecular drivers potentially contributing to cortical abnormalities and neurological manifestations in PKD–TSC overlap syndrome.

### 3.6 Interpretation of Results

The final part of the study paid a great attention on interpreting the molecular interactions among the genes and proteins encoded by them, also important hub genes were identified during the network analysis of PPI network. Therefore, the study was further carried out by carefully comparing the analyzed data with previously published reliable scientific literature and known biological pathway information, which helped in understanding the possible biological significance of the identified genes and how they may contribute to the development of PKD–TSC overlap syndrome.

The integrated network analysis suggested that PKD and TSC are not likely to function as separate disorders at the molecular level but, instead, both conditions appear to function in co-operation with one another with interconnected signaling pathways that are involved in cortical development, neuronal growth, cellular metabolism, and normal brain function. Any discrepancies observed in these pathways are highly likely to contribute to cortical abnormalities and neurological complications commonly observed in the overlap syndrome.

The gene MYC was identified as one of most the important hub genes in the network which is known for regulating cellular growth, proliferation, metabolism, and differentiation. In the present study, abnormal MYC-related signaling was interpreted as a key driver to excessive cellular proliferation and impaired neuronal differentiation because it may disturb cortical organization and affect the formation of normal neuronal connections and circuitry within the brain.

Another important hub gene identified in the study was EIF4EBP1 which is closely associated with the mTOR signaling pathway and is known for playing an important role in regulating protein synthesis, cellular growth, and synaptic development. Prolonged activation of EIF4EBP1-associated signaling pathway can result in enhanced abnormal protein translation and impaired synaptic maturation. Such abnormalities contribute to neuronal hyperexcitability, altered neuronal communication, and epilepsy-related manifestations commonly associated with TSC-related cortical dysfunction.

The gene NOS3 was also identified as an important molecular regulator in the network and is known to be involved in nitric oxide production, vascular regulation, and maintenance of normal blood flow. Disrupted NOS3 signaling contribute to neurovascular dysfunction and increased oxidative stress in brain tissues. Disturbance in nitric oxide signaling further affects cerebral blood circulation and metabolic balance, which can increase neuronal stress and make brain cells more vulnerable to damage and dysfunction.

Overall, the findings of the study suggest that these hub genes and signaling pathways may collectively contribute to the molecular mechanisms underlying cortical abnormalities and neurological dysfunction in PKD–TSC overlap syndrome. The study also highlights the importance of integrated bioinformatics and network analysis approaches in understanding complex disease mechanisms at the molecular level.

The combined interaction of proliferative dysregulation, translational imbalance, and neurovascular dysfunction supported a systems-level molecular model explaining neurological manifestations such as:

- Cortical abnormalities
- Epilepsy
- Cognitive impairment

- Synaptic dysfunction

The findings obtained from this study provide insight into the molecular convergence between PKD and TSC and may contribute to identification of future therapeutic targets for neurological complications associated with PKD–TSC overlap syndrome.

# CHAPTER 4

# RESULTS AND INTERPRETATION

## 4.1 Identification of Common Disease-Associated Genes

To get a better understanding of the molecular relationship between Polycystic Kidney Disease and Tuberous Sclerosis, we collected top 100 genes associated to both the diseases. They were retrieved based on their inference scores using Comparative Toxicogenomics Database (CTD), which significantly highlighted the molecular factors involved in the pathogenesis of both PKD and TSC.

Later, after collecting the gene datasets for both of the disorders, it was significant to perform a comparative analysis so that we could find out genes common to both conditions which on further analysis revealed several overlapping genes that are shared between PKD and TSC. However, both are genetically distinct disorders, they appear to converge through interconnected molecular pathways. So, the identification of these shared genes actually gave us the opportunity to think of common biological mechanisms that might contribute in the progression and development of PKD–TSC contiguous syndrome.

Moving forward, it was found that many of the genes were involved in pathways that are usually considered important for normal tissue development, neuronal differentiation and normal cortical organization. Such pathways are with cellular proliferation, mTOR signaling, angiogenesis, translational regulation, oxidative stress response, apoptosis, and synaptic signaling. Also, abnormal cortex development and neurological manifestations like epilepsy, cognitive dysfunction, and neuronal hyperecitability can be attributed to the dysregulation of these pathways.

Taking the research forward, studies revealed that there are several genes that associated with the pathways that are effectively known to regulate metabolic activities and cellular growth which provides the confirmation if dysregulation occurs, it may give rise to PKD-TSC associated pathology. Not just this, it also hints about the neuronal instability and abnormal cortical development due to the vascular dysfunction and disturbed metabolic activities as many of the genes are present that are actively involved in the oxidative stress response. Finding genes linked to translational regulation made the role of mTOR-mediated protein synthesis pathways even more clear. These pathways are already known to play a key role in TSC-related neurological disease.

So, it can be clearly stated that the two disorders PKD (Polycystic Kidney Disease) and TSC (Tuberous Sclerosis) don't exist as independent mechanisms but are strongly associated through various complex molecular interactions as the overlapping genes of both PKD and TSC seem to participate in common interconnected networks such as proliferation, metabolism, synaptic signalling and neurovascular homeostasis.

These findings provided the foundation for constructing a protein–protein interaction (PPI) network and its analysis. The identified common genes were investigated further, which recommended that their functional relationships, interaction patterns, and network connectivity are key regulatory proteins and major hub genes involved in PKD–TSC-associated cortical pathology.

### 4.2 Protein–Protein Interaction Network Analysis

To get a broader understanding of how different proteins within the cellular environment interact with each other a protein- protein interaction network (PPI) was created using the retrieved common genes between Polycystic Kidney Disease and Tuberous Sclerosis Complex. The network was generated using STRING which effectively showed us that the proteins were interconnected through several regulatory molecular systems associated with neurological function and cortical development.

In this network, every single point is an individual protein which is coded by the selected genes. The connections seen in this network are referred to as edges and represent the interactions between proteins. Proteins interact experimentally; functionally, they are co-expressed and have predicted signaling interactions. The edges or lines seen in the network are connecting proteins with one another and even one cluster to another cluster.

The PPI network shows large number of proteins connected to several other proteins and are denoted by different colours. The red colour nodes are the hub proteins shows the highest number of interactions and appear of playing key roles in the network. Yellow-coloured secondary nodes found around the hub proteins are representing different biological processes such as proliferation, apoptosis, metabolism, oxidative stress, and neuronal signal transduction.

Moreover, there are orange-coloured nodes present between major clusters. These proteins seem to act as bridges between hub proteins connecting several signaling pathways in the network. They suggest that signaling pathways related to proliferation, protein translation, and neurovascular communication are not independent of each other but work very closely together.

The PPI network showed a highly dense interaction pattern with three major hub genes occupying central positions within the molecular architecture:

- MYC
- EIF4EBP1
- NOS3

The gene products exhibited high interaction connectivity with adjacent proteins, and, therefore, might serve as key regulators of signaling networks associated with particular diseases.

In this regard, the MYC-associated cluster was found to be the largest cluster in the network and was associated with signaling networks controlling cell proliferation, metabolism, and transcription. Abnormal signaling involving the MYC protein product might lead to impaired proliferation and, therefore, might have a significant role in cortical malfunctioning and neuronal differentiation disorders.

On the other hand, the EIF4EBP1 gene product was also found to belong to a cluster of translational regulators associated with mTOR pathway and protein synthesis mechanisms. Abnormalities in these processes might lead to dysfunctional synapses and neuronal hyperactivity.

Lastly, the NOS3 protein was found to belong to a neurovascular signaling pathway, which involved regulation of endothelial cells, oxidative status management, and proper blood flow regulation to brain tissues. The highly interconnected network with regard to the NOS3 protein suggested the involvement of vascular complications and oxidative stress in the pathophysiology of brain tissue and neuron dysfunction.

To conclude, the PPI network had clear features of cluster formation in which various modules were linked to specific functions including proliferation, translation, neurovascular signaling, apoptosis/autophagy, and growth factor signaling pathways. The interaction among all these pathways shows that the cortical abnormalities associated with PKD-TSC overlap syndrome might be caused by a combination of dysfunctions in multiple biological pathways rather than a mere result of one malfunction at the molecular level.

### 4.3 Network Topology Analysis

In order to distinguish the proteins that have biological significance in the protein-protein interaction network, network topology analysis was carried out. Network topology analysis is used for determining which proteins have significant roles as regulators in the network and which proteins are responsible for facilitating communication among various signal transduction pathways. In conditions where complex disorders exist like Polycystic Kidney Disease and Tuberous Sclerosis Complex overlap syndrome, high network influencing proteins are generally deemed functional, as changes occurring in these proteins can impact various downstream biological functions simultaneously.

In order to assess the functional significance of the proteins in the interaction network, two network properties were considered, which were Degree Centrality and Betweenness Centrality.

These parameters were used to discover highly interconnected hub proteins, as well as proteins involved in regulating communications in various molecular pathways.

#### 4.3.1. Degree Centrality

Degree centrality is defined as the number of direct connections to which a particular protein belongs. Proteins with a high degree value are connected to a high number of neighbouring proteins, making them hub proteins. These proteins play important roles in the regulation of several physiological processes and may be essential for the pathogenesis of diseases.

The hub proteins MYC, EIF4EBP1, and NOS3 had the highest degree values among all other proteins which clearly suggest that these proteins play a very crucial role as key regulators of different biological processes connected to brain disorders.

Here in the PPI network, MYC because of its high degree centrality value, appears to be the key regulator of several mechanisms such cell cycle progression, metabolic signalin and proliferative regulation. It being a primary gene regulator associated with growth and metabolism, it may lead to neural precursor cell proliferation and neurogenesis, causing brain malfunctions and seizures if at all it malfunctions due to mutations.

Moreover, the high interaction density of EIF4EBP1 was observed within the network, especially among proteins related to mTOR signaling and protein translation. EIF4EBP1 is one

of the critical regulators of protein synthesis and neuronal translation processes. Therefore, the high level of connections indicates that mTOR-mediated hyperactivity of protein translation pathways might represent a potential pathological mechanism responsible for abnormal neuronal signaling and synaptic malfunction observed in patients with PKD–TSC overlap. As the proper development of neurons is critically dependent upon their ability to regulate protein synthesis and translation, its abnormality could lead to cognitive deficits and epilepsy.

The high degree centrality of NOS3 was also observed, together with its possible interaction with the proteins regulating neurovascular function, oxidative homeostasis, and endothelial cell signaling. Consequently, the central position of NOS3 in the network might indicate that cerebrovascular integrity and regulation of cerebral blood flow play a greater role in causing the disease's brain-related symptoms than expected before. The dysfunction of NOS3 signaling pathways can thus increase oxidative stress and lead to the lack of necessary support for neuronal function.

The high degree values observed for these proteins indicate that they are not merely individual signaling molecules but may instead function as major molecular regulators influencing multiple interconnected biological pathways simultaneously.

**4.3.2. Betweenness Centrality**

Along with degree centrality, betweenness centrality analysis helped find out the value of proteins for ensuring the communication between various parts of the network. This parameter shows how often a protein is located on the shortest path between two other proteins. Highly between proteins are called bridging molecules since they link separate signaling modules and ensure the flow of biological information throughout the network.

It turned out that hub genes have rather high betweenness centrality values as well. Thus, these proteins not only interact with other proteins but also are important connections between various biological signaling pathways.

The high betweenness centrality of MYC in the context of proliferative/metabolic and translational pathways suggests its role as the coordinator between proliferative, metabolic, and differentiation pathways through interaction with various proteins.

Similarly, the high betweenness centrality of EIF4EBP1 in terms of translational and mTOR-related pathways suggests that this protein can serve as a regulator connecting proliferative signaling pathways and neuronal translation. Hence, EIF4EBP1 is a major gene that plays an essential role in maintaining the stability of synapses and ensuring the well coordinated neuronal communications.

NOS3 protein exhibited relatively high betweenness centrality as well, especially concerning neurovascular and oxidative stress-related pathways. NOS3 localization in the network shows that vascular signaling pathways might be closely connected to proliferation and translation signaling pathways involved in the process of cortical development. This information gives additional evidence of the involvement of neurovascular communication dysfunction in PKD–TSC–related neurological disorders.

The appearance of high values of both degree and betweenness centrality for MYC, EIF4EBP1, and NOS3 shows that the proteins not only work as highly connected hubs but also act as

important pathway integrators of the network. These proteins are usually considered biologically significant since disruption of their functioning leads to the breakdown of signaling pathways' interactions.

In general, the network topology analysis revealed that the identified hub proteins have a central regulatory role in the interaction network, which coordinates the complex regulation of molecular mechanisms responsible for the cortical pathology caused by the PKD–TSC overlap syndrome. It could be stated that the pathogenic mechanisms associated with abnormal proliferation and translation regulations and neurovascular dysfunction are tightly interconnected by molecular signaling pathways.

Table 2: Following table shows the hub genes identified in the PPI network along with the parameters- Degree centrality and Betweenness centrality.

| Gene ID | Gene Name | Localization | Betweenness | Degree |
|---|---|---|---|---|
| 4846 | NOS3 | | 4561 | 48 |
| 4609 | MYC | Nucleoplasm | 3545 | 45 |
| 1978 | EIF4EBP1 | Cytosol | 1886 | 24 |

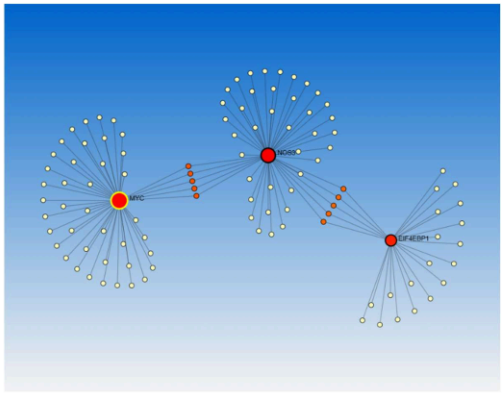



Figure 2. Protein- Protein Interaction network demonstrating how different proteins are connected to central hub proteins **MYC, NOS3 and EIF4EBP1** (red nodes), interactions between molecules are shown by the lines connecting different nodes (yellow, red and orange) which represent several proteins involved.

### 4.4 Identification of Major Hub Genes

The protein-protein interaction network analyses has shown that MYC, EIF4EBP1, and NOS3 are the main hub genes for the PKD-TSC overlap syndrome as it can be seen in the network

that they are also connected to several other genes already involved in the pathways. The selected genes hold a strong position in the network and are interacting with many other proteins, which is confirming the idea that these genes can be the key regulators of biological processes responsible for cortical pathology and neurological abnormalities.

The fact that these genes are located centrally is very significant, since the main role of highly interconnected genes is the regulation of many biological processes at once. This makes it possible to conclude that disturbances in these genes can lead to many different changes occurring at the cellular level.

#### 4.4.1. MYC

Among the hub genes identified, MYC became one of the most connected proteins in the network. MYC is a transcriptional factor responsible for regulation of proliferation, metabolism, differentiation, and progression through the cell cycle. In normal physiological state, MYC ensures balanced cellular proliferation and proper tissue development. Nevertheless, inappropriate activation of MYC-regulated pathways leads to unbalanced proliferation of cells.

MYC-related connectivity within the network indicates that proliferative dysfunction could be a considerable contributor to the cortical abnormalities observed in the PKD–TSC overlapping syndrome. Abnormal upregulation of MYC may stimulate the over proliferation of progenitor cells, which may result in disrupted differentiation and maturation of neurons. The abnormalities in cortical development affect normal neuronal organization and cortical structure.

Which implies that neuronal physiology can be affected if some changes occur in the cellular metabolism and proliferation because neurons largely depend on the well regulated cellular metabolism to mature and further survive. So, any disturbance in the processes can enhance the neuronal vulnerability.

One other significant finding was the correlation between MYC and neurogenic pathways associated with growth and synapse regulation. Overstimulation of growth and changes in neurogenesis could lead to cortical maldevelopment, hyper-excitability of neurons, and increased chances of developing seizures. This means that MYC, through its role in the pathway, might be responsible for the neurological problems associated with PKD–TSC overlap syndrome.

#### 4.4.2. EIF4EBP1

EIF4EBP1 was identified as another major hub protein within the interaction network and appeared to be strongly associated with mTOR-mediated translational regulation. EIF4EBP1 is mainly known to control translation initiation process and protein synthesis by controling eukaryotic initiation factor 4E's (eIF4E) activity. It is important for one's body's mechanism to ensure that there is proper regulation of protein synthesis in order to conduct neuronal differentiation, synaptic development, and maintenance of neuronal communication.

The high interaction around EIF4EBP1hub protein suggests that dysregulation in translation could be an important pathogenic mechanism in PKD–TSC overlap syndrome. Since EIF4EBP1 is a downstream effector of the mTOR pathway, continuous activation of mTOR

signaling may affect normal translational process and might also lead to excessive amounts of protein synthesis within the neuronal tissues.

Protein production in very large amount can influence synaptic maturation and stability of the neuron. The detailed investigation of the protein- protein interaction network recommended that the pathways associated with malfunctioned EIF4EBP1 can increase neuronal hyperexcitability as this malfunctioned hub protein can alter neuronal communication and synaptic organization.

The PPI network shows that the EIF4EBP1 cluster is majorly interacting with proteins that are responsible for growth signaling and regulation of the metabolism, proving the crosstalk communication between proliferative signaling mechanisms and translation pathways, which also reflects that excessive protein production instead of occurring independently, occurs by interacting with other disease associated pathways.

Overall, the central position of EIF4EBP1 within the network highlights the importance of mTOR-mediated translational control in maintaining neuronal homeostasis. Dysregulation of this pathway may therefore contribute significantly to cortical abnormalities and neurological manifestations associated with PKD–TSC overlap syndrome.

### 4.5. Cluster-Wise Network Interpretation

#### 4.5.1. MYC Cluster

The MYC centred cluster emerged as one of the most densely interconnected regions within the protein–protein interaction network and appeared to represent a major proliferative signaling module. The extensive connectivity surrounding MYC suggested active involvement of pathways associated with cell cycle progression, metabolic regulation, cellular growth, and neural progenitor expansion. Because MYC functions as a transcriptional regulator and control genes involved in proliferation and differentiation, its position in the centre within the network indicates that abnormal proliferative signaling may play a major role in PKD–TSC-associated cortical pathology.

The proteins interacting with MYC were mainly linked with pathways related to cell growth and metabolism. This shows that both cell proliferation and metabolic activity may work together during the progression of the disease. During normal brain development, neural progenitor cells divide in a controlled manner so that neurons can develop properly and form an organized cerebral cortex. But when this cell growth becomes excessive or uncontrolled, it can disturb normal neuronal development and affect the arrangement of cortical layers.

The MYC-related cluster in the network suggests that increased cell proliferation may play a role in causing cortical dysplasia and abnormal neuronal connections. These changes can disturb communication between neurons and may increase neuronal excitability. As a result, this may contribute to epilepsy and cognitive problems that are commonly seen in PKD–TSC overlap syndrome.

Another important observation within the MYC cluster was its association with metabolic signaling pathways. Developing neurons need a large amount of energy and metabolic support for proper differentiation and formation of synapses. Therefore, changes in MYC-related metabolic regulation may increase oxidative stress and make neurons more vulnerable to

damage. This may further contribute to abnormalities in the cerebral cortex and instability in neuronal function.

The strong interaction pattern seen around MYC in the network suggests that it may act as an important regulatory protein connecting uncontrolled cell proliferation with abnormal cortical development.

#### 4.5.2. EIF4EBP1 Cluster

The EIF4EBP1-centered cluster represent a translational regulatory module strongly associated with mTOR signaling pathways and also occupies a central position within this cluster and demonstrate high interaction with proteins involved in protein synthesis, translational control, synaptic signaling, and neuronal communication.

EIF4EBP1 is a crucial downstream effector of the mTOR pathway that appears to plays a critical role in regulating translation initiation and protein synthesis. Normal physiological conditions, controlled protein synthesis is essential for neuronal differentiation, synaptic maturation, and maintenance of neuronal connectivity. The strong connectivity within the EIF4EBP1 cluster reveals us that the disturbed translational activity may represent a significant molecular mechanism underlying PKD–TSC-associated neurological abnormalities.

However, continuous activation of mTOR signaling alter EIF4EBP1 regulation and excessive protein synthesis within neuronal tissues. These abnormalities can again synaptic organization and impair neuronal communication because synaptic development requires tightly regulated translational control, abnormal activation of these pathways may contribute to synaptic hyperexcitability and altered neuronal signaling.

The proteins present in the EIF4EBP1 cluster were also connected with pathways involved in neuronal growth and metabolic regulation. This shows a close relationship between protein synthesis and cell proliferation pathways. The observation suggests that abnormal protein synthesis does not happen alone, but works together with other dysregulated pathways to affect cortical development and pathology.

Overall, the EIF4EBP1-centered cluster effectively shows the importance of mTOR-mediated translational regulation in maintaining neuronal stability and cortical organization and the dysregulation of this pathway therefore contribute to epilepsy, cognitive dysfunction, and synaptic abnormalities associated with PKD–TSC overlap syndrome.

#### 4.5.3 NOS3 Cluster

The cluster in the protein- protein interaction containing NOS3 as a hub protein shows the neurovascular signaling module and is showing a great interaction with the proteins that are closely linked with processes such as endothelial regulation, oxidative stress response, vascular homeostasis, and metabolic signaling. One thing that can be clearly understood from the cluster in the network is that any abnormalities in the neurovascular systems is capable enough of causing cortical abnormalities that are associated with the PKD-TSC contiguous gene syndrome.

It is known that for the development of the cortex, proper vascular signaling is required for maintain oxygen delivery, nutrient exchange and neuronal survival. Endothelial nitric oxide synthase, encoded by NOS3 hub protein, is involved in nitric oxide production and regulation of cerebral blood flow. Therefore, malfunctioning of the above hub protein can effectively influence neuronal maturation and cortical organization. By closely observing the pattern around the NOS3, it is well understood that any lapses in the neurovascular system may sort of reduce cerebral perfusion and metabolic imbalance to a large extent within the neural tissues. Such abnormalities may result in increased oxidative stress and higher neuronal vulnerability during the development of the brain because neurons are extremely sensitive to metabolic changes, prolonged oxidative imbalance which will diturb the synaptic stability and the neuronal communication.

Another important finding is the way NOS3 and the proteins associated with it are interacting with the pathways that are closely linked to the proliferation and the regulation of the translation. It demonstrates that isn't acting independently but rather closely ineracting closely with with mTOR signaling and proliferative pathways. Therefore, it can be predicted that the combined effect of vascular dysregulation, oxidative stress and disrupted cellular signaling may contribute to cortical dysplasia and neuronal instability.

The NOS3-centered cluster highlights the importance of neurovascular signaling in maintaining cortical homeostasis and suggests that vascular dysfunction may represent an underexplored but biologically important component of PKD–TSC-associated neurological pathology. The findings further support the idea that impaired vascular regulation may contribute to epilepsy, cognitive dysfunction, and abnormal cortical development through its interaction with multiple interconnected molecular pathways.

### 4.6 Tissue-Specific Co-Expression Network Analysis

The tissue-specific co-expression network analysis has shown a well-coordinated pattern of gene expression where several genes are involved that are associated with cellular proliferation, apoptosis, translational regulation, and neurovascular signaling from which it is clear that these genes are not functioning independently but participating in interconnected molecular pathways that are collectively contributing to cortical abnormalities and neurological dysfunction in PKD–TSC overlap syndrome.

Several biologically important genes were identified within the co-expression network, including:

- AKT1
- PTEN
- EGFR
- SRC
- CASP3
- BCL2
- SQSTM1
- CCND2
- RB1
- AR

The co-expression pattern involving AKT1, PTEN, EIF4EBP1, and RPS6KB1 strongly suggesting the activation of the PI3K/AKT/mTOR signaling pathway that is playing a major role in regulating cellular growth, proliferation, metabolism, and protein synthesis. Balanced activation of this this pathway under normal physiological conditions is therefore required for normal neuronal differentiation and cortical development. If the pathway is dysregulated, excessive cellular proliferation and abnormal translational activity can be observed.

Close analysis of the co-expression pattern shows that AKT1 is a crucial signaling regulator that promotes cellular growth and cell survival whereas PTEN is behaving like a negative regulator that maintains pathway balance. If interaction between PTEN and AKT1 is disrupted then it will lead to continuous activation of mTOR-associated signaling. On the other hand, within the neuronal tissues, abnormal translation and abundant protein synthesis is expected to occur due to increased activity of EIF4EBP1 and RPS6KB1.

It is very likely that during the cortical brain development, dysregulation in the pathways may impair neuronal maturation and synaptic organization as both of these processes are dependent on the synthesis of the regulatory protein synthesis. The abnormal activation of these signaling pathways is very much possible that they contribute to neuronal hyperexcitability, epilepsy and cognitive dysfunction.

Moreover, it can be seen from the network that there is a well-coordinated expression pattern between EGFR and SRC which is pointing that it may activate the growth factor-mediated signaling pathways, where EGFR is regulating cellular proliferation, differentiation, and survival and SRC is participating in intracellular signaling associated with neuronal communication and cytoskeletal organization. The interaction between these proteins is promising enhanced proliferative and growth-related signaling within the cortical environment.

Therefore, EGFR- and SRC-associated signaling pathways may contribute to altered neuronal organization and abnormal cortical architecture. In addition, the interaction between these pathways and mTOR-associated signaling is further substantiating the fact that multiple interconnected molecular mechanisms together influence cortical pathology in PKD–TSC overlap syndrome.

The presence of genes closely linked to apoptosis and autophagy were also observed in the network. Those genes include CASP3, BCL2, and SQSTM1. CASP3, being a apoptotic regulator, is known for performing programmed cell death, whereas, BCL2 is an anti- apoptotic protein which means, it promotes cell survival. Keeping a balance between these two pathways is of paramount importance for maintain neuronal homeostasis during the brain development. Their altered expression may disturb the balance between neuronal survival and cell death, causing neuronal injury and impaired cortical organization.

In the same way SQSTM1 is a protein that is associated with autophagy and degradation of damaged cellular components. So, improper functioning of such pathways can become a major cause for the accumulation of damaged proteins, oxidative stress and synaptic dysfunction within the neuronal tissues.

The interaction of CCDN2 and RB1 can also be seen within the co-expression network. They are the important regulators of the cell cycle progression and proliferative control. Interaction between these two genes signifies and even confirms that they promote abnormal proliferative signaling in the cortical brain. While, signaling pathways associated with AR are pointing

towards the involvement of hormonal and metabolic regulatory mechanisms in the neuronal development and cellular homeostasis.

All in all, the tissue-specific co-expression network demonstrated extensive interaction among pathways associated with proliferation, translational regulation, apoptosis, oxidative stress response, and neurovascular signaling. Rather than functioning as isolated molecular mechanisms, these pathways appeared to form an interconnected regulatory system contributing to cortical pathology.

The coordinated expression patterns observed within the network suggest that cortical abnormalities associated with PKD–TSC overlap syndrome may result from the combined influence of abnormal cellular proliferation, dysregulated protein synthesis, impaired neuronal survival, and neurovascular dysfunction. These interconnected molecular disturbances may collectively contribute to neuronal instability, cortical dysplasia, epilepsy, and cognitive impairment.

Table 3: The table consists of the mechanisms, genes involved in those mechanisms and their biological effects that are highly likely to occur in the disease progression.

| **Identified Mechanism** | **Major Genes/Pathways Involved** | **Possible Biological Effect** |
|---|---|---|
| mTOR Hyperactivation | EIF4EBP1, AKT1, RPS6KB1 | Excessive protein synthesis, abnormal neuronal growth, impaired synaptic maturation. |
| Excessive Cellular Proliferation | MYC, CCND2, EGFR | Abnormal neural progenitor expansion, disrupted cortical organization. |
| Dysregulated Protein Translation | EIF4EBP1, mTOR signaling | Synaptic instability, altered neuronal communication, hyperexcitability. |
| Neurovascular Dysfunction | NOS3, endothelial signaling pathways | Impaired cerebral blood flow, metabolic imbalance, neuronal stress. |
| Oxidative Stress | NOS3-associated oxidative pathways, SQSTM1 | Increased neuronal injury, impaired cellular homeostasis. |
| Apoptotic Dysregulation | CASP3, BCL2 | Imbalance between neuronal survival and cell death. |
| Growth Factor Signaling Abnormalities | EGFR, SRC | Altered cellular differentiation and neuronal signaling. |
| Autophagy Dysfunction | SQSTM1 | Accumulation of damaged proteins and cellular stress. |

| | | |
|---|---|---|
| Synaptic Instability | mTOR-related translational pathways | Increased neuronal excitability and seizure susceptibility. |

**4.7 Systems-Level Interpretation of the Network**

The integrated network analysis suggested that PKD–TSC overlap syndrome is not caused by a single molecular defect but rather by the combined disturbance of several interconnected biological pathways. The interaction network showed close communication between pathways involved in cellular proliferation, protein translation, neurovascular regulation, oxidative stress response, and neuronal homeostasis.

One of the most important findings of the study was the strong relationship between MYC-associated proliferative signaling, EIF4EBP1-mediated translational regulation, and NOS3-associated neurovascular pathways. These pathways appeared to work together in influencing cortical development and neuronal function.

The network indicated that persistent activation of mTOR-related pathways may increase protein synthesis and uncontrolled cellular growth, while abnormal MYC signaling may interfere with proper neuronal differentiation and cortical organization. At the same time, altered NOS3-associated vascular regulation may impair oxygen delivery and metabolic support within developing cortical tissues, increasing neuronal stress and instability.

Together, these interconnected mechanisms may contribute to several neurological abnormalities observed in PKD–TSC overlap syndrome, including cortical dysplasia, abnormal neuronal migration, synaptic hyperexcitability, epilepsy, and cognitive dysfunction.

The major mechanisms identified in the network and their associated biological effects are summarized below.

Table1. The table consists of the mechanisms, genes involved in those mechanisms and their biological effects that are highly likely to occur in the disease progression.

Overall, the findings support a systems-level model in which abnormal cellular proliferation, dysregulated protein synthesis, oxidative imbalance, and impaired neurovascular homeostasis act together to drive cortical pathology and neurological dysfunction in PKD–TSC overlap syndrome.

**4.8 Biological Significance of the Findings**

The identification of MYC, EIF4EBP1, and NOS3 as major hub genes provides important insight into the molecular mechanisms underlying PKD–TSC-associated cortical abnormalities. Since these genes occupied central positions within the interaction network and showed extensive connectivity with multiple pathways, they are likely to play significant roles in disease progression and neurological dysfunction.

The findings suggest that these hub genes may potentially serve as biomarkers for monitoring disease progression and identifying molecular changes associated with neurological complications. Their strong association with pathways involved in proliferation, translational regulation, oxidative stress, and neurovascular signaling further indicates their possible role as indicators of cortical dysfunction and neuronal instability.

Another important aspect of the study is the therapeutic relevance of the identified pathways. The results suggest that targeting abnormal proliferative signaling pathways may help reduce excessive cellular growth and abnormal cortical organization. Similarly, regulating mTOR-associated translational pathways may improve synaptic stability and neuronal communication by restoring balanced protein synthesis within neuronal tissues. The identification of NOS3-associated neurovascular pathways also highlights the possible importance of vascular regulation in maintaining cortical homeostasis. Therapeutic approaches aimed at improving neurovascular signaling and reducing oxidative stress may help protect neuronal tissues and improve neuronal survival during disease progression.

Overall, the present findings provide a broader systems-level understanding of the molecular convergence between PKD and TSC. The study highlights how interconnected pathways related to proliferation, protein synthesis, oxidative balance, and vascular regulation collectively contribute to cortical abnormalities and neurological dysfunction in PKD–TSC overlap syndrome.

Table 4: The table summarizes the common genes identified from both PKD and TSC disorders, their function under normal physiological conditions and their possible role in the PKD-TSC contiguous gene syndrome.

| S.No. | Common Gene | Major Biological Function | Possible Role in PKD–TSC Overlap Syndrome |
|---|---|---|---|
| 1. | MYC | Regulates cell proliferation and metabolism | May contribute to abnormal neural progenitor proliferation and cortical dysplasia |
| 2. | EIF4EBP1 | Controls protein translation and mTOR signaling | May cause abnormal protein synthesis and synaptic dysfunction |
| 3. | NOS3 | Regulates vascular tone and nitric oxide production | May contribute to neurovascular dysfunction and oxidative stress |
| 4. | AKT1 | Controls cell survival and growth signaling | Associated with PI3K/AKT/mTOR pathway activation |
| 5. | PTEN | Negative regulator of PI3K/AKT signaling | Loss of regulation may promote excessive proliferation |
| 6. | EGFR | Mediates growth factor signaling | Mediates growth factor signaling |
| 7. | SRC | Regulates intracellular signaling pathways | Associated with proliferation and neuronal communication |
| 8. | CASP3 | Mediates apoptosis | Dysregulation may lead to neuronal injury |
| 9. | BCL2 | Anti-apoptotic regulator | May disturb balance between neuronal survival and cell death |
| 10. | SQSTM1 | Involved in autophagy and protein degradation | May contribute to oxidative stress and cellular damage |
| 11. | CCDN2 | Regulates cell cycle progression | May promote excessive cellular proliferation |

| | | | |
|---|---|---|---|
| 12. | RB1 | Controls cell cycle and proliferation | May influence abnormal cortical development |
| 13. | AR | Hormonal signaling and transcription regulation | May affect neuronal metabolism and cellular signaling |
| 14. | RPS6KB1 | Regulates protein synthesis downstream of mTOR | Associated with abnormal translational activity |

## 5. CONCLUSION OF THE STUDY

The present study that was carried out to understand the shared molecular mechanisms involved in PKD–TSC overlap syndrome using gene expression analysis and protein–protein interaction network analysis. Although Polycystic Kidney Disease and Tuberous Sclerosis Complex are caused by mutations in different genes and are generally studied as separate disorders, the findings of this study suggest that both diseases are connected through several common molecular pathways related to cortical development and neurological dysfunction.

The comparative gene analysis identified several overlapping genes between PKD and TSC, showing important molecular similarities between the two disorders. Many of these shared genes were involved in pathways related to cellular proliferation, mTOR signaling, protein translation, oxidative stress response, apoptosis, angiogenesis, and neuronal signaling. These pathways are important for normal brain development and proper neuronal function. Disturbance in these biological processes may therefore contribute to cortical abnormalities and neurological manifestations seen in PKD–TSC overlap syndrome. Further analysis using the protein–protein interaction network showed that the proteins formed a highly interconnected molecular network. Among all the identified proteins, MYC, EIF4EBP1, and NOS3 were identified as the major hub genes because they showed high connectivity and occupied central positions within the network. Since these genes were connected with multiple proteins and pathways, they may play important roles in disease progression and neurological abnormalities associated with PKD–TSC overlap syndrome.

The pathways associated with MYC suggested that abnormal proliferative signaling and disturbed neuronal differentiation may contribute to cortical pathology. Increased proliferative activity during brain development might cause the disturbance in the normal organization of the cortex and can even cause neurons to show hyperexcitability. Similarly, EIF4EBP1has showed a very strong connection with mTOR mediated translational regulation and protein synthesis pathways, however, if, these pathways are reported to have been disturbed, they are very much possible to affect proper synaptic maturation and neuronal communication which can further contribute to epilepsy and cognitive dysfunction.

Another important thing that was found in the study was the identification of NOS3 as a major neurovascular regulatory hub, which upon detailed analysis from the PPI network suggested that vascular dysfunction and oxidative imbalance are likely on the cards to play an important role in cortical abnormalities and neuronal instability because impaired neurovascular regulation can reduce proper oxygen and nutrient supply to developing neuronal tissues, thereby increasing oxidative stress and making neurons more vulnerable to damage.

The tissue-specific co-expression network has also showed that the coordinated interaction among genes is involved in proliferation, apoptosis, autophagy, translational regulation, and growth factor signaling which further provides an idea that the neurological manifestations associated with PKD–TSC overlap syndrome are probably not caused by a single molecular defect, but rather by the combined effect of several interconnected pathways working together.

All in all, the findings of the present study is supporting a systems-level model in which abnormal cellular proliferation, dysregulated protein synthesis, oxidative stress, and impaired neurovascular homeostasis come together to contribute towards cortical dysplasia, synaptic instability, epilepsy, and cognitive impairment. The study also highlights the importance of

understanding molecular interactions at the network level for better explanation of complex neurological disorders.

In addition, the identified hub genes are highly expected to serve as the potential biomarkers for disease development and progression and are very likely to act as possible therapeutic targets in future studies. Targeting pathways associated with proliferative signaling, mTOR-mediated translation, and neurovascular dysfunction may definitely help the researchers in developing better therapeutic strategies for combatting neurological complications associated with PKD–TSC overlap syndrome and improve the condition of those who generally suffer from this disease.

Although the present study was completely based on computational and bioinformatics approaches, it still provides an important foundation for future experimental validation studies. Further laboratory-based and clinical investigations will be necessary to confirm the exact role of the identified genes and pathways in cortical pathology and neurological dysfunction associated with PKD–TSC overlap syndrome.

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