

MODELING AND ANALYSIS OF CIRCADIAN RHYTHM DISORDER IN
WOMEN AND ITS CONNECTIONS TO AUTISM IN BABIES

by

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to my beloved mother...

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ABSTRACT

MODELING AND ANALYSIS OF CIRCADIAN RHYTHM DISORDER IN WOMEN AND ITS CONNECTIONS TO AUTISM IN BABIES

The circadian clock is a diurnal timer that regulates the biological processes of hormones in approximately 24-hour cycles. Melatonin, a sleep hormone, has a circadian rhythm, and environmental factors, like night shift work, disrupt the melatonin circadian rhythm, which might cause neurodevelopmental disorders. The maternal circadian rhythm is synchronized with the fetus via melatonin during the prenatal period. This thesis investigates how maternal circadian rhythm disorders affect fetal circadian rhythms and explores their potential impact on the risk of autism spectrum disorder (ASD) in the fetus, which constitutes the hypothesis of the thesis. The thesis established links between autism and circadian rhythm using two models: the steady-state model (protein-protein interaction(PPI)) and the dynamic model (using system dynamics methodology). The dynamic model examines the effects of the disruption of the circadian clock caused by night shift work on ASD. The night shift simulation results indicate that the Malondialdehyde level of the fetus increased by 47%, and the melatonin level decreased by 36% during the night shift which increases the risk of autism in the fetus. In the steady-state model, a PPI network was constructed in the research to understand the pathways between autism and the circadian clock and was evaluated using graph-theoretic analysis. In addition, potential hub proteins and pathways such as ribosome biogenesis, oxidative stress, insulin/IGF pathway, Wnt pathway, and mTOR pathway on the development of ASD were examined. Considering these findings, the impacts of circadian disorders should be examined in more detail, and further clinical studies should be conducted on fetal health.

ÖZET

KADINLARDAKİ SİRKADİYEN RİTİM BOZUKLUĞUNUN VE BUNUN BEBEKLERDEKİ OTİZM İLE BAĞLANTISININ MODELLENMESİ VE ANALİZİ

Sirkadiyen saat, hormonların biyolojik süreçlerini yaklaşık 24 saatlik döngülerde düzenleyen günlük bir zamanlayıcıdır. Uyku hormonu melatoninin sirkadiyen ritmi vardır ve gece vardiyası gibi çevresel faktörler sirkadiyen ritmini bozar, ve nörogelişimsel bozukluklara neden olabilir. Anne sirkadiyen ritmi, doğum öncesi dönemde melatonin aracılığıyla fetüsle senkronize edilir. Bu tezde, maternal sirkadiyen ritim bozukluklarının fetal sirkadiyen ritmi nasıl etkilediği incelenmiş ve tezin hipotezi olan fetüste otizm spektrum bozukluğu (OSB) riski üzerindeki potansiyel etkileri araştırılmıştır. Çalışmada, otizm ve sirkadiyen ritim arasındaki bağlantı, iki model kullanılarak kurulmuştur: statik ağ modeli (protein-protein etkileşim ağı kullanılarak) ve dinamik model (sistem dinamiği metodolojisi kullanılarak). Dinamik model, gece vardiyasında çalışmanın neden olduğu anne sirkadiyen saatinin bozulmasının fetüsteki OSB riskine etkilerini modellemektedir. Gece vardiyası simülasyon sonuçlarına göre, gece vardiyası esnasında fetüsün malondialdehit düzeyinin %47 arttığı, melatonin düzeyinin ise %36 azaldığı bulunmuştur ve bu da fetüste otizm riskini artırmaktadır. OSB ve sirkadiyen saat arasındaki yolları anlamak için bir protein-protein etkileşim ağı oluşturulmuş ve graf-teoretik analizi kullanılarak değerlendirilmiştir. Ayrıca, OSB gelişiminde ribozom biyogenezisi, oksidatif stres, insülin/IGF yolu, Wnt yolu ve mTOR yolu gibi potansiyel merkez proteinler ve yollar incelenmiştir. Bu bulgular dikkate alındığında sirkadiyen bozuklukların etkilerinin daha detaylı incelenmesi ve fetal sağlık üzerine ileri klinik çalışmaların yapılması gerekmektedir.

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LIST OF ACRONYMS/ABBREVIATIONS

ASD	Autism Spectrum Disorder
ASMT	N-acetylserotonin O-methyltransferase
GO	Gene Ontology
MDA	Malondialdehyde
OSB	Otizm Spektrum Bozukluğu
PPI	Protein-Protein Interaction
SCN	Suprachiasmatic Nucleus

1. INTRODUCTION

Autism spectrum disorder (ASD) is a neurodegenerative disorder, and research is ongoing into the environmental and genetic factors that cause the disorder. In recent years, an increase in the prevalence of autism has been observed, which was found to be 62/10,000 in a study conducted in 2012 [1], and 100/10,000 in a study conducted between 2012 and 2022 [2]. This rate means that about one child out of every 100 is diagnosed with autism. Studies are being conducted on the effect of environmental factors exposure during pregnancy on the development of ASD. Research [3, 4] has found that the exposure to traffic-related air pollution or prenatal stress due to family conflict in the second and third trimesters of pregnancy may increase the risk of autism in the fetus.

The suprachiasmatic nucleus (SCN), located in the anterior hypothalamus, is responsible for the regulation of the circadian clock of mammals and the periods of cortisol and melatonin during the day [5]. When SCN perceives darkness, it would increase the secretion of the melatonin hormone [5] which plays a crucial role in the sleep-wake cycle. Exposure to light during sleep can suppress the melatonin hormone which results in the disruption of the circadian rhythm. For instance, the circadian rhythm of night shift workers can be disrupted due to exposure to artificial light during the night [6]. Oxidative stress and DNA damage have also been associated with exposure to artificial light during nighttime [6]. According to a clinical research study, people with autism spectrum disorder (ASD) have different melatonin secretion patterns, which can have an impact on sleep difficulties and disruption of circadian rhythm [7].

Melatonin circadian rhythm is transferred from the mother to the fetus; maternal Melatonin quickly passes to the fetus via the placenta, binds to Melatonin receptors, and adjusts the circadian rhythm of the fetus [8,9]. Maternal Melatonin transferred to the fetus prevents the fetus from being exposed to oxidative stress and the resulting oxidative damage. It is also responsible for forming the basis of the programming

of the fetal suprachiasmatic nucleus (SCN) and the circadian rhythm of the newborn after birth [10]. In an experiment performed by Torres-Farfan et al. in capuchin monkeys [11], it was observed that suppressing maternal Melatonin by exposing the monkey to constant light altered the fetal circadian gene expression. In another hamster experiment [12], disruptions in the fetus' circadian rhythms are observed when the maternal hamster's SCN is lesioned.

This thesis includes two modeling approaches: steady-state modeling and dynamic modeling. The steady-state modeling focuses on reconstructing the protein-protein interaction (PPI) networks of ASD and circadian rhythm. The PPI is used to understand the connection between the circadian clock and autism. Up to now, most of the published research has concentrated on the behavioral characteristics of people with ASD. Animal models have been employed to explore the complex history of ASD in experiments because it was impossible to create human cell cultures that could proliferate indefinitely [13]. Hence, capturing the comprehensive overview of these two networks in the combined form is of utmost significance to understanding the crosstalk between ASD and circadian rhythm. A graph theoretical analysis determines whether the network properties are biologically feasible. This part presents a comprehensive circadian-ASD network indicating key components that facilitate a deeper understanding of shared mechanisms underlying circadian rhythm disorder related to the pathophysiology of ASD.

The dynamic modeling focuses on the dynamics of autism risk in the fetus in the third trimester of a pregnant woman who has a disrupted circadian clock due to working night shift. By simulation experiments, we examine how and to what extent the risk of autism in the fetus may increase due to circadian disruptions in the maternal melatonin.

2. LITERATURE REVIEW

The effects of melatonin and oxidative stress on autism are still hot topic for many researchers from different domains. Melatonin is secreted in the pineal gland, and the suprachiasmatic nucleus located in the anterior part of the hypothalamus arranges the secretion after the retina perceives light. Melatonin has light-dark (circadian) cyclical levels that are normally highest at night, but light exposure suppresses melatonin production and shifts the nightly peak phase [14]. It has been found that the amount of melatonin was lower in nurses working the night shift than in those working the day shift [15]. In research conducted by Folkard [16], only 3% of the night shift workers could adapt to the night shift, which may cause a health risk. A related line of research found that melatonin levels in mothers of children with autism are approximately 40 percent lower compared to age-adjusted melatonin levels in mothers of children without autism [17]. In another study it has been observed that melatonin levels are lower in children with autism than in children without autism, which results in sleep problems and poor sleep quality in children with ASD [18].

Melatonin reduces oxidative stress by showing antioxidant properties and ensuring the secretion of antioxidant enzymes [19]. Melatonin is important in adjusting the circadian clock (zeitgeber) and its antioxidant properties. Malondialdehyde (MDA), a marker of oxidative stress and lipid peroxidation [20], exhibits a circadian rhythm that inversely correlates with melatonin secretion [21]. In contrast to the melatonin level, MDA levels are higher in children with autism compared to children without autism [22]. Compared with melatonin levels, MDA levels are also lower in nurses working the night shift than those working the day shift [23].

The primary causes of the neuropsychiatric disorder ASD are the combination of genetic factors, including mutations in genes, chromosomal abnormalities, copy number variations (CNVs), and environmental factors. Several researches have demonstrated a direct correlation between ASD and circadian rhythm disorders [24, 25]. Changes

in melatonin and cortisol levels may be observed in pregnant women working night shifts because they may experience stress and disruption of the circadian rhythm. These altered hormone levels are considered risk factors for ASD [24, 26–28]. Both maternal circadian rhythm [29] and the development of fetal circadian rhythm [30] can be affected by environmental conditions such as shift work and sleep disorders. Some research indicates a close association between ASD and preterm birth [31, 32]. Constant exposure to bright light and loud noises in neonatal intensive care units can disrupt sleep patterns in preterm babies, and the disruption in sleep patterns can impact their circadian development [30]. The ultimate effect is the dysregulation of hormones such as melatonin and glucocorticoids. The irregularity in the mentioned hormone levels impacts growth, development, and other health outcomes, such as the increased risk of autism and impaired learning, vision, and neurobehavioral abnormalities [30, 32].

ASD and attention deficit hyperactivity disorder have also been associated with changes in the functioning of various neurotransmitters like gamma-aminobutyric acid (GABA), norepinephrine, dopamine, and serotonin [33, 34]. These studies show a causal link between the HPA axis, circadian rhythm disruption, and ASD. Another clinical research showed that normal and abnormal sleep-wake rhythms in individuals with ASD whose ages range from 18 to 41 years could be associated with specific gene variants in the circadian clock (PER1) and the melatonin pathway system named N-acetylserotonin O-methyltransferase (ASMT) [35]. An increasing amount of evidence indicates the relationship between the circadian rhythm misalignment and various chronic diseases, including cardiovascular, metabolic, and neuropsychiatric disorders such as schizophrenia and ASD [36].

Sleep and circadian rhythm disturbances in ASD patients are indicated as reasons for several behavioral complications [37]. Melatonin synthesis, which plays a significant role in neurodevelopment, is low in children with autistic spectrum disorder [38]. Mutations in the ASMT enzyme of the melatonin synthesis pathway are considered genetic factors among ASD patients [39]. Melatonin synthesis begins with tryptophan, followed by intermediates of 5-hydroxytryptophan, serotonin, and N-acetyl serotonin,

respectively [40]. It is observed that the blood level of serotonin is differentiated between children with ASD. It is higher in children with advanced ASD than in children with less advanced autism [41]. Disruption in melatonin secretion and the serotonin system in melatonin pathways are observed in patients with ASD. This observation revealed the association between ASD and circadian rhythm. The rhythmicity in the secretion of neurohormones, including cortisol, prolactin, and thyroid-stimulating hormone, also has a role in the pathology of autism [26, 27]. A dysfunctional HPA axis and abnormal cortisol levels are observed in children with ASD [36]. Activity-dependent changes in the efficacy of synaptic communication become significant in circadian rhythm disorders, which can increase the susceptibility to ASD during early neurodevelopment [42, 43].

3. STEADY STATE MODEL - MATERIALS AND METHOD

3.1. Protein-Protein Interaction Data in Homo Sapiens

BioGRID database is used as resource for Homo sapiens PPI data [44]. Only physical interactions in the data are used, on the other hand genetic interactions are excluded. Interactions with confidence above 0.85 are kept during the research because the confidence score is correlated to the reliability of PPI from physical data. In cases where a different scoring system produces data with scores exceeding 1, the average of interaction scores within the same publication is calculated. Interaction data with scores higher than 1.5 times the calculated average in the previous step are selected. After PPI selection and filtration processes, 102,991 PPIs are obtained for further analysis. A Python script is developed and utilized to facilitate the filtering process. Additionally, a dataset of physical PPIs with confidence scores higher than 0.85 is obtained from the String database. The new data contain 106,669 PPIs. These two data are combined, and the result contains unique 209,658 PPIs.

3.2. Development of the Circadian PPI Network

The term "circadian" is used to search the Gene Ontology Consortium web page [45] to generate a dataset containing GO-terms associated with circadian functions. All three categories of GO terms -biological process, molecular function, and cellular component- are carefully analyzed to identify the terms that demonstrate significant connections with each set of studied proteins. For Homo sapiens, a total of 7253 GO-terms are linked to the "Circadian" term. These include 34,926 proteins associated with molecular function, 38,012 proteins associated with the cellular component, and 35,556 proteins associated with biological processes. Among these proteins, there are 26,504 proteins which are common across all three types of GO-terms under the "circadian" query. The interactions of 26,504 common proteins within the interacting

protein set are selected, leading to 77,594 protein-protein interactions (PPIs). A PPI network, called "circadian PPI network 1 (CPPIN1), is reconstructed using these pair-wise proteins." Subclusters within this network are identified using Cytoscape software for further analysis.

Furthermore, following a careful review of existing literature, an additional dataset consisting of 1435 human circadian gene products is refined [46]. The same approach mentioned earlier is used to process these gene products. Finally, there are 1417 PPIs referred to as "CPPIN2".

3.3. Development of the Autism-Related PPI Network

According to our literature review, there are 106 genes in homo sapiens associated with susceptibility to autism [47]. A list of 3590 GO ID numbers linked to these susceptibility genes is also compiled. A network is created on top of 106 gene products. The network is referred to as "Autism PPI Network 1 (APPIN1)". The first neighbors (1444 proteins) of these 106 gene products by collecting unique PPI data (comprising 27,008 interactions) are identified. By first neighbors, proteins that directly interact with the gene products are referred to. After searching the complete set of autism-related proteins (totaling 1550), 31,382 PPIs are discovered. Then, the PPI network is reconstructed and named "APPIN2" on top of these PPIs.

This study examined another autism dataset containing 191 autism risk factors [48]. The dataset is then combined with the existing 106 proteins in APPIN1. A new network, named "APPIN3", is reconstructed by the combination. This new network has 287 unique proteins connected by 193 PPIs. Furthermore, other data from Corominas et al. [48], 291 autism-related risk proteins are selected and then combined with 106 proteins of Iakoucheva et al. [47] to construct the network referred to as "APPIN4". APPIN4 had 397 unique proteins and 263 PPIs connecting the proteins. Finally, APPIN4, APPIN3, and APPIN1 are combined to create the APPIN5 network, which contains 484 PPIs among 559 unique proteins.

3.4. Integration of Circadian and Autism PPI Networks

The proteins from APPIN5 and CPPIN2 are combined to construct the autism and circadian joint network. There are 1844 proteins and 2394 pairwise protein interactions within the joint network. A more comprehensive network, APPIN5, has been chosen, including clusters of circadian proteins. CPPIN2 is selected over CPPIN1 because it focuses more on the circadian network. In other words, CPPIN2 contains interactions more directly related to circadian rhythms than CPPIN1. This combined network is named combined circadian-autism PPI (CAPPIN) and is included in further investigations.

3.5. Topological Analysis Cluster Identification and Gene Ontology (GO) Enrichment Analysis

Graph theoretic analysis is used to conduct topological analysis of the reconstructed PPI networks. Graph properties are used for our analysis: node degree, hubs (highly connected nodes), shortest path lengths among indirectly connected nodes, network diameter, and mean path length. In graph analysis, the Network Analyzer plugin (ver. 2.7 and 4.4.6) [49] within Cytoscape (ver. 3.6.1 and 3.9.0) [50] is utilized. The list containing protein pairs interacting with each other is given as input. The tool gave us different topological network parameters such as network clustering coefficient, network diameter, network radius, characteristic path length, average number of neighbors, network density, and node degrees as output.

Additionally, the MCODE plugin (v1.5.1 and v2.0.0) is used to identify highly interconnected clusters within the network [51]. The node score cutoff is set to 0.2 for cluster expansion, the K-core value is set to 2, and the maximum depth from seed is set to 100. The GO Tool developed by Princeton University [52] is used to analyze the GO terms in enrichment analysis. The significance level is set to 0.05, meaning only terms enriched with a p-value of .05 or lower are considered.

3.6. Network Decomposition Analysis (NDA)

To understand the information flow (topology) within the CAPPIN, linear paths are identified that connect a central circadian rhythm protein to an autism-related transcription factor in the nucleus. This is achieved thanks to the NetSearch algorithm [53]. The algorithm draws all possible linear paths of a specified length through the interaction map, starting at any membrane protein and ending at any DNA-binding protein. The PPI network is used as input for the NetSearch algorithm. In addition, path calculations must specify the starting protein (associated with circadian rhythms) and the ending protein (an autism-related transcription factor). This linear path analysis is conducted to obtain insight into network crosstalk and the involvement of molecules in the CAPPIN pathways.

4. STEADY STATE MODEL - RESULTS

4.1. Reconstructing and Analyzing the Topology of the Protein-Protein Interaction Network in Circadian and Autism Systems

The properties of the nine main circadian genes PER1, PER2, PER3, CLOCK, ARNTL [BMAL1], CRY1, CRY2, CSNK1E, and NPAS2) in CPPIN2 are tabulated in Table 4.4. Closeness centrality demonstrates the distance of a protein to other proteins [54], which indicates the effective spread of information through the network. Among these nine main circadian proteins, CSNK1E stood out with the highest closeness centrality value (0.219), which signified its role in facilitating the flow of information between proteins. In PPI networks, the radiality is related to the probability of a protein being functionally connected to other proteins, and thus, the CSNK1E protein with the highest radiality value among others is the functionally relevant one to other primary circadian proteins.

Table 4.1. MCODE cluster analysis results for APPIN5.

	APPIN1	APPIN2	APPIN3	APPIN4	APPIN5
Total protein count	106	1550	287	397	559
Number of nodes	31	1537	101	165	259
P-p interaction count	44	27008	193	263	484
Network density (%)	5.4	1.3	2.1	1.4	1.5
Clustering coefficient	0.12	0.36	0.17	0.18	0.22
Network diameter	6	11	13	15	18
Characteristic path length	2.60	3.58	4.28	6.14	6.74
Network heterogeneity	0.70	1.17	0.78	0.69	0.69

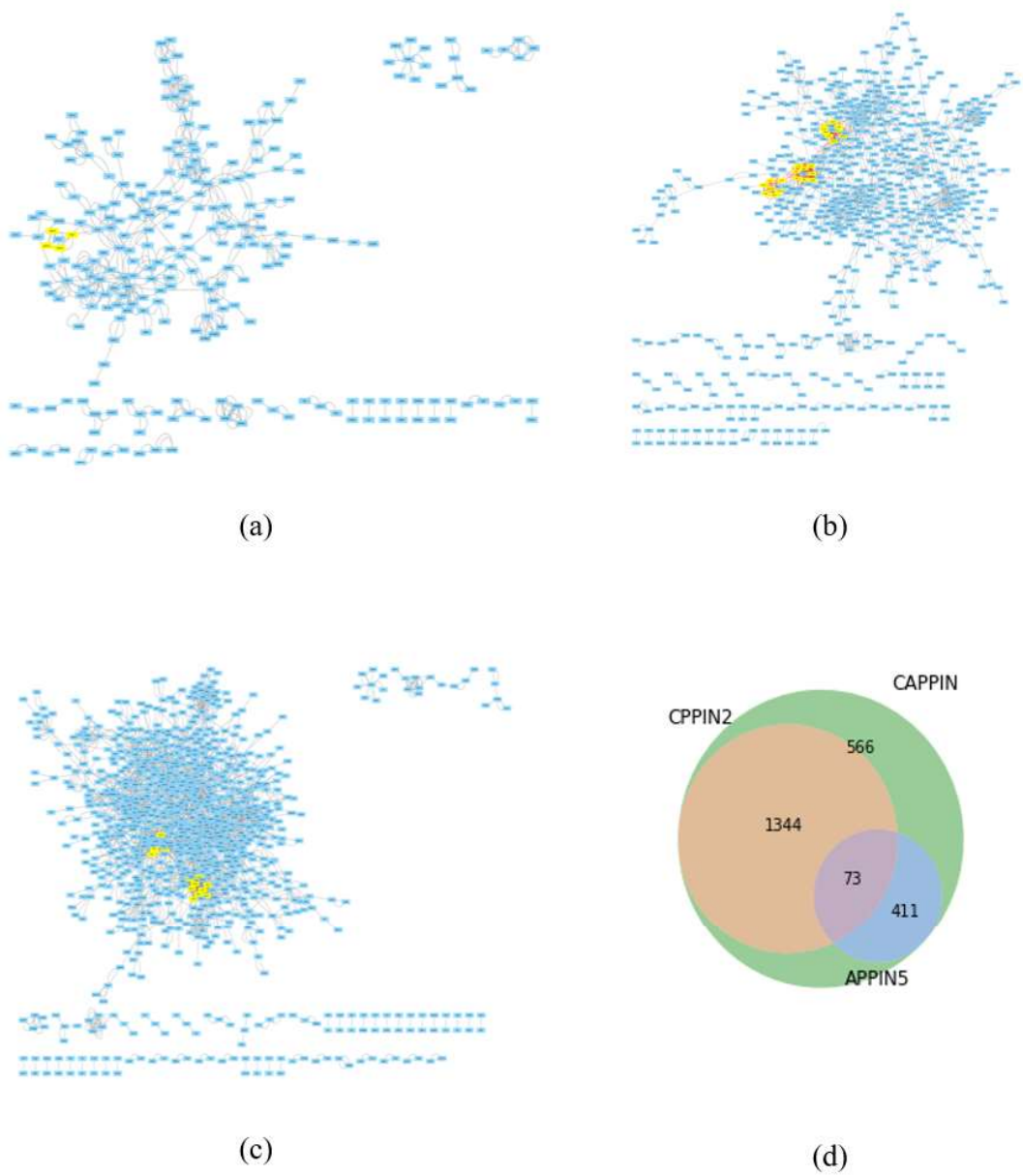


Figure 4.1. Generated protein-protein interaction networks and venn diagram (a)APPIN5 (b)CPPIN2 (c)CAPPI (d)venn diagram of interactions (Reproduced from [55]).

Table 4.2. Details of circadian and circadian-autism PPI networks.

	CPPIN1	CPPIN2	New CAPPIN (APPIN5 + CPPIN2)
Total protein count	26504	1435	1844
Number of nodes	9333	657	980
P-P interaction count	77594	1417	2394
Network density (%)	0.1	0.5	0.4
Network diameter	13	18	14
Characteristic path length	4.61	6.73	5.90
Network heterogeneity	1.67	1.03	0.95

Table 4.3. Human PPI network graph properties from literature.

Model PPI network	Insulin	Wnt/PCP	Random	Hypertension	DIP
Clustering coefficient	-	-	0.16	-	0.20
Number of nodes	498	1952	8448	535	1065
Network diameter	5	13	13	12	21
Characteristic path length	2.9	4.61	4.39	5.23	6.80
Reference	[56]	[57]	[58]	[59]	[60]

Table 4.4. Key properties of nine core circadian genes in the PPI network.

Gene Name	CSNK1E	CRY1	CRY2	ARNTL	PER1	CLOCK	NPAS2	PER2	PER3
Average Shortest Path Length	4.56	5.51	5.51	5.51	5.51	5.51	5.51	5.51	6.51
Clustering Coefficient	0.28	0.75	0.75	0.93	0.95	0.95	0.95	0.95	1.00
Closeness Centrality	0.22	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.15
Degree	34	21	19	18	18	15	15	15	4
Neighborhood Connectivity	8.56	8.44	8.44	9.38	9.86	9.86	9.86	9.86	9.00
Radiality	0.80	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.69

4.2. Analysis of Hub Proteins in the Network

The node connectivity measures lists of highly connected proteins that participate significantly in the organization of the cellular protein interaction network. The top ten hub proteins are listed in Table 4.5 and Table 4.6. In the circadian network CPPIN1, ubiquitin C or polyubiquitin-C (UBC) is one of the primary hub proteins. The protein known for its stress-protective functions impacts the circadian network [61]. Circadian rhythm translation–transcription feedback loops are mediated by ubiquitination and intermediates created during this process to help the stability of the central circadian proteins [62]. The ribosomal proteins are among the highly connected proteins in

CPPIN2. So, the relationship between the circadian and ribosomal proteins deserves further investigation. UBE2A, the hub enzymes UBE2B and UBE2C are involved in the pathway protein ubiquitination, specifically as ubiquitin-conjugating enzymes E2A, E2B, and E2C. Mutations in the UBE2A gene are known to cause X-linked intellectual disability [63], indicating the link to ASD. The central component of the circadian clock, CSNK1E (Casein Kinase 1, Epsilon), determines the circadian period length. It had a high degree in the circadian network, CPPIN, and the joint network, CAPPIN. Thirty-four degrees in CPPIN and 37 degrees in CAPPIN are observed. It is the most interacting protein among the nine primary circadian proteins. Research has shown that CSNK1E is a gene that damages de novo mutations in ASD [64,65].

Table 4.5. Top-degree proteins in autism PPI networks.

	APPIN1		APPIN2		APPIN3		APPIN4		APPIN5
HubProt	Dg.	HubProt	Dg.	HubProt	Dg.	HubProt	Dg.	HubProt	Dg.
CREBBP	11	RPS27A	496	CTNNB1	18	CREBBP	22	CREBBP	24
CTNNB1	8	UBB	302	DLG2	16	CTNNB1	13	CTNNB1	21
ANK2	6	RPS3	297	NRXN1	14	CTBP1	13	CTBP1	17
SIN3A	6	UBA52	296	CREBBP	14	DYNC1H1	12	DLG2	16
TBL1XR1	5	RPS2	292	HRAS	10	SEPT6	12	EIF4E	16
TCF7L2	4	RPS8	290	SHANK2	10	TCF4	11	NRXN1	16
PHF12	4	RPS6	286	QPRT	9	PSME3	10	STX1A	15
PHF21A	4	UBC	279	STX1A	8	STX1A	10	DYNC1H1	14
TCF4	4	RPS16	279	APC	8	SNAP29	10	SNAP29	13
DYNC1H1	4	RPSA	274	ANK2	8	SIN3A	10	HRAS	12

CTNNB1 and CREBBP proteins are common in all autism networks except for the APPIN2 network. DYNC1H1 and STX1A proteins existed in three of the five networks. ANK2, SIN3A, DLG2, NRGXN1, HRAS, SNAP29, CTBP1, and TCF4 are found in two of the five autism networks. Changes in the synapse function and gene regulation may contribute to the development of ASD. The ANK2 gene plays a role in cell motility, activation, and proliferation processes [66,67]. Syntaxin-1A (STX1A) is a nervous system protein involved in docking synaptic vesicles and the presynaptic membrane. Additionally, it affects the release of serotonin (5-HT) in the brain and GABAergic neurotransmission. This is why it possibly contributes

to attention-deficit/hyperactivity disorder [68]. The common ASD-related proteins SIN3A, CTNNB1, and CTBP1 interact with other shared proteins, namely MECP2, CHD8-PTK7-TCF7L2, and EHMT1-FOXP1 respectively. Different publications have indicated that mutations in the CTNNB1 gene are associated with cancer and autism [69]. The interesting point from these highly connected proteins is that ribosomal proteins form most of the hub proteins in the APPIN networks. The CREBBP protein takes part in the transcriptional coactivation of numerous transcription factors. Rubinstein–Taybi syndrome (RTS), which is classified within the ASD family, arises from a mutation in the gene coding for CREBBP [70]. Similar to ASD, the primary symptoms of Rubinstein–Taybi disorder encompass the gradual development of cognitive and motor skills [71]. The hub protein CREBBP (Table 4.5) also appears in several clusters of GO Enrichment analysis of 49 common proteins of CPPIN2 and APPIN5. The relationship between autism and ribosomal genes/proteins requires further investigation. Another protein listed in Table 4.5, DYNC1H1, is linked to regulating cellular processes. Mutations in this DYNC1H1 gene may potentially contribute to neurological disorders [72], such as neuromuscular and sensory deficits [73]. TCF7L2 is linked to the Wnt-signaling pathway and is associated with schizophrenia [74]. With a prominent expression in the brain, TCF4 actively participates in neurodevelopment. It collaborates with class II bHLH transcription factors Math1, HASH1, and neuroD2 [75]. The TCF4 gene is also associated with memory function and regulates neurodevelopmental pathways [76]. Haploinsufficiency in TBL1XR1 leads to ID with dysmorphism, though it does not consistently result in autistic behavior [77]. TBL1XR1 contributes to the regulation of the Wnt/ β -catenin signaling pathway. It influences various brain development stages, processes linked to cell fate determination, and stem cell renewal [78]. APC protein is critical in multiple cellular processes by interacting with beta-catenin signaling pathways. Deleting the APC gene is observed in individuals with autism [79]. NRXN1, associated with neurodevelopmental disorders, also plays a role in ASD due to CNVs in the NRXN1 gene [80].

Table 4.6. Top-degree proteins in circadian and combined circadian-autism PPI networks.

	CPPIN1		CPPIN2		CAPPIN
HubProt	Degree	HubProt	Degree	HubProt	Degree
TRIM28	656	UBE2B	41	UBE2B	56
RPS27A	625	UBE2C	41	UBE2C	56
UBB	456	RPL14	41	RPL14	47
GNB4	424	RPS5	38	RPS23	41
GNB3	422	RPS23	37	RPS5	41
GNB5	421	CSNK1E	34	GNG2	40
UBC	404	RPS25	33	CSNK1E	37
RPS16	373	CSNK1D	29	RPS25	36
GNGT1	352	RPL3	28	CREBBP	35
RPS12	348	RPL21	28	CTNNB1	34

4.3. Detection and Analysis of Modules in Protein-Protein Interaction Networks

In biological systems, scale-free networks comprise clustered regions called modules [51]. CPPIN2 is divided into modules using the “Cytoscape” Network Analyzer MCODE plug-in. Twenty clusters, characterized by highly associated domains, are obtained and visualized in Cytoscape (Table 4.7). The cluster with the significantly higher score, Cluster 1, comprises 24 proteins and 218 interactions (edges) between these proteins (Figure 4.2). Additionally, this cluster has nine primary circadian clock gene products and ribosomal proteins related to the circadian system. APPIN5 network is segmented into sub-clusters via the “Cytoscape” Network analyzer MCODE plug-in to conduct a comprehensive analysis. Highly associated domains characterized 16 clusters. These clusters are extracted and visualized via Cytoscape.

The most significant cluster, Cluster 1, had four proteins and a higher score

in MCODE results. Cluster 1 included the primary circadian clock gene products, ARNTL, ARNTL2, PER1, and NPAS2. Identifying a cluster associated with circadian clock proteins within the pure autism network holds considerable significance. However, the circadian clock and ASD are not directly connected due to the small size of Cluster 1. Nevertheless, several studies [35, 81, 82] have indicated the interrelation between the circadian clock and autism. The thalamic-cortical-amygdala pathway, essential for complex emotional memory [83], is dysfunctional in autism [84]. These studies on neuronal PAS domain protein 2 (NPAS2) reveal that NPAS2-deficient mice subjected to behavioral tests exhibit deficits in long-term memory. NPAS2 plays a regulatory role in acquiring specific types of memory, linking NPAS2 to autistic disorder. Moreover, a study [84] on the hypothesis that clock genes are implicated in autistic disorder revealed noteworthy associations in the PER1 and NPAS2 genes. Each gene has two single nucleotide polymorphisms (SNPs) that are statistically significant at the usual levels. Another significant finding emerged in APPIN5. Cluster 16 encompassed SHANK2 and NLGN1 (Table 4.11). Neurexin/neurologin interactions in the neuronal synaptic cleft are crucial for adequately functioning the synaptic network and neurotransmission [85]. A specific mRNA or translation process stimulates the local translation of significant ASD-related proteins in the synaptic domain. Translation of the mRNAs for NLGN1, NLGN2, and NLGN3 is negatively regulated by Fragile X mental retardation protein (FMRP). The loss of this translational repressor, FMRP, leads to Fragile X syndrome, associated with ASD [86]. Loss-of-function mutations in NRXN1, NLGN3, and NLGN4 genes in ASD [86, 87], and disruption of SH3 and SHANK (multiple ankyrin repeat domains) lead to abnormal behaviors in ASD [86, 88]. Some gene mutations known to modulate synaptic plasticity are associated with rare cases of autism [89]. Therefore, the contribution of these synaptic plasticity-related genetic factors to developing abnormal central nervous system (CNS) in ASD requires further research.

Table 4.7. MCODE cluster analysis results for CPPIN2.

Cluster	Cluster Score	Cluster Nodes	Cluster Edges	Cluster Node IDs
1	7.48	24	218	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/RPS5/WDR3/WDR75
2	5	5	23	ARID1B/BCL7B/SMARCB1/SMARCC2/SMARCE1
3	4.8	6	24	CUL3/KLHL20/KLHL3/UBE2B/UBE2C/ZBTB16
4	4	4	13	AP2S1/RALBP1/REPS1/REPS2
5	4	4	27	EXOSC2/EXOSC7/EXOSC8/EXOSC9
6	4	4	12	NUP107/NUP155/NUP54/NUP98
7	3.8	9	27	AXIN1/CTNNB1/DVL3/GSK3B/HLX/LDB1/LRP6/TLE3/TLE4
8	3.5	9	19	DNAJC19/GRPEL1/HSPA9/LRPPRC/LUC7L/MRPS27/MRRF/PPA2/TBRG4
9	3.3	4	8	LGALS9/LGALS9C/SLC12A6/TSPAN3
10	3.3	4	5	AKAP1/FKBP8/GK/LARP4
11	3.3	4	9	PACSIN1/PACSIN2/RAB5C/SYNJ1
12	3.3	4	10	FPR2/GNA11/GNG2/LPAR2
13	3.1	8	22	DDX42/HNRNPA1/HNRNPA3/KMT2C/POLR2K/RXRA/SNRPF/TRA2B
14	3	3	15	EIF3E/EIF3G/EIF3M
15	3	3	3	FABP5/FKBP1B/TAGLN2
16	3	3	6	PHF12/PHF21A/SIN3A
17	3	3	6	CACNA1C/CACNA2D3/CACNB2
18	3	3	6	ARRB1/MAPK1/QKI
19	3	3	7	PSMA3/PSMD8/USP14
20	3	3	3	BRIX1/GPATCH4/MAK16

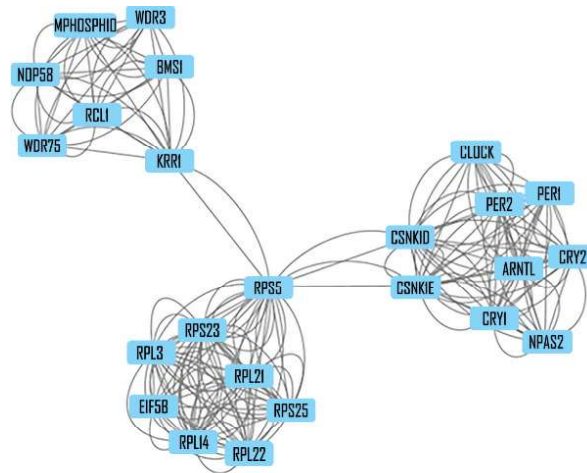


Figure 4.2. CPPIN2 Cluster 1 extracted from Cytoscape including circadian main genes (Reproduced from [55]).

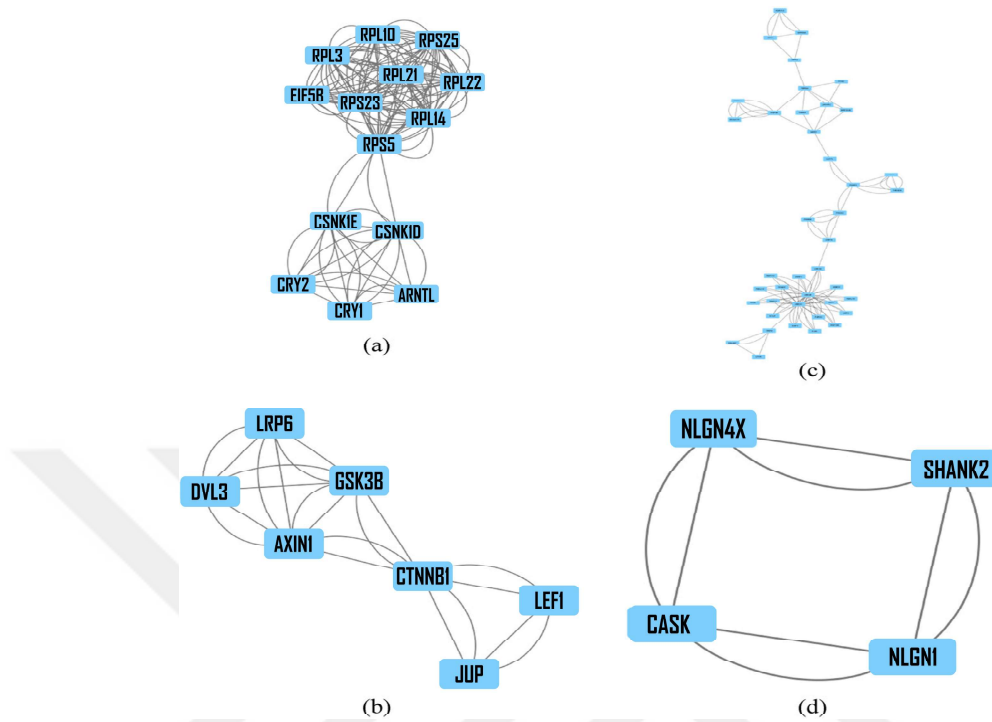


Figure 4.3. CAPPIN clusters (a) Cluster 1 (b) Cluster 10 (c) Cluster 12 (d) Cluster 30 extracted from Cytoscape (Reproduced from [55]).

The CAPPIN joint network is divided into clusters using the “Cytoscape” Network Analyzer MCODE plug-in to understand the network better. There are 30 identified unique clusters in total. The highest-scoring Cluster 1, which contained 14 proteins and 145 edges, is also included in the highest-scoring cluster in CPPIN2 (Table 4.10). Additionally, seven more clusters are identified within this combined network (Figure 4.3). Analyzing these clusters, a significant correlation between autism and circadian proteins is detected in DNA and RNA transcription processes. The identification of Cluster 10, which is directly connected to the Wnt signaling pathway, pointed out the importance of this pathway (Figure 4.3b). The Wnt signaling pathway is crucial in tumorigenesis [90].

Numerous studies [91–93] have demonstrated a connection between the Wnt signaling pathway and autism spectrum disorder (ASD). Increased transcription activity within the Wnt pathway is strongly associated with the development of ASD [94]. The

binding of β -catenin to LEC/TCF plays a significant role in regulating the transcription of genes within the Wnt pathway [94]. The CTNBB1 protein, which functions as a central hub in the CAPPIN network, is essential in the canonical Wnt signaling pathway and has a link to autism [92]. CTNNB1 interacts with various products of genes associated with ASD risk within these protein interaction networks. Both nonsense and missense mutations in the CTNNB1 gene have been found in individuals diagnosed with ASD. This gene is recognized as a significant modulator among specific ASD risk genes [95]. Additionally, GSK3B-mediated Wnt signaling has been implicated in amyloid peptide toxicity; however, inhibiting GSK3B activity may help reduce neurodegeneration [96].

Table 4.8. MCODE cluster analysis results for APPIN5.

Cluster	Cluster Score	Cluster Nodes	Cluster Edges	Cluster Node IDs
1	4	4	10	ARNTL/ARNTL2/NPAS2/PER1
2	4	4	16	SEPT1/SEPT2/SEPT5/SEPT6
3	4	4	12	APC/CTNNB1/JUP/TCF7L2
4	3.3	4	15	LDB1/MYOG/TCF12/TCF4
5	3.3	4	12	AP2B1/AP2S1/HGS/NTRK1
6	3.3	4	19	PRKAA1/PRKAA2/PRKAB2/PRKAG1
7	3.3	4	7	NDE1/NDEL1/NUP54/SNAP29
8	3	3	6	DLG2/NLGN3/NRXN1
9	3	3	3	GATAD2B/IKZF1/IKZF3
10	3	3	4	LPXN/PDLIM7/TRIP6
11	3	3	8	MBIP/PSME3/TADA2A
12	3	3	6	PHF12/PHF21A/SIN3A
13	3	3	5	RABGEF1/RBCK1/RNF31
14	3	3	6	ERBB2/NRG1/NRG3
15	3	3	6	HRAS/ITGB3/MAPK3
16	2.7	4	8	CASK/NLGN1/NLGN4X/SHANK2

4.4. Gene Ontology (GO) Enrichment Analysis

The Gene Ontology Tool provided by Princeton University is used to identify significant GO terms of the proteins within all clusters. These enriched GO terms of the circadian proteins in CPPIN2-Cluster1 are presented along with their corresponding p-values (Table 4.7). The molecular function of blue light photoreceptors denotes a series of molecular signals initiated upon sensing blue light by photoreceptor molecules

at a wavelength between 400nm and 470nm [97, 98]. The GO category of "blue light photoreceptor activity" exhibited a shallow frequency within the genome, with only 2 out of 19,751 genes associated with it. Thus, the blue light receptor's function holds significance in comprehending light's impact on the circadian clock, particularly given the correlation of the CRY1 and CRY2 genes with specific molecular functions.

Other GO terms with higher cluster frequencies also include transcription regulatory region sequence-specific metabolic pathways. Furthermore, metabolic pathways specific to transcription regulatory region sequences are among the other GO keywords with greater cluster frequencies. This word refers to proteins that engage selectively and non-covalently with particular DNA and RNA sequences in regulatory areas, hence regulating the transcription of specific DNA and RNA molecule segments. The most significant finding is the following: GO terms with high corrected p-values are associated with circadian rhythm, glucocorticoid receptor modulation, ribosome biogenesis, and circadian control of gene expression (Table A.1).

Table 4.9. Key GO term functions of Cluster 1 proteins in APPIN5 with the core circadian genes.

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
Process	Sample Genes (%)	Total Genes (%)	p-value	Genes
circadian regulation of gene expression	4/4 (100.0%)	69/19810 (0.3%)	2.08×10^{-8}	ARNTL/ARNTL2/NPAS2/PER1
circadian rhythm	4/4 (100.0%)	207/19810 (1.0%)	1.79×10^{-6}	ARNTL/ARNTL2/NPAS2/PER1
corticosteroid receptor signaling pathway	2/4 (50.0%)	14/19810 (0.1%)	4.3×10^{-4}	ARNTL/PER1
entrainment of circadian clock	2/4 (50.0%)	35/19810 (0.2%)	2.81×10^{-3}	ARNTL2/PER1

Table 4.9. Key GO term functions of Cluster 1 proteins in APPIN5 with the core circadian genes (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
glucocorticoid receptor signaling pathway	2/4 (50.0%)	13/19810 (0.1%)	3.6×10^{-4}	ARNTL/PER1
negative regulation of glucocorticoid receptor signaling pathway	2/4 (50.0%)	6/19810 (0.0%)	7.1×10^{-5}	ARNTL/PER1
negative regulation of intracellular steroid hormone receptor signaling pathway	2/4 (50.0%)	38/19810 (0.2%)	3.32×10^{-3}	ARNTL/PER1
positive regulation of circadian rhythm	2/4 (50.0%)	17/19810 (0.1%)	6.4×10^{-4}	ARNTL/ARNTL2
positive regulation of nucleic acid-templated transcription	4/4 (100.0%)	1616/19810 (8.2%)	6.84×10^{-3}	ARNTL/ARNTL2/NPAS2/PER1
positive regulation of RNA biosynthetic process	4/4 (100.0%)	1622/19810 (8.2%)	6.94×10^{-3}	ARNTL/ARNTL2/NPAS2/PER1
positive regulation of RNA metabolic process	4/4 (100.0%)	1750/19810 (8.8%)	9.4×10^{-3}	ARNTL/ARNTL2/NPAS2/PER1

Table 4.9. Key GO term functions of Cluster 1 proteins in APPIN5 with the core circadian genes (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
positive regulation of transcription by RNA polymerase II	4/4 (100.0%)	1200/19810 (6.1%)	2.07×10^{-3}	ARNTL/ARNTL2/NPAS2/PER1
positive regulation of transcription, DNA-templated	4/4 (100.0%)	1616/19810 (8.2%)	6.84×10^{-3}	ARNTL/ARNTL2/NPAS2/PER1
regulation of circadian rhythm	3/4 (75.0%)	118/19810 (0.6%)	1.2×10^{-4}	ARNTL/ARNTL2/PER1
regulation of glucocorticoid receptor signaling pathway	2/4 (50.0%)	7/19810 (0.0%)	9.95×10^{-5}	ARNTL/PER1
regulation of hair cycle	2/4 (50.0%)	28/19810 (0.1%)	1.78×10^{-3}	ARNTL/PER1
response to redox state	2/4 (50.0%)	13/19810 (0.1%)	3.6×10^{-4}	ARNTL/NPAS2
rhythmic process	4/4 (100.0%)	300/19810 (1.5%)	7.99×10^{-6}	ARNTL/ARNTL2/NPAS2/PER1

The significant GO terms of the proteins in Clusters 1 of APPIN5 were tabulated with p-values in Table 4.9. These findings are of significant importance as they provide a deeper understanding of the biological processes involved. The enriched GO terms for Cluster 1 are mainly related to circadian rhythm and its regulation.

The enriched GO terms for CAPPIN Cluster 1 primarily pertain to cytoplasmic

translation or translation, glucocorticoid receptor signaling pathway and its regulation, ribonucleoprotein complex biogenesis, cellular response to endogenous stimulus, histone acetylation, and peptidyl-lysine acetylation processes (Table 4.10). MAPK3, existing in both APPIN and CAPPIN and RPS6KA4, existing in both CPPIN2 and CAPPIN, are involved in the positive regulation of peptidyl-lysine acetylation. Additionally, it is found that antidepressants and related molecular pathways analogs influence these genes [99]. The enriched GO terms for CAPPIN Cluster 10 are primarily related to the Wnt signaling pathway, macromolecule biosynthetic process regulation, RNA biosynthetic process, and midbrain dopaminergic neuron differentiation.

Table 4.10. MCODE cluster analysis results for CAPPIN.

Cluster	Cluster Score	Cluster Nodes	Cluster Edges	Cluster Node IDs
1	7.38	14	145	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/RPS5
2	7	7	42	BMS1/KRR1/MPHOSPH10/NOP58/RCL1/WDR3/WDR75
3	6.86	8	49	DDX42/HNRNPA1/HNRNPA3/PPIL1/PPIL4/SNRPF/SNRPN/TRA2B
4	5	5	23	ARID1B/BCL7B/SMARCB1/SMARCC2/SMARCE1
5	5	5	21	NUP107/NUP155/NUP54/NUP62/NUP98
6	4.80	6	24	ARRB1/HRAS/ITGB3/MAPK1/MAPK3/QKI
7	4	4	16	SEPT1/SEPT2/SEPT5/SEPT6
8	4	4	17	MCL1/PRKAA1/PRKAB2/PRKAG1
9	4	4	27	EXOSC2/EXOSC7/EXOSC8/EXOSC9
10	3.67	7	22	AXIN1/CTNNB1/DVL3/GSK3B/JUP/LEF1/LRP6
11	3.60	6	9	BRX1/DGCR8/GPATCH4/MAK16/MECP2/MYBBP1A
12	3.40	41	119	ASB13/DNAJC19/DZIP3/FBXL13/FBXL16/GPR89A/GRPEL1/HSPA9/ITCH/LNX1/LPXN/LRPPRC/LUC7L/MBIP/MRPS18C/MRRF/MYLIP/P2RY12/PARK2/PDLIM7/PJA1/PPA2/PSMA3/PSMD8/PSME3/RNF111/RNF19B/TADA2A/TBRG4/THEM4/TRIM37/TRIP12/TRIP6/UBE2B/UBE2C/UBE3A/UBR2/USP14/VIPR2/WWP1/YIPF3
13	3.33	4	10	AES/LDB1/TLE3/TLE4
14	3.33	4	5	JUNB/RFWD2/TRIB1/TRIB2

Table 4.10. CODE cluster analysis results for CAPPIN (cont.).

Cluster	Cluster Score	Cluster Nodes	Cluster Edges	Cluster Node IDs
15	3.33	4	8	LGALS9/LGALS9C/SLC12A6/TSPAN3
16	3.33	4	5	AKAP1/FKBP8/GK/LARP4
17	3.33	4	10	CACNA1C/CACNA1F/CACNA2D3/CACNB2
18	3	3	15	EIF3E/EIF3G/EIF3M
19	3	3	6	DLG2/NLGN3/NRXN1
20	3	3	7	RALBP1/REPS1/REPS2
21	3	3	6	EPS15L1/HGS/OCRL
22	3	3	8	AP2B1/AP2S1/NTRK1
23	3	3	3	GATAD2B/IKZF1/IKZF3
24	3	3	3	FABP5/FKBP1B/TAGLN2
25	3	3	6	PHF12/PHF21A/SIN3A
26	3	3	5	IRF7/TRAF2/TRAF6
27	3	3	3	FBXO28/IKBIP/TRAF7
28	3	3	5	NDE1/NDEL1/SNAP29
29	2.67	4	8	CLOCK/NPAS2/PER1/PER2
30	2.67	4	8	CASK/NLGN1/NLGN4X/SHANK2

There are 49 common proteins in CPPIN2 and APPIN5. The enriched GO terms for these common proteins are mainly related to regulation processes such as regulation of transcription by RNA polymerase II, regulation of RNA metabolic process, regulation of transcription, DNA templated, regulation of transport, regulation of localization, regulation of ion transport, histone modification and acetylation, peptidyl-lysine modification and acetylation, multicellular organism development (Table A.2).

Table 4.11. MCODE cluster analysis results for APPIN5.

Cluster	Cluster Score	Cluster Nodes	Cluster Edges	Cluster Node IDs
1	4	4	10	ARNTL/ARNTL2/NPAS2/PER1
2	4	4	16	SEPT1/SEPT2/SEPT5/SEPT6
3	4	4	12	APC/CTNNB1/JUP/TCF7L2
4	3.3	4	15	LDB1/MYOG/TCF12/TCF4
5	3.3	4	12	AP2B1/AP2S1/HGS/NTRK1
6	3.3	4	19	PRKAA1/PRKAA2/PRKAB2/PRKAG1
7	3.3	4	7	NDE1/NDEL1/NUP54/SNAP29
8	3	3	6	DLG2/NLGN3/NRXN1
9	3	3	3	GATAD2B/IKZF1/IKZF3
10	3	3	4	LPXN/PDLIM7/TRIP6
11	3	3	8	MBIP/PSME3/TADA2A
12	3	3	6	PHF12/PHF21A/SIN3A
13	3	3	5	RABGEF1/RBCK1/RNF31
14	3	3	6	ERBB2/NRG1/NRG3
15	3	3	6	HRAS/ITGB3/MAPK3
16	2.7	4	8	CASK/NLGN1/NLGN4X/SHANK2

Table 4.12. Significant GO terms for Cluster 1 proteins in CAPPIN (Princeton Gene Ontology Tool).

GO term	Genes (annotated to the term)
amide biosynthetic process	EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
biosynthetic process	ARNTL/CRY1/CRY2/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
blue light signaling pathway	CRY1/CRY2
cellular amide metabolic process	EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
cellular biosynthetic process	ARNTL/CRY1/CRY2/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
cellular macromolecule biosynthetic process	ARNTL/CRY1/CRY2/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
cellular macromolecule metabolic process	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
cellular nitrogen compound biosynthetic process	ARNTL/CRY1/CRY2/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25

Table 4.12. Significant GO terms for Cluster 1 proteins in CAPPIN (Princeton Gene Ontology Tool) (cont.).

GO term	Genes (annotated to the term)
cellular nitrogen compound metabolic process	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
cellular protein metabolic process	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
cellular response to blue light	CRY1/CRY2
circadian regulation of gene expression	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E
circadian rhythm	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E
corticosteroid receptor signaling pathway	ARNTL/CRY1/CRY2
cytoplasmic translation	RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
gene expression	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
glucocorticoid receptor signaling pathway	ARNTL/CRY1/CRY2
glucocorticoid secretion	CRY1/CRY2
macromolecule biosynthetic process	ARNTL/CRY1/CRY2/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
macromolecule metabolic process	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
negative regulation of corticosteroid hormone secretion	CRY1/CRY2
negative regulation of glucocorticoid receptor signaling pathway	ARNTL/CRY1/CRY2
negative regulation of glucocorticoid secretion	CRY1/CRY2
negative regulation of intracellular steroid hormone receptor signaling pathway	ARNTL/CRY1/CRY2

Table 4.12. Significant GO terms for Cluster 1 proteins in CAPPIN (Princeton Gene Ontology Tool) (cont.).

GO term	Genes (annotated to the term)
negative regulation of steroid hormone secretion	CRY1/CRY2
non-membrane-bounded organelle assembly	CSNK1D/RPL10/RPL3/RPS5/RPS23
organic substance biosynthetic process	ARNTL/CRY1/CRY2/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
organonitrogen compound biosynthetic process	EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
organonitrogen compound metabolic process	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
peptide biosynthetic process	EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
peptide metabolic process	EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
positive regulation of dopaminergic neuron differentiation	CSNK1D/CSNK1E
positive regulation of midbrain dopaminergic neuron differentiation	CSNK1D/CSNK1E
positive regulation of Wnt-mediated midbrain dopaminergic neuron differentiation	CSNK1D/CSNK1E
protein metabolic process	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
regulation of circadian rhythm	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E
regulation of dopaminergic neuron differentiation	CSNK1D/CSNK1E
regulation of glucocorticoid receptor signaling pathway	ARNTL/CRY1/CRY2
regulation of glucocorticoid secretion	CRY1/CRY2
regulation of intracellular steroid hormone receptor signaling pathway	ARNTL/CRY1/CRY2

Table 4.12. Significant GO terms for Cluster 1 proteins in CAPPIN (Princeton Gene Ontology Tool) (cont.).

GO term	Genes (annotated to the term)
regulation of midbrain dopaminergic neuron differentiation	CSNK1D/CSNK1E
regulation of Wnt-mediated midbrain dopaminergic neuron differentiation	CSNK1D/CSNK1E
response to blue light	CRY1/CRY2
rhythmic process	ARNTL/CRY1/CRY2/CSNK1D/CSNK1E
ribonucleoprotein complex biogenesis	RPL10/RPL14/RPL3/RPS5/RPS25
ribosomal large subunit biogenesis	RPL10/RPL14/RPL3
ribosome assembly	RPL10/RPL3/RPS5
ribosome biogenesis	RPL10/RPL14/RPL3/RPS5/RPS25
translation	EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25

Recent research has clarified the critical function that histone acetylation plays in the setting of ASD, and more thorough research is necessary. Histone acetylation changes may lead to how genes are transcribed, which is essential for the development of ASD. The mRNA levels of ASD risk genes, such as NLGN1, SHANK2, SHANK3, and CNTNAP2, are altered by prenatal HDAC (histone deacetylase family) suppression. These genes' changes are implicated in neurodevelopmental disorders [100]. Furthermore, exposure to environmental stimuli during pregnancy may modify epigenetic markers in utero through various processes, including chemical changes like DNA methylation or protein changes like histone acetylation/deacetylation. These changes have been implicated in contributing to neurodevelopmental consequences associated with ASD [100].

4.5. Network Decomposition Analysis (NDA)

Network decomposition analysis (NDA) involves breaking down a network into fundamental pathways that capture the time-invariant topological structure. NDA serves the purpose of defining the overall capabilities of a cell. CPPIN is dissected into linear pathways connecting transcription factors and various proteins within the PPI network to conduct a detailed information flow analysis. Linear pathways that originate from the central circadian gene product (ARNTL [known as BMAL1]) to the transcriptional factor gene (TCF4) are examined using the NetSearch Algorithm. TCF4 functions as a transcription factor that regulates a wide array of developmental processes such as schizophrenia and autism [76]. Deficiencies in TCF4 are associated with various human disorders, including Pitt–Hopkins syndrome, an intellectual disability condition, and autism spectrum disorder (ASD). The total count of pathways from ARNTL to TCF4 is estimated to be 1350.



Figure 4.4. 6-proteins length pathways of CAPPIN from ARNTL to TCF4
(Reproduced from [55]).

The participation of proteins in these linear paths indicates their significance in signal transduction, as any signaling network is composed of linear paths. Therefore, the participation percentages of each protein in linear paths of the CAPPIN are calculated to get an insight into the roles of the proteins in the information flow from ARNTL to TCF4. MATLAB finds the most frequently encountered proteins in these linear paths. Among these findings, CSNK1E protein is recognized as the most interactive protein with the initial ARNTL protein. The AXIN1 protein is the most

interactive protein with CSNK1E when applied to all proteins with path lengths of 6, 7, and 8. The characteristic path length of CAPPIN was 5.896. Therefore, the pathways of 6 steps are visualized in Figure 4.4, and pathways with steps of 5-6-7-8 are shown in Figure 4.5.

The key connectors central to many shortest paths in an interaction network are known as bottleneck proteins. Bottleneck proteins control most information flow from ARNTL to the TCF4 protein. The ARNTL and CSNK1E are the hub/bottleneck proteins in our network. Thus, their mutations may disrupt the circadian system, resulting in diseases such as ASD. A recent study on AXIN1 has indicated a correlation with autistic traits [101]. Furthermore, some research reports the role of the CTNNB1 gene in regulating cognitive and autistic-like behaviors [69]. TLE3 is a target of FOXP2, a transcription factor located on chromosome 7q31 related to autistic disorder [102].

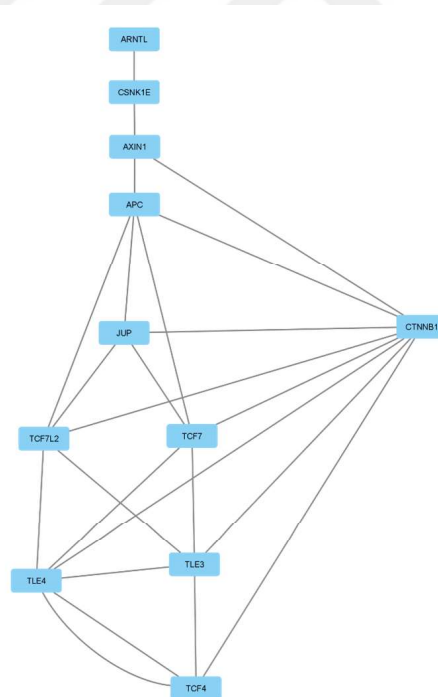


Figure 4.5. Probable CAPPIN pathways (5-8 steps) from ARNTL to TCF4
(Reproduced from [55]).

5. STEADY STATE MODEL - DISCUSSION

5.1. Interactions Between the Circadian Clock and Various Pathways

The human circadian clock orchestrates many physiological activities at every organizational level in the body and maintains a 24-hour feedback loop of transcription and translation. There are nine core gene products related to circadian rhythms: Period 1 (PER1), Period 2 (PER2), Period 3 (PER3), Casein Kinase I ϵ (CKI ϵ), CLOCK, ARNTL (BMAL1), TIM, CRY1 and CRY2 [103]. These gene products are also part of the regulation of cell proliferation and could be critical to the cell cycle. Moreover, a few studies on micro-peripheral tissues show that the biological clock is stimulated in case of DNA damage. PER2 is primarily involved in the DNA damage response process in rodents [104].

The light activates the BMAL1 (ARNTL) gene that interacts with the CLOCK or NPAS2 genes to form BMAL1:NPAS2 or BMAL1:CLOCK in the cytoplasm. These resulting compounds activate other circadian genes in the nucleus and the E-box: PER1/2, CRY1/2, and DEC1/2. PER1/2-CRY1/2 and DEC1/2 inhibit the heterodimeric transcription factors BMAL1:NPAS2 or BMAL1:CLOCK according to the tissue; then, these heterodimers activate their transcription in the negative loop [105].

An important point to mention here is that the P53 gene is also affected by PER1 and PER2. The lack of P53 changes PER2 expression in SCN [106]. P53 and P21 (activated by P53) are both involved in damaged DNA repair pathways and inhibition of tumor formation pathways. By looking at the main circadian pathway (Figure 5.1), it is possible to observe that damaged circadian genes/gene products cannot activate the P53 [105]. UV lights or stress (external effects) affect the expression of the P53, leading to P53 gene damage and an increase in cancer risk [107].

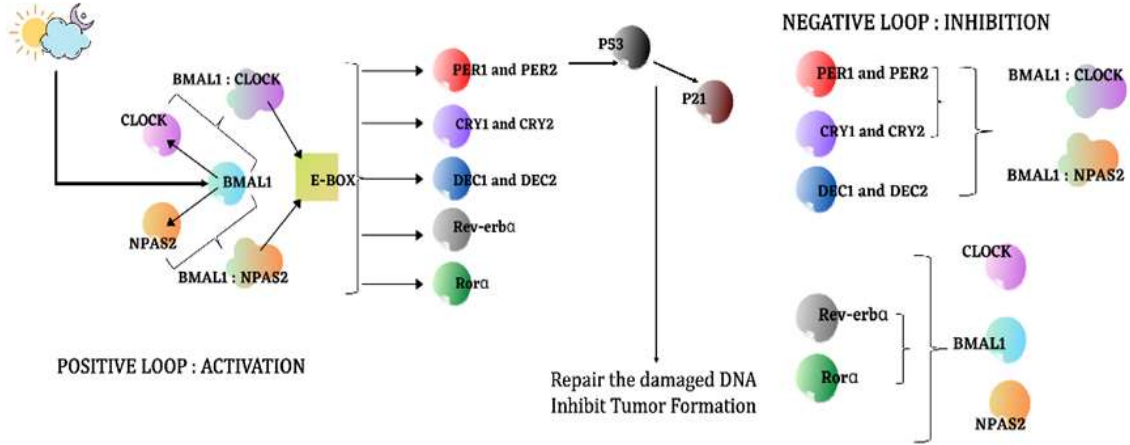


Figure 5.1. Mammalian protein signaling pathway with positive and negative feedback loops (Reproduced from [55]).

BMAL1:CLOCK heterodimer whose activation is altered by CREB-binding protein (CBP) acts as the transcriptional activator in the circadian rhythm and regulates the expression of circadian genes [108]. However, the MYC protein, known as an oncogene due to its potential to cause cancer, disrupts the formation of the BMAL1-CLOCK dimers by binding to the E-box. This disruption has an impact on the activation of other negative regulators, such as REV-ERB. Thus it suppresses the BMAL1 expression and consequently impacts the circadian clock [109]. A well-functioning circadian rhythm is necessary for good health and its maintenance.

5.2. Metabolic Pathway Dysfunctions Caused by Mutations in Clock-Controlled Genes

Autism is influenced by the dysfunction of significant pathways within the central nervous system (CNS). Neuronal PAS domain protein 2 (NPAS2), a member of PAS protein 4 (MOP4), is a transcription factor protein, so it plays a vital role in sleep homeostasis and maintaining circadian rhythms in normal light/dark conditions. NPAS2 protein emerges prominently in clusters of excessively associated proteins (MCODE

clusters) and as a result of GO enrichment analysis. Some ASD symptoms, such as impaired memory and sleep disturbances, have been investigated using mouse models. *Npas2* (-/-) knockout mice have shown disruptions in complex emotional memory [83], indicating the critical role of non-REM sleep homeostasis and a reduction in total sleep time [110]. Furthermore, a specific protein variant, NPAS2 471 Leu/Ser, has been associated with seasonal affective disorder and diurnal preference in humans [111]. In the case of an impaired transcription/translation feedback loop, the binding of CLOCK:BMAL1 or NPAS2:BMAL1 is inhibited, resulting in the downregulation of both the period and cryptochrome genes' transcription [36, 112].

Mutations in the clock-controlled genes are frequently observed in individuals with ASD. Although individuals with ASD have been studied for genes strongly associated with circadian rhythm, statistical analyses have revealed variations among these ASD individuals [82]. The polymorphisms in NPAS2 (cytosine/thymine SNP in intron 3-NPAS2.X3.C.T) [84], mutations in PER1 (cytosine $\rightarrow$ guanine SNP (Per1_rs885747) and a cytosine/adenine SNP (Per1_rs6416892)) [84], mutations in PER2 (proline/alanine substitution at amino acid 1228), and mutations in PER3 (an arginine/glutamine substitution at amino acid 366) have been identified in ASD patients. These variations have a detrimental impact on gene function. These clinical findings underscore the association of circadian rhythm genes/gene products PER2 and PER and the pathogenesis of ASD. Moreover, mutations in NR1D1 are also linked to abnormal ASD brain development [36]. $ROR\alpha/\beta$ is known to activate the transcription of BMAL1 and NFIL3 [113, 114]. However, $ROR\alpha$ cannot function properly in the case of impaired NR1D1. Another pathway linked to ASD is the canonical WNT/ β -catenin pathway, which becomes upregulated under the dysregulation of circadian rhythm (sleep disorder) [81]. This pathway initiates the metabolic reprogramming of cellular energy metabolism in individuals with developmental cognitive disorders [81].

5.3. Association of Oxidative Stress with ASD and the Circadian Clock

Oxidative stress is one of the mechanisms by which prenatal and postnatal problems contribute to the development of ASD. In more than 100 publications investigating any relationship between oxidative stress and ASD/Rett syndrome, an association has been reported (e.g., studies on abnormalities in microglial activation, maternal antibodies to fetal brain tissue, genetic mutations affecting the immune system, and cytokine abnormalities). The effects of oxidative stress on ASD may potentially explain the influence of parental age on ASD, as older men's spermatozooids are more sensitive to oxidative stress. Thus, their kids are more likely to experience DNA fragmentation, which can lead to neuropsychiatric diseases [115].

5.4. Link Between the Circadian Clock and Autism Through the mTOR Signaling Pathway

The mTOR signaling pathway is essential in controlling significant functions within neurons, encompassing neurodevelopment and intracellular metabolism [116, 117]. Recent studies [118, 119] have demonstrated the influence of the circadian clock on mTOR activities. These activities exhibit robust diurnal oscillations across various systems. Furthermore, mTOR signaling impacts central and peripheral circadian clocks' duration, synchronization, and entrainment [120, 121]. The study, which focused on the association between autism and circadian genes in mice, demonstrated that mice that lacked the *Bmal1* gene exhibited differences in the expression of genes associated with autism and ataxia and also displayed dysregulated pathways, including overactive mTORC1 signaling [122]. Moreover, mTOR creates a complex signaling network that combines external and internal signals to influence mRNA translation [116, 117].

The study on autism [123] demonstrates that the eukaryotic initiation factor 4E (EIF4E) gene, which plays a role in translation control, is implicated in the disorder. Specifically, specific mutations in the EIF4E promoter region have been associated with autism. EIF4E is a hub protein in APPIN5 and is closely interconnected with

the mTOR signaling pathway and circadian clock. One of the downstream processes of this pathway is the activation of the EIF4E. This study suggests additional evaluation of the mTOR pathway and a review of ribosomal proteins.

5.5. The Role of Ribosome Biogenesis in ASD and the Circadian Clock

The GO enrichment analyses point out the importance of ribosome biogenesis cross-talking with ASD (which might be one of its causative factors) and related neurodevelopmental diseases. In autism, the ribosomal protein-encoding genes have a high expression (upregulated), pointing to enhanced ribosome biogenesis and, consequently, to high protein synthesis [124]. The high copy number of ribosomal genes in the dendrites alters the level of dendritic translation and thus is considered a risk factor for neuropsychiatric disorders [125]. Thus, due to disturbed “translational homeostasis,” ASD may develop in subjects with abundant ribosomes due to moderate or high copy numbers of ribosomal genes. Tran et al. [126] identified new variants in six new candidate genes (CHM, ENPP1, IGF1, LAS1L, SYP, and TBX22) enriched in an ASD cohort. These genes are linked with several neurological conditions and have roles in neuronal biological processes, such as synaptic processes, regulation of transport, and ribosome maturation. Therefore, the genetic predispositions in these genes are reported to be causative factors for ASD [126]. Another ribosomal gene with missense mutations is the RPL10 gene, whose expression is high in the mouse hippocampus, which is the site of action for learning, memory, and social and affective functions. Thus, the functionally altered RPL10 protein causes cognitive malfunction, which is also impaired in autism [89]. On the other hand, another study mentioned that RPL10 has no significant impact on ASD susceptibility [127].

The circadian clock controls the transcription of translation initiation factors and the clock-dependent rhythmic activation of signaling pathways involved in their regulation, thereby regulating the temporal translation of a subset of mRNAs involved in ribosome biogenesis. Consequently, the circadian clock is crucial in synchronizing the transcription and translation processes leading to ribosome biogenesis [128, 129].

The profile of downregulated genes for RNA-binding proteins in the transcriptome of induced pluripotent stem cell neurons (iNeurons) is examined in modeling the Rubinstein Taybi Syndrome [130]. According to research, a high number of people with Rubinstein-Taybi syndrome exhibit traits similar to those of autistic people. Several ribosomal subunits and nucleolar proteins, such as NOP58 and fibrillarin, are down-regulated gene products in RTS. These proteins form complexes with snoRNAs and play a key role in driving posttranscriptional changes required for rRNA maturation. Fibrillarin is necessary for epigenetic regulation of ribosomal genes, and NOP58 inter-actor BMAL1, a transcriptional regulator of the circadian rhythm, controls NOP58-associated snoRNA levels.

The involvement of ribonucleoprotein particles in the production of rhythmic gene expression is still a mystery. Informatics efforts to integrate these findings with circadian transcriptome datasets are needed to elucidate the posttranscriptional processes of circadian control while the target RNAs for many of the RNA-binding proteins are being discovered.

6. DYNAMIC MODEL - METHODOLOGY

6.1. System Dynamics Methodology

System dynamics is a model-based method to capture the cause-effect relationships between factors in a system, simulate how external and/or internal interactions determine the dynamics, and thus understand complex systems' behavior over time [131–133]. The essential elements in system dynamics models are stocks, flows, feedback loops, and time delays. Stocks are the main accumulations that express the system's state, which can decrease or increase by their flows. For instance, hormones are among the main stocks in the human body (Figure 6.1). Flows directly affect the stocks and cause them to decrease or increase over time, determining the stock dynamics. In Figure 6.1, hormone production increases the hormone stock, and degradation decreases it. The relationship between a stock and its flows is given by the corresponding differential (or integral) equation as

$$\frac{dHormone}{dt} = Production - Degradation. \quad (6.1)$$

The equations for the flow variables must reflect to causal relations in the real system as follows

$$Production = \frac{Ref_Level - Hormone}{Production_Adjustment_Time} \quad (6.2)$$

$$Degradation = Degradation_Fraction \times Hormone. \quad (6.3)$$

Finally, a third type of conventional 'convertor' (or auxiliary) variable is used to identify the construction of functional relations between variables. In Figure 6.1, Production Fraction and Degradation Fraction are convertor variables (in this example constants), represented by circles.

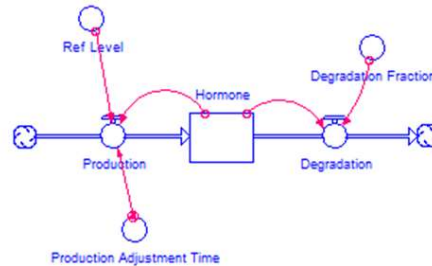


Figure 6.1. Illustration of hormone model structure

Another key concept in system dynamics is feedback loops, which are of two types: negative (stabilizing) and positive (compounding). In Figure 1, the feedback loop between Hormone and Production is negative, goal-seeking: When Production goes up, so does Hormone level; but an increase in Hormone results in reduced production. This feedback loop operates to keep Hormone level at the desired level (RefLevel). In the human body, negative feedback loops generally provide homeostasis. Positive feedback loops on the other hand compound, reinforce an initial change in the system. Tumor growth results from the operation of a harmful positive loop. Not all positive loops are harmful, they can be used to accelerate a goal-seeking action, when implemented together with negative feedback loops. There are countless positive and negative feedback in the human body. For instance, in the hypothalamus–pituitary–adrenal axis, when there is stress, corticotropin-releasing hormone (CRH) initiates the secretion of adrenocorticotropin hormone (ACTH), ACTH in turn increases the secretion of cortisol, and increased cortisol inhibits the secretion of ACTH and CRH hormones through a negative feedback loop. On the other hand, when cortisol levels are too low, cortisol increases the secretion of CRH and ACTH hormones, constituting positive feedback [134].

6.2. Description of the Model

The model aims to represent the dynamics and effects of the altered melatonin circadian rhythms in pregnant women working night shifts on fetal melatonin levels and oxidative stress marker Malondialdehyde (MDA) and the associated risk of autism. The time unit of the model is an hour. Stella modeling and simulation software version 10.0.6 is used for model construction and simulations. The boundaries of the problem suggest two subsystems in the model: maternal-fetal melatonin and maternal-fetal MDA, as shown in Figure 6.2.

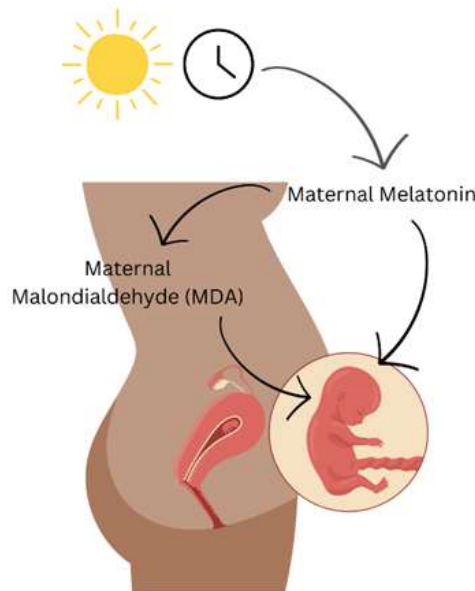


Figure 6.2. Illustration of maternal-fetal MDA and melatonin relations.

The main stock variables are Melatonin and MDA. The stock-flow diagram of the model is shown in Figure 3 and 4. The equations for all stock variables are obvious from the stock-flow diagrams: For each stock, the rate of change per unit time (hour) is given by net sum of its flows. As for the flow and convertor equations, the connecting red arrows in the diagram show the functional relations such as $F_i = f_i(S_j, F_j, C_j)$ ve $C_i = g_i(S_j, F_j, C_j)$ for all flow variables F_i and convertor variables C_i . Flow and convertor equations are not automated, the modeler writes the specific f and g formulations for all flows and convertors, using data and other information from literature. All model

equations are given in the Appendix. In this section, we describe only some important and representative flow and convertor formulations. The main goal is to implement night shift scenarios to simulate the impacts of circadian disruptions in the model. To this end, we model how the maternal-fetal Melatonin and maternal-fetal MDA levels are affected by circadian disruptions from night shift work (model sector in Figure 6.3). Finally, we model and experiment with how the risk of autism may change as a result of melatonin and MDA disruptions (Figure 6.4).



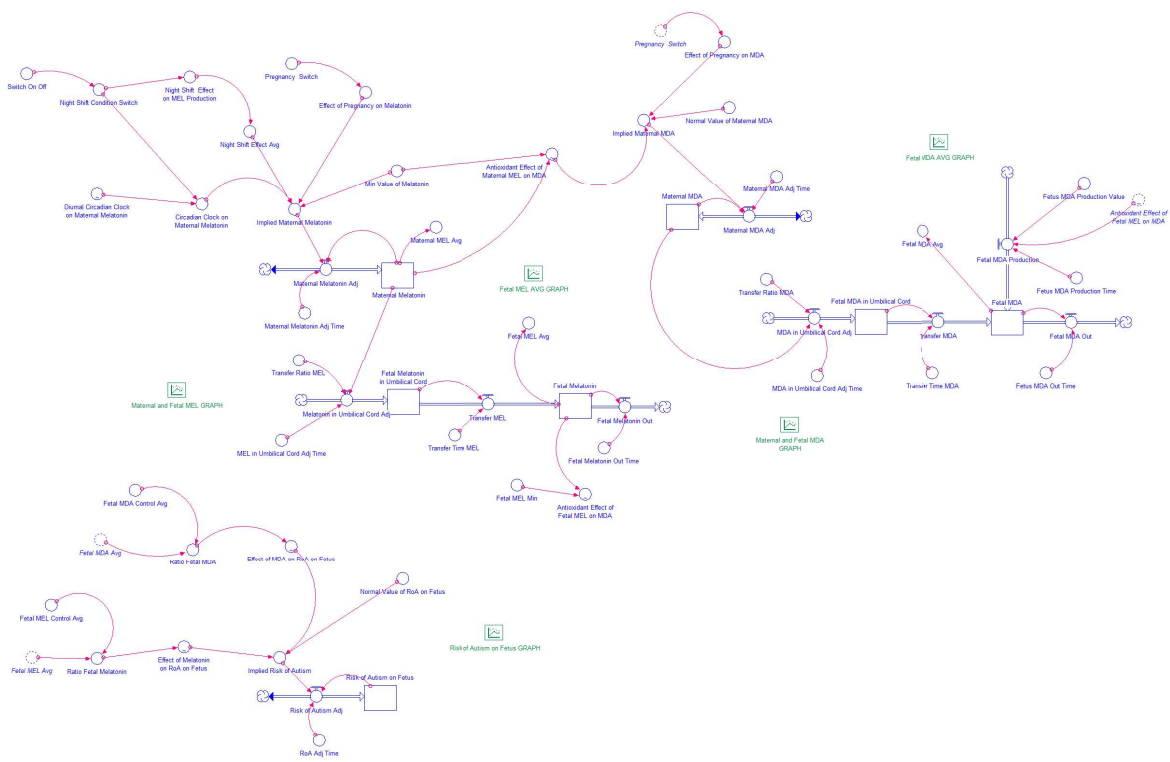


Figure 6.3. Stock flow diagram of maternal-fetal melatonin and MDA sector.

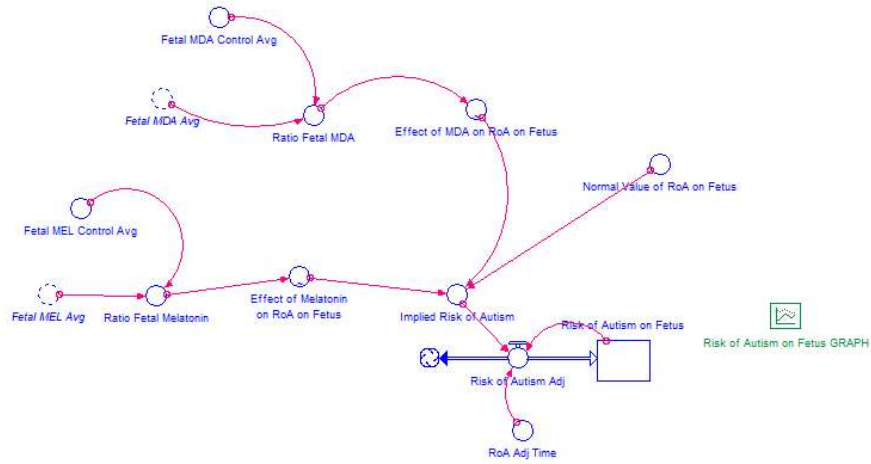


Figure 6.4. Stock flow diagram of risk of autism sector.

6.3. Key Variables and Formulations

Maternal Melatonin: Melatonin is known as the hormone of darkness, and its levels are measured nearly 10 pg/ml during daytime hours and increase to approximately 60 pg/ml at night [135]. The role of the circadian clock on melatonin is modeled by *Diurnal Circadian Clock on Maternal Melatonin*, a graphical effect function, and it has 24-hour cycles using a time mod formula, as shown in Figure 6.6. The diurnal clock graphical function is drawn based on the melatonin circadian clock from Rajaratnam and Arendt's research as shown in Figure 6.5 [135], and the melatonin level peaks at 2 a.m [136].

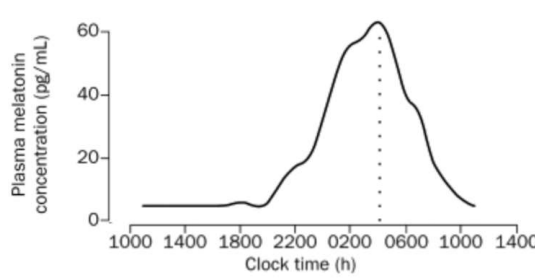


Figure 6.5. Circadian rhythm of plasma melatonin levels over 24 hours (Reproduced from [135].)

During the third trimester of pregnancy, melatonin levels increase to about 1.8 times a non-pregnant woman's daytime and nighttime melatonin levels [137,138]. We have modeled the effect of pregnancy by multiplying *Implied Maternal Melatonin* by 1.8, using *Effect of Pregnancy on Melatonin* as follows

$$\begin{aligned}
 \text{Implied_Maternal_Melatonin} &= \text{Circadian_Clock_on_Maternal_Melatonin} \\
 &\times \text{Min_Value_of_Melatonin} \\
 &\times \text{Night_Shift_Effect_Avg} \\
 &\times \text{Effect_of_Pregnancy_on_Melatonin},
 \end{aligned} \tag{6.4}$$

$$\text{Circadian_Clock_on_Maternal_Melatonin} = \begin{cases} \text{DELAY}(\text{Diurnal_Circadian_Clock_on_Maternal_Melatonin}, 1), \\ \text{if } \text{Night_Shift_Condition_Switch} = 1 \\ \text{Diurnal_Circadian_Clock_on_Maternal_Melatonin}, \\ \text{otherwise,} \end{cases} \tag{6.5}$$

and

$$\text{Effect_of_Pregnancy_on_Melatonin} = \begin{cases} 1.8 & \text{Pregnancy_Switch} = 1, \\ 1 & \text{ELSE.} \end{cases} \tag{6.6}$$

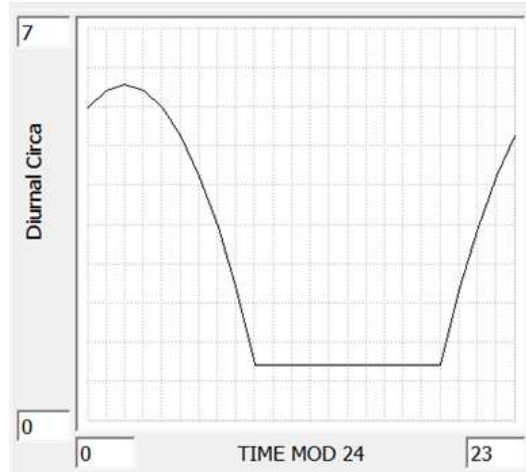


Figure 6.6. Melatonin diurnal circadian clock (24h) graphical function: *Diurnal circadian clock on maternal melatonin*.

Antioxidant Effect of Maternal Melatonin on Oxidative Stress: Melatonin has an antioxidant property that lowers Malondialdehyde (MDA) levels as a marker for oxidative stress and cellular damage. Malondialdehyde (MDA) levels are reported to be lower at night and higher during the day, which correlates with the diurnal clock of melatonin [139]. Chakravarty et al. [21] indicates that melatonin generates a diurnal rhythm in MDA levels, with MDA levels at night reaching nearly 65% of daytime MDA levels [139]. Nighttime melatonin levels increase approximately six times the daytime melatonin levels (minimum level), while MDA levels decrease by 65% [135].

Antioxidant Effect of Maternal MEL on MDA influences *Maternal MDA* levels. The antioxidant effect is illustrated in the model through the graphical function of the *Antioxidant Effect of Maternal MEL on MDA* as a function of $\frac{\text{Maternal Melatonin}}{\text{Min Value of Melatonin}}$, as shown in Figure 6.7.

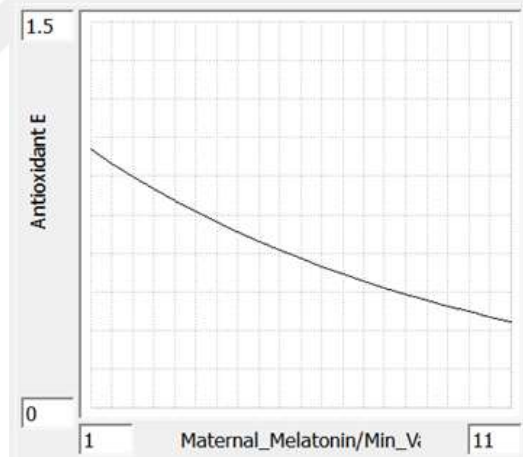


Figure 6.7. Antioxidant effect of melatonin on malondialdehyde graphical function.

Fetal Melatonin: The fetus barely produces any melatonin. Therefore, it is assumed in the model that the fetus does not produce any on its own, and instead, fetal melatonin is transferred from the mother [10]. In the experiment performed on sheep, the circadian rhythm of the fetus develops 1 to 1.5 hours later than the maternal circadian rhythm. Additionally, the melatonin level in the fetus is close to 60% of the maternal melatonin level, indicating that the amplitude of fetal melatonin is lower than that of the mother as shown in Figure 6.8 [140]. A study on rhesus monkeys found that

maternal melatonin transfers to the fetus, with nearly 85% of the $[3H]$ -melatonin passing into the fetal circulation within the first three minutes [141]. In humans, the only available data on fetal melatonin levels comes from samples taken from the umbilical cords during labor, and the values are close to those of maternal melatonin levels [142]. Unfortunately, there is no available data for fetal melatonin levels during pregnancy.

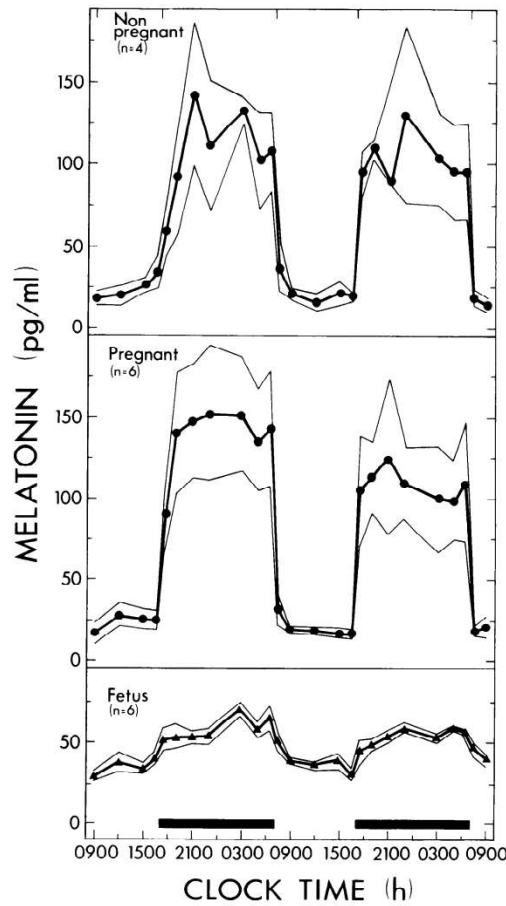


Figure 6.8. Circadian patterns of plasma melatonin concentrations over 48 hours in nonpregnant ewes (top), pregnant ewes (middle), and their fetuses (bottom) (Reproduced from [140]).

In the model, the maternal melatonin transfer ratio is considered to be 85% due to the genetic similarities between humans and rhesus monkeys close to 90% [143]. We have formulated the obtaining rate using *Transfer Ratio MEL*, which states that 85% of maternal melatonin is achieved in the *Fetal Melatonin through the Umbilical Cord* without reducing the maternal melatonin concentration with the following set of

equations

$$\text{MEL_in_Umbilical_Cord_Adj} = \frac{\text{Maternal_Melatonin} \times \text{Transfer_Ratio_MEL}}{\text{Melatonin_in_Umbilical_Cord_Adj_Time}}, \quad (6.7)$$

$$\text{Transfer_Ratio_MEL} = 0.85, \quad (6.8)$$

$$\text{Transfer_MEL} = \frac{\text{Fetal_Melatonin_in_Umbilical_Cord}}{\text{Transfer_Time_MEL}}. \quad (6.9)$$

Maternal MDA: Malondialdehyde shows a circadian rhythm under the effect of melatonin [21]. During the third trimester of pregnancy, maternal malondialdehyde levels increase by approximately 50% due to lipid peroxidation in the placenta [144,145]. We have formulated *Implied Maternal MDA* by multiplying the *Effect of Pregnancy on MDA*, *Normal Value of Maternal MDA*, and *Antioxidant Effect of Maternal MEL on MDA* (Equation 6.11). We considered the *Effect of Pregnancy on MDA* as 1.63 (Equation 6.12) instead of 1.5 because the melatonin level increases by 1.8, and MDA increases even more. However, melatonin's antioxidant property will reduce its level.

The changes in maternal MDA levels, except those influenced by maternal melatonin level, are neglected. Time-dependent changes in maternal MDA levels are simulated using following equations

$$\text{Maternal_MDA_Adj} = \frac{\text{Implied_Maternal_MDA} - \text{Maternal_MDA}}{\text{Maternal_MDA_Adj_Time}}, \quad (6.10)$$

$$\begin{aligned} \text{Implied_Maternal_MDA} = & \text{Normal_Value_of_Maternal_MDA} \\ & \times \text{Effect_of_Pregnancy_on_MDA} \\ & \times \text{Antioxidant_Effect_of_Maternal_MEL_on_MDA}, \end{aligned} \quad (6.11)$$

$$\text{Effect_of_Pregnancy_on_MDA} = \begin{cases} 1.63, & \text{if Pregnancy_Switch} = 1, \\ 1, & \text{ELSE.} \end{cases} \quad (6.12)$$

Fetal MDA: The studies conducted by Madazli indicated that the malondialdehyde level in the umbilical blood plasma is approximately 60% of the maternal MDA

level [146]. A study conducted on fetuses indicated that the MDA level in the umbilical artery is higher than that in the umbilical vein, demonstrating that the fetus also produces its own MDA [147]. We have modeled the obtaining rate using the *Transfer Ratio MDA*, which indicates that 50% of maternal MDA is obtained to the *Fetal MDA through the Umbilical Cord* without reducing the maternal MDA levels (Equation 6.13 and Equation 6.14), defined as

$$Transfer_MDA = \frac{Fetal_MDA_in_Umbilical_Cord}{Transfer_Time_MDA}, \quad (6.13)$$

$$Fetal_MDA_Production = Antioxidant_Effect_of_Fetal_MEL_on_MDA \times \frac{Fetus_MDA_Production_Value}{Fetus_MDA_Production_Time}, \quad (6.14)$$

$$Fetal_MDA_Out = \frac{Fetal_MDA}{Fetus_MDA_Out_Time}, \quad (6.15)$$

$$Fetus_MDA_Production_Value = 0.2 \text{ nmol/ml}. \quad (6.16)$$

The production of fetal MDA is formulated by *Fetus MDA Production Value* at a constant rate (Equation 6.16). Additionally, *Fetal MDA Production* is influenced by graphical function of the *Antioxidant Effect of Maternal MEL on MDA* depends on $\frac{Fetal_Melatonin}{Fetal_MELMin}$.

Risk of Autism: Risk of Autism is our hypothesis stock to determine the changes in the fetal risk of autism depending on *Fetal Melatonin* and *Fetal MDA* levels that can be seen in Equations 6.17 and Equation 6.18. The *Risk of Autism* is formulated by the *Effect of MDA on RoA on Fetus* and the *Effect of Melatonin on RoA on Fetus* on the Fetus graphical functions of the *Ratio of Fetal MDA* and *Ratio of Fetal Melatonin*, as shown in Figure 6.9. The *Ratio of Fetal MDA* and *Ratio of Fetal Melatonin* are calculated using Equation 6.19 and Equation 6.20. The difference in MDA and melatonin levels between autistic and non-autistic children is a potential factor worth researching about autism. There are many oxidative stress markers, and MDA represents the marker of oxidative stress in this study. In a study conducted with autistic and control group (non-autistic) children [22], the MDA level in autistic children was found to be 2.8 times higher than in the control group of non-autistic children, where approximately 1.5 is the threshold as the diagnostic value of autism.

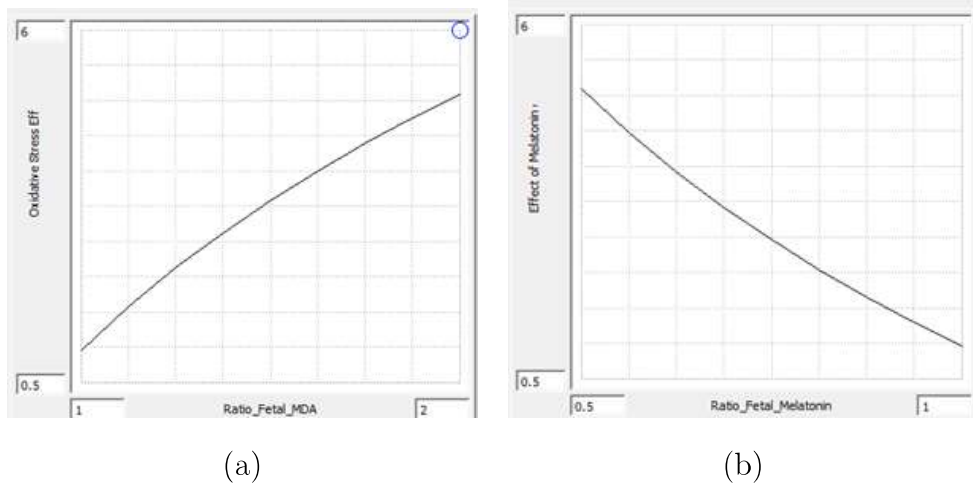


Figure 6.9. Effects of oxidative stress and melatonin on autism risk in the fetus (a) *effect of MDA on RoA on fetus* and (b) *effect of melatonin on RoA on fetus*.

In the study conducted by Melke and their team [148], it was indicated that the melatonin levels in individuals with autism were 50% lower than those in the control group; a similar research result was demonstrated in Tordjman's study [18].

Risk of Autism is formulated to understand the dynamics of the effect of maternal MDA and Melatonin disruptions on fetal MDA and Melatonin and how the "disrupted fetal MDA and Melatonin level" to "control group level" ratio creates a risk factor. The assumption is that melatonin levels at half the amount and MDA levels at double that of the control group represent the diagnostic threshold, indicating a higher *Effect of MDA on RoA on Fetus* and *Effect of Melatonin on RoA on Fetus* at the impact of 5. The following equations are used

$$Risk_of_Autism_Adj = \frac{Implied_Risk_of_Autism - Risk_of_Autism_on_Fetus}{RoA_Adj_Time}, \quad (6.17)$$

$$\begin{aligned} Implied_Risk_of_Autism &= Effect_of_MDA_on_RoA_on_Fetus \\ &\times Normal_Value_of_RoA_on_Fetus \\ &\times Effect_of_Melatonin_on_RoA_on_Fetus, \end{aligned} \quad (6.18)$$

$$Ratio_Fetal_MDA = \frac{Fetal_MDA_Avg}{Fetal_MDA_Control_Avg}, \quad (6.19)$$

$$Ratio_Fetal_Melatonin = \frac{Fetal_MEL_Avg}{Fetal_Melatonin_Control_Avg}. \quad (6.20)$$

In our simulation, we apply a 5-fold increased risk of autism based on the existing literature that reported a 5-fold increased risk of autism associated with low iron intake with advanced maternal age [149].

6.4. Night Shift Scenario Simulations for Maternal and Fetal Circadian Clock

A scenario comparing melatonin levels between night shift and day shift workers is created based on data from studies summarized in Table 6.1. The data indicates that the melatonin levels in women working night shifts are lower than in women working day shifts. Melatonin suppression and phase shift are formulated by the *Night Shift Condition Switch*, *Night Shift Effect on MEL Production*, and the *Night Shift Effect Avg*, as given

$$Night_Shift_Condition_Switch = \begin{cases} 1 & Night_Shift_Switch = 1 \text{ and} \\ & 21 > TIMEMOD168 < 117, \\ 1 & \text{ELSE,} \end{cases} \quad (6.21)$$

$$Night_Shift_Effect_on_MEL_Production = \begin{cases} 0.60 & Night_Shift_Condition_Switch = 1 \\ 1 & \text{ELSE,} \end{cases} \quad (6.22)$$

and

$$Night_Shift_Effect_Avg = SMTH1(Night_Shift_Effect_on_MEL_Production, 24).$$

respectively. The effect of the night shift on melatonin levels is modeled to decrease gradually, reaching a minimum level by the third day of working night shifts, and then increasing on days when the night shift is not worked, consistent with the literature (Table 6.1).

Table 6.1. Effect of night shift on melatonin levels: literature-based findings.

Reference	Participants	Findings
[150]	1,845 night-shift workers and 3,414 controls	Night-shift workers had lower levels of 6-sulfatoxymelatonin (aMT6s) in their 24-hour urine samples compared to day workers. The standardized mean difference (SMD) was -0.264, with a 95% confidence interval of -0.473 to -0.056 ($P = 0.013$).
[151]	172 nightshift and 151 dayshift nurses	Night-shift workers had significantly lower aMT6 levels than day-shift workers. Their levels were 62% lower during daytime sleep, 69% lower during night work, and 42% lower during off-night sleep.
[152]	80 pre-menopausal women	The aMT6 levels in women who worked night shifts were 56% lower than in women who did not.
[153]	72 night-shift workers and 41 day-shift workers	Night-shift workers had 33.8% lower aMT6 levels than day workers over 24 hours. The timing of melatonin peak production was delayed by three hours for night-shift workers.
[154]	8 participants (15 men and 23 women)	aMT6 levels declined gradually over three simulated continuous days of simulated night shifts. A significant decrease was observed on the third night.
[155]	61 rotating shift nurses	For nurses, the aMT6 levels of those working at night were 63.6% lower than nurses working during the day.

The simulation begins at midnight, followed by four nights of work and three days off. The melatonin peak time delay for night shift workers is two hours, formulated by

Circadian Clock on Maternal Melatonin and Night Shift Effect Avg.

Comparative Analysis of the Effect of Different Night Shift Working Conditions on Maternal and Fetal Circadian Clock: We create a base model for night shift work consisting of 4 nights of work followed by 3 days off, all within a 7-day cycle. This model is simulated over 3 months. To observe the effects of more intense and soft night shift conditions, we perform experiments in which 5 nights of work followed by 2 days off and 2 nights of work followed by 5 days off, all within a 7-day cycle. Experiments are conducted to understand the change in the risk of autism after quitting the night shift after 1.5 months and for the last two weeks using *Switch On Off* given as

$$Switch_On_Off = \begin{cases} 0 & TIME > 1824 | OR | TIME > 1080 \\ 11 & ELSE. \end{cases} \quad (6.24)$$

6.5. Scenario Analysis of Risk Adjustment Time (*RoA Adjustment Time*)

Two different risk adjustment times (*RoA Adjustment Time*) are tested in the model to understand the timeline of the development of autism. We modelled the base adjustment time as 360 hours. Adjustment times of risk of autism are simulated for 720 hours (1 month) and 360 hours (2 weeks) as the following equations

$$RoA_Adjustment_Time = 360 \text{ hours}, \quad (6.25)$$

$$RoA_Adjustment_Time = 720 \text{ hours}. \quad (6.26)$$

6.6. The Role of Oxidative Stress and Melatonin in Autism Risk in the Fetus

We simulated how the model's different quantitative risk ratio behaves as formulated by the graphical functions in Figures 6.10. We conducted experiments assuming that the diagnostic limits increase the risk of autism by a factor of 10 instead of 5, which is the base parameter. The initial autism rate was set at 0.01, the prevalence rate in recent years [2].

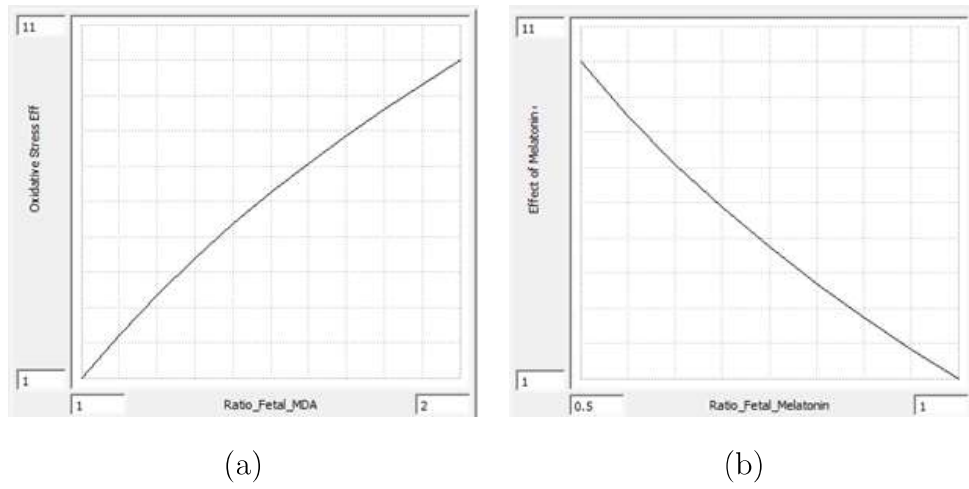


Figure 6.10. Scenario-based: effects of oxidative stress and melatonin on risk of autism in the fetus (a) *effect of MDA on RoA on fetus(10x)* (b) *effect of melatonin on RoA on fetus (10x)*.

7. DYNAMIC MODEL - RESULTS AND DISCUSSION

The melatonin circadian rhythm is approximately 24 hours, and an increase in melatonin levels is observed in the third trimester of pregnancy [137]. Melatonin transfers to the fetus rapidly via the placenta and synchronizes the fetal circadian rhythm to the maternal melatonin circadian rhythm but at a lower amplitude. Fetal circadian rhythms in sheep have been observed in different research [140]. The 48-hour maternal-fetal melatonin output is illustrated in Figure 7.1. The fetal circadian rhythm has a lower amplitude than maternal melatonin and lags behind by approximately one hour. The instantaneous level difference between fetal and maternal circadian rhythms is approximately 12%, and the difference between peaks is 18%. The result is consistent with the studies in the literature [140–142].

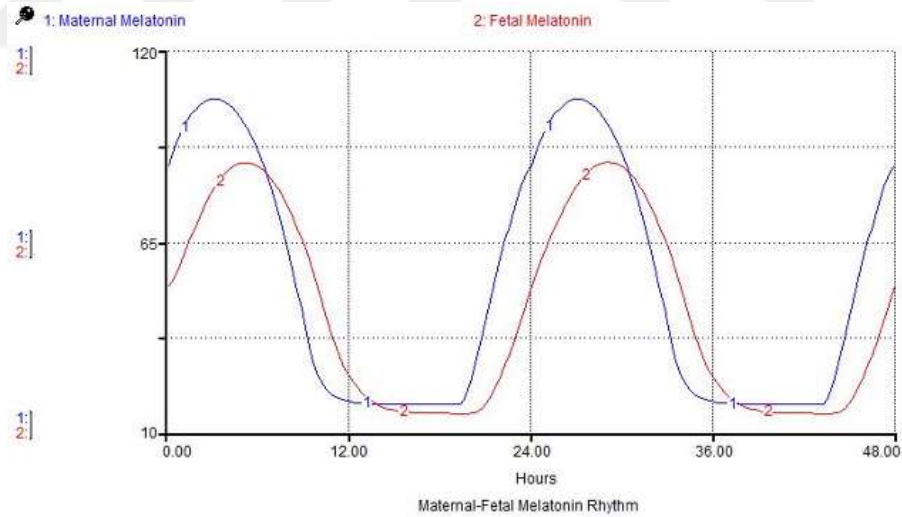


Figure 7.1. Maternal and fetal melatonin circadian rhythm (pg/ml) over a 48-hour period.

Malondialdehyde (MDA) is an indicator of lipid peroxidation and is closely associated with oxidative stress. MDA levels in the body fluctuate depending on the melatonin levels due to the antioxidant properties of melatonin [139]. In the third trimester of pregnancy, the level of MDA reaches 1.8 mol/ml [145]. The fetus's MDA

level is approximately 65% of the maternal MDA level [146], of which 10% is assumed to be produced in the model. The 48-hour maternal-fetal MDA simulation result is given in Figure 7.2, which is consistent with the literature [21, 139]. In the model, MDA levels decrease more during the third trimester because melatonin levels increase significantly at night, and melatonin has antioxidant properties [137].

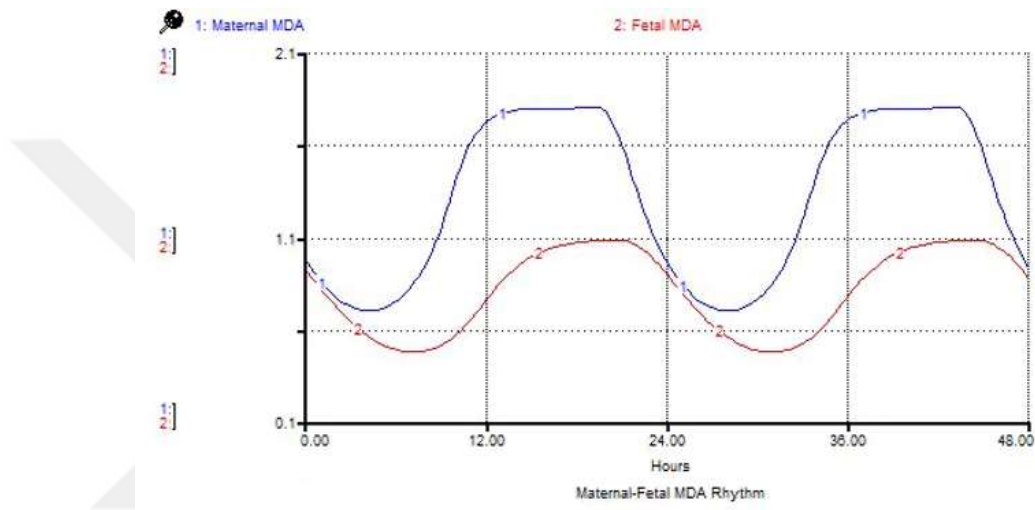


Figure 7.2. Maternal and fetal MDA circadian rhythm (nmol/ml) over a 48-hour period.

7.1. Dynamic Effects of Night Shift on Melatonin and Malondialdehyde during Pregnancy

According to statistics published by the U.S. Bureau of Labor Statistics in May 2007 [156], approximately 46.5 percent of total employees are women, and approximately 2.8 percent work the night shift (Figure 12). 2.9 percent of women with children under 18 work the night shift. The total number of women in the workforce is given as 43,366,000, and the number of women working the night shift is 1,214,00 based on statistics in the U.S.

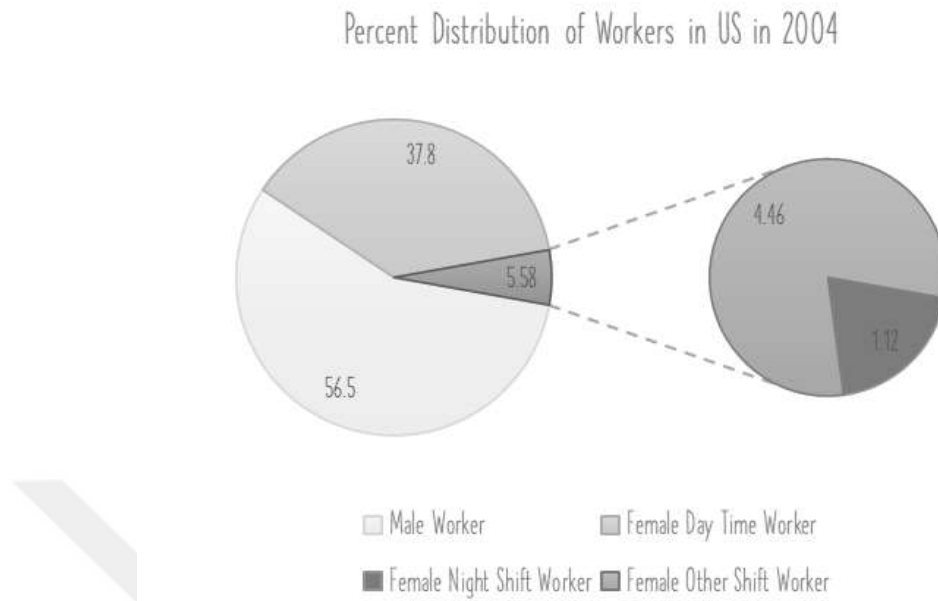


Figure 7.3. Gender-based percent distribution of workers and women's night shift participation [156].

On the night shift, workers are exposed to constant light; melatonin secretion is suppressed and secreted at much lower levels than sleeping in the dark. A study on Danish nurses shows that night shift nurses have approximately 30% lower melatonin levels on working days and approximately 15% lower melatonin levels on days off than day shift nurses [15, 157]. Another study indicates that night-shift female workers had approximately 70% lower melatonin levels than day-shift female workers. In comparison, women working the night shift have 35% lower melatonin levels when sleeping on their days off than day-shift female workers [151]. Another study observed that night-shift female workers have decreased melatonin levels by approximately 45% compared to day-shift female workers [152]. The study by Gómez-Acebo et al. [158] compares the circadian rhythm of aMT6s production with amplitude between day shift and rotating-night shift workers, as illustrated in Figure 7.4(a). The literature data aligns with our simulation results (blue line control group, red line night shift worker on the third night), as shown in Figure 7.4(b). The simulation results show that the peak times of melatonin differ from the literature data, with the peak time derived from another study (Tamarkin et al., 1982). However, the acrophase and the dynamics

of these levels remain consistent within the simulation.

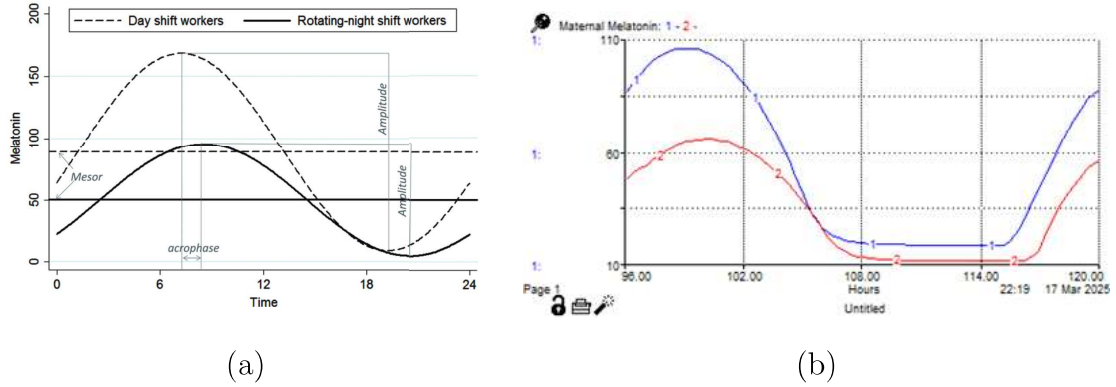


Figure 7.4. Melatonin rhythm in shift workers: day vs. rotating-night shifts (a) the literature data (Reproduced from [158]) (b) simulation result

In our model, women working on the night shift have approximately a 45% decrease during the night shift and 1-1.5-hour phase shift and a 15% decrease in days off. As a result, the maternal and fetal 7-days melatonin circadian rhythm is as shown in Figure 7.5.

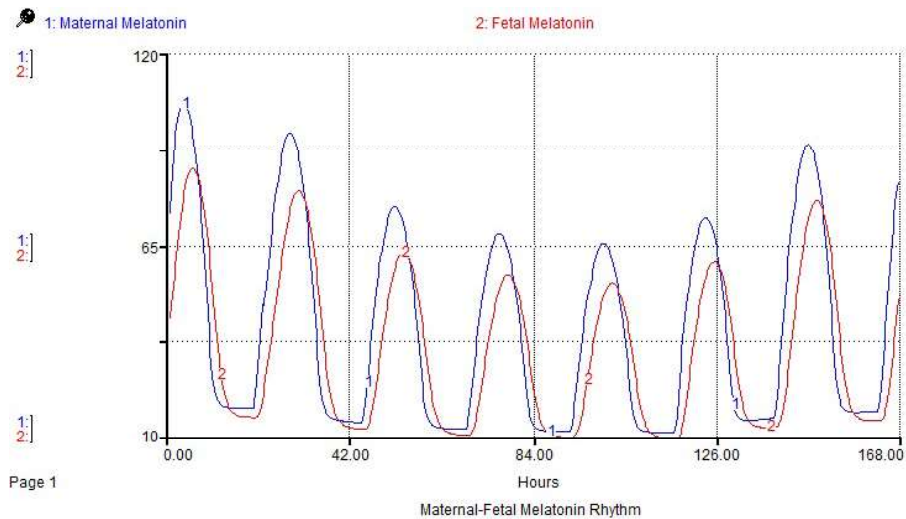


Figure 7.5. Base experiment: maternal and fetal melatonin circadian rhythm (pg/ml) across a 7-day cycle: 4 night shift and 3 off-day.

In the studies on MDA levels, an approximately 20% increase in MDA levels

was found in nurses working at night compared to nurses working during the day [23], and this rate was 50% in male nurses [159]. In our model, since melatonin levels are low during the day, their effect on MDA levels is less, while at night, the decreasing melatonin levels due to working in the night shift affect MDA more. As a result of the model, a 45% increase was observed during the night shift. The maternal and fetal 7-day MDA circadian rhythm is shown in Figure 7.6.

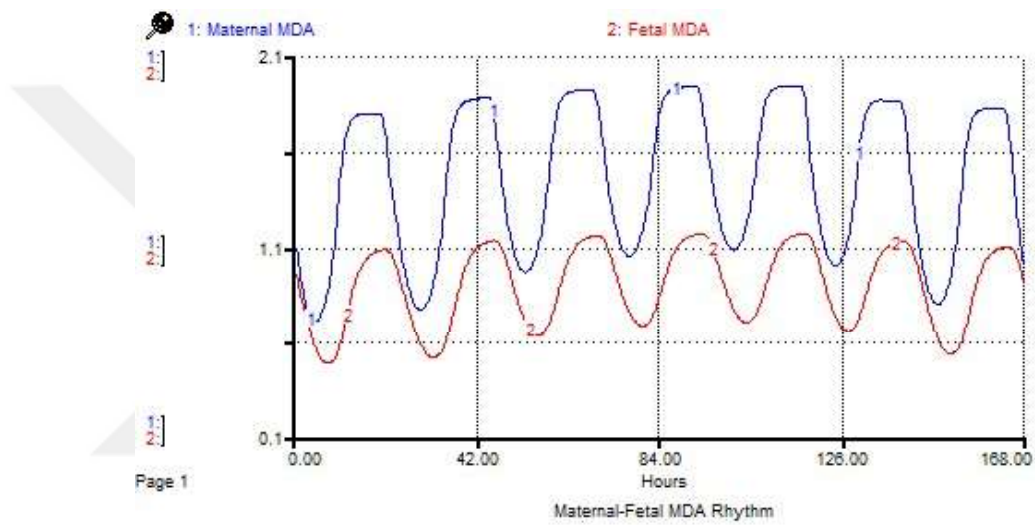


Figure 7.6. Base experiment: maternal and fetal MDA circadian rhythm (nmol/ml) across a 7-day cycle: 4 night shift and 3 off-day.

7.2. Effect of Night Shift on Autism Risk in Fetus and Disturbance in the Circadian Clock of Melatonin and Malondialdehyde

To examine the effects of night shift work on the fetus, fetal melatonin and MDA levels are simulated for an average value of every 2 week for 3 months and compared for women on night shift vs day shift, we tabulate the experiments details in Table 7.1. The circadian periods and average levels of fetal MDA and melatonin comparison of these groups are given in Table 7.3. During the night shift (according to third night shift results), the fetus is exposed to 47% more MDA at night compared to the when the mother is not working at night shift in simulation results; however, the fetus has 36% less melatonin at night under the night shift scenario compared. Fetal and maternal

MDA and melatonin values for both day and night periods for night shift and day shift are separately tabulated in Table 7.2.

Table 7.1. Night shift experiments details.

Experiment Number	Night Shift Condition	RoA Adjustment Time	Effect of MDA and Melatonin	Night Shift Period
Experiment Base Run	4 nights of work followed by 3 days off	360 Hours	x5	2160 Hours
Experiment 1	5 nights of work followed by 2 days off	360 Hours	x5	2160 Hours
Experiment 2	3 nights of work followed by 4 days off	360 Hours	x5	2160 Hours
Experiment 3	2 nights of work followed by 5 days off	360 Hours	x5	2160 Hours

An examination of the simulation results indicates that the average fetal melatonin levels in women working on the night shift decreased by 22% in the existence of the scenarios of 4 Days of Work-3 Days Off (Base Experiment). Additionally, the MDA level increased by 11% in night shift scenario of 4 Days of Work-3 Days Off (Base Experiment). Although changes in fetal MDA and melatonin levels are not indicative by themselves, the results suggest an increased risk of autism because the circadian rhythm of fetal melatonin, interrupted during night shifts, continues to be affected after birth. The experimental results for other night shift conditions are tabulated in Table 7.3.

Table 7.2. Comparison of maternal and fetal melatonin and MDA levels during 3rd night (at peak time) and 3rd day (at noon) between base night shift experiment and control group: simulation results.

	Night Shift		Control	
	Day Time (at noon)	Nighttime (at peak time)	Day Time (at noon)	Nighttime (at peak time)
Maternal Melatonin (pg/ml)	12.00	68.20	18.00	106.20
Fetal Melatonin (pg/ml)	10.32	56.44	15.30	88.00
Maternal MDA (nmol/ml)	1.92	1.05	1.80	0.70
Fetal MDA (nmol/ml)	1.16	0.66	1.09	0.45

As a result of the simulation, the risk of autism rate increases from 0.01 to 0.041 for the base night shift experiment based on the quantitative risk effect parameter set at 5. According to the simulation result, working one more night shift per week is associated with a 27% increased risk of autism in the fetus, and working two fewer night shifts per week decreases the risk of autism by 44%. The numbers and rates are based on assumptions, but they indicate that the risk of autism increases over time due to night shifts. When the circadian rhythm is undisturbed, the risk of autism remains constant at 0.01. The simulation results provide insight; detailed and long-term clinical studies need to be conducted to determine the effects of night shifts on the fetus.

Quitting the Night Shift Work Before Labor: When quitting night shift work two weeks before the end of the third trimester, the risk of autism initially increases from 0.01 to a peak at 0.041 before gradually decreasing to 0.033 as shown in Figure 7.7(a). Similarly, night shifts are ended during the second half of the third trimester (45 days

before the end of the third trimester), and the risk of autism increases from 0.01 to 0.038 and then gradually decreases to 0.015, as given in Figure 7.7(b). However, the risk of autism does not return fully to its base level, according to our experiments. These results suggest that even after stopping night shift work during the third trimester of the pregnancy, there remains an increase in autism risk.

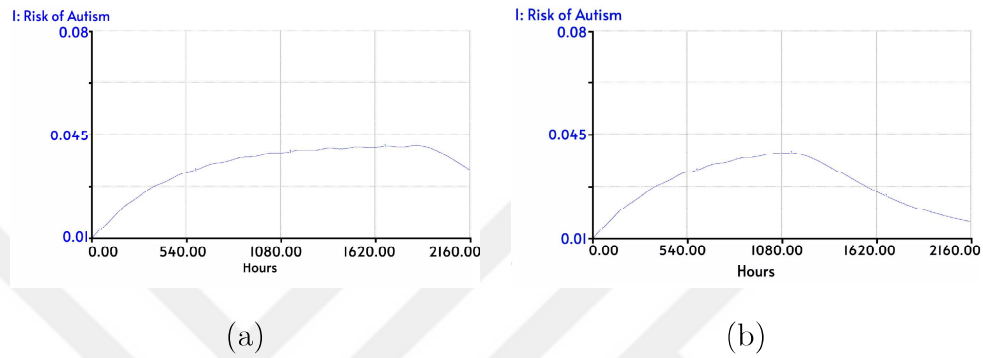
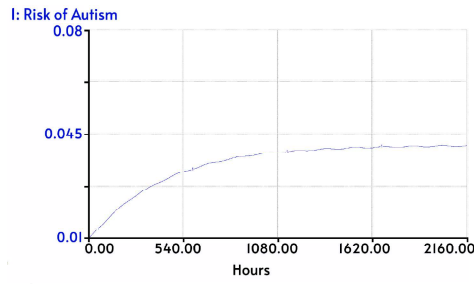


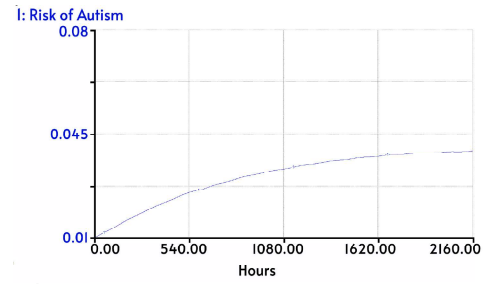
Figure 7.7. The risk of autism after quitting night shifts before the end of the third trimester of the pregnancy (a) two weeks before (b) 1.5 months before.

Comparative Impact of Different Risk Adjustment Times and Different Autism Risk Effects: When simulating the adjustment time parameter for the development of risk of autism over two different periods—720 hours (1 month) and 360 hours (2 weeks)—the changes in risk factors for a base schedule of 4 days of work followed by 3 days off are illustrated in Figure 7.8(a). The result demonstrates that increasing the adjustment time parameter to 720 hours (1 month) allowed the autism risk to reach 0.039 more gradually than 360 hours (2 weeks), as shown in Figure 7.8(b). However, the risk remained stable.

Quantitative Effect of MDA and Melatonin on Risk of Autism: When we double the effect of the graphical functions used in the base model, the *Oxidative Stress Effect of MDA on the Risk of Autism in Fetus* and the *Effect of Melatonin on the Risk of Autism in Fetus* formulated by Figure 8(a) and (b) reduces the risk of autism by 60% in the simulation. The risk of autism plateaued at 0.107 instead of 0.041 (Figure 7.9(b)).

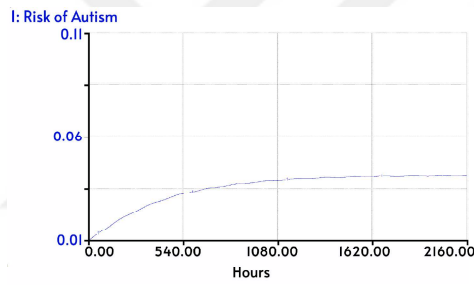


(a)

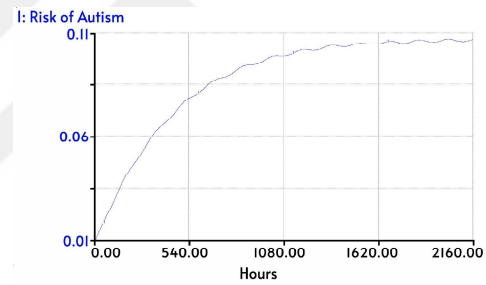


(b)

Figure 7.8. Adjustment time scenario-based: risk of autism during the third trimester
 (a) 360 hours (2 weeks) RoA adjustment time (b) 720 hours (1 months) RoA adjustment time.



(a)



(b)

Figure 7.9. Autism risk effect scenario-based: effects of oxidative stress and melatonin on autism risk in the fetus (a) 5x (b) 10x.

Table 7.3. Night shift experiment results.

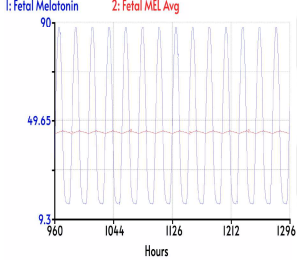
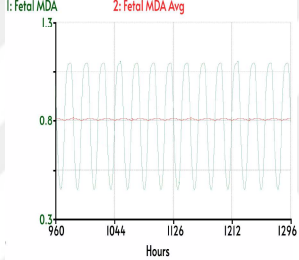
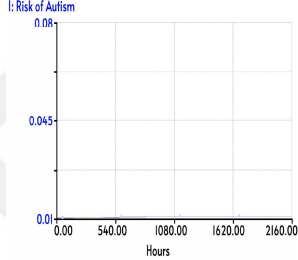
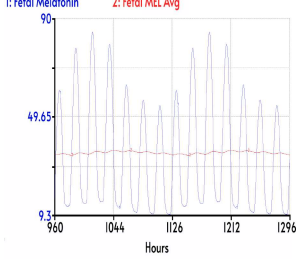
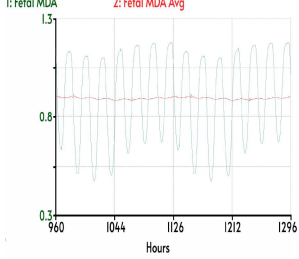
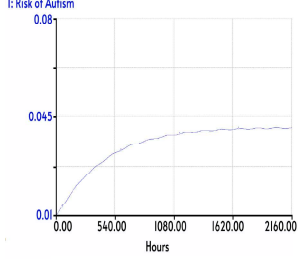
Experiment Number	Average Fetal Melatonin Levels (pg/ml)	Average Fetal MDA Levels (nmol/ml)	Risk of Autism
Control			
	44.5	0.80	0.01
Base			
	34.5	0.89	0.041

Table 7.3. Night shift experiment results (cont.).

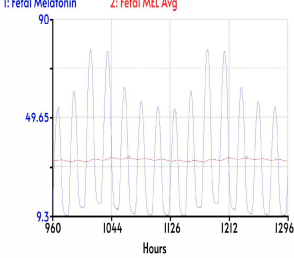
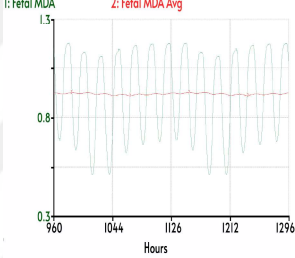
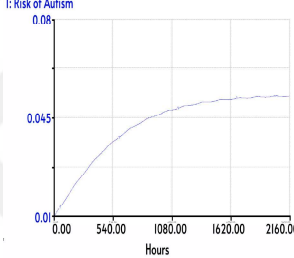
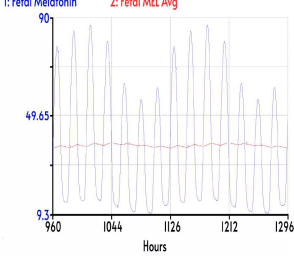
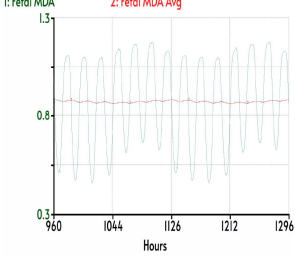
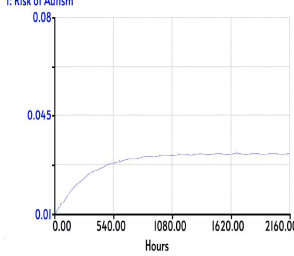
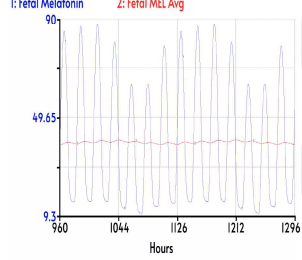
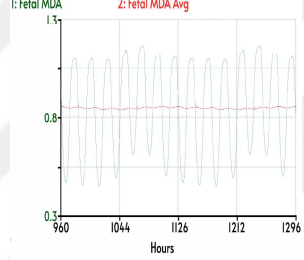
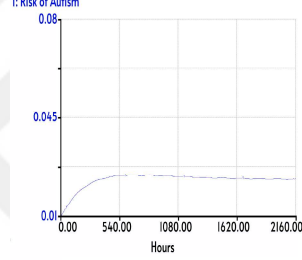
Experiment Number	Average Fetal Melatonin Levels (pg/ml)	Average Fetal MDA Levels (nmol/ml)	Risk of Autism
#1			
	32.0	0.91	0.052
#2			
	36.2	0.87	0.031

Table 7.3. Night shift experiment results (cont.).

Experiment Number	Average Fetal Melatonin Levels (pg/ml)	Average Fetal MDA Levels (nmol/ml)	Risk of Autism
#3			
	39.0	0.85	0.023

8. CONCLUSIONS AND RECOMMENDATIONS

8.1. Conclusion

Autism is a complex disorder that is affected by more than 1000 genes. However, ASD is not only controlled by genes or gene products. In addition to genetic aspects, various environmental factors have impacts on ASD. Maternal nutritional factors and obesity, maternal stress, maternal immunological response/dysfunction, early life immune insults, parental age, heavy metals, and air pollution can be addressed as ASD-causing environmental factors. The steady-state modeling part of the thesis uses a network-based approach to identify significant pathways and genes/gene products. Different networks, such as pure autistic, pure circadian, and combined networks, are built in this research. Numerous essential hub gene products are highlighted, showing significant enrichment in areas such as ribosomal proteins, protein synthesis, regulatory pathways, and the Wnt/ β -catenin signaling pathway. These results should be investigated further using experimental validation studies. It also turns out that specific genes linked to ASD control several basic processes related to synaptic function. These processes encompass ribosome maturation and mRNA regulation. Thus, changes in synaptic function and gene regulation may cause the development of ASD. Because essential proteins linked to ASD are translated in the synaptic region, preserving translational homeostasis may prevent or lessen this complex and multifactorial disorder.

The enriched GO terms for joint network CAPPIN are mainly related to cytoplasmic translation or translation, glucocorticoid receptor signaling pathway and its regulation, ribonucleoprotein complex biogenesis, cellular response to endogenous stimulus, histone acetylation and peptidyl-lysine acetylation processes. Histone deacetylases have recently been implicated in ASD and other neurodevelopmental disorders characterized by similar clinical and diagnostic comorbidities (e.g., epilepsy, anxiety, and intellectual impairment). The use of drugs, such as histone deacetylase inhibitors [130] in the therapy of neurodevelopmental disorders caused by RNA-binding proteins could

restore the activity of lysine (K) acetyltransferases (KAT) in several neurodegenerative conditions, including autism.

There is a significant link between circadian clock disruption and the risk of autism. Circadian genes involved in Autism and Circadian PPIs play a role in photoperiodism and response to redox state processes. The processes mean these genes regulate the internal clock and biological processes in response to the light and dark cycle. Involvement in oxidative stress regulation is related to melatonin antioxidant properties. As a result of PPIs, disruptions in melatonin production caused by the night shift increase oxidative stress due to weakened oxidative stress properties and further increase the risk of autism.

The steady-state model analysis introduces a novel perspective on metabolic mechanisms and unveils potential key proteins/pathways such as ribosome biogenesis, redox, and oxidative stress. Consequently, it highlights that it could mitigate the development of ASD triggered by environmental factors like maternal stress or advanced parental age (which is linked to spermatozooids in older men and oxidative stress). Ultimately, comprehending the connection between circadian rhythm and ASD provides valuable insights into the role of these pathways in the etiology and pathogenesis of ASD. Initiating antioxidant treatment early on could enhance the trajectory of the medical condition by mitigating oxidative stress before it leads to irreversible brain damage. Furthermore, emerging treatments that target epigenetic marks, like DNA methylation and histone acetylation, are under investigation with the hope of addressing or preventing autism.

The dynamic modeling analysis of the thesis aims to conduct simulation experiments to understand the effects of changes in melatonin circadian rhythms of pregnant women working night shifts and its contribution to the possible risk of autism in fetuses. According to the simulation results, the night shift disrupts the fetal melatonin cycle and causes an increase in Malondialdehyde (MDA) levels, which is an oxidative stress parameter. In the 4-day night-shift work-3-day off, a decrease of 22% in aver-

age fetal melatonin levels and an increase of 11% in average fetal MDA levels were obtained, respectively. During the night shift, the fetus is exposed to 47% more MDA, and the melatonin level is 36% lower, which causes disruptions in the circadian clock programming of the fetus. As a result, the risk of autism increased from 0.01 to 0.041. Working one more night shift per week increased the risk of autism in the fetus by 27%, according to simulation results. Ending the night shift after some time reduces the risk of autism, but it never returns the risk of autism to the base level. When the adjustment time for the development of autism risk of the fetus was increased from 360 hours (2 weeks) to 720 hours (1 month), it is indicated that the autism risk reached about the same level more slowly and remained stable thereafter.

This is an initial simulation modeling study to understand the neurodevelopmental disorders that night shift work may cause in the fetus. The literature has limited data on fetal circadian rhythms or maternal melatonin transfer to the fetus. This study is the first to model fetal melatonin levels through simulation, giving information about fetal circadian regulation dynamics. The impact of night shifts on fetal health is not yet fully understood. Night shifts reduce fetal melatonin levels and increase fetal MDA (a marker of oxidative stress), notably raising the risk of autism. The study can provide a different dynamic perspective on protecting fetal health and developing work-shift related actions to prevent neurodevelopmental disorders like autism. Further clinical studies on effects of night shifts are important to understand autism pathways to protect fetal health, also to provide more data for improved simulation models and experiments.

8.2. Recommendations

This dissertation consists of two main parts: protein-protein interaction networks between circadian and autism spectrum disorder and disruptions of the circadian rhythm of melatonin and its impact on the fetus, especially on the risk of autism factor. There are some recommendations to improve the existing protein-protein interaction networks and dynamic simulations.

First, protein-protein interaction networks can be created with more detailed circadian and autism genes. Since genetic studies on autism are ongoing, autism PPIs were created with a limited number of genes in this dissertation.

Second, to enhance the dynamic simulation model, it is recommended to consider adding a cortisol part and the impact of night shifts on fetal and maternal cortisol levels. The enhanced system dynamics model will provide a more comprehensive understanding of how night shift work affects the hormone dynamics in the body.

Finally, clinical studies need to be conducted to validate our study and other studies. In this way, our hypothesis could be verified, and/or our models could be improved.

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APPENDIX A: GO TERMS AND ENRICHMENT ANALYSIS

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool).

GO term	Cluster fre- quency	Genome fre- quency	Corrected P-value	Genes (annotated to the term)
amide biosyn- thetic process	10/24 (41.7%)	828/19751 (4.2%)	9.41×10^{-6}	EIF5B/PER1/PER2/RPL10/RPL14/RPL21/RPL22/ RPL3/RPS23/RPS25
blue light sig- naling pathway	2/24 (8.3%)	2/19751 (0.0%)	7.3×10^{-4}	CRY1/CRY2
cellular amide metabolic pro- cess	10/24 (41.7%)	1118/19751 (5.7%)	1.5×10^{-4}	EIF5B/PER1/PER2/RPL10/RPL14/RPL21/RPL22/ RPL3/RPS23/RPS25
cellular aromatic compound metabolic process	22/24 (91.7%)	6151/19751 (31.1%)	4.89×10^{-7}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1E/KRR1/ MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/ RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/ WDR75
cellular macro- molecule catabolic process	11/24 (45.8%)	1204/19751 (6.1%)	2.54×10^{-5}	ARNTL/CLOCK/CSNK1D/CSNK1E/RPL14/RPL21/ RPL22/RPL3/RPS23/RPS25
cellular metabolic process	24/24 (100.0%)	10825/19751 (54.8%)	2.7×10^{-4}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/ CSNK1E/EIF5B/KRR1/MPHOSPH10/NOP58/NPAS2/ PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/ RPS23/RPS25/WDR3/WDR75
cellular nitro- gen compound catabolic process	7/24 (29.2%)	721/19751 (3.7%)	8.7×10^{-3}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
cellular nitrogen compound metabolic process	23/24 (95.8%)	6649/19751 (33.7%)	1.09×10^{-7}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1E/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
cellular response to blue light	2/24 (8.3%)	2/19751 (0.0%)	7.3×10^{-4}	CRY1/CRY2
cellular response to steroid hormone stimulus	5/24 (20.8%)	209/19751 (1.1%)	2.35×10^{-3}	ARNTL/CLOCK/CRY1/CRY2/PER1
circadian regulation of gene expression	9/24 (37.5%)	66/19751 (0.3%)	1.90×10^{-14}	ARNTL/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/NPAS2/PER1/PER2
circadian regulation of translation	2/24 (8.3%)	4/19751 (0.0%)	4.37×10^{-3}	PER1/PER2
circadian rhythm	9/24 (37.5%)	220/19751 (1.1%)	1.30×10^{-9}	ARNTL/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/NPAS2/PER1/PER2
corticosteroid receptor signaling pathway	5/24 (20.8%)	14/19751 (0.1%)	1.74×10^{-9}	ARNTL/CLOCK/CRY1/CRY2/PER1
cotranslational protein targeting to membrane	7/24 (29.2%)	100/19751 (0.5%)	1.14×10^{-8}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
entrainment of circadian clock	4/24 (16.7%)	35/19751 (0.2%)	4.41×10^{-5}	CRY1/CRY2/PER1/PER2
entrainment of circadian clock by photoperiod	4/24 (16.7%)	30/19751 (0.2%)	2.32×10^{-5}	CRY1/CRY2/PER1/PER2

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
establishment of protein localization to endoplasmic reticulum	7/24 (29.2%)	114/19751 (0.6%)	2.91×10^{-8}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
establishment of protein localization to membrane	7/24 (29.2%)	335/19751 (1.7%)	5.28×10^{-5}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
establishment of protein localization to organelle	8/24 (33.3%)	552/19751 (2.8%)	9.04×10^{-5}	ARNTL/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
gene expression	24/24 (100.0%)	5612/19751 (28.4%)	3.81×10^{-11}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
glucocorticoid receptor signaling pathway	5/24 (20.8%)	13/19751 (0.1%)	1.12×10^{-9}	ARNTL/CLOCK/CRY1/CRY2/PER1
heterocycle catabolic process	7/24 (29.2%)	720/19751 (3.6%)	8.62×10^{-3}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
heterocycle metabolic process	22/24 (91.7%)	6102/19751 (30.9%)	4.13×10^{-7}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1E/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
hormone-mediated signaling pathway	5/24 (20.8%)	202/19751 (1.0%)	1.99×10^{-3}	ARNTL/CLOCK/CRY1/CRY2/PER1

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
intracellular receptor signaling pathway	5/24 (20.8%)	267/19751 (1.4%)	7.72×10^{-3}	ARNTL/CLOCK/CRY1/CRY2/PER1
intracellular steroid hormone receptor signaling pathway	5/24 (20.8%)	119/19751 (0.6%)	1.4×10^{-4}	ARNTL/CLOCK/CRY1/CRY2/PER1
macromolecule catabolic process	11/24 (45.8%)	1438/19751 (7.3%)	1.5×10^{-4}	ARNTL/CLOCK/CSNK1D/CSNK1E/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
macromolecule localization	14/24 (58.3%)	3121/19751 (15.8%)	1.22×10^{-3}	ARNTL/CLOCK/CRY1/CSNK1D/CSNK1E/MPHOSPH10/NOP58/PER2/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
macromolecule metabolic process	24/24 (100.0%)	9565/19751 (48.4%)	1.40×10^{-5}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
metabolic process	24/24 (100.0%)	11677/19751 (59.1%)	1.69×10^{-3}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
mRNA catabolic process	7/24 (29.2%)	377/19751 (1.9%)	1.1×10^{-4}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
ncRNA metabolic process	8/24 (33.3%)	586/19751 (3.0%)	1.4×10^{-4}	BMS1/KRR1/MPHOSPH10/NOP58/RCL1/RPL14/WDR3/WDR75
ncRNA processing	8/24 (33.3%)	400/19751 (2.0%)	7.54×10^{-6}	BMS1/KRR1/MPHOSPH10/NOP58/RCL1/RPL14/WDR3/WDR75

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
negative regulation of circadian rhythm	3/24 (12.5%)	15/19751 (0.1%)	3.6×10^{-4}	CRY1/CRY2/PER2
negative regulation of gene expression	13/24 (54.2%)	1984/19751 (10.0%)	4.53×10^{-5}	ARNTL/CLOCK/CRY1/CRY2/PER1/PER2/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
negative regulation of glucocorticoid receptor signaling pathway	5/24 (20.8%)	6/19751 (0.0%)	5.25×10^{-12}	ARNTL/CLOCK/CRY1/CRY2/PER1
negative regulation of intracellular steroid hormone receptor signaling pathway	5/24 (20.8%)	36/19751 (0.2%)	3.22×10^{-7}	ARNTL/CLOCK/CRY1/CRY2/PER1
negative regulation of macromolecule metabolic process	13/24 (54.2%)	2845/19751 (14.4%)	3.01×10^{-3}	ARNTL/CLOCK/CRY1/CRY2/PER1/PER2/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
negative regulation of metabolic process	14/24 (58.3%)	3098/19751 (15.7%)	1.12×10^{-3}	ARNTL/CLOCK/CRY1/CRY2/MPHOSPH10/PER1/PER2/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
nitrogen compound metabolic process	24/24 (100.0%)	10332/19751 (52.3%)	8.98×10^{-5}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
nucleobase-containing compound catabolic process	7/24 (29.2%)	673/19751 (3.4%)	5.56×10^{-3}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
nucleobase-containing compound metabolic process	22/24 (91.7%)	5936/19751 (30.1%)	2.30×10^{-7}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1E/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
organic cyclic compound metabolic process	22/24 (91.7%)	6369/19751 (32.2%)	1.02×10^{-6}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1E/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
organic substance metabolic process	24/24 (100.0%)	11184/19751 (56.6%)	6.0×10^{-4}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
peptide biosynthetic process	10/24 (41.7%)	683/19751 (3.5%)	1.49×10^{-6}	EIF5B/PER1/PER2/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
peptide metabolic process	10/24 (41.7%)	841/19751 (4.3%)	1.09×10^{-5}	EIF5B/PER1/PER2/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
photoperiodism	5/24 (20.8%)	34/19751 (0.2%)	2.38×10^{-7}	ARNTL/CLOCK/CRY1/CRY2/PER1
positive regulation of dopaminergic neuron differentiation	2/24 (8.3%)	4/19751 (0.0%)	4.37×10^{-3}	CSNK1D/CSNK1E

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
positive regulation of midbrain dopaminergic neuron differentiation	2/24 (8.3%)	3/19751 (0.0%)	2.18×10^{-3}	CSNK1D/CSNK1E
positive regulation of Wnt-mediated midbrain dopaminergic neuron differentiation	2/24 (8.3%)	2/19751 (0.0%)	7.3×10^{-4}	CSNK1D/CSNK1E
primary metabolic process	24/24 (100.0%)	10810/19751 (54.7%)	2.6×10^{-4}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
protein localization to endoplasmic reticulum	7/24 (29.2%)	141/19751 (0.7%)	1.31×10^{-7}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
protein localization to membrane	7/24 (29.2%)	645/19751 (3.3%)	4.21×10^{-3}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
protein localization to organelle	9/24 (37.5%)	946/19751 (4.8%)	4.4×10^{-4}	ARNTL/CSNK1D/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
protein targeting	7/24 (29.2%)	436/19751 (2.2%)	3.1×10^{-4}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
protein targeting to ER	7/24 (29.2%)	110/19751 (0.6%)	2.26×10^{-8}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
protein targeting to membrane	7/24 (29.2%)	202/19751 (1.0%)	1.62×10^{-6}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
regulation of circadian rhythm	8/24 (33.3%)	123/19751 (0.6%)	6.27×10^{-10}	ARNTL/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/PER1/PER2
regulation of gene expression	18/24 (75.0%)	4660/19751 (23.6%)	7.68×10^{-5}	ARNTL/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/MPHOSPH10/NPAS2/PER1/PER2/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR75
regulation of glucocorticoid receptor signaling pathway	5/24 (20.8%)	8/19751 (0.0%)	4.89×10^{-11}	ARNTL/CLOCK/CRY1/CRY2/PER1
regulation of hair cycle	3/24 (12.5%)	29/19751 (0.1%)	2.91×10^{-3}	ARNTL/CLOCK/PER1
regulation of intracellular steroid hormone receptor signaling pathway	5/24 (20.8%)	80/19751 (0.4%)	1.98×10^{-5}	ARNTL/CLOCK/CRY1/CRY2/PER1
regulation of metabolic process	19/24 (79.2%)	6910/19751 (35.0%)	6.24×10^{-3}	ARNTL/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/EIF5B/MPHOSPH10/NPAS2/PER1/PER2/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR75
regulation of midbrain dopaminergic neuron differentiation	2/24 (8.3%)	5/19751 (0.0%)	7.28×10^{-3}	CSNK1D/CSNK1E
regulation of Wnt-mediated midbrain dopaminergic neuron differentiation	2/24 (8.3%)	2/19751 (0.0%)	7.3×10^{-4}	CSNK1D/CSNK1E

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
response to blue light	2/24 (8.3%)	2/19751 (0.0%)	7.3×10^{-4}	CRY1/CRY2
response to redox state	3/24 (12.5%)	14/19751 (0.1%)	2.9×10^{-4}	ARNTL/CLOCK/NPAS2
rhythmic process	9/24 (37.5%)	307/19751 (1.6%)	2.59×10^{-8}	ARNTL/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/NPAS2/PER1/PER2
ribonucleoprotein complex biogenesis	11/24 (45.8%)	483/19751 (2.4%)	1.61×10^{-9}	BMS1/KRR1/MPHOSPH10/NOP58/RCL1/RPL14/RPL3/RPS5/RPS23/WDR3/WDR75
ribosomal small subunit biogenesis	4/24 (16.7%)	75/19751 (0.4%)	9.9×10^{-4}	BMS1/RCL1/RPS5/WDR3
ribosome biogenesis	10/24 (41.7%)	310/19751 (1.6%)	6.54×10^{-10}	BMS1/KRR1/MPHOSPH10/NOP58/RCL1/RPL14/RPL3/RPS5/WDR3/WDR75
RNA catabolic process	7/24 (29.2%)	417/19751 (2.1%)	2.3×10^{-4}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
RNA metabolic process	21/24 (87.5%)	4729/19751 (23.9%)	4.26×10^{-8}	ARNTL/BMS1/CLOCK/CRY1/CRY2/CSNK1D/CSNK1E/KRR1/MPHOSPH10/NOP58/NPAS2/PER1/PER2/RCL1/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25/WDR3/WDR75
RNA processing	8/24 (33.3%)	959/19751 (4.9%)	5.67×10^{-3}	BMS1/KRR1/MPHOSPH10/NOP58/RCL1/RPL14/WDR3/WDR75
rRNA metabolic process	8/24 (33.3%)	265/19751 (1.3%)	2.97×10^{-7}	BMS1/KRR1/MPHOSPH10/NOP58/RCL1/RPL14/WDR3/WDR75
rRNA processing	8/24 (33.3%)	227/19751 (1.1%)	8.71×10^{-8}	BMS1/KRR1/MPHOSPH10/NOP58/RCL1/RPL14/WDR3/WDR75

Table A.1. Significant GO terms for Cluster 1 in CPPIN2 (Princeton Gene Ontology Tool) (cont.).

GO term	Cluster frequency	Genome frequency	Corrected P-value	Genes (annotated to the term)
SRP-dependent co-translational protein targeting to membrane	7/24 (29.2%)	95/19751 (0.5%)	7.94×10^{-9}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
steroid hormone mediated signaling pathway	5/24 (20.8%)	142/19751 (0.7%)	3.5×10^{-4}	ARNTL/CLOCK/CRY1/CRY2/PER1
translation	10/24 (41.7%)	658/19751 (3.3%)	1.04×10^{-6}	EIF5B/PER1/PER2/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
translational initiation	8/24 (33.3%)	193/19751 (1.0%)	2.38×10^{-8}	EIF5B/RPL10/RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
viral gene expression	7/24 (29.2%)	195/19751 (1.0%)	1.27×10^{-6}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25
viral transcription	7/24 (29.2%)	178/19751 (0.9%)	6.73×10^{-7}	RPL14/RPL21/RPL22/RPL3/RPS23/RPS25

Table A.2. Enrichment analysis of 49 common proteins in CPPIN2 and APPIN5 (Princeton Gene Ontology Tool).

GO term	Genes (annotated to the term)
action potential	ANK2/CACNA1C/NRCAM/NUP155/SCN1A/SCN2A
aromatic compound biosynthetic process	ARID1B/ARNTL/CHD8/CTBP1/CTNBN1/CREBBP/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/PPP5C/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4

Table A.2. Enrichment analysis of 49 common proteins in CPPIN2 and APPIN5
(Princeton Gene Ontology Tool) (cont.).

GO term	Genes (annotated to the term)
biological regulation	ANK2/ARID1B/ARNTL/BMS1/CACNA1C/CACNA1E/CHD8/CLOCK/ CORO1A/CREBBP/CSNK1E/DYNC1H1/ESR1/FOXP1/FOXP2/ GABRB2/GABRB3/GFAP/GNAI1/HTR1B/KMT2C/LDB1/MED13L/ MKX/NCOA1/NDEL1/NRCAM/NPAS2/NUP155/NUP54/PER1/ PHF12/PHF21A/PPP2R5D/PPP5C/RCL1/SCN1A/SCN2A/SIN3A/ SMARCC2/STX11/STXBP1/TBL1XR1/TCF4/TCF7L2/TSC22D4/TRAF7/ UBR1/WDR3
canonical Wnt signaling pathway involved in positive regulation of epithelial to mesenchymal transition	CTNNB1/TCF7L2
cardiac muscle cell action potential involved in contraction	ANK2/CACNA1C/NUP155/SCN1A
cell-cell signaling	ANK2/ARNTL/CACNA1C/CACNA1E/CHD8/CTNNB1/GABRB2/ GABRB3/GFAP/HTR1B/LDB1/NUP155/NRCAM/STX11/STXBP1/ TBL1XR1/TCF7L2
cellular response to endogenous stimulus	ARNTL/CHD8/CORO1A/CREBBP/CTNNB1/ESR1/FOXP1/GABRB2/ GABRB3/HTR1B/NCOA1/NDEL1/PER1/SIN3A/UBR1
cellular response to organic cyclic compound	ARNTL/CTNNB1/ESR1/FOXP1/GABRB2/GABRB3/GNAI1/HTR1B/ PER1/SIN3A
chromatin organization	ARID1B/CHD8/CTBP1/ESR1/KMT2C/PHF21A/SIN3A/SMARCC2/ TBL1XR1
chromosome organization	ARID1B/CHD8/CTBP1/CTNNB1/ESR1/KMT2C/LDB1/NUP155/PHF21A/ SIN3A/SMARCC2/TBL1XR1/TCF7L2
heterocycle biosynthetic process	ARID1B/ARNTL/CHD8/CTBP1/CTNNB1/CREBBP/ESR1/FOXP1/ FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/ PHF12/PHF21A/PPP5C/SIN3A/SMARCC2/TBL1XR1/TCF4/ TCF7L2/TSC22D4
histone acetylation	CREBBP/CTBP1/LDB1/NCOA1/PER1/SIN3A
histone modification	ARID1B/CHD8/CREBBP/CTBP1/CTNNB1/KMT2C/LDB1/NCOA1/PER1/ SIN3A/TBL1XR1
internal peptidyl-lysine acetylation	CREBBP/CTBP1/LDB1/NCOA1/PER1/SIN3A

Table A.2. Enrichment analysis of 49 common proteins in CPPIN2 and APPIN5
(Princeton Gene Ontology Tool) (cont.).

GO term	Genes (annotated to the term)
internal protein amino acid acetylation	CREBBP/CTBP1/LDB1/NCOA1/PER1/SIN3A
membrane depolarization during action potential	ANK2/CACNA1C/SCN1A/SCN2A
multicellular organism development	ANK2/ARID1B/ARNTL/CACNA1C/CHD8/CREBBP/CTNNB1/ESR1/FOXP1/FOXP2/GABRB2/GABRB3/GFAP/KMT2C/LDB1/MKX/NCOA1/NPAS2/NRCAM/NUP155/PER1/PPP2R5D/SMARCC2/STXBP1/TBL1XR1/TCF4/TCF7L2
multicellular organismal process	ANK2/ARID1B/ARNTL/C3orf62/CACNA1C/CHD8/CREBBP/CTNNB1/ESR1/FOXP1/FOXP2/GABRB2/GABRB3/GFAP/HTR1B/KMT2C/LDB1/MKX/NCOA1/NDEL1/NPAS2/NRCAM/NUP155/PER1/PPP2R5D/SCN1A/SCN2A/SMARCC2/STX11/STXBP1/TBL1XR1/TCF4/TCF7L2
multicellular organismal signaling	ANK2/CACNA1C/NRCAM/NUP155/SCN1A/SCN2A
negative regulation of biosynthetic process	ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/NCOA1/PER1/PHF12/PHF21A/SMARCC2/TBL1XR1/TCF7L2/TSC22D4
negative regulation of cellular biosynthetic process	ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/NCOA1/PER1/PHF12/PHF21A/SMARCC2/TBL1XR1/TCF7L2/TSC22D4
negative regulation of cellular macromolecule biosynthetic process	ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/NCOA1/PER1/PHF12/PHF21A/SMARCC2/TBL1XR1/TCF7L2/TSC22D4
negative regulation of macromolecule biosynthetic process	ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/NCOA1/PER1/PHF12/PHF21A/SMARCC2/TBL1XR1/TCF7L2/TSC22D4
negative regulation of nucleic acid-templated transcription	ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/NCOA1/PER1/PHF12/PHF21A/SMARCC2/TBL1XR1/TCF7L2/TSC22D4
negative regulation of transcription by RNA polymerase II	ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/LDB1/PHF12/PHF21A/PER1/SIN3A/TBL1XR1/TCF7L2

Table A.2. Enrichment analysis of 49 common proteins in CPPIN2 and APPIN5
(Princeton Gene Ontology Tool) (cont.).

GO term	Genes (annotated to the term)
negative regulation of transcription, DNA-templated	ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/NCOA1/PER1/PHF12/PHF21A/SIN3A/SMARCC2/TBL1XR1/TCF7L2/TSC22D4
nervous system development	ANK2/ARID1B/ARNTL/CACNA1C/CHD8/CTNNB1/ESR1/FOXP1/FOXP2/GABRB2/GABRB3/GFAP/LDB1/NCOA1/NPAS2/NRCAM/NDEL1/PPP2R5D/SCN2A/SMARCC2/STXBP1/TCF4
nucleic acid-templated transcription	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/PPP5C/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4
nucleobase-containing compound biosynthetic process	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/PPP5C/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4
organic cyclic compound biosynthetic process	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/PPP5C/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4
peptidyl-lysine acetylation	CREBBP/CTBP1/LDB1/NCOA1/PER1/SIN3A
peptidyl-lysine modification	CREBBP/CTBP1/CTNNB1/KMT2C/LDB1/NCOA1/PER1/SIN3A
positive regulation of cellular process	ANK2/ARID1B/ARNTL/CACNA1C/CHD8/CREBBP/CTBP1/CTNNB1/DYNC1H1/ESR1/FOXP1/GABRB2/GABRB3/GFAP/GNAI1/HTR1B/KMT2C/LDB1/NCOA1/NPAS2/NRCAM/NDEL1/PPP2R5D/PPP5C/SIN3A/SMARCC2/STXBP1/TBL1XR1/TCF4/TCF7L2/TRAFF7
positive regulation of macromolecule biosynthetic process	ARID1B/ARNTL/CHD8/CREBBP/CTNNB1/ESR1/KMT2C/LDB1/NCOA1/NPAS2/PER1/SMARCC2/SIN3A/TBL1XR1/TCF4/TCF7L2
positive regulation of nucleic acid-templated transcription	ARID1B/ARNTL/CHD8/CREBBP/CTNNB1/ESR1/KMT2C/LDB1/NCOA1/NPAS2/PER1/SMARCC2/SIN3A/TBL1XR1/TCF4/TCF7L2
positive regulation of nucleobase-containing compound metabolic process	ARID1B/ARNTL/CHD8/CREBBP/CTNNB1/ESR1/KMT2C/LDB1/NCOA1/NPAS2/PER1/SMARCC2/SIN3A/TBL1XR1/TCF4/TCF7L2

Table A.2. Enrichment analysis of 49 common proteins in CPPIN2 and APPIN5
(Princeton Gene Ontology Tool) (cont.).

GO term	Genes (annotated to the term)
positive regulation of RNA biosynthetic process	ARID1B/ARNTL/CHD8/CREBBP/CTNNB1/ESR1/KMT2C/LDB1/NCOA1/NPAS2/PER1/SMARCC2/SIN3A/TBL1XR1/TCF4/TCF7L2
positive regulation of RNA metabolic process	ARID1B/ARNTL/CHD8/CREBBP/CTNNB1/ESR1/KMT2C/LDB1/NCOA1/NPAS2/PER1/SMARCC2/SIN3A/TBL1XR1/TCF4/TCF7L2
positive regulation of transcription by RNA polymerase II	ARID1B/ARNTL/CHD8/CREBBP/CTNNB1/ESR1/KMT2C/LDB1/NCOA1/NPAS2/PER1/SIN3A/TBL1XR1/TCF4/TCF7L2
positive regulation of transcription, DNA-templated	ARID1B/ARNTL/CHD8/CREBBP/CTNNB1/ESR1/KMT2C/LDB1/NCOA1/NPAS2/PER1/SMARCC2/SIN3A/TBL1XR1/TCF4/TCF7L2
protein acetylation	ARNTL/CREBBP/CTBP1/LDB1/NCOA1/PER1/SIN3A
protein acylation	ARNTL/CREBBP/CTBP1/LDB1/NCOA1/PER1/SIN3A
protein localization to nuclear inner membrane	NUP155/NUP54
regulation of biological process	ANK2/ARID1B/ARNTL/CACNA1C/CACNA1E/CHD8/CREBBP/CTBP1/CTNNB1/DYNC1H1/ESR1/FOXP1/FOXP2/GABRB2/GABRB3/GFAP/GNAI1/HTR1B/KMT2C/LDB1/MED13L/MKX/NCOA1/NDEL1/NPAS2/NRCAM/NUP155/NUP54/PER1/PHF12/PHF21A/PPP2R5D/PPP5C/SCN1A/SCN2A/SIN3A/SMARCC2/STX11/STXBP1/TBL1XR1/TCF4/TCF7L2/TSC22D4/TRAFF7/UBR1
regulation of cellular biosynthetic process	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4
regulation of ion transport	ANK2/CACNA1C/CACNA1E/CACNA2D3/CORO1A/CTNNB1/HTR1B/PER1/SCN1A/SCN2A/STXBP1
regulation of membrane potential	ANK2/CACNA1C/GABRB2/GABRB3/NRCAM/NUP155/SCN1A/SCN2A
regulation of transcription by RNA polymerase II	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4
regulation of transcription, DNA-templated	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4

Table A.2. Enrichment analysis of 49 common proteins in CPPIN2 and APPIN5
(Princeton Gene Ontology Tool) (cont.).

GO term	Genes (annotated to the term)
regulation of transport	ANK2/ARNTL/CACNA1C/CACNA1E/CACNA2D3/CORO1A/CTNNB1/DYNC1H1/GFAP/HTR1B/NDEL1/NUP54/PER1/SCN1A/SCN2A/STXBP1/TCF7L2
response to endogenous stimulus	ARNTL/CHD8/CORO1A/CREBBP/CTNNB1/ESR1/FOXP1/GABRB2/GABRB3/GNAI1/HTR1B/NCOA1/NDEL1/PER1/SIN3A/UBR1
response to organic cyclic compound	ARNTL/CTNNB1/ESR1/FOXP1/GABRB2/GABRB3/GNAI1/HTR1B/PER1/PPP5C/SIN3A/STXBP1
RNA biosynthetic process	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/PPP5C/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4
system development	ANK2/ARID1B/ARNTL/CACNA1C/CHD8/CTNNB1/ESR1/FOXP1/FOXP2/GABRB2/GABRB3/GFAP/LDB1/NCOA1/NPAS2/NRCAM/NDEL1/PPP2R5D/SCN2A/SMARCC2/STXBP1/TBL1XR1/TCF4/TCF7L2
transcription by RNA polymerase II	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4
transcription, DNA-templated	ARID1B/ARNTL/CHD8/CREBBP/CTBP1/CTNNB1/ESR1/FOXP1/FOXP2/KMT2C/LDB1/MED13L/MKX/NCOA1/NPAS2/PER1/PHF12/PHF21A/PPP5C/SIN3A/SMARCC2/TBL1XR1/TCF4/TCF7L2/TSC22D4

APPENDIX B: MODEL EQUATIONS

$$\text{Fetal_MDA}(t) = \text{Fetal_MDA}(t - dt) +$$

$$(\text{Transfer_MDA} + \text{Fetal_MDA_Production} - \text{Fetal_MDA_Out}) * dt$$

INIT Fetal_MDA = 0.9

UNITS: nmol/ml

INFLOWS:

$$\text{Transfer_MDA} = \text{Fetal_MDA_in_Umbilical_Cord} / \text{Transfer_Time_MDA}$$

$$\text{Fetal_MDA_Production} = (\text{Antioxidant_Effect_of_Fetal_MEL_on_MDA} *$$

$$\text{Fetus_MDA_Production_Value}) / \text{Fetus_MDA_Production_Time}$$

OUTFLOWS:

$$\text{Fetal_MDA_Out} = \text{Fetal_MDA} / \text{Fetus_MDA_Out_Time}$$

$$\text{Fetal_MDA_in_Umbilical_Cord}(t) = \text{Fetal_MDA_in_Umbilical_Cord}(t - dt) +$$

$$(\text{MDA_in_Umbilical_Cord_Adj} - \text{Transfer_MDA}) * dt$$

INIT Fetal_MDA_in_Umbilical_Cord = 0.57

UNITS: nmol/ml

INFLOWS:

$$\text{MDA_in_Umbilical_Cord_Adj} = (\text{Maternal_MDA} *$$

$$\text{Transfer_Ratio_MDA}) / \text{MDA_in_Umbilical_Cord_Adj_Time}$$

UNITS: nmol/ml/Hour

OUTFLOWS:

$$\text{Transfer_MDA} = \text{Fetal_MDA_in_Umbilical_Cord} / \text{Transfer_Time_MDA}$$

UNITS: nmol/ml/Hour


```

Fetal_Melatonin(t) = Fetal_Melatonin(t - dt) +
                      (Transfer_MEL - Fetal_Melatonin_Out) * dt
INIT Fetal_Melatonin = 52
UNITS: pg/ml

INFLOWS:
Transfer_MEL = Fetal_Melatonin_in_Umbilical_Cord/Transfer_Time_MEL
UNITS: pg/ml/Hour

OUTFLOWS:
Fetal_Melatonin_Out = Fetal_Melatonin/Fetal_Melatonin_Adj_Time
UNITS: pg/ml/Hour

Fetal_Melatonin_in_Umbilical_Cord(t) =
                      Fetal_Melatonin_in_Umbilical_Cord(t - dt) +
                      (Melatonin_in_Umbilical_Cord_Adj - Transfer_MEL) * dt
INIT Fetal_Melatonin_in_Umbilical_Cord = 64.5
UNITS: pg/ml

INFLOWS:
Melatonin_in_Umbilical_Cord_Adj =
                      (Maternal_Melatonin * Transfer_Ratio_MEL) /
                      MEL_in_Umbilical_Cord_Adj_Time
UNITS: pg/ml/Hour

OUTFLOWS:
Transfer_MEL = Fetal_Melatonin_in_Umbilical_Cord / Transfer_Time_MEL
UNITS: pg/ml/Hour

Maternal_MDA(t) = Maternal_MDA(t - dt) + (Maternal_MDA_Adj) * dt
INIT Maternal_MDA = 0.97

```

UNITS: nmol/ml

INFLOWS:

Maternal_MDA_Adj =

$$(\text{Implied_Maternal_MDA} - \text{Maternal_MDA}) / \text{Maternal_MDA_Adj_Time}$$

UNITS: nmol/ml/Hour

Maternal_Melatonin(t) = Maternal_Melatonin(t - dt) +

$$(\text{Maternal_Melatonin_Adj}) * dt$$

INIT Maternal_Melatonin = 87

UNITS: pg/ml

INFLOWS:

Maternal_Melatonin_Adj =

$$(\text{Implied_Maternal_Melatonin} - \text{Maternal_Melatonin}) / \text{Maternal_Melatonin_Adj_Time}$$

UNITS: pg/ml/Hour

Risk_of_Autism_on_Fetus(t) = Risk_of_Autism_on_Fetus(t - dt) +

$$(\text{Risk_of_Autism_Adj}) * dt$$

INIT Risk_of_Autism_on_Fetus = 0.01

UNITS: Unitless

INFLOWS:

Risk_of_Autism_Adj =

$$(\text{Implied_Risk_of_Autism} - \text{Risk_of_Autism_on_Fetus}) / \text{RoA_Adj_Time}$$

UNITS: Unitless /Hour

Antioxidant_Effect_of_Maternal_MEL_on_MDA =

$$\text{GRAPH}(\text{Maternal_Melatonin} / \text{Min_Value_of_Melatonin})$$

```
(1.00, 1.01), (1.50, 0.952), (2.00, 0.901), (2.50, 0.853),
(3.00, 0.808), (3.50, 0.765), (4.00, 0.724), (4.50, 0.685),
(5.00, 0.649), (5.50, 0.614), (6.00, 0.581), (6.50, 0.55),
(7.00, 0.521), (7.50, 0.493), (8.00, 0.467), (8.50, 0.442),
(9.00, 0.418), (9.50, 0.396), (10.0, 0.375), (10.5, 0.355),
(11.0, 0.336)
```

UNITS: Unitless

Antioxidant_Effect_of_Fetal_MEL_on_MDA =

```
GRAPH(Fetal_Melatonin / Fetal_MEL_Min)
(1.00, 1.01), (1.50, 0.952), (2.00, 0.901), (2.50, 0.853),
(3.00, 0.808), (3.50, 0.765), (4.00, 0.724), (4.50, 0.685),
(5.00, 0.649), (5.50, 0.614), (6.00, 0.581), (6.50, 0.55),
(7.00, 0.521), (7.50, 0.493), (8.00, 0.467), (8.50, 0.442),
(9.00, 0.418), (9.50, 0.396), (10.0, 0.375), (10.5, 0.355),
(11.0, 0.336)
```

UNITS: Unitless

Circadian_Clock_on_Maternal_Melatonin =

```
IF Night_Shift_Condition_Switch = 1 THEN
    DELAY(Diurnal_Circadian_Clock_on_Maternal_Melatonin,1)
ELSE
    Diurnal_Circadian_Clock_on_Maternal_Melatonin
```

UNITS: Unitless

Diurnal_Circadian_Clock_on_Maternal_Melatonin =

```
GRAPH(TIME MOD 24)
(0.00, 5.59), (1.00, 5.90), (2.00, 6.00), (3.00, 5.90),
(4.00, 5.59), (5.00, 5.08), (6.00, 4.37), (7.00, 3.45),
(8.00, 2.33), (9.00, 1.00), (10.0, 1.00), (11.0, 1.00),
(12.0, 1.00), (13.0, 1.00), (14.0, 1.00), (15.0, 1.00),
```

```
(16.0, 1.00), (17.0, 1.00), (18.0, 1.00), (19.0, 1.00),
(20.0, 2.33), (21.0, 3.45), (22.0, 4.37), (23.0, 5.08)
```

UNITS: Unitless

Effect_of_MDA_on_RoA_on_Fetus =

```
GRAPH(Ratio_Fetal_MDA)
(1.00, 1.00), (1.13, 1.68), (1.25, 2.29), (1.38, 2.84),
(1.50, 3.34), (1.63, 3.80), (1.75, 4.23), (1.88, 4.63),
(2.00, 5.00)
```

UNITS: Unitless

Effect_of_Melatonin_on_RoA_on_Fetus =

```
GRAPH(Ratio_Fetal_Melatonin)
(0.5, 5.00), (0.563, 4.32), (0.625, 3.71), (0.688, 3.16),
(0.75, 2.66), (0.813, 2.20), (0.875, 1.77), (0.938, 1.37),
(1.00, 1.00)
```

UNITS: Unitless

Effect_of_Pregnancy_on_MDA =

```
IF Pregnancy__Switch = 1 THEN
    1.63
ELSE
    1
```

UNITS: Unitless

Effect_of_Pregnancy_on_Melatonin =

```
IF Pregnancy__Switch = 1 THEN
    1.8
ELSE
    1
```

UNITS: Unitless

Fetal_MDA_Avg = SMTH1(Fetal_MDA,336,0.88)

UNITS: nmol/ml

Fetal_MDA_Control_Avg = 0.8

UNITS: nmol/m

Fetal_Melatonin_Out_Time = 1

UNITS: Hour

Fetal_MEL_Avg = SMTH1(Fetal_Melatonin,336,35)

UNITS: pg/ml

Fetal_MEL_Control_Avg = 45

UNITS: pg/ml

Fetal_MEL_Min = 10

UNITS: pg/ml

Fetus_MDA_Out_Time = 1

UNITS: Hour

Fetus_MDA_Production_Time = 1

UNITS: Hour

Fetus_MDA_Production_Value = 0.2

UNITS: nmol/ml

Implied_Maternal_MDA = Normal_Value_of_Maternal_MDA *
Effect_of_Pregnancy_on_MDA *

Antioxidant_Effect_of_Maternal_MEL_on_MDA

UNITS: nmol/ml

Implied_Maternal_Melatonin = Circadian_Clock_on_Maternal_Melatonin *
 Min_Value_of_Melatonin *
 Night_Shift_Effect_Avg *
 Effect_of_Pregnancy_on_Melatonin

UNITS: pg/ml

Implied_Risk_of_Autism = Effect_of_MDA_on_RoA_on_Fetus *
 Normal_Value_of_RoA_on_Fetus *
 Effect_of_Melatonin_on_RoA_on_Fetus

UNITS: Unitless

Maternal_MDA_Adj_Time = 1

UNITS: Hour

Maternal_Melatonin_Adj_Time = 1

UNITS: Hour

MDA_in_Umbilical_Cord_Adj_Time = 1

UNITS: Hour

MEL_in_Umbilical_Cord_Adj_Time = 1

UNITS: Hour

Min_Value_of_Melatonin = 10

UNITS: pg/ml

Night_Shift_Condition_Switch =

IF Switch_On_Off = 1 THEN

```

        IF TIME MOD 168 > 21 AND TIME MOD 168 < 117 THEN
            1
        ELSE
            0
        ELSE
            0

```

UNITS: Unitless

```

Night_Shift_Effect_Avg =
    SMTH1(Night_Shift__Effect_on_MEL_Production,24,
        Night_Shift__Effect_on_MEL_Production)

```

UNITS: Unitless

```

Night_Shift__Effect_on_MEL_Production =
    IF Night_Shift_Condition_Switch = 1 THEN
        0.60
    ELSE
        1

```

UNITS: Unitless

Normal_Value_of_Maternal_MDA = 1.2

UNITS: nmol/ml

Normal_Value_of_RoA_on_Fetus = 0.01

UNITS: Unitless

Pregnancy__Switch = 1

UNITS: Unitless

Ratio_Fetal_MDA = Fetal_MDA_Avg / Fetal_MDA_Control_Avg

UNITS: Unitless

Ratio_Fetal_Melatonin = Fetal_MEL_Avg / Fetal_MEL_Control_Avg

UNITS: Unitless

RoA_Adj_Time = 360

UNITS: Hour

Switch_On_Off =

IF TIME > 2160 THEN

0

ELSE

1

UNITS: Unitless

Transfer_Ratio_MDA = 0.5

UNITS: Unitless

Transfer_Ratio_MEL = 0.85

UNITS: Unitless

Transfer_Time_MDA = 1

UNITS: Hour

Transfer_Time_MEL = 1

UNITS: Hour

APPENDIX C: FIGURE PERMISSIONS



Circadian clock crosstalks with autism
Author: Kutlu O. Ulgen, Yaman Barlas, Ekin Yurdakul
Publication: BRAIN AND BEHAVIOR
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Association between exposure to rotating night shift versus day shift using levels of 6-sulfatoxymelatonin and cortisol and other sex hormones in women

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