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# Investigating the role of schizophrenia-associated gene expression in the developing human brain using Machine Learning

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# *Investigating the role of schizophrenia-associated gene expression in the developing human brain using Machine Learning*

*A Thesis Presented for the Award of Masters by Research*

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*For Research Carried Out Under the Guidance of*

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**Submitted to Technological University Dublin**

**July 2021**

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*Dedicated to Jim and Siobhan Kelly who have always had faith that I could do anything I put my mind to. I love you both.*

## *Abstract*

Schizophrenia is a debilitating condition that affects 1% of the population, causes significant hardship and though there are treatments available they are characterised by several limitations. It is a complex mental disorder where some individuals show mild subclinical cognitive symptoms before psychosis onset in adolescence. The treatments available only target a portion of the symptoms and although extensive research has been conducted, a comprehensive understanding of the nature of schizophrenia remains elusive. Unlike other neurodevelopmental disorders, schizophrenia symptoms do not typically present themselves until adolescence. This study aimed to discover gene co-expression networks at multiple developmental stages to identify candidate therapeutic targets to better treat and manage schizophrenia.

Recent genome-wide association studies have identified 145 genetic loci associated with schizophrenia. Allen Brain Atlas's BrainSpan resource provides brain development data from neurotypical brains. Using this resource, it was possible to study the gene expression of 316 schizophrenia-associated genes, identified previously in a large-scale GWAS, across each of the developmental stages available in the Allen Brain Atlas. K means Clustering and a systems biology approach (WGCNA) was applied to these schizophrenia-associated genes at each developmental stage where modules within networks were created by grouping co-expressed genes. To facilitate biological interpretation of these modules co-expressed genes were visualised using Cytoscape and gene ontology pathway enrichment analysis was applied.

We identified 21 hub genes using WGCNA. Of the 316 schizophrenia-associated genes, 27 modules were identified and 3 hub genes *GPR52, INA, SATB2* were common in multiple developmental stages. Our results suggest that *GPR52, INA, SATB2* represent candidate genes for future evaluation of their potential as therapeutic targets of schizophrenia. Additional hub genes included *TRANK1* and *ALMS1*, genes which were previously identified as expression quantitative trait loci. Taken together our results add further evidence that these genes could be good candidates for further research as they may regulate several schizophrenia-related genes in their respective modules. Finally, our enrichment analysis implicated a role for positive regulation of macrophage proliferation and cellular response to catecholamine stimulus, and cellular response to diacyl bacterial lipopeptide at each developmental stage. The immune system and catecholamines, including dopamine, have long been associated with schizophrenia and our results provide further support for these hypotheses.

## *List of Abbreviations*

| | | |
|---|---|---|
| 5HT | - | 5- hydroxytryptamine receptors |
| ABA | - | Allen Brain Atlas |
| AHBA | - | Allen Human brain Atlas |
| ADHD | - | Attention-Deficit Hyperactivity Disorder |
| ANPs | - | Antipsychotic Naïve Patients |
| ASD | - | autism spectrum disorder |
| BP | - | bipolar disorder |
| CNVs | - | Copy Number Variants |
| DMAs | - | Dopamine modulating antipsychotics |
| eQTL | - | Expression quantitative trait loci |
| FDR | - | False Discovery Rate |
| GABA | - | Gamma aminobutyric acid |
| GCN | - | Gene co-expression networks |
| GMV | - | Grey Matter volume |
| GO | - | Gene Ontology |
| GS | - | Gene significance |
| GWAS | - | Genome Wide Association Study |
| HCs | - | Healthy controls |
| HG | - | Hub Genes |
| IBD | - | Inflammatory bowel disease |
| ID | - | Intellectual disability |
| IL1β | - | Interleukin 1 beta |
| IL6 | - | Interleukin 6 |
| INDELS | - | Insertions and deletions |
| ISH | - | In Situ Hybridisation |
| LD | - | Linkage disequilibrium |
| LoF | - | Loss of Function |
| LMD | - | Laser Microdissection |
| LSD | - | Lysergic acid diethylamide |
| ME | - | Module eigengene |
| MIA | - | Maternal Immune activation |
| MK-801 | - | Dizocilpine |
| MM | - | Module membership |
| MNI | - | Montreal Neurological Institute |
| MS | - | Module significance |
| MRI | - | Magnetic Resonance Imaging |
| mRNA | - | Messenger ribonucleic acid |
| NS | - | node significance |
| NMDAR | - | N-methyl-D-aspartate receptors |
| OTU | - | Operational Taxonomic Units |
| PCA | - | Principal component analysis |
| PCP | - | Phencyclidine |
| PEN | - | Polyethylene naphthalene |
| PFC | - | Prefrontal cortex |
| PNNs | - | perineuronal nets |
| PV | - | Parvalbumin |
| QC | - | Quality control |

| | | |
|---|---|---|
| RNA | - | Ribonucleic acid |
| SNPs | - | Single Nucleotide Polymorphisms |
| TO | - | Topological Overlap |
| TOM | - | Topological Overlap Matrix |
| TNF-α | - | Tumour necrosis factor alpha |
| TNF-β | - | Tumour necrosis factor beta |
| UHR | - | Ultra high risk |
| VTA | - | Venteral Tegmental Area |
| WES | - | Whole Exome Sequencing |
| WGS | - | Whole Genome Sequencing |
| WGCNA | - | Weighted Correlation Network Analysis |

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# Chapter 1 – Introduction to schizophrenia

Schizophrenia is a debilitating psychiatric condition that manifests itself early in adolescence and can last a lifetime. It has a 1% global prevalence and comes with significant societal and economic costs as well as substantial mortality and morbidity (1). Its complex nature is believed to originate from a mixture of genetic and environmental factors including prenatal exposure to infection and lack of nutrients which cause disruptions during early brain development *in utero* (2,3). There are many hypotheses about the causes of schizophrenia, but the aetiology is unknown. This lack of understanding of schizophrenia is evident in its treatments which haven't significantly advanced since the introduction of the first-generation antipsychotic medication such as Chlorpromazine in the 1950s (4). This complex condition presents with three modes of clinical features namely: positive symptoms (psychoses manifesting as delusions and hallucinations, paranoia, hyperactivity and agitation), negative symptoms (social withdrawal, lack of motivation, asociality, avolition, affective flattening, consummatory and anticipatory anhedonia, and alogia), and cognitive symptoms (trouble with critical thinking, working memory and difficulty integrating feelings, thoughts and behaviour, attention and vigilance, verbal learning, reasoning and problem solving, and social cognition) as well as motor disturbances which regularly results in a poor quality of life (5–7). The presentation of symptoms is heterogeneous which makes schizophrenia both difficult to diagnose and treat (8). The negative and cognitive symptoms are chronic and are closely related to functional outcomes, and contribute greatly to illness burden, (6) the positive symptoms usually relapse and remit (8). Despite some progress in the understanding of several of the fundamental mechanisms involved in schizophrenia's

aetiology, the current treatments available come with serious side effects, inconsistent efficacy, and lack of evidence that they substantially improves the outcomes (9,10). At present, schizophrenia's treatments consist of antipsychotic drugs, social support, rehabilitation, and psychological therapies (8). Current antipsychotics are associated with serious limitations. Firstly, around 30% of sufferers are treatment-resistant, secondly, they mainly ameliorate positive symptoms only leaving cognitive and negative symptoms untreated and lastly, antipsychotics trigger both neurological and metabolic side effects (11). As a result, there is a clear need for more efficient and effective treatments as well as uncovering a model for prediction of efficacy as currently determining the most effective treatment of schizophrenia is a trial and error method (7,12). It is important to study patients at several clinical stages to give insight into the effects of schizophrenia itself, its progression and what alterations are caused by the pharmacological treatments (13).

## *1.1 Schizophrenia's aetiology*

At present, there are no clinical diagnostic tests available for schizophrenia so diagnosis relies on clinical observations and self-report (14). Schizophrenia remains incurable and the best outcome continues to be managing symptoms and preservation of independence and functionality (9). Until there is a more complete understanding of schizophrenias aetiology, there is little hope for improving diagnosis, predicting susceptibility, management, and treatments for those with schizophrenia.

## *1.2 Understanding of schizophrenia to date*

Schizophrenia's complicated and unknown underlying mechanisms has meant that there has been no fundamental innovation in schizophrenia treatments since the

introduction of first-generation antipsychotics in the 1950s (15). As treatments mainly target the positive symptoms there is a clear need for a focus on cognitive and negative symptom domains. These types of studies could lead to new endophenotypic markers which could promote novel treatment discovery and could initiate concurrent medication strategies with current antipsychotics (15).

### 1.2.1 Immune System

Schizophrenias pathogenesis is elusive, and though animal models have been used to understand elements, the human central nervous system (CNS) and immune system are much more complex and intricate (16). Both systems share common features in developmental mechanisms, so therefore CNS and immune system dysregulation should be studied in humans (16,17). The immune hypothesis of schizophrenia has been around for a long time and is supported by epidemiological, genetic, imaging and biomarker studies (17). The accumulating evidence that anti-inflammatory and immunosuppressive medications are effective treatments and that autoimmune conditions and immune activation are risk factors for developing schizophrenia provides perhaps the most convincing evidence of the immunes system involvement (18,19).

Dysregulation of the innate and adaptive immune system has been identified by epidemiological, genetic, postmortem and therapeutic studies and are likely to contribute to some of the symptoms of schizophrenia (20). Though there have been a large number of studies with significant funding devoted to better understand schizophrenia outcomes remain poor and hope remains in advances in psychoneuroimmunology and other advanced technological research areas to provide more consistent and successful management of schizophrenia (12). Several

autoimmune conditions display neuropsychiatric symptoms suspected to be caused by brain reactive antibodies (21). Schizophrenia and autoimmune diseases are often comorbid likely because of some genetic overlap, (22) affecting common underlying pathways which entail inflammatory immune response antibodies which can attack brain tissue (23)(22). A national cohort found if a patient had a prior autoimmune disease they are 29% more likely to develop schizophrenia in adolescence (21).

Maternal Immune Activation (MIA) can disrupt normal fetal brain development and has been linked to schizophrenia for over a century, it is estimated that if MIA could be avoided that 30% of schizophrenia cases would be averted (20). Lower levels of acute-phase proteins in neonates which increases the susceptibility of infection have also been hypothesized to increase the risk of psychosis in adulthood (22). Patients experiencing acute episodes of schizophrenia often have increased levels of Interleukin-1- beta (IL-1 β), Interleukin-6 (IL-6), and transforming growth factor-beta (TGF- β) (23). In unmedicated patients, tumour necrosis factor-alpha (TNF α) protein levels and *IL-1β* messenger RNA (mRNA) is seen to be elevated (24).

Within the body exists a dynamic population of gut microbes which houses many bacteria approximately $10^{14}$ cells. The biological biodiversity is established in the first couple of months of existence, and has a continuous role throughout life, and is very susceptible to environmental factors (25). The gut microbiome can control how the brain behaves and functions via the microbiota-gut-brain (MGB) and it has been reported to be related to changes in cognition, anxiety, and memory, as well as development, maturation of immune, neural and endocrine systems in animal models (26). These physiological and behavioural processes are often impaired in people with schizophrenia. A high α-diversity score is usually a sign of good health (27). In

a study performed by Zheng et al. medicated and unmedicated patients with schizophrenia, it has been observed that they have a decreased α-diversity in their microbiome when compared to healthy controls (HCs) (26). It was also found that Veillonellaceae and Lachnospiraceae found in the microbiome environment were associated with symptom severity in schizophrenia (26). β- diversity analysis of schizophrenia patients and HCs found clear differences in the compositions of each microbiome by looking at operational taxonomic units (OTU) levels (26). In one study when the linear discriminating analysis effect size was applied to 77 differential OTUs it was observed that 23 out of the 77 OTUs saw an increase in patients with schizophrenia patients when compared to controls. The OTUs belonged to the bacterial families Veillonellaceae, Coriobacteriaceae, Bacterioidaceae, and Prevotellaceae, the other 54 OTU levels were seen to be decreased in patients with schizophrenia (Lachnospiraceae, Norank, Ruminococcaceae and Enterobacteriaceae) (26).

### 1.2.2 Neurodevelopmental hypothesis

Epidemiological, basic, and clinical neuroscience research has presented evidence that schizophrenia is of neurodevelopmental origin (28). This hypothesis is now widely accepted but what differentiates schizophrenia from other neurodevelopmental conditions is its time of onset, in adolescence (29). Autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD) and intellectual disabilities (ID) characteristically present themselves much earlier in childhood (29). Schizophrenia shares many phenotypic and clinical similarities and is often comorbid with these neurodevelopmental disorders but because of its delayed presentation, they were not initially linked (29). Before they were connected it was

then hypothesised that schizophrenia may be a neurodegenerative disorder, but when post-mortem studies failed to identify traumatic, neurotoxic, or neurodegenerative mechanisms in the brain this theory was disproven, and the neurodevelopmental hypothesis replaced it (29). In neonatal primates and rodents, prenatal cortical lesions were shown to lead to the emergence of abnormalities that mimicked schizophrenia in early adolescence, proving that early developmental abnormalities could have an impact on cortical function in later life, making the neurodevelopmental hypotheses for schizophrenia plausible (30). Instead of each neurodevelopmental disorder being viewed independently an alternative view was proposed, that these neurodevelopmental disorders lie on an etiological continuum with a diverse range of outcomes that follows from early brain development disturbances because of shared genetic variants and environmental factors (29). These neurodevelopmental disorders are diagnosed based on symptoms, the timing of onset, severity/persistence, and abnormal brain development (29). Before the first psychotic episode, schizophrenia presents itself very similarly to the other neurodevelopmental disorders, but only the negative and cognitive symptoms. There are several rare copy number variants (CNVs), genes affected by loss of function (LoF) mutations, genes enriched with *3* nonsynonymous mutation and alleles that have significant associations with schizophrenia, ASD, ADHD and ID which represent direct outcomes of the rare pathogenic mutations that they share (29). This would also suggest that the risk of developing positive symptoms is not mediated by cognitive impairment.

### 1.2.3 Dopamine hypothesis

Dopamine is a catecholamine neurotransmitter in the brain which regulates critical neurological processes such as cognition, motor control, reward and learning (31). In

the 1950s Chlorpromazine an antipsychotic drug and affective antagonist for the D2, D3, and D5 receptors was released and the treatments dopamine receptor antagonists have remained the most prevalent therapeutic (11). Chlorpromazine controlled the positive symptoms of schizophrenia patients and the theory that dopamine alterations in the mesolimbic pathway caused positive symptoms was strengthened and confirmed (11). Other key evidence supporting the dopamine hypothesis was when amphetamines were administered which increase the extracellular concentrations of dopamine and psychotic symptoms like schizophrenias appeared (32). This evidence was reinforced when treatments that depleted the concentration of dopamine such as alpha-methyl-para-tyrosine and reserpine were shown to reduce psychotic symptoms (32). These antipsychotics target other dopamine receptors, serotonin, norepinephrine, acetylcholine and histamine as well (32). It is often seen has that in the associative striatum there is an increased dopamine synthesis capacity for people who have psychotic disorders including schizophrenia (33). The increased dopamine synthesis is detectable in ultra-high-risk (UHR) subjects and before early symptoms of people who eventually develop schizophrenia thus are not a consequence of antipsychotic exposure or psychotic episodes (33). Rodent models have been able to replicate this, these models have also shown that increased synthesis and release of striatal dopamine can be a result of acute stressors and inflammatory challenges *in utero* (34). These developmental disruptions cause the dopamine system to become hyper-responsive later in life, in the rodent equivalent of adolescence (34). Recent studies have pinned that part of the cause for schizophrenia to be a combination of an increased spontaneous dopamine release and decreased dopamine release for relevant stimuli (34). Studies using amphetamines have been important for proving this. At moderate doses, amphetamines act as a reward predicting cue by increasing the

levels of striatal dopamine appropriately while at larger doses, the amphetamines blunt adaptive responses, which alters the behavioural response and increases the spontaneous transients (spikes in the levels of dopamine) (35). These spontaneous transients may explain the inappropriate phasic firing of dopamine neurons known to be part of schizophrenia (35). All psychostimulants including amphetamines have the effect of increasing spontaneous transients in the striatum which correlate and could explain some of the positive symptoms of schizophrenia. Some of the primary negative symptoms of schizophrenia could be explained by the decreased adaptive transients in the striatum. (35) It is thought that many antipsychotic drugs perform in the same manner and affect the adaptive and spontaneous transients similarly, where one cannot be fixed without aggravating the other (34).

Around 30% of patients with schizophrenia do not respond to antipsychotics with high D2 occupancy and do not respond to treatments that diminish the levels of presynaptic dopamine concentrations. (32,36) Demjaha et.al found that people who responded to typical antipsychotic treatment had higher dopamine synthesis capacity and that increased synaptic dopamine may be used to predict treatment responsiveness. (36). Treatment-resistant patients did not have this capacity, this demonstrated that there may be a subtype of schizophrenia which is non-dopaminergic. Accumulating evidence has shown that schizophrenia's core pathophysiology may also involve dysfunction in glutamatergic, serotonergic and gamma-aminobutyric acid (GABA). (37)

### 1.2.4 Glutamate hypothesis

The dopamine hypothesis can account for a portion of the psychopathology of schizophrenia, in particular positive symptoms (38). Atypical antipsychotic drugs apart from Clozapine have little to no effect on negative and cognitive symptoms (11). Negative and cognitive symptoms are neglected by antipsychotics and persist causing chronic disability (4). In patients with chronic schizophrenia cortical atrophy correlates with the negative and cognitive symptoms but not with the severity of the psychosis, (39) showing that although some of the cognitive and negative symptoms may be caused by dysregulation in dopamine pathways, not all are.

Glutamatergic pathways are primarily the excitatory neurotransmitters in the brain and glutamatergic neurons utilise between 60-80% of the total brain metabolic activity (32). Glutamate pathways have been linked to the limbic system, cortex, thalamus and are mediated by N-methyl-D-aspartate receptors (NMDARs) (37). Glutamate was originally associated with schizophrenia because it was observed that there were decreased levels of glutamate in cerebrospinal fluid (CSF) of patients with schizophrenia (32). There is now mounting evidence that glutamatergic dysregulation in the prefrontal cortex causes dopamine hyperactivity in the ventral tegmental area (VTA) which causes auditory hallucinations and paranoid delusions (40). Studies using NMDAR antagonists (Ketamine and phencyclidine (PCP), dizocilpine (MK-801)) on HCs induce schizophrenia-like symptoms (negative and cognitive) and increased prefrontal glutamine levels, these can last up to two weeks (41). PCP and Ketamine are non-competitive antagonists that bind at the NMDA subtype of glutamate receptor (39,42). From observing the effects of the NMDAR antagonists on healthy individuals, it has been proposed that certain symptoms of

schizophrenia may result from the hypofunction of NMDAR (43). It has also been observed that patients with schizophrenia undergoing long term treatment have increased levels of glutamine in the anterior dorsal cingulate cortex which was linked with the severity of psychotic symptoms (41), suggesting that despite the treatment with antipsychotic treatments there is a basal increase of presynaptic glutamate which is consistent with the NMDAR hypofunction pathophysiological model of schizophrenia (41). It has been seen in patients with schizophrenia that increased synaptic release of glutamine is associated with psychosis, while glutamate metabolism is related to cognitive impairments (41). In one metanalysis it was observed that glutamate in the frontal region was lower but glutamine is higher in people with schizophrenia when compared with controls, over time the levels of both reduce which could suggest a progressive load of synaptic activity (44). Patients with schizophrenia who don't respond to typical antipsychotic treatment seem to have more marked glutamatergic abnormalities while treatment responders have dopaminergic abnormalities (36).

The role of glutamate in the pathophysiology of schizophrenia has been investigated in Genome-wide association studies (GWAS) they have highlighted several genes associated with glutamatergic neurotransmission or with downstream mediators (*GRM3, GRIN2A, and GRIA1*) (32).

### 1.2.5 Gamma-aminobutyric Acid (GABA)

GABA is a major inhibitory neurotransmitter located in the CNS (37). Results from animal models and postmortem studies suggest that part of schizophrenias pathophysiology is caused by both dysfunctions of GABAergic interneurons and

NMDARs(45,46). In human postmortem studies in individuals with schizophrenia, alterations were seen in GABA-related epigenetic, transcript, synaptic, and protein markers especially evident was the subpopulation of GABA neurons which encompass calcium-binding protein parvalbumin (PV) (47). GABA interneurons are an important part of the brains rhythm generating network, they are also important in controlling neural oscillations which are fundamental mechanisms for memory, perception and consciousness (5). The third layer of the prefrontal cortex houses a microcircuit where GABAergic PV cells and glutamatergic cells synchronize neural oscillations (11). The PFC PV neurons have lower levels of PV and proteins and a GABA synthesizing enzyme GAD67 (45). These structural and molecular alterations are hypothesised to alter GABA neurotransmission and weaken the PFC gamma oscillations in people with schizophrenia (47). GABA antagonists have been shown to effective in improving some of the core symptoms of schizophrenia in clinical studies (48). Benzodiazepine which works on the GABA-A receptor allosteric site is used often with antipsychotic medications to treat schizophrenia (5).

### 1.2.6 Serotonin Hypothesis (5-hydroxytryptamine, 5-HT)

Although the serotonin hypotheses are one of the oldest in regards to schizophrenias pathogenesis it remains highly topical because of the lack of reproducible results (49). Serotonin has been linked to schizophrenia's pathophysiology since studies looking at the interaction between 5-HT and the hallucinogenic drug lysergic acid diethylamide (LSD) which resulted from antagonism of 5-HT in the CNS (37,50). Psychotic symptoms due to dementia and Parkinson's are successfully treated with 5HT2A antagonists without D2 antagonism which halts excess serotonin being released which stops the downstream release of glutamate which can activate the

mesolimbic dopamine pathway (40). Sizeable evidence from multiple methods suggests that a subpopulation of patients with schizophrenia display serotonergic function abnormalities (49). It is believed that 5-HT receptors (5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_{2C}$) may underlie cognitive symptoms and motivational disabilities and this shown by atypical antipsychotics which act on 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors and how they ameliorate negative symptoms and mood disturbances (51). In mutant mice who display decreased willingness to work for a reward, there were $D_2$ receptors overexpression and up-regulation of 5-$HT_{2C}$ receptors in the striatum (51). The extrapyramidal effects of antipsychotics can be ameliorated by serotonin antagonists, (37) though the pathogenesis of schizophrenia has not been explicitly linked with serotonergic dysfunction, 5HT-3 and 5HT-6 continue to be looked into as potential therapeutic targets.

### *1.3 Environmental risks for schizophrenia*

Many epidemiological studies have investigated the impact of the environment on the development of schizophrenia. Several risk factors have been highlighted, such as being raised in an urban environment, early life adversities, and the use of cannabis (especially compounds with high tetrahydrocannabinol levels) early and frequently which has an impact on the developing social brain (2,3,8). Immigration (first and second-generation) has also been studied as well as an increase in the rate of incidence in individuals with young parents or with relatively old parents. (2,8,52,53). Prenatal exposure to infection, preterm births, social disadvantage, and lack of nutrients in the womb have all been linked to heightening the risk of developing schizophrenia (28,54). Prenatal stress increases the basal secretion of glucocorticoid hormones which can reprogram the hypothalamic-pituitary-adrenal

axis (3). In rodent models, following prenatal stress malformations in the DNA methylation in GABAergic neurons were observed and connected to schizophrenia-like symptoms (3). Exposure to prenatal infection has been shown to induce epigenetic modifications which can cause the downregulation of genes essential for synaptic plasticity, transmission, working memory, and social cognition (55). Obstetric complications such as bleeding during pregnancy, pre-eclampsia and traumatic births can also increase the risk of developing schizophrenia (3). Severe famine at the time of conception or early in the pregnancy increased the risk of developing schizophrenia two-fold, while mothers with inadequate weight gain increased the risk of psychosis for their offspring by 9-fold (56). Prenatal immune system activation can affect brain development negatively and can slow or alter the neurodevelopmental trajectories which can cause behavioural and cognitive impairments later in life (3). The brain is especially vulnerable in the first and second trimester of pregnancy during critical brain development so maternal stress such as bereavements, unwanted pregnancies and other serious life events are positively linked with the development of schizophrenia and other serious mental disorders (3). Particular childhood and adolescent risk factors are capable of predicting the age of manifestation in patients with and without a relative who has schizophrenia (53). Studies have shown that some patients who develop schizophrenia during adolescence experienced delayed developmental milestones in their first year, had hearing impairments, emotional problems, low IQ in childhood, and interpersonal difficulties (2).

Some of the positive symptoms of schizophrenia have been linked to developmental trauma, cannabis use, living in an urbanised area and the minority group position in

that area, for these studies cultural bias and selective migration were inspected and found to not impact these association (56). Living in an area densely populated with the same ethnic group and moving from an urban area to a rural environment decreases the risk of developing any kind of psychosis (2,56). The accumulating evidence that environmental exposures occurring preconception through to adolescence and adulthood play a role in the susceptibility of schizophrenia, as well as ample evidence that exposures to environmental factors *in utero* produce brain anomalies as well as phenotypes similar to schizophrenia (56). Though the associations with environmental factors are robust the observational epidemiology cannot distinguish true causation from the association as a result of pleiotropy or reverse causation (8).

The role of environment in schizophrenia has been hypothesised for decades but the lack of biological models and methodologic limitations has made it a difficult test to what extent they are involved (52). In recent years genetics has dominated the discourse of schizophrenia's aetiology (9,52). Twin studies have shown a discordance rate for monozygotic twins who develop schizophrenia of 40-55%. Monozygotic twins have identical genomes this illustrates that the risk of developing schizophrenia is not solely genetic but plays an important role (56). The most plausible explanation for this discordance is exposure to environmental factors which are likely to occur as early as *in utero*, or gene-environment interactions during crucial brain development (2,56).

## *1.4 MRI Findings*

The first MRI (magnetic resonance imaging) study focused on schizophrenia was conducted in 1984 and with advancements in technology over recent years many more followed. These studies have shown that there is not distinct diagnostic neuropathology for schizophrenia, but any of the subtle changes which are evident is apparent when a patient first becomes symptomatic (49,57). The lack of evidence of distinct neuropathology for schizophrenia could be explained by schizophrenias diverse presentation, range and severity of the symptoms and if the patient has been treated with antipsychotic medications before the time of MRI scan (48,57). Studies have found that several of the brain abnormalities which can occur are evident before any symptoms appear, hinting at schizophrenias neurodevelopmental nature and that these abnormalities may change over time (21,26). Understanding these changes in brain structure could prove most valuable in prognosis, treatment, and intervention. Across studies, reduced volume in the intracranial is seen especially with male patients and because 90% of the intracranial volume is usually reached at the age of five this suggests that there is an early developmental cause (59). Reduced total brain volume has also been observed consistently with a marked reduction in grey matter volume (GMV), while cerebrospinal fluid, third and lateral ventricles, and the left side of the planum temporale have increased volumes and are associated with more severe symptoms (60). The levels of GMV reduction is associated with elevated doses of antipsychotics and duration of illness (27). In antipsychotic naive patients (ANPs) most of the brain abnormalities observed are the same as those found in medicated patients but to a lesser extent, in ANPs GMV and total brain volume, the effect size was up to 30% less (24). Conversely thalamic and caudate nucleus volumes are more prominent in ANPs which strengthens the evidence that typical

antipsychotic medication enlarges the volume of the basal ganglia (61). The levels of white matter reductions are similar in medicated patients and ANPs which suggests that the levels of white matter do not considerably change after onset (60). Some post-mortem studies have also uncovered neuroinflammation in the brains of schizophrenia patients which are unrelated to treatments (9,20).

## *1.5 Genetics of Schizophrenia*

Schizophrenia is heritable meaning having a family member with the disorder heightens the odds of developing it during a lifetime (53,62). Offspring with one parent with schizophrenia have a risk rate of 7% while offspring, where neither parent has schizophrenia, is 0.86% (63). Twin studies have been pivotal in furthering our understanding of the role that genetics plays in schizophrenia's aetiology (62,63). A study performed using the Danish Wide Twin Register found that the propandwise concordance for schizophrenia is 7% for dizygotic twins (Fraternal) and 33% for monozygotic twins (Identical) in terms of disease liability (8). Although previous studies have found higher rates of concordance, with monozygotic twins achieving 30-40% and heritability estimates for schizophrenia >80% (6,21). Longitudinal twin studies have shown that children of the unaffected monozygotic twin have a similar risk as to the affected twins' children of developing schizophrenia or a schizophrenia-related disease in their lifetimes (9). Taken together, these findings highlighted a clear genetic susceptibility to schizophrenia.

As a result of multiple technological advances and extensive collaboration, there have been remarkable advances in the genetics of schizophrenia in the past decade (64). Genome-wide association studies (GWAS) have been valuable in uncovering

many schizophrenia risk loci, including single nucleotide polymorphisms (SNPs), copy number variants (CNVs) and insertions or deletions in bases in the genome (INDELs) (48). These GWA studies aim to identify areas of the genome that increase an individual's risk for developing schizophrenia (65). Schizophrenia is a complex polygenic psychotic disorder meaning it is not caused by one genetic variation with a large effect but rather by a combination of multiple genetic variants that each subtly increases the risk of the disease developing (66,67).

Genome-wide association studies (GWAS) are a powerful tool used for studying the genetic architecture of diseases (68). It is an experimental design used to uncover associations between traits of interest and genetic variants, with the aim of better understanding the underlying biology which could lead to better treatments and prevention strategies (69). GWASs have also been successful in uncovering disease-associated biological processes and assisting in risk prediction. (69). GWAS exploits linkage disequilibrium (LD) to measure an association at one genetic variant as a proxy for other genetic variants, the statistical power of these studies depends on the sample size, the distribution of effect size of the casual genetic variants, their frequency in the general population and the LD between genotyped DNA variants and unknown causal variants (65,69). In 2009 the first robustly associated loci linked with schizophrenia were identified using a sample size of 3000, and in 2014 using a sample size of 35,000 cases the number of genetic variants increased to 128 common variant associations across 108 genetic loci (1). In 2018 a schizophrenia meta-analysis identified 179 independent significant SNPs which mapped to 145 loci (1,66).

The 2018 meta-analysis GWA study for schizophrenia had 11,260 people with schizophrenia (cases) and 25,542 healthy controls (HCs). Potential schizophrenia risk genes were generated by taking proximity of a gene to SNPs into account but also the kind of genetic variant, expression quantitative trait loci (eQTLs), chromosome conformation data and genomic finemapping (70). Despite the large sample size, there was not a huge overlap of results from the previous GWAS, which can be expected from studying a complex, polygenic disorder like schizophrenia. (1,66). This study found that associations converge in specific cell types like pyramidal cells, some interneurons and medium spiny neurons (67). It was estimated that from a third to a half of genetic liability derive from common alleles, and a large portion of rare variant architecture comes from mutation intolerant genes which have also consistently been observed in other neurodevelopmental disorder (1,66). It was also noted that in the case of schizophrenia there was an enrichment of common variants associated with loss of function (LoF) and mutation intolerant genes and that these genes accounted for 30% of SNPs based heritability (66). People with schizophrenia have decreased fecundity and early mortality, but the common risk alleles persist in the population which could be because of 1) balance selection that schizophrenia-related alleles have reproductive advantages so are preserved because of their association with positively selected alleles or 2) the effects of gene-environment interaction on these rare variants (8).

Despite this improved knowledge, the understanding of the underlying biological mechanism has not progressed far enough to develop new treatments or cultivate preventative strategies.

## *1.6 Aim of the project*

Understanding how gene expression and regulation differ between individuals has advanced the understanding of healthy tissues and the origins of diseases and complex traits (71). To get a better understanding of the control of gene expression it's important to understand the relationship between genotype and phenotype and RNA sequencing which is a more quantitatively accurate absolute transcript (72).

This study aims to examine gene expression of the schizophrenia-associated genetic loci, identified by Pardiñas et al., in the developing human brain using the BrainSpan Atlas of the ABA repository. Of the 145- independent schizophrenia-associated loci provided by Pardiñas et al. 316 genes were available on ABA's resource, BrainSpan for further investigation. The expression profile of the 316 schizophrenia-associated genes was investigated using 1) K-means to identify underlying patterns in the genetic data and 2) network analysis using weight-gene co-expression network analysis (WGCNA) in the developmental stages available in ABA's Brainspan resource. Both steps were performed across the five developmental stages of the ABA dataset. Gene modules identified using WGCNA were further characterized by identifying hub genes and performing enrichment analysis to identify schizophrenia-related biological pathways. Identifying relevant biological pathways can further our understanding of disease aetiology and present new targets for novel therapeutics which could provide better outcomes for schizophrenia patients.

# Chapter 2 – Methods

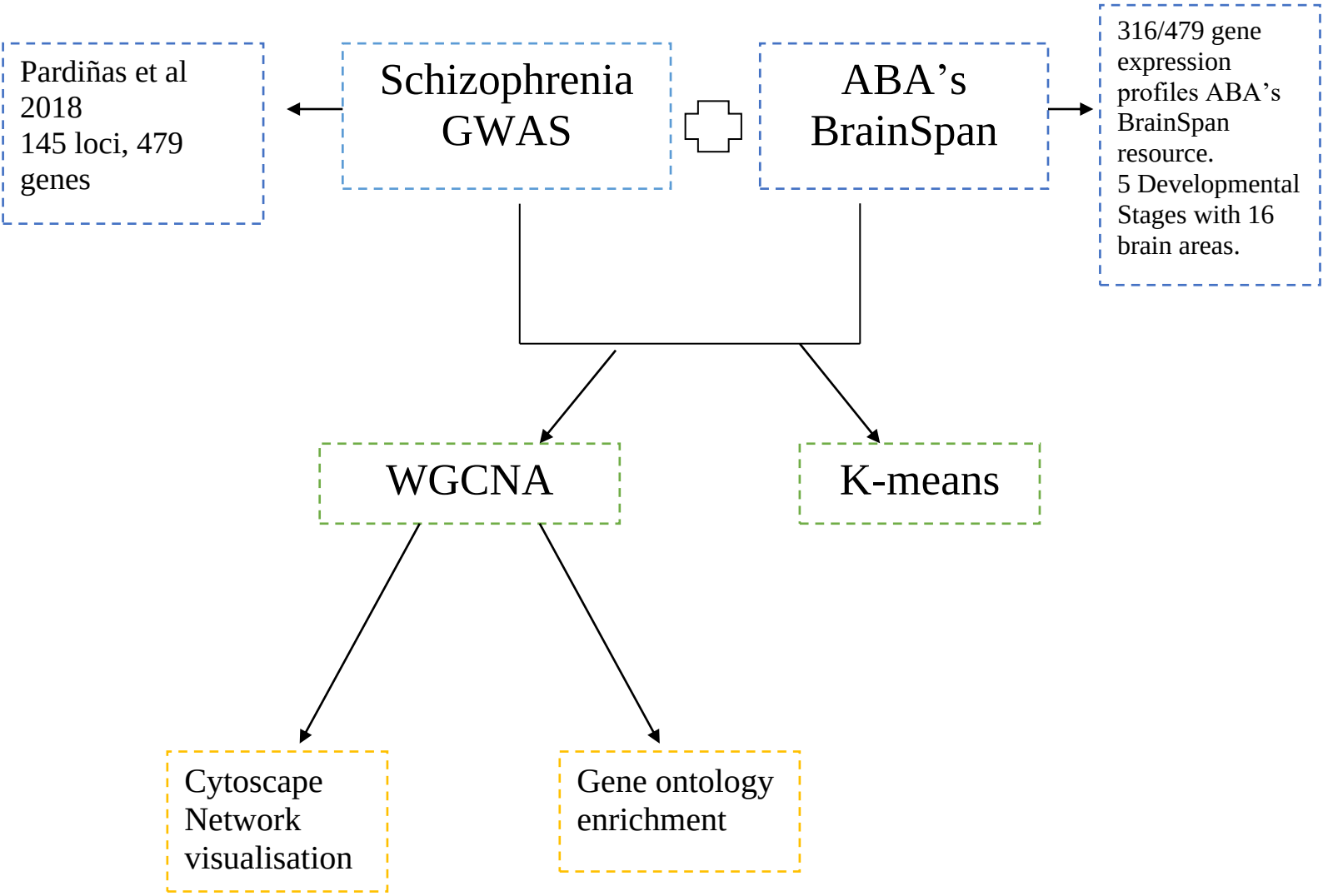



Figure 1.6.1 Diagram of methods performed in this study.

## *2.1 Collation of Schizophrenia-associated Genes*

Our knowledge of schizophrenia's genetics has vastly improved in the past decade, however, identifying gene targets has proven difficult (1). Pardiñas et al. completed a GWAS meta-analysis in 2018 which had 11,260 cases obtained from a CLOZUK sample on genetic information from people with schizophrenia in the UK and 24,542 controls (66). The meta-analysis used cases from the CLOZUK GWAS and combined them with Psychiatric Genetic Consortium (PGC) datasets from the 2014 GWAS excluding any overlapping samples which brought the total number of cases to 40,657 cases and 64,643 controls, the meta-analysis highlighted 179 independent genome-wide SNPs which were significant, which mapped to 145 loci (66). 93 of the 145 loci had been previously identified by the PGC GWAS in 2014 and had shown an increased association in the 2018 meta-analysis (1,66). Summary statistics were added to the ClOZUK genes so a combined analysis could occur. The PGC data was re-examined with a fixed-effects procedure derived from standard errors and polygenic risk scores were calculated for the whole dataset (66). In this study, schizophrenia-associated genes (n=316) identified by the GWAS meta-analysis conducted in 2018 by Pardiñas et al. who identified 145 loci total with $P < 5 \times 10^{8}$. If the loci did not overlap with a gene, the closest gene within a 500kb radius was used. Of the 145 loci, 316 genes were available from ABA's BrainSpan resource (66).

## *2.2 Allen Brain Atlas*

The brain is the most complex system in the human body with approximately 86 billion neurons and around a trillion synapses per cubic centimetre of the cortex (73). Its circuitry, cellular and structural diversity and the regulation of its transcriptome are far from being completely understood. One billion people are suffering

worldwide with brain diseases and disorders but there is a lack of diseased tissue to study. There is a need for another approach to uncovering their aetiologies (74). The challenge of this type of research is the scarcity of high-quality post-mortem human brains. These brains are normally dissected at brain banks and distributed to various research groups and thus data derived from these analyses have diversified hypotheses that are non-parallel as well as different types of research methods, which has hindered the analysis of brain disorders (73). Although other species model systems have been useful, analyses of the human brain itself are essential to get a true understanding (73).

ABA is a public resource that gives access to gene expression, connectivity, and neuroanatomical data for mouse, primate, adult humans, and developing brains for humans and mice which integrates MRI, genomic and anatomic information, histology, diffusion tensor imaging and gene expression data derived from ISH and microarray methods (75). The original Allen Human Brain Atlas (AHBA) uses high-quality post-mortem brains from males and females between the ages of 18-68 with no known neuropathological or neuropsychiatric history and maps the genes expression to the stereotaxic space. (76) This valuable tool can help researchers trying to comprehend how spatial variation on the molecular scale associate with macroscopic neuroimaging phenotypes (76). While there are other human atlases, only Allen Human Brain Atlas (AHBA) possesses high-resolution coverage of the majority of the brain (76).

The brain tissue underwent several tests including serology, toxicology and tested the RNA quality to determine if it meets the inclusion factors (74). If the brain tissue

samples passed, they were then sent to tissue repositories for initial tissue processing. After which the brain tissue was frozen, after and sent to the Allen Institute where thorough quality control (QC) tests were performed, the brain tissues that passed this threshold have histological data collected from them and the tissues were subdivided and categorised based on if they contain cortical or subcortical substructures. Additional tissue containing subcortical structures were collected and then placed on membrane slides so laser microdissection (LMD) could occur. Both cortical and subcortical tissue samples were collected for microarray analysis. The microarray analysis quantified the expression levels of thousands of genes at once by measuring the hybridisation of Cy3-labelled RNA (cRNA) to a probe on a microarray chip (Agilent 8 X 60K custom design arrays) (74). The probes were mapped to a specific location of DNA that contains single-stranded nucleic acid profiles which recombine with their complementary targets during hybridisation. The gene expression levels in the tissue samples were quantified by measuring the fluorescence at the sequence-specific locations which correlate to the levels of mRNA (76).

### 2.2.1 Brainspan Atlas of the Developing Human Brain

The human brain develops following a complex series of histogenic occurrences that depend on differential gene expression and its complex development is not fully understood (77). During the first 6 months of embryonic life, the brains general architecture is formed this is driven by strong genetic influences which are silenced in the third trimester allowing for environmental factors to influence the last trimester (78). Mice and non-human primates' models have been useful in developing some knowledge of the brain but the differences between species is a huge limitation. Firstly, because of the difference in size, in addition to this the

evolutionary differences which are seen in the superficial layers of the neocortex and secondly the developmental differences in the evolution of GABAergic interneurons (77). The shortage of human prenatal tissue and the use of different species models which have their restrictions has hindered the development of an anatomically comprehensive atlas of the prenatal human brain which could be used for studying the roots of neurodevelopmental and psychiatric disorders (79). The ABA resource BrainSpan transcriptional atlas of developing human brain is a repository of RNA sequences expression profiles of 16 brain structures from 8 weeks post-conception (prenatal) to 40 years of age (80). The stages are outlined in Table 1. The prenatal stage is made up of four high quality mid gestational brains, two from fifteen to sixteen post-conceptual weeks, and two twenty-one post-conceptual weeks specimens. These tissues had no history of maternal drug or alcohol abuse or potential agents that could disturb their development or relations with HIV 1 or 2 or HepB or HepC (77). The specimens were donated from the birth defects research lab at Washington University and the Advanced Biosciences resource in California (77). The left hemispheres were coronally, serially cytosectioned onto polyethylene naphthalene (PEN) membrane slides for LMD and histologically stained for detailed structure identification, and three hundred regions per specimen were isolated (77). The right hemisphere of two of the specimens was handled similarly and was used further for In situ hybridisation and Nissl staining for structure identification (81). The sample locations were mapped to MRI coordinates and then to the Montreal Neurological Institute (MNI) coordinate space (81). This data was anatomically delineated to create a digital reference atlas which allows for the visualisation of transcriptome data in its exact coordinates. The atlas resources also include MRI, diffusion-weighted MRI from three brains with the approximate same post-

conceptual weeks as well as the white matter reconstruction for three additional brains (77).

**Table 2.1: Age categories from the developmental stages for ABA's resource BrainSpan available for download in R.**

| Stage | Age category |
|---|---|
| 1 | Prenatal |
| 2 | Infant (0-2 years) |
| 3 | Child (3-11 years) |
| 4 | Adolescent (12-19 years) |
| 5 | Adult (>19 years) |

### 2.2.2 ABA and its application in research

The scarcity of suitable brain tissue available for research led scientists to develop Allen Brains Atlas human brain resource. ABA's gene expression data being accessible at high neuroanatomical data makes it possible to identify intricate gene expression patterns for healthy human brains, these profiles for healthy brains can be used as a baseline to identify genes involved in neurological conditions by using machine learning techniques which could relate to a neurological condition. This approach was successfully applied by Negi et al. where they applied machine learning methods such as hierarchical clustering and weighted co-expression on ABAs gene expression profiles across brain regions (82). From there they were able to build supervised classification models for Autism and Parkinson's with 84% and 81% accuracies respectively (82). Researchers can solely use ABAs resources alone or can apply external data from GWAS or MRI studies to aid their analysis.

McCarthy et al. applied the latter technique when investigating Bipolar disorder (BD), they took 58 genes identified to be involved with BD from a previously published GWAS and looked at their expression pattern across 900 areas (83). They also compiled a meta-analysis of MRI studies looking for structural abnormalities across patients diagnosed with BD (83). They aimed to see if they could link unusual gene expression in the BD genes with the brain structural differences (83). Using ABA's Brainspan human brain transcriptome database Mahfouz et al. hypothesised that understanding the functional relationships between ASD candidate genes during normal development could provide insight into ASD's genetic heterogeneity (80). Over human development, the heterogeneous ASD candidate genes share transcriptional networks related to protein turnover, mitochondrial function and synapse elimination and formation (80).


Figure 2.2.2.1 Schizophrenia-associated genes identified by Pardiñas et al and their position on the chromosomes.

## 2.2.3 Collation of schizophrenia-associated genes from ABA

The ABA data was downloaded into R (version 4.0.0) using the R packages ABAData and ABAEnrichment (52,74). The complete ABA dataset has 17,245 genes expressed in 16 distinct areas over five developmental stages from prenatal to adulthood (See Table 2.1 for more detail). The genes found to be significantly related to schizophrenia identified by Pardiñas et al. determined by their p-values (66) were exported by CSV file into R. The 17,245 genes available in ABAData were filtered into five dataframes for each of the developmental stages for further analysis. The dataframes were shaped into a wide format using the `pivot_wider` function in R

where the brain areas are the column names, and the row names were converted to the schizophrenia-associated gene names using `column_to_rowname` function in R and the gene expression for each gene was scaled. The schizophrenia-associated genes are available in Table 37 in the Appendix.

Figure 2.2.2.1 shows the schizophrenia-associated genes identified by Pardiñas et al. which were available on ABA's resource and where they lie on the chromosome.

## *2.3 Machine Learning and Clustering*

Regression analysis, feature selection methods, and classification are elements of the term Machine Learning (84). Classification can be subdivided into supervised, semi-supervised, and unsupervised. Supervised classification deals with objects that are labelled beforehand and build a learning algorithm which is then used to predict the classification of unlabelled data. Semi-supervised uses labelled and unlabelled data to train an algorithm (85). Unsupervised classification defines classes without help from previously known labels (84), clustering is a form of unsupervised learning.

In genetics, large datasets of genes and their expression are given to a clustering algorithm to cluster genes whose expression are similar to each other. These algorithms can be used for prediction, classification, and identification in DNA sequences but can also be taught to distinguish between phenotypes and identify possible biomarkers (85).

### 2.3.1 Unsupervised Learning and clustering for gene expression data

Unsupervised learning is a machine learning technique that looks for natural structures in data and groups them without classifying them (85). Gene expression data is

massively complex. Clustering is an unsupervised learning approach capable of discovering subgroups within a dataset, each of these subgroups or clusters have similar observations within them. This type of analysis has been a cornerstone for interpreting biological information from large gene datasets (86). Clustering can group genes based on their similar expression across brain areas and discover patterns in the data. Clustering can suggest regulatory relationships between genes and transcription factors and can further genome annotation by using the principle of guilt by association, as well as give a better understanding of how diseases manifest and can progress over time (87).

### 2.3.2 K-means clustering and NbClust

One of the most fundamental modes of understanding learning is to organise data into sensible groupings (88). K-means is a numerical, unsupervised, iterative, non-deterministic method that is classified as a partitional clustering algorithm (89). The k-means algorithm finds a split so the squared error between the points in a cluster and the empirical mean is minimized in each of the clusters. To perform k-means the number of clusters (k), distance metric and cluster initialisation must be pre-specified before the algorithm can be run (88). The goal of k-means is to produce groupings each with a high degree of similarity and a low degree of similarity with the other groupings (90). One of the issues with k-means is deciding the number of clusters (k) that are suitable for a dataset, there are many different indices to determine this but a package in R called NbClust integrates thirty different indices in one package to determine the optimal number of clusters in a dataset (91).

### 2.3.3 Kmeans analysis of schizophrenia-associates genes

In this study, unsupervised machine learning techniques were performed on the schizophrenia-associated gene set to identify underlying patterns. The NbClust package in R was used to determine the optimal number of clusters for each of the developmental stages. NbClust uses thirty different methods of determining cluster number and produces a bar chart to visualise which cluster number fits best (91). The optimal cluster number was put into the kmeans function in R for the centres. Twenty-five was selected to be the optimum number for initial configurations (nstart) and Euclidean distance was used. Each cluster is filled with genes with similar expression patterns. After the K-means analysis was performed each of the clusters was visualised using the `fvis_cluster` from the factoextra package in R (92). A table to show module assignment for each of the genes is available in table 17 in the appendix.

## *2.4 Co-expression Network Analysis*

The information found in gene expression data can be used to link genes with unknown function to biological processes, identify candidate genes for disease, determine transcriptional regulatory systems, and identify novel targets for therapeutics (93). Co-expression network analysis recognises genes that show coordinated expression patterns, and the networks can be shown as gene-gene similarity matrixes in later analyses. Co-expression looks to identify relationships between pairs of genes by using mutual background information or correlation (93). These pair-wise correlations between them are then rolled out to the other genes in the dataset until a network is formed where multiple modules are fashioned and each node signifies a gene, and the edges represent the presence and strength of the relationship. Functional enrichment analysis can be

applied to the modules formed after applying the co-expression network analysis method, these modules can often represent biological processes (93).

## *2.5 Weighted correlation network analysis (WGCNA)*

Genes do not work alone, and each gene can work with between four to eight genes which in turn could be involved in up to ten biological processes (94). Any dysfunction in these pathways can potentially lead to diseases. There are many ways to analyse complex, multi-dimensional genetic data, and one of the most popular methods are correlation networks. This technique is a useful way of discovering the underlying intrinsic organisation of the transcriptome. Constructing gene co-expression networks (GCN) for complex diseases is an important method of identifying genes involved in disease, highlighting highly connected genes within the networks and modules that can lead to novel therapeutics or biomarkers for diagnosis. WGCNA is an unsupervised learning systems biology network analysis method for associating correlation patterns among genes across gene expression microarray samples. The WGCNA package which is available for download in R can construct gene networks, identify modules, and can detect highly connected genes that are representative of the module using hierarchical clustering (95). When WGCNA is performed the algorithm evaluates the expression for each gene, pairing them based on topological overlap (TO) and then considering the degree of shared neighbours looking for consistent gene expression patterns and placing them into modules (82). Once the modules are defined the module eigengene (ME) which is the first principal component of the module is isolated and centralised. Highly connected nodes which are most like the ME and that are representative of the modules are specified and these are called hub genes (HG). The module membership (MM) calculates the degree of correlation between the genes within a module and the ME (96).

Using module significance (MS) methods can help detect important modules which contain high average node significance (NS) and the gene significance (GS) which is the correlation between a node and a phenotype of interest (95). The WGCNA algorithm can execute network construction, module detection, gene selection, data simulation, visualisation, and calculate topological properties (95). WGCNA has been applied successfully with cancers, mice and yeast genetics, and brain imaging data.

### 2.5.1 Network analysis using WGCNA

After the initial unsupervised learning analysis looking for underlying patterns, network analysis was performed using WGCNA in R. WGCNA was performed on the developmental stages because it is an effective way to characterise correlation patterns within the schizophrenia gene set, genes that correlate sometimes are related biologically. Networks were constructed using an adjacency matrix which looked at the co-expression similarity between a pair of genes and constructed a hierarchical graph. Pairwise correlations were used to identify modules where genes with similar gene expression are grouped into modules. To construct the weighted gene network, a soft threshold power analysis was first performed using the `pickSoftThreshold` function within the WGCNA package to calculate the adjacency by using gene co-expression, the power in the `pickSoftThreshold` function was calculated independently for each developmental stage and verbose was set at five (94,95). Once the power is chosen to calculate adjacencies, the adjacency is transformed into Topological Overlap Matrix (TOM) and used to calculate the dissimilarity. A clustering dendrogram is made from the genes using TOM-based dissimilarity and subsequently a minimum module size of 10 was chosen the genes were assigned to modules with genes of similar expression profiles. HG and ME were identified in each of the modules using the `moduleEigengenes` function and the

`chooseTopHubInEachModule` function in WGCNA. Each gene in the module is annotated to its distance from the ME this is the MM.

### 2.5.2 Networks and their applications

Networks are abstract models made up of nodes, vertices, and a set of edges. The nodes are the entities and the edges are the information that connects them (97). There are different types of networks for different situations that can yield different outputs, directed networks are formed when nodes are asymmetrical and mean one can influence the second, but the second cannot influence the first. Undirected networks are when the relationship between the nodes is symmetrical and is most useful for exploratory analysis of genes (97). Understanding the intricate relationship between diseases or disorders and their underlying mechanism is a subject that continues to challenge the areas of medicine and biology. There is clear evidence that there are disease-disease associations where two or more conditions can have similar or identical underlying mechanisms and understanding one can further the understanding of the other (80). The advancements of high throughput technologies like DNA microarray and next-generation sequencing have given researchers large scale genomic datasets (98). Constructing new biological pathways is generally achieved by using the interactions found from previous studies with gene regulation information for specific diseases or tissues, using system-level biological data is predicted to improve current knowledge of underlying mechanisms and lead to improvements in diagnosis, prognosis and treatments (99).

### 2.5.3 Cytoscape

Cytoscape is a free software project which combines expression data with biomolecular networks and aids in visualising, querying, and linking the data to functional annotation

databases (100). Functional proteomics and genomics techniques allow for measurements of expression profiles and interactions between cells and tissue to be collected which could potentially map cellular processes and their dynamics. From these expression profiles active biological processes can be identified using enriched gene annotation and by combining expression profiles and cellular network interactions changes in biological activity could be explained (101). Cytoscape allows for protein or gene properties to be associated with the nodes and edges by changing their appearances which allows for numerous types of data to be seen in a network context. It also includes a range of environments that can model gene transcription kinetics, biochemical reactions, and metabolic control, which advance biological research (100). To gain insight into the structure and organisation of a network Cytoscape's NetworkAnalyzer plugin was developed for visualisation and analysis (102). NetworkAnalyzer computes a set of topological parameters, including, the number of nodes, edges, network diameter, radius, density, centralization, heterogeneity, connected components, clustering coefficient and shortest path lengths (103).

### 2.5.4 Visualisation of modules using data from WGCNA in Cytoscape

Gene relationship data, module membership, weight and direction of the edge was saved to csv files Node files for each module in the developmental stage, this data was generated using WGCNA analysis in R. These CSV files were exported into Cytoscape (104) (version 3.7.1) using the `exportNetworkToCytoscape` function so each of the modules for the developmental stages could be visualised. Firstly, the edge data was exported, source and target node columns were selected, and the p-value for SNP inclusion determined by Pardiñas et al. (66) was marked as the source node attribute and weight which was filtered to 0.8 and above was selected as the edge attribute. To the node file

produced by Cytoscape, the node table for each of the modules was loaded in for the module membership of each node. Cytoscape's NetworkAnalyser was applied to each of the modules, each of the modules was treated as undirected. Using the visualise parameters function in NetworkAnalyzer the size of the Node (which referred to the genes) used the MM, edge width was mapped to the weight of the schizophrenia genes and the node colours were charted to the p-value determined by Pardiñas et al. (66).

## *2.6 Gene Ontologies (GO)*

A gene ontology defines a gene's function and how the functions of other genes are related to each other (105). GO is described with respects to three features: molecular function (the activities performed at a molecular level by the gene products), cellular components (where the gene product performs a function relative to a cellular structure), and, biological processes (the biological programs which are completed by several molecular activities) (105,106). As the knowledge of gene ontology is expanding so too are the databases that house them.

anRichment a package available in R is used to calculate ontology enrichment within the modules provided when compared to known reference gene sets such as KEGG, GO, Reactome, etc. (107). By using the function `enrichmentAnalysis` in anRichment and providing a module in the classes input and a collection reference gene set, GO enrichment analysis was applied (107). GO enrichment using anRichment was performed on each of the modules to identify biological processes which are over-represented in each of the modules (107). To run the analysis using `anRichment` the `enichmentAnalysis` function was run using GOcollection which is built using `org.Hs.eg.db` R package (108) and specifying species as human, the threshold was set at 1e-4, the threshold type

was Bonferroni, getOverlapEntrez = TRUE, getOverlapSymbols = TRUE and ignoring the grey module.

# Chapter 3 -Results

## *3.1 Data Pre-processing*

The schizophrenia-associated genes (genes tagged) from the GWAS meta-analysis by Pardiñas et al. (66) are found in the supplementary materials section NIHMS958804-supplement-Supplementary_Table.xlsx on the sheet titled "Supplementary Data Table 4: Independent genome-wide significant association signals from the CLOZUK + PGC meta-analysis, clumped and amalgamated into loci". This data was downloaded into R (66). The `dataset_5_stages` function available within the ABAData package was loaded into R, each of the five age categories was separated into their data frame and then filtered so they only included the schizophrenia-associated genes identified by Pardiñas et al (66). This gave five dataframes for each developmental Stage with 316 schizophrenia-associated genes expression over 16 distinct brain regions. Each of the dataframes was placed in a wide format where the gene names were rows, and the brain regions were columns. This is illustrated below in Table 3.1.

**Table 3.1: Wide-format of the schizophrenia-associated genes data frame. Brain areas available from ABAs Brainspan are the names of the columns in bold and the schizophrenia-associated genes are the row names. Each cell contains the scaled gene expression for each brain area**

| | **10163** | **10173** | **10185** | **10194** | **10209** | **10225** |
|---|---|---|---|---|---|---|
| **ABCB1** | 0.645635 | 0.193074 | 0.103555 | -1.09892 | 0.05936 | -1.24916 |
| **ABCB9** | -0.62942 | 0.871415 | 1.128378 | -0.6889 | -0.32854 | 0.351194 |
| **ABCD2** | 1.410626 | 0.488282 | 0.541653 | 0.88314 | 1.281896 | 0.40718 |
| **ACO2** | -0.66424 | -0.09813 | -0.02023 | -0.50082 | -0.88345 | -1.04544 |
| **ACP2** | -0.5259 | -0.74472 | -0.28589 | -0.63244 | -0.70708 | -0.90644 |
| **ACTR1A** | 0.84579 | 0.475071 | 0.904972 | 0.463709 | 1.186486 | 0.717958 |
| **ACTR5** | -0.97613 | 0.244193 | 0.215631 | -0.26563 | -1.25836 | -0.95691 |

## *3.2 Unsupervised learning using K-means analysis on the schizophrenia-associated genes*

Unsupervised learning looks for patterns in data. During this project, a large amount of gene expression data from the ABA was used to associate schizophrenia-associated genes based on the similarity of their expression profiles across brain areas. Unsupervised learning allows users to group the schizophrenia-associated genes into clusters.

## 3.3 Determining Optimal cluster Number using NbClust

K-means analysis was used to cluster each developmental stage to show the schizophrenia-associated genes cluster. As K-means requires the number of clusters to be prespecified, the `NbClust` function in R was used to determine the optimum number of clusters in each developmental stage as is illustrated in Figure 3.3.1.

`NbClust` uses thirty optima (91) ways of determining cluster number and produces a bar plot to represent how many times each number of clusters appeared. The optimal cluster numbers for developmental stages 1-5 are as seen in Figure 3.3.1. The `kmeans` function available in R was used to run the analysis and the package `factoextra` was used to visualise the clusters (109).

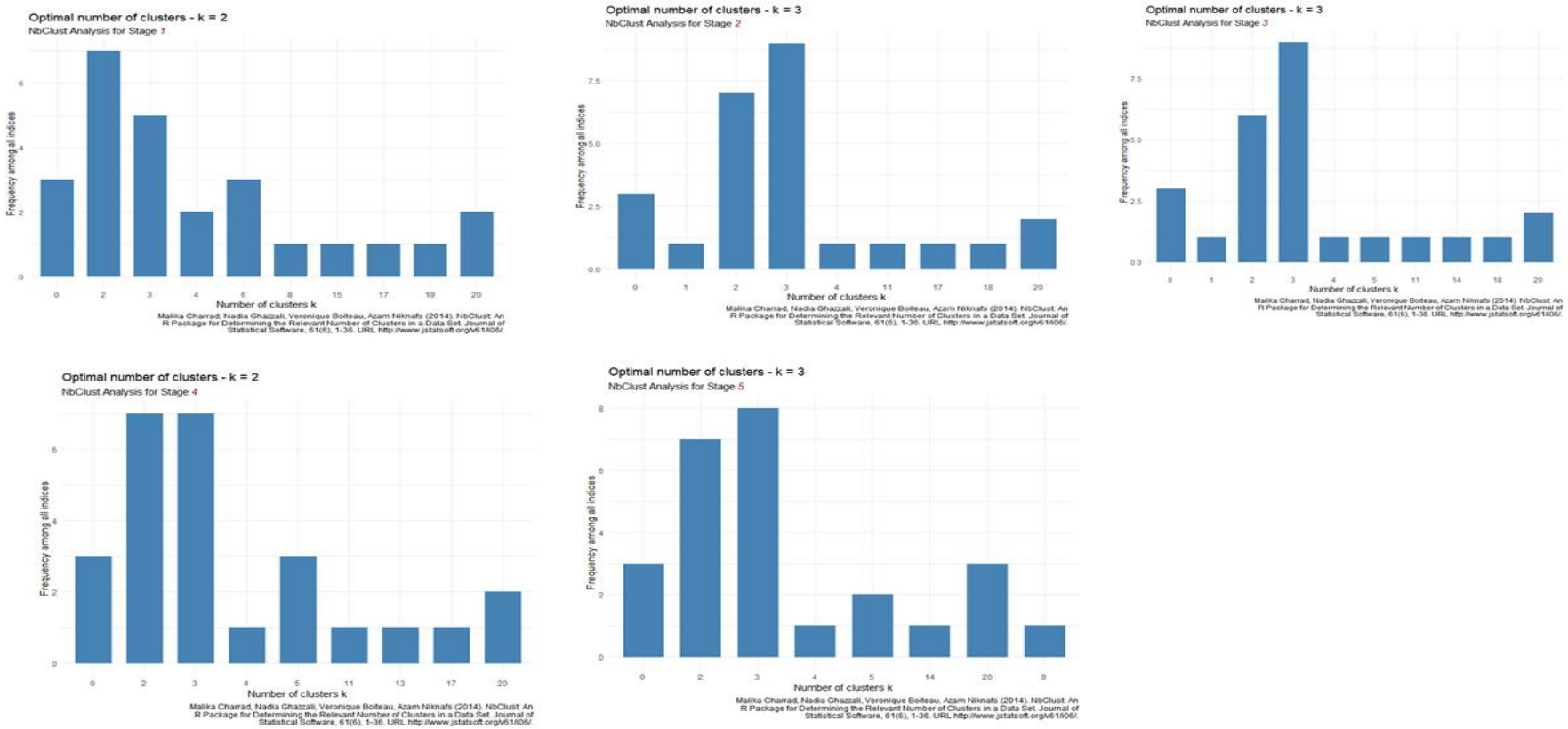



Figure 3.3.1 NbClust analysis performed on the schizophrenia genes identified by Pardiñas et al. to identify the optimal number of clusters (looking between 1-10 clusters) for K-means clustering on each of the five developmental stages available on ABA's BrainSpan resource

From the results in Figure 3.3.1, there is a clear indication of the optimal number for all clusters except Stage Four where 2 and 3 are both optimal.

Figure 3.3.2.-3.3.6 visualise the cluster assignments for each of the schizophrenia-associated genes in each developmental stage. Figure 3.3.7 exhibits the clusters of each developmental stage side by side for comparison.

The sum of squares of a cluster measures the total variance within a cluster. A smaller sum of squares means a more compact cluster which means there is internal cohesion in the cluster. A low total variance tells us that the genes in the Developmental stage are like each other, in good clustering, a total variance would achieve a high percentage where the difference between the groups would explain a majority of the total variance and the within-cluster variance would explain the small fraction left.

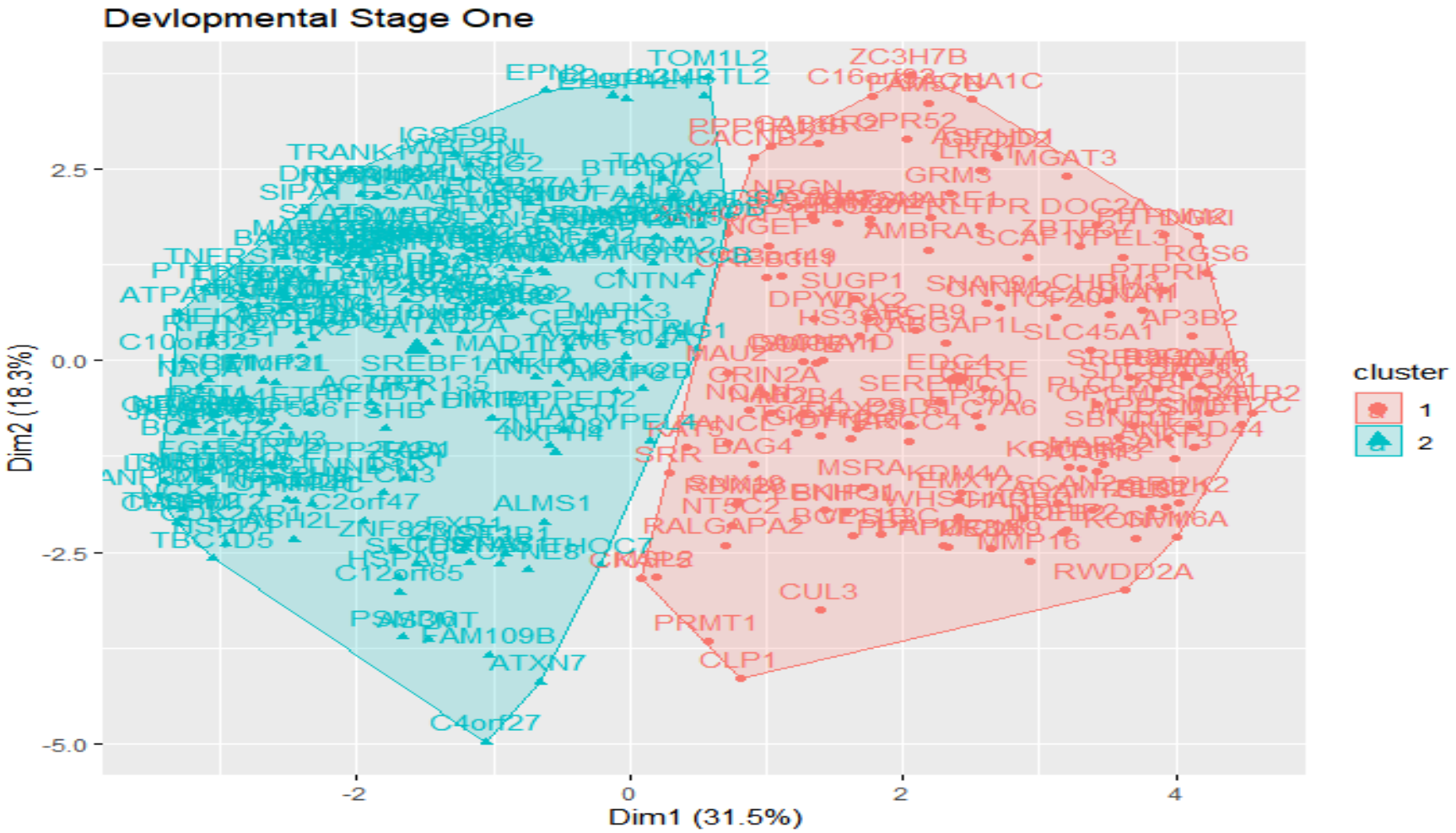



***Figure 3.3.2: K-means analysis on the Developmental Stage One genes using the kmeans function in R with 2 centres selected as per the NbClust recommendation and nstart= 25***

**Table 3.2: Sum of squares for each module in Developmental Stage One determined using the `k-means` function in R.**

| ***Cluster*** | ***1*** | ***2*** |
|---|---|---|
| **Sum of Squares per cluster** | 1714.970 | 2115.253 |

The total variance in the data (Between SS/ Total SS) = 24%

In Figure 3.3.2 cluster One is the most compact cluster, and this is confirmed by it having a smaller sum of squares seen in Table 3. Both cluster One and cluster Two have a very high within-cluster variance. A total variance value of 24% shows that the gene expression data in all of the genes are similar and most of the variance is explained by within-cluster variance. A higher total variance is more desirable with only a portion of the variance being explained within the clusters.

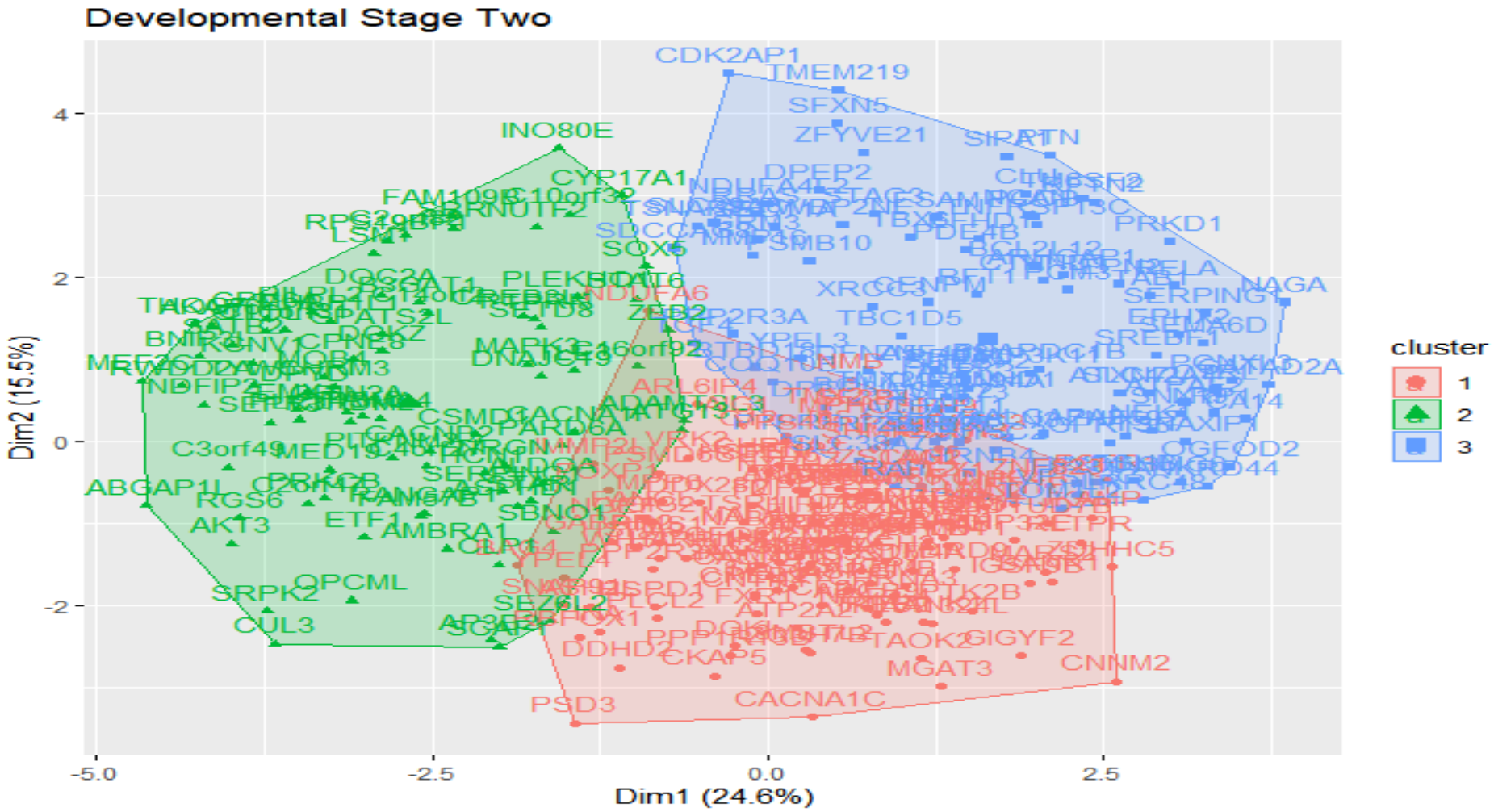



***Figure 3.3.3: K-means analysis on the Developmental Stage Two genes using the kmeans function in R with 3 centres selected as per the NbClust recommendation and nstart= 25.***

**Table 3.3: Sum of squares for each module in Developmental Stage Two determined using the kmeans function in R**

| ***Cluster*** | ***1*** | ***2*** | ***3*** |
|---|---|---|---|
| **Sum of squares of cluster** | 1339.484 | 1128.824 | 1247.380 |

Total variance in the data = 26.3%

In the clusters in Figure 3.3.3 each of the sum of squares is high, this tells us that there is a lot of in-cluster variation, the total variance of the data is also low at 26.3%. The high sum of squares within the clusters explains a lot of the total variance in the dataset.

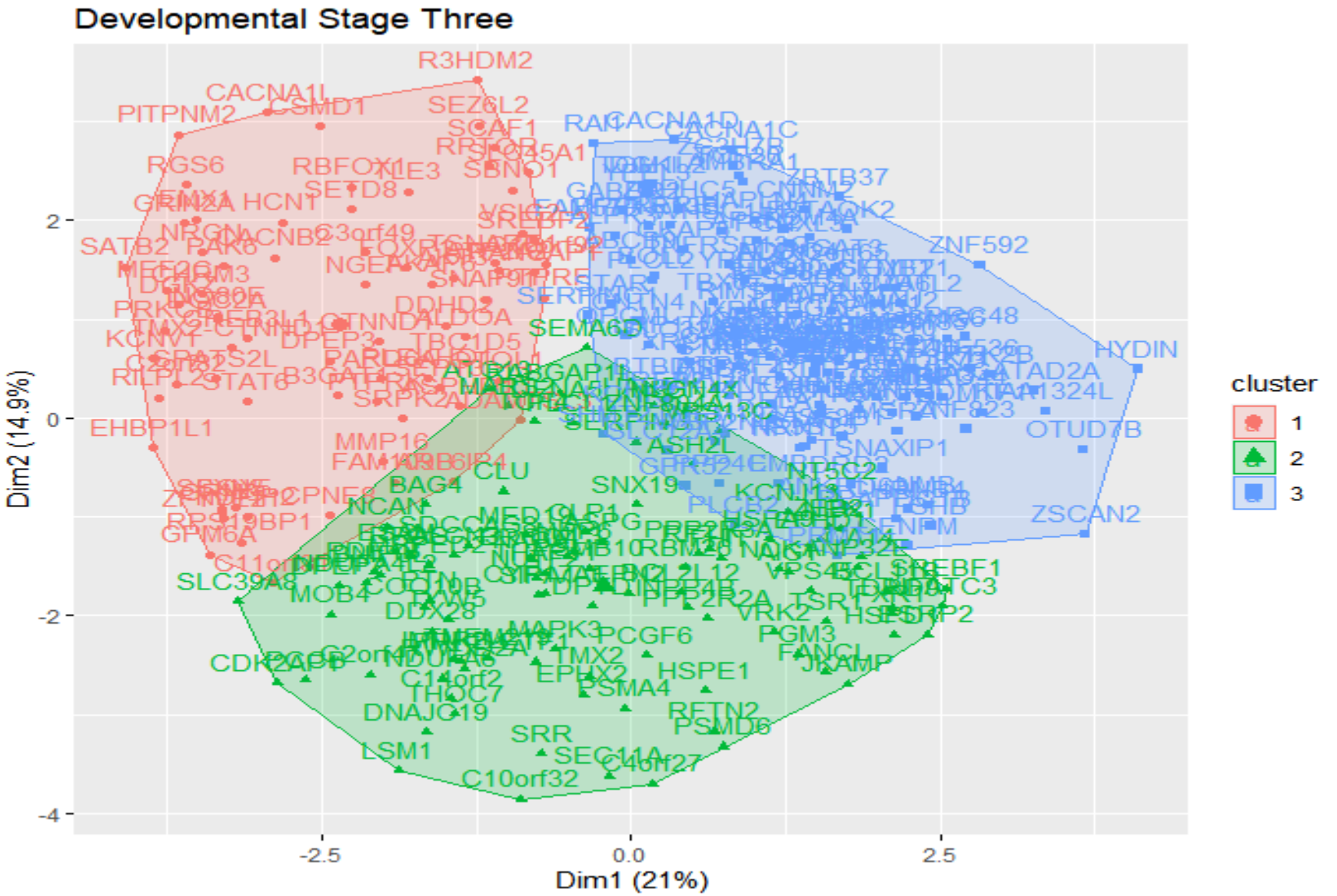



***Figure 3.3.4: K-means analysis on the Developmental Stage Three genes using the kmeans function in R with 3 centres selected as per the NbClust recommendation and nstart= 25***

**Table 3.4: Sum of squares for each module in Developmental Stage Three determined using the kmeans function in R**

| ***Cluster*** | ***1*** | ***2*** | ***3*** |
|---|---|---|---|
| **Sum of squares of cluster** | 925.8711 | 1544.4462 | 1389.3763 |

The total variance of data in module = 23.4%

The sum of squares of both clusters in Figure 3.3.4 tells us that again there is some variance in the clusters. The low total variance in the data also tells us that the clusters are similar. Ideally, the properties of good clustering would have clusters that are alike, and the other clusters would be very different giving a total variance of the data a percentage closer to 1.

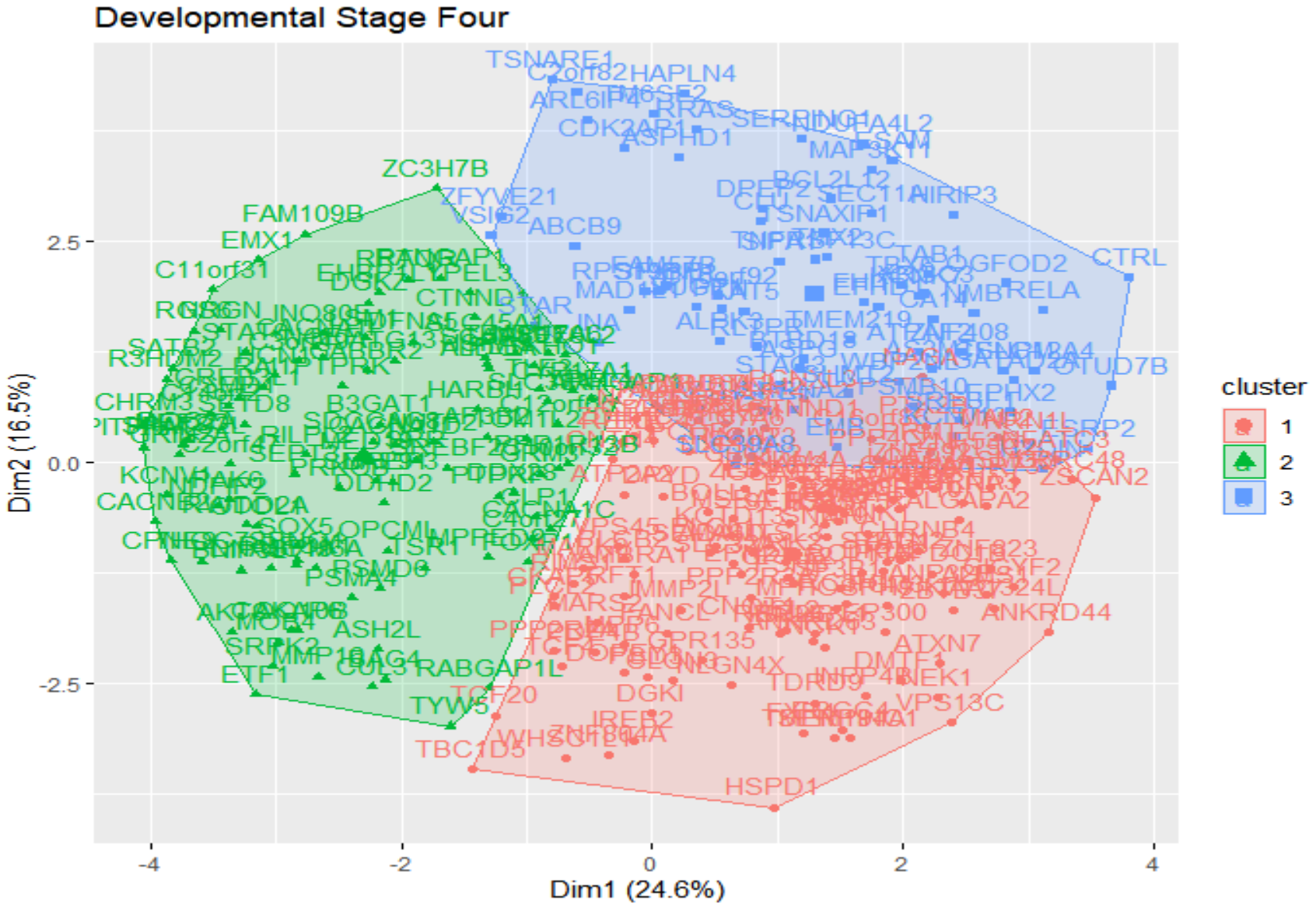



***Figure 3.3.5:** K-means analysis on the Developmental Stage Four genes using the kmeans function in R with 3 centres selected as per the NbClust recommendation and nstart= 25.*

**Table 3.5: Sum of squares for each module in Developmental Stage Four determined using the kmeans function in R**

| ***Cluster*** | ***1*** | ***2*** | ***3*** |
|---|---|---|---|
| **Sum of squares of cluster** | 1430.5423 | 1308.7500 | 993.0729 |

The total variance of data in module = 25.9%

The in-cluster variation in Figure 3.3.5 is especially high for cluster One whereas cluster Two and Three there has a similar cluster sum of squares. A large portion of the total variance in the module would be explained by cluster One.

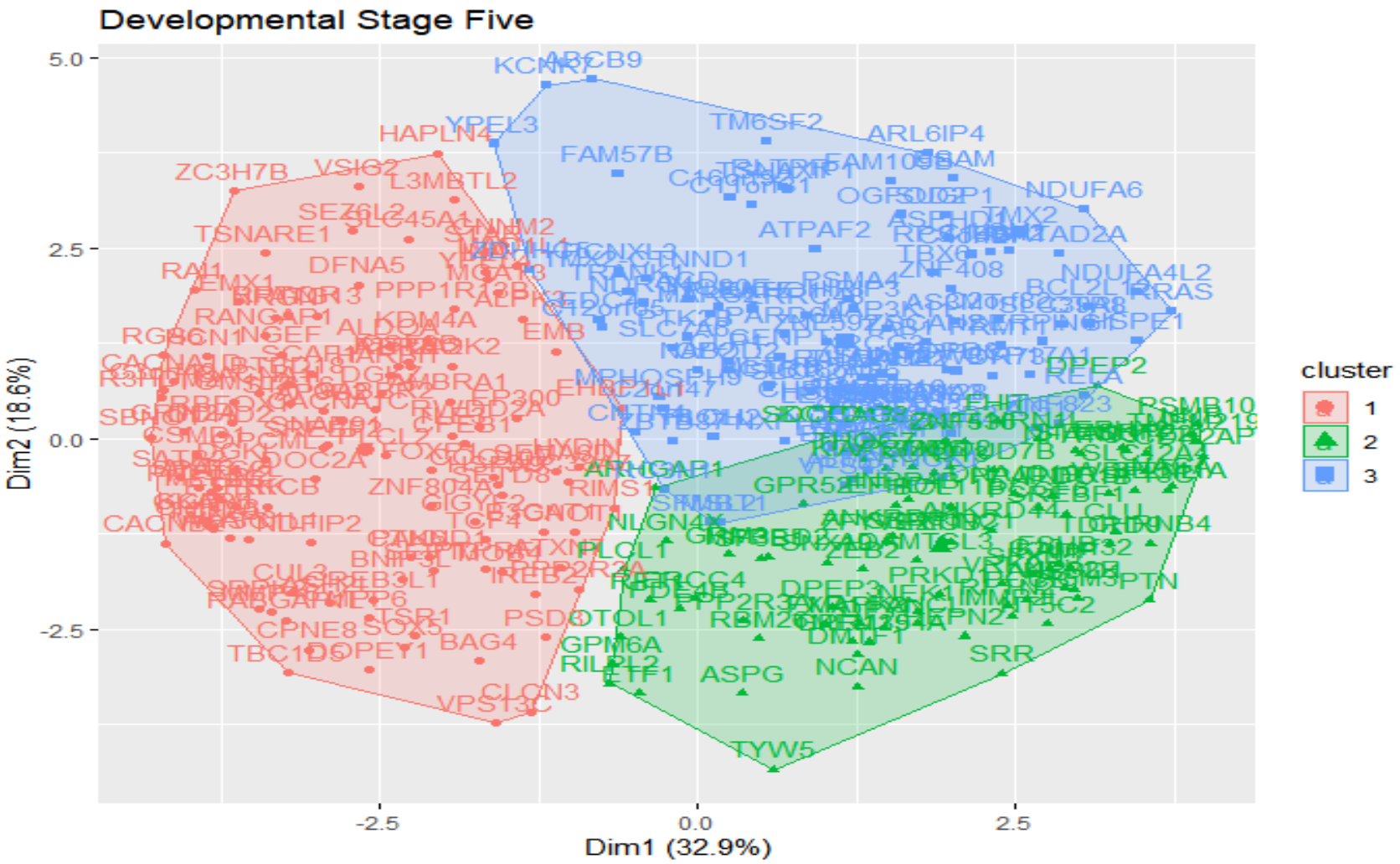



***Figure 3.3.6 K-means analysis on the developmental stage Five genes using the kmeans function in R with 3 centres selected as per the NbClust recommendation and nstart= 25***.

**Table 3.6: Sum of squares for each module in developmental stage Five determined using the kmeans function in R**

| ***Cluster*** | ***1*** | ***2*** | ***3*** |
|---|---|---|---|
| **Sum of squares of cluster** | 1268.6299 | 952.0207 | 1120.2446 |

The total variance of data in module = 33.7 %

In Figure 3.3.6 all of the clusters have high in-cluster variation and a cluster that is not compact, and it can be seen the total variance in the data is low at 33.7% meaning both clusters are similar so a large portion of the 33.7% variation will be explained by the within-cluster variance.

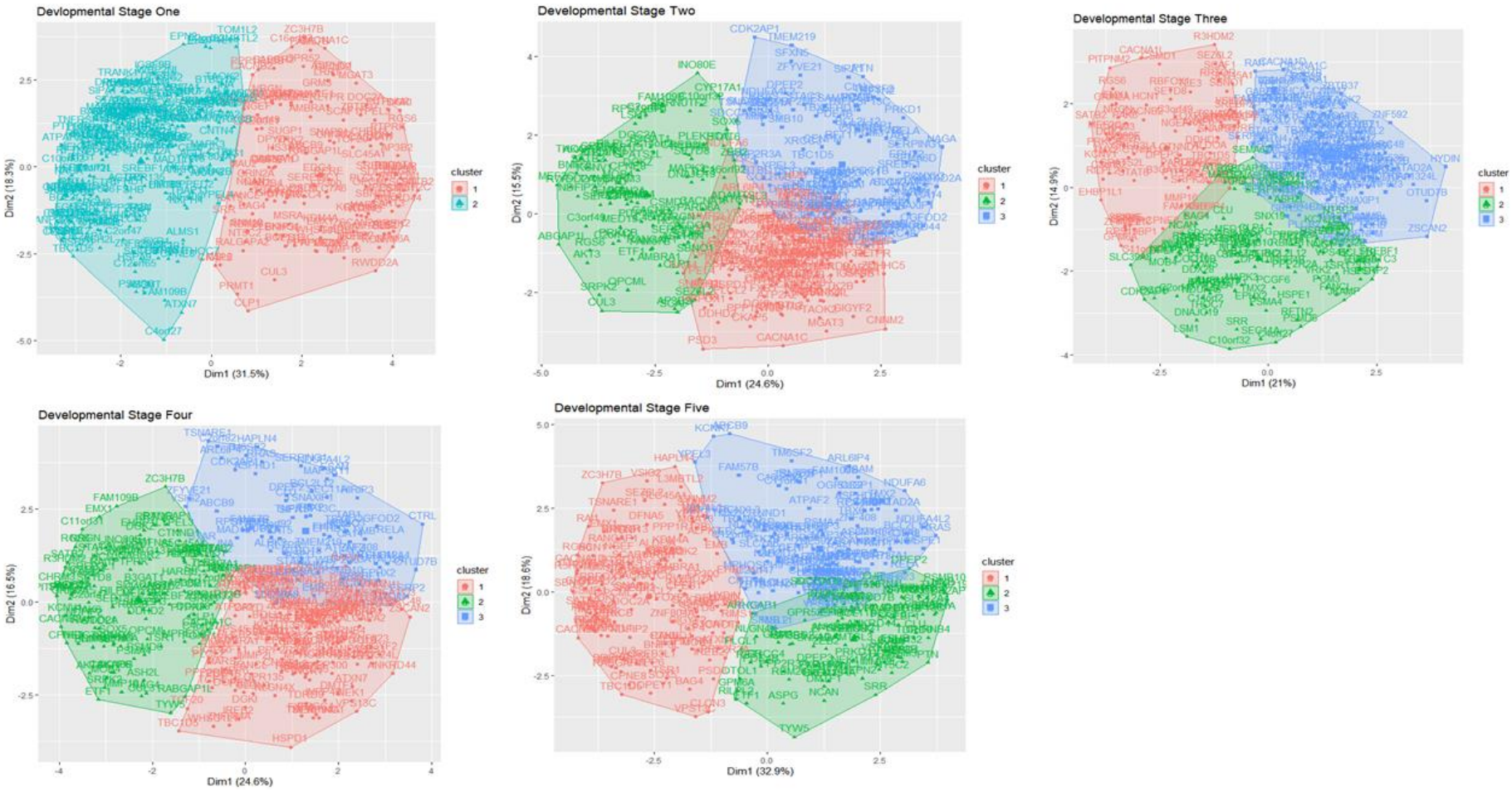



Figure 3.3.7: Kmeans analysis run on schizophrenia genes identified by Pardiñas et al. to determine intrinsic patterns within the genes at each of the developmental stages from ABAs Brainspan resource

## *3.4 WGCNA on schizophrenia-associated genes and Network Visualisation using Cytoscape*

We next employed WGCNA to undertake a system-level approach to identify networks of co-expressed modules of schizophrenia-associated genes. WGCNA is performed to organise highly correlated genes into gene modules.

First, an analysis of the topology of the networks at various soft thresholding powers was performed separately for developmental stages (results are illustrated in Figure 3.4.1). The soft threshold is calculated to identify the power of the gene correlation should be raised. By raising the correlation to this power, it will reduce the noise of any correlations in the adjacency matrix**.**

**Table 3.7: The Soft Thresholding power of each developmental stage calculated using WGCNA and shown in Figure 3.4.1**

| Developmental Stage | Soft Thresholding power |
|---|---|
| Stage One | 7 |
| Stage Two | 6 |
| Stage Three | 7 |
| Stage Four | 9 |
| Stage Five | 9 |

WGCNA is used to organise highly correlated genes into modules. Below a gene co-expression network is constructed which is represented by an adjacency matrix which signifies similar co-expression between a gene pair. Hierarchical clustering is used to identify modules and uses topological overlap to measure dissimilarity. Once the schizophrenia-associated genes are separated into modules these modules were summarised by calculating a module eigengene and defining an intramodular hub

gene. Then the modules were and visualised using Cytoscape's `NetworkAnalyzer`. Next, GO enrichment was performed on each of the modules using `anRichment` an R package (107).

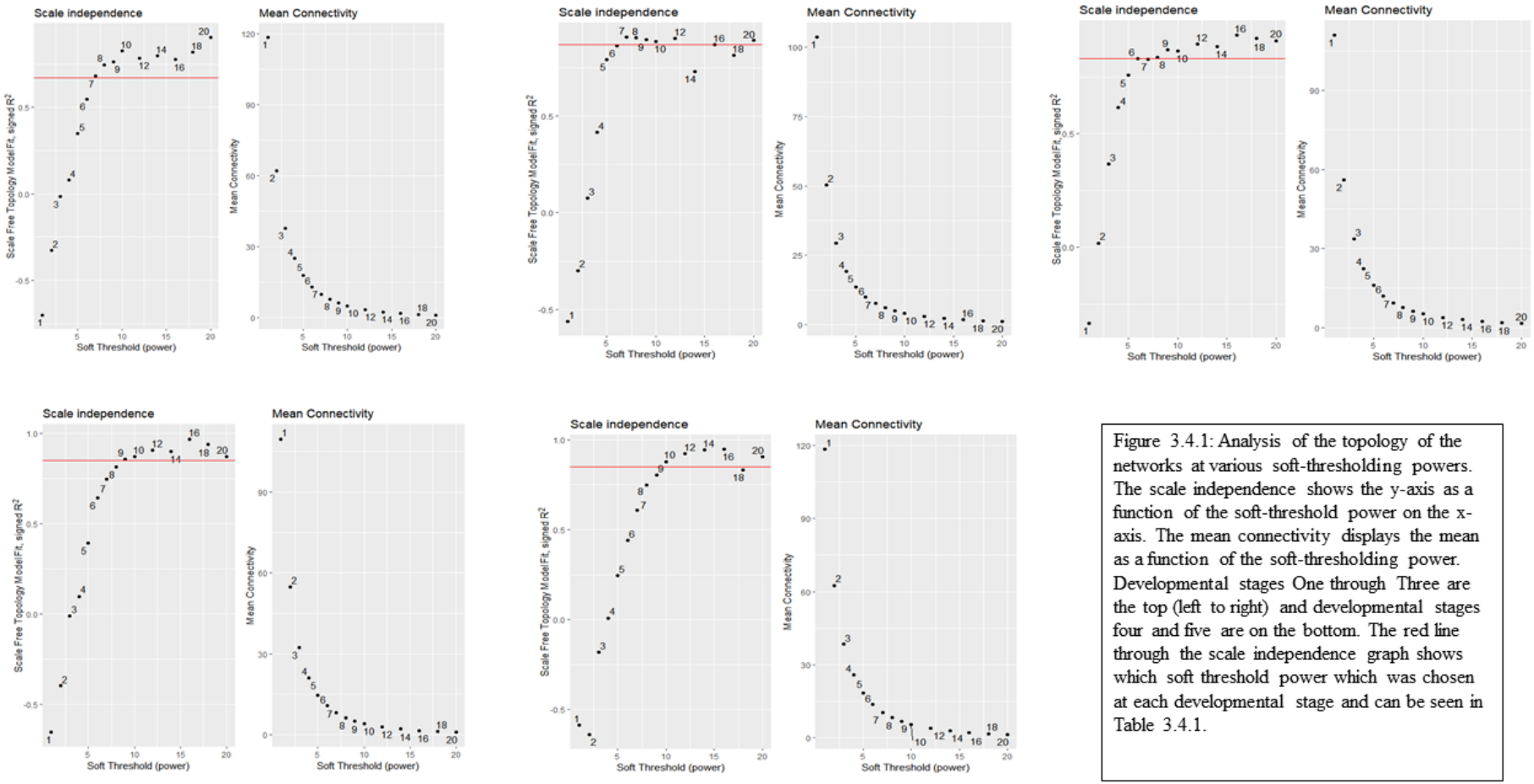



Figure 3.4.1: Analysis of the topology of the networks at various soft-thresholding powers. The scale independence shows the y-axis as a function of the soft-threshold power on the x-axis. The mean connectivity displays the mean as a function of the soft-thresholding power. Developmental stages One through Three are the top (left to right) and developmental stages four and five are on the bottom. The red line through the scale independence graph shows which soft threshold power which was chosen at each developmental stage and can be seen in Table 3.4.1.

After the differential topological matrix is calculated, a gene clustering dendrogram is plotted using the `hclust` function in R. Each leaf of the dendrogram is a gene and after the minimum module is set, the genes may remerge. The dendrogram clusters the branches into coloured modules but some of the modules may need to merge because their genes are highly co-expressed. This is done by calculating eigengenes of each module and re-clustering based on the module eigengene dissimilarity correlations using the `mergeCloseModules` function in WGCNA. Once the modules have merged the module eigengene is re-calculated. WGCNA identified 7 modules in Stage One, 3 modules in Stage Two, 5 modules in Stage Three, 6 modules in Stage Four and 9 modules in Stage Five.

The merged modules and initial modules are illustrated in Figure 3.4.2. After the modules are merged the eigengene is recalculated and in Figure 3.4.3 the adjacency of the eigengene compared to the other eigengenes in the developmental stage is shown.

Network heatmap plot for the developmental stages one through five, using the function `TOMplot` in `WGCNA`, were created to visualise the topological overlap matrix (illustrated in Figure 3.4.4). This TOM matrix uses the adjacency matrix to build another adjacency matrix which takes topological overlap (the number of shared neighbours of the nodes).

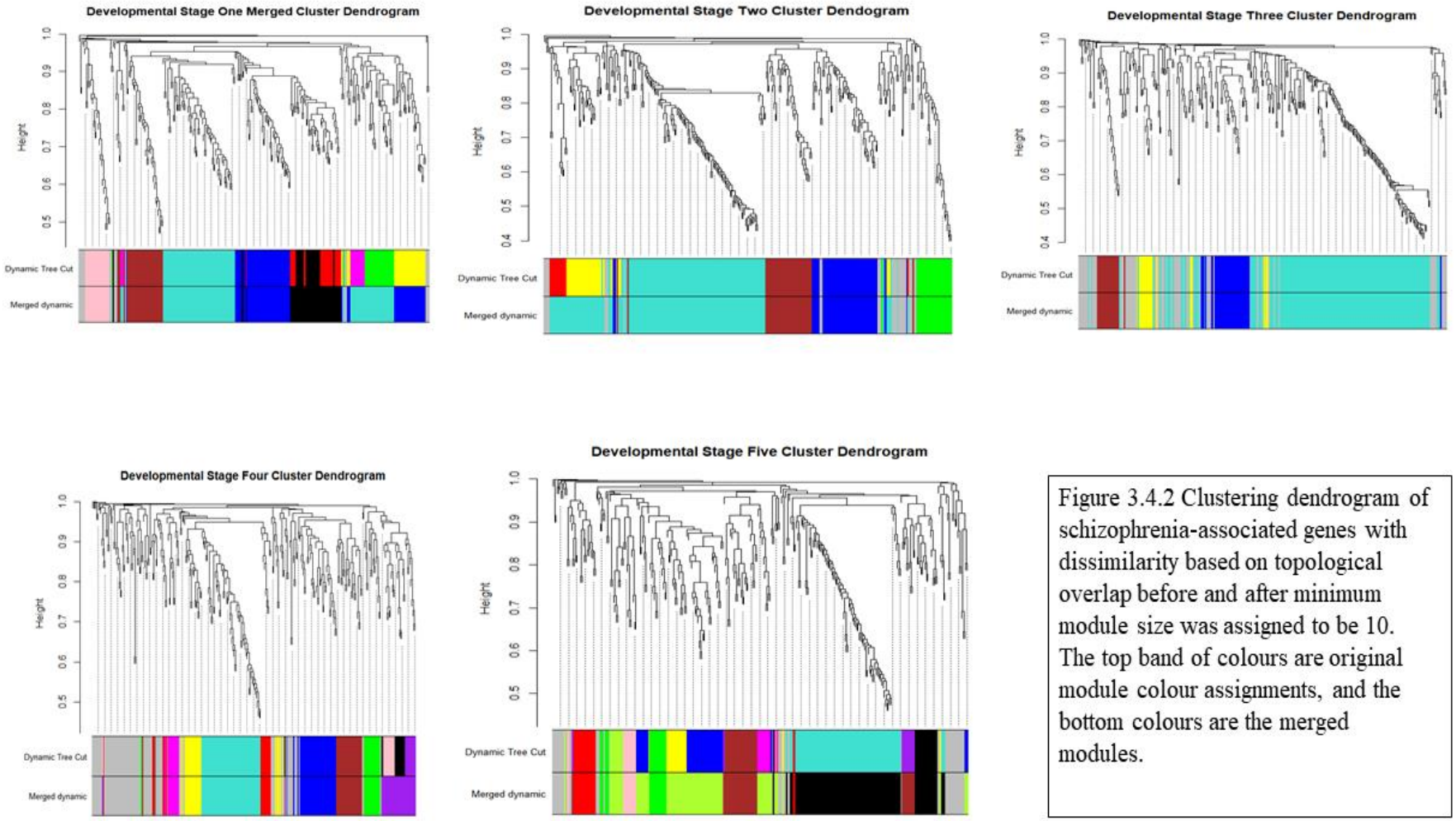
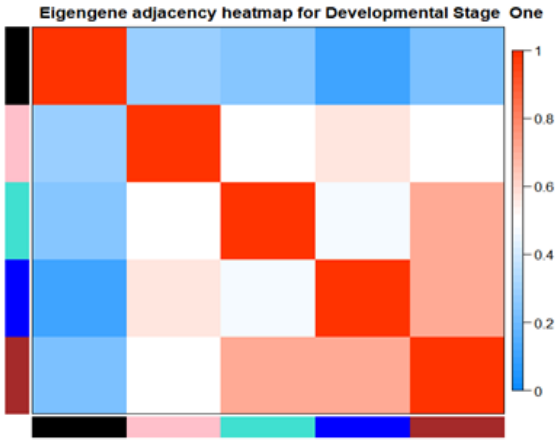
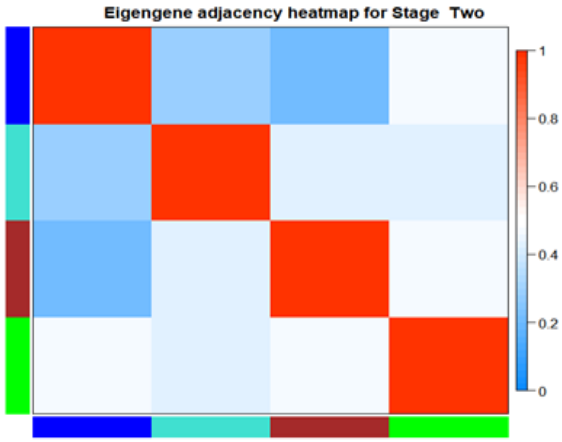
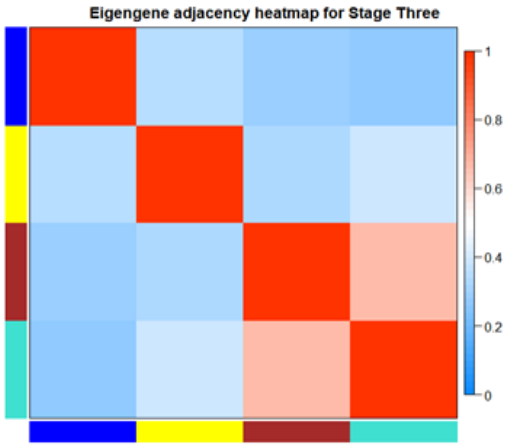
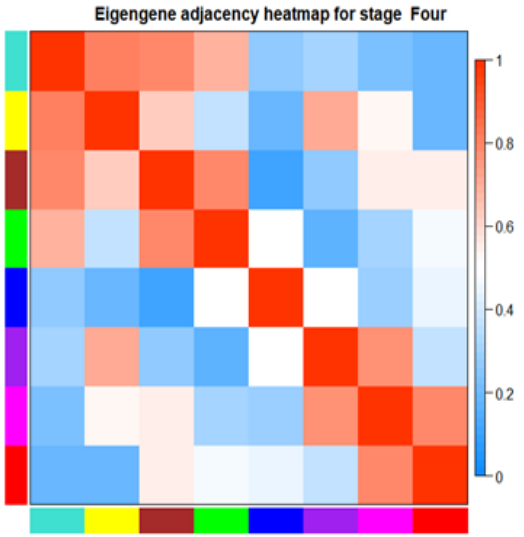



Figure 3.4.2 Clustering dendrogram of schizophrenia-associated genes with dissimilarity based on topological overlap before and after minimum module size was assigned to be 10. The top band of colours are original module colour assignments, and the bottom colours are the merged modules.



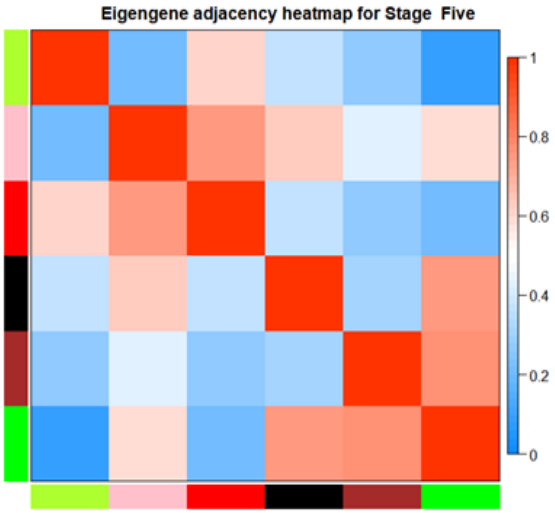




Figure 3.4.3 Eigengene adjacency heatmap for each eigengene network in each developmental stage. Each row and column indicate an eigengene labelled by its module colour. Orange indicates high correlation and blue indicates low correlation.

## *3.5 Intramodular Hub Genes and Network Analysis*

Hub genes (absolute module membership $\geqslant 0.8$) for each module within each stage were identified using the function `chooseTopHubInEachModule` in the `WGCNA` package. Identified HGs for each module, the function of each gene, as defined by NCBI and their association with disease phenotypes are outlined in Table 9.

**Table 3.8: Gene Functions and the phenotypes they are involved in for each hub genes identified by the WGCNA function in R when performed on the schizophrenia-associated genes identified by Pardiñas et al. for the five developmental stages available on ABA's Brainspan.**

| Module Colour | Hub Gene | Gene Name | Genomic Location | Function (NCBI gene and Gene ontology) | Association with other Conditions | References (PMID ID) |
|---|---|---|---|---|---|---|
| | | | | ***Stage One*** | | |
| *Black* | *SOX5* | SRY-Box Transcription Factor 5 | NC_000012.12 | This gene encodes a member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of embryonic development and the determination of cell fate. The encoded protein may act as a transcriptional regulator after forming a protein complex with other proteins. The encoded protein may play a role in chondrogenesis | Lamb-Shaffer Syndrome and Optic Nerve Hypoplasia, Bilateral. | 31578471 |
| Blue | *FHIT* | Fragile Histidine Triad Diadenosine Triphosphatase | NC_000003.12 | The protein encoded by this gene is a P1-P3-bis(5'-adenosyl) triphosphate hydrolase involved in purine metabolism. This gene encompasses the common fragile site FRA3B on chromosome 3, where carcinogen-induced damage can lead to translocations and aberrant transcripts. Aberrant transcripts from this gene have been found in about half of all oesophageal, stomach, and colon carcinomas. The encoded protein is also a tumour suppressor, as loss of its activity results in replication stress and DNA damage. | Renal Cell Carcinoma, Nonpapillary and Sporadic Breast Cancer. | 28404875 |
| Brown | *SLC12A4* | Solute Carrier Family 12 Member 4 | NC_000016.10 | The encoded protein controls the movement of potassium and chloride ions across the plasma membrane. | Sickle Cell and Fisheye disease | 31792382 |
| Pink | *OTOL1* | Otolin 1 | NC_000003.12 | Secreted glycoprotein with a C-terminal complement Cq1-like globular domain that belongs to the C1q/tumour necrosis factor-related protein (CTRP) family. The encoded protein is expressed in the inner ear and forms a multimeric complex called the otoconia, together with cerebellin-1 and otoconin-90, as part of the otoconial membrane. It contains extensive posttranslational modifications including hydroxylated prolines and glycosylated lysine's | Benign Paroxysmal Positional Nystagmus and Vestibular Disease | 29533337, 31120422 |

| Module Colour | Hub Gene | Gene Name | Genomic Location | Function (NCBI gene and Gene ontology) | Association with other Conditions |
|---|---|---|---|---|---|
| | | | ***Stage Two*** | | |
| Blue | *KCNV1*-Potassium Voltage-Gated Channel Modifier Subfamily V Member 1 | NC_000008.11 | Potassium channel subunit that does not form functional channels by itself. Modulates KCNB1 and KCNB2 channel activity by shifting the threshold for inactivation to more negative values and by slowing the rate of inactivation. Can down-regulate the channel activity of KCNB1, KCNB2, KCNC4 and KCND1, possibly by trapping them in intracellular membranes. | Atrial Septal Defect 5 and Familial Adult Myoclonic Epilepsy | 25969726 |
| Brown | *NDRG4*-NDRG Family Member 4 | NC_000016.10 | This gene is a member of the N-myc downregulated gene family which belongs to the alpha/beta hydrolase superfamily. The protein encoded by this gene is a cytoplasmic protein that is required for cell cycle progression and survival in primary astrocytes and may be involved in the regulation of mitogenic signalling in vascular smooth muscles cells | Infantile Myofibromatosis and Pulmonary Atresia With Ventricular Septal Defect. | 31832525, 19711485 |
| Green | *GPR52*- G Protein-Coupled Receptor 52 | NC_000001.11 | Members of the G protein-coupled receptor (GPR) family play important roles in signal transduction from the external environment to the inside of the cell | Huntington's disease | 33796846, 24587241 |
| Turquoise | *STAG1*-Stromal Antigen 1 | NC_000003.12 | SCC3 family and is expressed in the nucleus. It encodes a component of cohesin, a multi-subunit protein complex that provides sister chromatid cohesion along the length of a chromosome from DNA replication through prophase and prometaphase, after which it is dissociated in preparation for segregation during anaphase. | Mental Retardation, Autosomal Dominant 47 and Cornelia De Lange Syndrome. | 2467316, 28430577, 28119487, 32778134 |

| Module Colour | Hub Gene | Gene Name | Genomic Location | Function (NCBI gene and Gene ontology) | Association with other Conditions | References (PMID ID) |
|---|---|---|---|---|---|---|
| | | | ***Stage Three*** | | | |
| Blue | *SATB2* | SATB homeobox 2 | NC_000002.12 (199269500.199471266, complement) | SATB2 encodes for a DNA binding protein that binds specifically at nuclear matrix attachment regions. These regions are involved in chromatin remodelling and transcription regulation. | Glass syndrome (with intellectual disability) | 24301056 |
| Brown | *INA* | Internexin Neuronal Intermediate Filament Protein Alpha | NC_000010.11 | Neurofilaments are type IV intermediate filament heteropolymers composed of light, medium, and heavy chains. Neurofilaments comprise the axoskeleton and they functionally maintain the neuronal calibre. They may also play a role in intracellular transport to axons and dendrites. This gene is a member of the intermediate filament family and is involved in the morphogenesis of neurons | Gastroenteropancreatic Neuroendocrine Neoplasm and Medulloepithelioma. | 29339073 |
| Turquoise | *TRANK1* | Tetratricopeptide repeat and ankyrin repeat-containing 1 | NC_000003.12 (36826817.36945662, complement) | | Associated with BPD | 24309898 |
| Yellow | *ANKRD63* | Ankyrin repeat domain 63 | NC_000015.10 (40278372.40282586, complement) | | | |

| Module Colour | Hub Gene | Gene Name | Genomic Location | Function (NCBI gene and Gene ontology) | Association with other Conditions | References (PMID ID) |
|---|---|---|---|---|---|---|
| ***Stage Four*** | | | | | | |
| Blue | *MEF2C* | Myocyte Enhancer Factor 2C | NC_000005.10 | Encodes a member of the MADS-box transcription enhancer factor 2 (MEF2) family of proteins, which play a role in myogenesis. The encoded protein, MEF2 polypeptide C, has both trans-activating and DNA binding activities. This protein may play a role in maintaining the differentiated state of muscle cells. Mutations and deletions at this locus have been associated with severe cognitive disability, stereotypic movements, epilepsy, and cerebral malformation | Mental Retardation, Autosomal Dominant 20 and autism spectrum disorder. | 32418612, 27779093 |
| Brown | *SMG6* | SMG6 Nonsense Mediated mRNA Decay Factor | NC_000017.11 | This gene encodes a component of the telomerase ribonucleoprotein complex responsible for the replication and maintenance of chromosome ends. The encoded protein also plays a role in the nonsense-mediated mRNA decay (NMD) pathway, providing the endonuclease activity near the premature translation termination codon that is needed to initiate NMD | Pancreatic Adenosquamous Carcinoma and Lissencephaly. | 25770585 |
| Green | *TAOK2* | TAO Kinase 2 | NC_000016.10 | Involved in many different processes, including, cell signalling, microtubule organization and stability, and apoptosis. | Wilson-Turner X-Linked Mental Retardation Syndrome and Syndromic X-Linked Intellectual Disability | 29467497 |
| Magenta | *OPCML* | Opioid Binding Protein/Cell Adhesion Molecule Like | NC_000011.10 | Bind's opioids in the presence of acidic lipids; probably involved in cell contact. | Ovarian Cancer and Hypogonadotropic Hypogonadism 14 With Or Without Anosmia. | 29907679, 33777925, 31577955 |
| Purple | *GPR52* | G Protein-Coupled Receptor 52 | NC_000001.11 | Members of the G protein-coupled receptor (GPR) family play important roles in signal transduction from the external environment to the inside of the cell | Psychiatric disorders | 33796846, 24587241 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Red | *WHSC1L1/NSD3* | Nuclear Receptor Binding SET Domain Protein 3 | NC_000008.11 | Histone methyltransferase. Preferentially dimethylates 'Lys-4' and 'Lys-27' of histone H3 forming H3K2me2 and H3K27me2. H3 'Lys-4' methylation represents a specific tag for epigenetic transcriptional activation, while 'Lys-27' is a mark for transcriptional repression | Wolf-Hirschhorn Syndrome and Nut Midline Carcinoma | 31190890, 27285764, 25942451 |
| Turquoise | *CA8* | Carbonic anhydrase 8 | NC_000008.11 (60185412.60281400, complement) | In the carbonic anhydrase family but carbonic anhydrase activity (i.e., the reversible hydration of carbon dioxide) The absence of CA8 gene transcription in the cerebellum of the lurcher mutant in mice with a neurologic defect suggests an important role for this acatalytic form. | Mutations in this gene are associated with cerebellar ataxia, mental retardation, and disequilibrium syndrome 3 (CMARQ3). Polymorphisms in this gene are associated with osteoporosis, and overexpression of this gene in osteosarcoma cells suggests an oncogenic role. | 19461874 |
| Yellow | *ALMS1* | ALMS1 Centrosome and Basal Body Associated Protein | NC_000002.12 | Involved in PCM1-dependent intracellular transport. Required, directly or indirectly, for the localization of NCAPD2 to the proximal ends of centrioles. Required for proper formation and/or maintenance of primary cilia (PC), microtubule-based structures that protrude from the surface of epithelial cells. | Alstrom Syndrome and Premature Ovarian Failure 1. | 30421101, 32808654 |

| Developmental Stage | Hub Gene | Gene Name | Genomic Location | Function (NCBI gene and Gene ontology) | Association with other Conditions | References (PMID ID) |
|---|---|---|---|---|---|---|
| ***Stage Five*** | | | | | | |
| Black | *C16orf86* | Chromosome 16 Open Reading Frame 86 | NC_000016.10 | Protein Coding Gene | | 33639916 |
| Brown | *RFTN2* | Raftlin Family Member 2 | NC_000002.12 | Upon bacterial lipopolysaccharide stimulation, mediates clathrin-dependent internalization of TLR4 in dendritic cells, resulting in activation of TICAM1-mediated signalling and subsequent IFNB1 production. May regulate B-cell antigen receptor-mediated signalling. | Glass Syndrome. | |
| Green | *NFATC3* | Nuclear Factor of Activated T Cells 3 | NC_000016.10 | Acts as a regulator of transcriptional activation. Plays a role in the inducible expression of cytokine genes in T-cells, especially in the induction of the IL-2 (PubMed:18815128). Along with NFATC4, involved in embryonic heart development | Crouzon Syndrome with Acanthosis Nigricans and Leukostasis | 31249342, 33520407 |
| Greenyellow | *SATB2* | SATB homeobox 2 | NC_000002.12 (199269500.199 471266, complement) | SATB2 encodes for a DNA binding protein that binds specifically at nuclear matrix attachment regions. These regions are involved in chromatin remodelling and transcription regulation. | Glass syndrome (with intellectual disability) | 24301056 |
| Pink | *CHRNA5* | Cholinergic Receptor Nicotinic Alpha 5 Subunit | NC_000015.10 | The protein encoded by this gene is a nicotinic acetylcholine receptor subunit and a member of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses. | Smoking as a Quantitative Trait Locus 3and Tobacco Addiction | 33752734, 30366711, 32817066, 33511332 |
| Red | *INA* | Internexin Neuronal Intermediate Filament Protein Alpha | NC_000010.11 | Neurofilaments are type IV intermediate filament heteropolymers composed of light, medium, and heavy chains. Neurofilaments comprise the axoskeleton and they functionally maintain the neuronal calibre. They may also play a role in intracellular transport to axons and dendrites. This gene is a member of the intermediate filament family and is involved in the morphogenesis of neurons | Gastroenteropancreatic Neuroendocrine Neoplasm and Medulloepithelioma. | 29339073 |

## 3.6 Cytoscape and Network Visualisation

`NetworkAnalyzer`, a Cytoscape plugin, aids in visualisation but can also calculate a network's topological properties (103). Using data loaded into Cytoscape including source nodes, target nodes, the p-value for SNP inclusion identified by Pardiñas et al, weights and node attributes, the network could be visualised using `NetworkAnalyzer`. `NetworkAnalyzer` also calculates the properties of a network as shown in Figure 3.6.1, treating the network as undirected. The clustering coefficient is a ratio of closed triangles over the total open and closed triangles (110). Connected components measure the number of separated fragments in the overall network. Network diameter is the largest number of edges to transverse the network. Network centralization measures the centrality of each node in the network, a network that is highly centralized (= 1) contains a few nodes that dominate the network and without these nodes, the network would become fragmented and leave unconnected sub-networks (111). Network density is a measure of how densely a network is filled with edges, where 0 means there are no edged and 1 means the network is highly populated with edges. Network heterogeneity is a measure of the diversity of the number of connections a node has shown by the node degrees where a homogenous network is equal to 0 and 1 is heterogeneous (112). Networks containing biological data are usually very heterogeneous where most nodes have very few edges apart from HGs which are highly connected (113). The network visualisations for the modules can be seen in the appendix and the network topological parameters are below in Table 3.9 -3.13.

**Table 3.9 Network topology parameters calculated by NetworkAnalyzer in Cytoscape for Developmental Stage One.**

| Module Colour | Cluster Coefficient | Diameter | Network Centralization | Number of Nodes | Network Density | Network Heterogeneity |
|---|---|---|---|---|---|---|
| Black | 0.61 | 5 | 0.414 | 47 | 0.299 | 0.63 |
| Blue | 0.55 | 6 | 0.271 | 74 | 0.161 | 0.777 |
| Brown | 0.634 | 5 | 0.378 | 32 | 0.323 | 0.66 |
| Pink | 0.72 | 4 | 0.421 | 20 | 0.411 | 0.551 |
| Turquoise | 0.477 | 7 | 0.258 | 101 | 0.127 | 0.805 |

**Table 3.10 Network topology parameters calculated by NetworkAnalyzer in Cytoscape for Developmental Stage Two.**

| Module Colour | Cluster Coefficient | Diameter | Network Centralization | Number of Nodes | Network Density | Network Heterogeneity |
|---|---|---|---|---|---|---|
| Blue | 0.595 | 5 | 0.356 | 33 | 0.197 | 0.654 |
| Brown | 0.585 | 4 | 0.308 | 14 | 0.429 | 0.554 |
| Turquoise | 0.57 | 11 | 0.281 | 144 | 0.177 | 0.922 |
| Yellow | 0.365 | 6 | 0.21 | 16 | 0.217 | 0.62 |

**Table 3.11 Network topology parameters calculated by NetworkAnalyzer in Cytoscape for Developmental Stage Three**

| Module Colour | Cluster Coefficient | Diameter | Network Centralization | Number of Nodes | Network Density | Network Heterogeneity |
|---|---|---|---|---|---|---|
| Blue | 0.595 | 5 | 0.356 | 33 | 0.197 | 0.654 |
| Brown | 0.585 | 4 | 0.308 | 14 | 0.429 | 0.554 |
| Turquoise | 0.57 | 11 | 0.281 | 144 | 0.177 | 0.922 |
| Yellow | 0.365 | 6 | 0.21 | 16 | 0.217 | 0.62 |

**Table 3.12 Network topology parameters calculated by NetworkAnalyzer in Cytoscape for Developmental Stage Four.**

| Module Colour | Cluster Coefficient | Diameter | Network Centralization | Number of Nodes | Network Density | Network Heterogeneity |
|---|---|---|---|---|---|---|
| Blue | 0.636 | 4 | 0.348 | 38 | 0.265 | 0.558 |
| Brown | 0.578 | 5 | 0.274 | 25 | 0.29 | 0.417 |
| Green | 0.373 | 4 | 0.2125 | 17 | 0.25 | 0.522 |
| Magenta | 0.397 | 4 | 0.327 | 12 | 0.273 | 0.593 |
| Purple | 0.582 | 4 | 0.338 | 32 | 0.264 | 0.553 |
| Red | 0.37 | 4 | 0.359 | 14 | 0.308 | 0.598 |
| Turquoise | 0.708 | 4 | 0.316 | 58 | 0.379 | 0.537 |
| Yellow | 0.648 | 5 | 0.379 | 21 | 0.357 | 0.512 |

**Table 3.13 Network topology parameters calculated by NetworkAnalyzer in Cytoscape for Developmental Stage Five.**

| Module Colour | Cluster Coefficient | Diameter | Network Centralization | Number of Nodes | Network Density | Network Heterogeneity |
|---|---|---|---|---|---|---|
| Black | 0.643 | 5 | 0.289 | 104 | 0.202 | 0.781 |
| Brown | 0.642 | 5 | 0.244 | 36 | 0.256 | 0.522 |
| Green | 0.614 | 4 | 0.379 | 19 | 0.327 | 0.634 |
| Greenyellow | 0.544 | 6 | 0.262 | 77 | 0.179 | 0.678 |
| Pink | 0.538 | 4 | 0.309 | 12 | 0.379 | 0.425 |
| Red | 0.44 | 4 | 0.288 | 19 | 0.298 | 0.51 |

## 3.7 Gene Ontologies

Gene ontologies that are enriched for each module within each stage were identified using the function `enrichmentAnalysis` which uses the GO collection database in the anRichment Bioconductor package in R. Finding GOs which are enriched in each of the modules can deepen the understanding of schizophrenia and lead to the discovery of novel therapeutic targets.

Table 10 – Table 14 displays the most enriched gene ontologies per module per stage. In the appendix Tables 10 – 27 illustrate the full gene ontologies identified by anRichment. It is clear from the ontology results that positive regulation of macrophage proliferation is very important as it appears in the top 3 ontologies in a significant number of the modules per Developmental Stage. Cellular response to catecholamine stimulus and cellular response to diacyl bacterial lipopeptide appear in the top three ontologies at least once per stage also. These enriched ontologies are candidates for future investigation.

**Table 3.9: Gene Ontologies of the top enriched gene ontologies in Stage One using anRichment**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process/ Term | FDR |
|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Black | positive regulation of macrophage proliferation | 2.92E-15 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Black | binding | 1.88E-10 |
| GO:0043231 | The organised structure of distinctive morphology and function bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Black | intracellular membrane-bounded organelle | 6.74E-10 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Blue | positive regulation of macrophage proliferation | 1.75E-29 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Blue | intracellular organelle lumen | 2.74E-14 |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl) benzene-1,2-diol] and derivatives formed by substitution. | BP | Blue | cellular response to catecholamine stimulus | 4.03E-13 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 2.10E-15 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Brown | binding | 3.36E-10 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Brown | intracellular organelle lumen | 4.32E-09 |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Pink | protein binding | 1.61E-08 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Pink | positive regulation of macrophage proliferation | 2.30E-07 |
| GO:0008152 | The chemical reactions and pathways, including anabolism and catabolism, by which living organisms transform chemical substances. Metabolic processes typically transform small molecules, but also include macromolecular processes such as DNA repair and replication, and protein synthesis and degradation. | BP | Pink | metabolic process | 6.41E-07 |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Turquoise | positive regulation of macrophage proliferation | 9.49E-49 |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to diacyl bacterial lipopeptide | 1.07E-17 |
| GO:0070016 | Interacting selectively and non-covalently with the armadillo repeat domain | MF | Turquoise | armadillo repeat domain binding | 4.10E-17 |

| | | | | | |
|---|---|---|---|---|---|
| | of a protein, an approximately 40 amino acid long tandemly repeated sequence motif first identified in the Drosophila segment polarity protein armadillo. Arm-repeat proteins are involved in various processes, including intracellular signalling and cytoskeletal regulation. | | | | |

**Table 3.10: Gene Ontologies for the most enriched ontologies in Stage Two using anRichment**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process/ Term | FDR |
|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Blue | positive regulation of macrophage proliferation | 9.38E-19 |
| GO:0043231 | Organized structure of distinctive morphology and function bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Blue | intracellular membrane-bounded organelle | 2.90E-12 |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Blue | cytoplasm | 1.74E-10 |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 7.94E-15 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Brown | intracellular organelle lumen | 2.07E-09 |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Brown | cytoplasm | 9.37E-09 |
| GO:0003674 | A molecular process that can be carried out by the action of a single macromolecular machine, usually via direct physical interactions with other molecular entities. Function in this sense denotes an action, or activity, that a gene product (or a complex) performs. These actions are described from two distinct but related perspectives: (1) biochemical activity, and (2) role as a component in a larger system/process. | MF | Green | molecular function | 2.31E-12 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Green | positive regulation of macrophage proliferation | 7.16E-10 |
| GO:1902644 | The chemical reactions and pathways involving tertiary alcohol | BP | Green | tertiary alcohol metabolic process | 2.55E-09 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Turquoise | positive regulation of macrophage proliferation | 1.10E-69 |
| GO:0070016 | Interacting selectively and non-covalently with the armadillo repeat domain of a protein, an approximately 40 amino acid long tandemly repeated sequence motif first identified in the Drosophila segment polarity protein armadillo. Arm-repeat proteins are involved in various processes, including intracellular signalling and cytoskeletal regulation. | MF | Turquoise | armadillo repeat domain binding | 3.94E-28 |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to diacyl bacterial lipopeptide | 4.57E-25 |

**Table 3.11: Gene Ontologies for the most enriched ontologies in Stage Three using anRichment**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process/ Term | FDR |
|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Blue | positive regulation of macrophage proliferation | 2.37E-12 |
| GO:0043231 | Organized structure of distinctive morphology and function bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Blue | intracellular membrane-bounded organelle | 1.70E-10 |
| GO:0044238 | The chemical reactions and pathways involving those compounds are formed as a part of the normal anabolic and catabolic processes. These processes take place in most, if not all, cells of the organism. | BP | Blue | primary metabolic process | 1.03E-09 |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 4.06E-08 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Brown | intracellular organelle lumen | 8.00E-06 |
| GO:0099582 | Any neurotransmitter receptor activity that is involved in regulating the concentration of calcium in the presynaptic cytosol. | MF | Brown | neurotransmitter receptor activity involved in the regulation of presynaptic cytosolic calcium ion concentration | 1.25E-05 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Turquoise | positive regulation of macrophage proliferation | 5.64E-77 |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl) benzene-1,2-diol] and derivatives formed by substitution. | BP | Turquoise | cellular response to catecholamine stimulus | 4.05E-29 |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to diacyl bacterial lipopeptide | 1.01E-23 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Yellow | binding | 3.20E-07 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Yellow | positive regulation of macrophage proliferation | 3.44E-07 |
| GO:0044237 | The chemical reactions and pathways by which individual cells transform chemical substances. | BP | Yellow | cellular metabolic process | 1.60E-06 |

**Table 3.12: Gene Ontologies for the most enriched ontologies in Stage Four using anRichment**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process/ Term | FDR |
|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Blue | positive regulation of macrophage proliferation | 1.70E-10 |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Blue | cytoplasm | 4.22E-09 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Blue | binding | 4.46E-08 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 1.89E-08 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Brown | binding | 4.85E-07 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Brown | intracellular organelle lumen | 2.09E-06 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Green | positive regulation of macrophage proliferation | 3.43E-06 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Green | intracellular organelle lumen | 9.16E-05 |
| GO:0035373 | Interacting selectively and non-covalently with a chondroitin sulphate proteoglycan, any proteoglycan containing chondroitin sulphate as the glycosaminoglycan carbohydrate unit. | MF | Green | chondroitin sulphate proteoglycan binding | 0.000174 |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Magenta | cytoplasm | 7.51E-05 |
| GO:0003674 | A molecular process that can be carried out by the action of a single macromolecular machine, usually via direct physical interactions with other molecular entities. Function in this sense denotes an action, or activity, that a gene product (or a complex) performs. These actions are described from two distinct but related perspectives: (1) biochemical activity, and (2) role as a component in a larger system/process. | MF | Magenta | molecular function | 0.000264 |
| GO:0019222 | Any process that modulates the frequency, rate or extent of the chemical reactions and pathways within a cell or an organism. | BP | Magenta | regulation of metabolic process | 0.000537 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Purple | positive regulation of macrophage proliferation | 4.37E-10 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Purple | intracellular organelle lumen | 6.00E-08 |
| GO:1901666 | Any process that activates or increases the frequency, rate, or extent of NAD+ ADP-ribosyl transferase activity | BP | Purple | positive regulation of NAD+ ADP-ribosyltransferase activity | 2.40E-07 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Red | positive regulation of macrophage proliferation | 3.43E-06 |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Red | protein binding | 0.0004 |

| | | | | | |
|---|---|---|---|---|---|
| GO:0043412 | The covalent alteration of one or more monomeric units in a polypeptide, polynucleotide, polysaccharide, or other biological macromolecules, resulting in a change in its properties. | BP | Red | macromolecule modification | 0.000479 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Turquoise | positive regulation of macrophage proliferation | 3.42E-25 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Turquoise | intracellular organelle lumen | 2.23E-12 |
| GO:0005634 | A membrane-bounded organelle of eukaryotic cells in which chromosomes are housed and replicated. In most cells, the nucleus contains all of the cell's chromosomes except the organellar chromosomes and is the site of RNA synthesis and processing. In some species of specialized cell types, RNA metabolism or DNA replication may be absent. | CC | Turquoise | nucleus | 5.73E-11 |
| GO:0003674 | A molecular process that can be carried out by the action of a single macromolecular machine, usually via direct physical interactions with other molecular entities. Function in this sense denotes an action, or activity, that a gene product (or a complex) performs. These actions are described from two distinct but related perspectives: (1) biochemical activity, and (2) role as a component in a larger system/process. | MF | Yellow | molecular function | 6.12E-08 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Yellow | positive regulation of macrophage proliferation | 2.55E-07 |
| GO:0043227 | Organized structure of distinctive morphology and function bounded by a single or double lipid bilayer membrane. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Yellow | membrane-bounded organelle | 9.65E-07 |

**Table 3.13: Gene Ontologies for the most enriched ontologies in Stage Five using anRichment**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process/ Term | FDR |
|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Black | positive regulation of macrophage proliferation | 2.43E-38 |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) because of a diacylated bacterial lipopeptide stimulus. | BP | Black | cellular response to diacyl bacterial lipopeptide | 3.66E-14 |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) because of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl) benzene-1,2-diol] and derivatives formed by substitution. | BP | Black | cellular response to catecholamine stimulus | 3.49E-13 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 1.14E-11 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Brown | binding | 1.07E-09 |
| GO:0044237 | The chemical reactions and pathways by which individual cells transform chemical substances. | BP | Brown | cellular metabolic process | 9.83E-09 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Green | positive regulation of macrophage proliferation | 5.52E-06 |
| GO:0098809 | Catalysis of the reaction: nitrite + acceptor = product(s) of nitrate reduction + reduced acceptor. | MF | Green | nitrite reductase activity | 1.04E-05 |
| GO:0043226 | Organized structure of distinctive morphology and function. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton, and prokaryotic structures such as anammoxosomes and pirellulosomes. Excludes the plasma membrane. | CC | Green | organelle | 1.33E-05 |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Greenyellow | positive regulation of macrophage proliferation | 1.22E-34 |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) because of a diacylated bacterial lipopeptide stimulus. | BP | Greenyellow | cellular response to diacyl bacterial lipopeptide | 3.08E-18 |
| GO:0070052 | Interacting selectively and non-covalently with a type V collagen trimer. | MF | Greenyellow | collagen V binding | 7.65E-15 |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Pink | positive regulation of macrophage proliferation | 0.000262 |
| GO:0019866 | The inner, i.e. lumen-facing, the lipid bilayer of an organelle envelope; usually highly selective to most ions and metabolites. | CC | Pink | organelle inner membrane | 0.000645 |
| GO:0031966 | Either of the lipid bilayers surrounds the mitochondrion and form the mitochondrial envelope. | CC | Pink | mitochondrial membrane | 0.001661 |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Red | positive regulation of macrophage proliferation | 1.84E-07 |

| | | | | | |
|---|---|---|---|---|---|
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Red | protein binding | 1.43E-05 |
| GO:0070013 | An organelle lumen is part of an intracellular organelle. | CC | Red | intracellular organelle lumen | 3.82E-05 |

# *4.0 Discussion*

Schizophrenia is a chronic and disabling disorder that affects 1% of the general population whose causes remain unclear even with much research into disease aetiology (4). Schizophrenia is a neurodevelopmental disorder, therefore, constructing and exploring networks of genes, previously identified as associated with schizophrenia, over key developmental stages could aid our understanding of schizophrenia's aetiology and identify novel therapeutic targets of disease.

Although schizophrenia is researched extensively from many angles its mechanisms remain elusive. It is difficult to predict who will develop schizophrenia because of the complex interactions between genetic and environmental factors. Schizophrenia is classed as a neurodevelopmental disorder but as it doesn't manifest until adolescence it's very important to study it at multiple clinical stages (13,53,162). Insults to brain development *in utero* can have an impact on the severity of schizophrenia's symptoms later in life. Changes in gene expression throughout schizophrenia have been observed, the study performed by Ota et al. looked at participants blood and compared expression levels of schizophrenia genes in clinically high-risk patients through to chronic schizophrenia. They observed changes in gene expression profiles at different clinical stages (13). Studies like these highlight the importance of looking at schizophrenia at multiple stages to captures its heterogeneity.

In this study, genes previously identified in a large-scale schizophrenia GWAS as being significantly associated with schizophrenia were used to filter ABA's BrainSpan resource to include gene expression data for these genes across 16 brain areas and five developmental stages. To find underlying patterns in the gene expression data K-means analysis was utilised. A systems biology approach (WGCNA) was used to describe the pairwise relationship between genes at each development stage and to create networks (i.e., modules) of schizophrenia-associated genes which were co-expressed in each

Developmental Stage. Next, GO enrichment analysis was applied to each of the modules using anRichment to aid biological interpretation of the identified networks in each developmental stage.

## 4.1 K-means analysis on the schizophrenia-associated genes

K-means clustering was performed on each Developmental Stage. Each schizophrenia-associated gene was assigned to the closest centre (k). Developmental Stage One to Five can be seen in Figure 3.3.2 to 3.3.7 and a comparison of the five stages in 3.3.7.

If a cluster is filled with genes that are very similar the within-cluster sum of squares of the cluster will be small and when the cluster is visualised it will appear small and compact. Each of the clusters in this analysis had a large within-cluster sum of squares, this means there is variance within the cluster. The total within variance measures the deviation from the mean. The total within variance in this dataset is low which means that the dataset is very similar. Usually, only a small amount of the total variance is explained by the within-cluster sum of squares but in this case, the total variance is low and the within the sum of squares is high. This tells us the schizophrenia-associated genes expression profile in the brain are similar to each other. From the k-means results, it is clear that these genes do not cluster well together, if you increase the number of clusters for each of the Developmental Stages the sum of squares with each cluster decreases and the total variance increases but this would mean ignoring the optimal cluster number for each stage previously calculated.

## 4.2 Weighted Gene Correlations Network Analysis on schizophrenia-associated genes

WGCNA is a systems biology method that uses gene transcripts to describe pairwise relationships between the genes. WGCNA was used to calculate modules of

schizophrenia-associated genes which were co-expressed for each developmental stage. WGCNA identified three modules in developmental stage One and Five, four in developmental stages Two and two in developmental Stages Three and Four. The ME of each cluster was identified and the gene most like the ME is classed as the HG. Each HG can be seen in Table 9. WGCNA relies strongly on the assumption that gene co-expression networks follow a scale-free topology where highly connected genes are essential for a functioning system (163). It assumes that gene products associated with the same phenotype usually participate in the same module (163). When WGCNA was applied to the expression profiles of the 316 schizophrenia-associated genes in the brain, each gene was assigned to a module and each module was given a colour. A hierarchical clustering graph of initial and final (after minimum module size of 10) module assignments for each Developmental Stage can be seen in Figure 3.4.2. After module construction, each genes module membership and weight were calculated for downstream visualisation using Cytoscape.

## 4.3 Visualisation of modules using data from WGCNA.

First, genes were assigned to modules. Using each gene's MM the ME and thus HG was identified, and the weight calculated. This information was collated into csv files, which were uploaded to Cytoscape so the modules could be visualised. Cytoscape allows for the important attributes to be highlighted in the network graph. In each graph node size is shown by the module membership, edge width signifies weight and node colour displays p-value as calculated by Pardiñas et al. These p-values range from $2.12x10^{-44}$ – $4.88x10^{-08}$ the lower the value the greater the association. Each Cytoscape network can be seen in Figure 3.6.1 to 3.6.27. Only the edges width of 0.8 and over were included to capture the most important edges.

### 4.3.1 Developmental Stage One - Prenatal

BrainSpan's Developmental Stage One includes gene expression for healthy prenatal brains. Figure 3.6.1 shows the Black module which contains 47 genes. The HG for this module is *SOX5*. This gene encodes a transcription factor that mediates DNA binding and nuclear trafficking (114). *SOX5* modulates the timing of important processes during corticofugal neuron production so consequently neocortical neuron diversity and sub-type specific differentiation (164). *SOX5* has been linked to Lamb Shaffer syndrome which is a developmental disorder that involves ID, language, and motor deficits and serves various roles in multiple cancer types (165,166). Figure 3.6.2 shows the blue module and contains 74 genes, two of which have split into a small module. The HG for this module is *FHIT*, this genes inactivation, deletion and decreased expression is seen in most cancers (116). It can induce cell apoptosis, stunt the cell growth cycle which can impede tumour proliferation (167). Figure 3.6.3 displays the Brown module which contains 32 genes and whose HG is *SLC12A4* codes for KCC1. KCC1 is a protein that facilitates the symport of chlorine and potassium through cells surfaces and is involved in cancer growth, bone turnover and sickle cell formation (117). Na-K-Cl and K-Cl co-transporters regulate Cl levels, shifts in chloride electrochemical

gradients can affect GABAergic transmission which is seen to be impaired in schizophrenia especially in ANPs (168,169). Figure 3.6.4 shows the pink module with 20 genes, the HG *OTOL1* which is essential for hearing and vestibular function (119,120). It is not known what function *OTOL1* has in the brain. Figure 3.6.5 shows the large turquoise module which contains 101 genes. The HG for the Turquoise module is *TMEM194A* also known as *NEMP1*. This gene is poorly understood but the NEMP1 protein has been linked to eye development from the gastrula through to the neurula stage (123).

Stage One is the prenatal stage, *SOX5* being associated with a neurodevelopmental disorder and *NEMP1* being linked to eye development are interesting. There are several physiological and structural impairments in the eye associated with schizophrenia. The retina and the brain develop from the same tissue the neuroectoderm and it has been proposed that retinal changes can be a marker for progressive brain tissue loss and function (170). This may mean that retinal structure changes could parallel the brain (171). Performing a longitudinal study observing retinal changes and linking them to schizophrenia symptoms could potentially map the progression of schizophrenia.

### 4.3.2 Developmental Stage Two – Infant (0-2 years)

BrainSpan's Developmental Stage Two includes gene expression for a healthy infant's brain from 0-2 years old. 3.6.6 shows the blue module which has 43 genes, the HG is *KCNV1*, this gene is involved in another neurodevelopmental disorder autism spectrum disorder (ASD) (124). It codes for a subunit that regulates potassium channels which regulates neurotransmission release, neuronal excitability, and epithelial electrolyte transport controlled by BK channels (125). The BK channels have been linked to schizophrenia (172). Figure 3.6.7 displays the brown module with 31 genes and whose HG is *NDRG4* which is involved in cell proliferation, differentiation, and development (127). *NDRG4* deficient mice are at a greater risk of cerebral ischemia and exhibit poor spatial learning (127,173). Knockout of *NDRG4*

induces glioblastoma cell apoptosis which contributes to neurological damage (127). Figure 3.6.8 shows the green module and has 26 genes, two of the genes in the modules have split into their submodule. The HG for the module is *GPR52* which is a G protein-coupled receptor, it is thought that it could regulate dopaminergic and glutamatergic transmission which is responsible for cognitive function and is affected in schizophrenia (128). Figure 3.6.9 displays the turquoise module; this module has six sub-modules and contains 147 genes in total. The HG for the module is *STAG1* is needed for cohesion at telomeres and DNA replication (131). STAG1 deletions and point mutations can attribute to syndromic unspecific ID (132).

### 4.3.3 Developmental Stage Three – Child (3- 11 years)

Developmental Stage Three contains gene expression across the brains of children between the ages of 3 and 11 years. Figure 3.6.10 displays the blue module and contains one connected component and contains 33 genes. The HG for the module is *SATB2,* which is involved in transcription regulation and chromatin remodelling (133). *SATB2* is a transcription factor that regulates neocortical circuitry and organisation (133). *SATB2* has been shown to cause *SATB2*-associated syndrome and developmental delays (133,134). Figure 3.6.11 shows the brown module which contains 14 genes and whose HG is *INA.* It is found in developing neuroblasts and in cerebellar granule in the adult CNS (135,174). *INA* maintains the morphogenesis of neurons (135). Figure 3.6.12 shows the turquoise module and has 144 genes and whose HG is *TRANK1*. *TRANK1* encodes a protein in the brain with unknown function (175). Decreased expression of *TRANK1* affected the expression of several genes which are involved in neural development (175). Low levels of *TRANK1* mRNA expression is a BP risk factor (175,176). The yellow module is shown in Figure 3.6.13 and has16 genes in the module. The HG is *ANKRD63* but not much is known about the function of this gene.

There is not a clear link between the HGs in Developmental Stage Three. *SATB2*-syndrome is a neurodevelopmental disorder that leads to developmental delays, it also regulates chromatin remodelling. Defects in chromatin remodelling are often seen in neurodevelopmental disorders,

these defects can compound over time and impair brain circuit establishment (177). These impairments in neurodevelopmental disorders can lead to decreased cognitive function. Although not much is known about the exact function of *TRANK1* it has been linked to BP, schizophrenia and BP share some symptomatology and significant genetic overlap with a genetic correlation from common risks estimated to be 0.6-0.7, and 114 loci contributing to both (178).

### 4.3.4 Developmental Stage Four – Adolescent (12-18 years)

Developmental Stage Four captures gene expression in the brain for adolescents. Typically schizophrenia manifests its symptoms during this time frame (179). Figure 3.6.14 illustrates the blue Module with 38 genes. The HG for this module is *MEF2C*, this gene regulates expression across development in processes such as synapse formation and development, neuronal differentiation (140). It has also been shown to be involved in numerous neurodevelopmental disorders including ASD (180). Figure 3.6.15 displays the brown module with 25 genes. This module's HG is *SMG6*, although there is not much known about this gene's exact function. Still, it has been linked to pancreatic adenosquamous carcinoma and lissencephaly (181). *SMG6* hyperfunction has been shown in epileptic seizures (182). Figure 3.6.16 displays the green module with 17 genes, this module has very few edges and is filled with genes with very low p-values showing a statistically strong link to schizophrenia. The HG for this module is *TAOK2* which regulates neurodevelopment, synapse formation and the development of synapses through modulation of the cytoskeleton which can be linked to schizophrenia (141). *TAOK2* has also been seen to regulate apoptosis by activating T cells and is a neurodevelopmental risk gene (141). Figure 3.6.17 shows the magenta module which contains only 12 genes. *OPCML* is the HG for this module and has been connected to the lung, brain, and cervical cancers (143,145). This gene regulates synaptogenesis and synaptic plasticity. Figure 3.6.18 shows the purple module with 32 genes. The HG is *GPR52* which is a G protein-coupled receptor, it may regulate dopaminergic and glutamatergic transmission which is

responsible for cognitive function (129). Figure 3.6.19 displays the red module which is a small module with 14 genes. The HG of this module is *WHSC1L1/NSD3*. This gene has been linked to numerous cancers, cell cycle progression and promotes antiviral innate immunity (146,147). Figure 3.6.20 presents the turquoise module with 58 genes. The HG is *CA8* which is a member of the carbonic anhydrase family, it is expressed in Purkinje cells in the cerebellum. It is a $IP_3R1$ inhibitor that regulates calcium levels which help key cellular processes (150,183). Figure 3.6.21 shows the yellow module with 21 genes. The HG is *ALMS1* which is involved in the maintenance of centromere cohesion, transcription and actin organisation and endosomal trafficking. Mutations in ALMS1 can cause Alström syndrome which can sometimes include ID and can cause psychotic like symptoms in adults which is also when psychotic symptoms appear in schizophrenia (184).

*MEF2C* and *TAOK2* are both involved in synapse formation and development. Dysregulated synaptic development has been hypothesised as underlying altered neuronal function in schizophrenia (185). Synaptic pruning occurs during adolescence Developmental Stage Four (12 – 18 years old) 30% of synapses which are formed during adolescence in the dorsolateral prefrontal cortex are lost but in people with schizophrenia it is closer to 60% (186). The synaptic pruning roughly ends at the time of schizophrenia onset leading to the hypothesis that altered synaptic pruning may be a part of the pathophysiology of schizophrenia (185).

### 4.3.5 Developmental Stage Five – Adult (>19 years)

Figure 3.6.22 shows the Black module which has 104 genes that have formed their submodule. The HG for this module is *C16orf86* which is associated with insulin sensitivity in human skeletal muscles, insulin sensitivity in a subgroup of patients with schizophrenia who are anti-psychotic resistant (155). Figure 3.6.23 displays the brown module with 36 modules. The modules HG is *RFTN2* is implicated in glass syndrome which is characterised by developmental delay, speech development and ID. Glass syndrome has some symptom similarities to schizophrenia which would suggest some

shared underlying pathways. Figure 3.6.24 shows the green module with 19 genes. Its HG is *NFATC3*, a member of the calcineurin nuclear factor of activation of T cells who play an essential role in the immune system (187). It is a transcription factor that is involved in the development and progression of tumours and is important for brain tissue homeostasis (158) and is a prompter of neural progenitor cell differentiation into neurons and astrocytes (188). Figure 3.6.25 shows the Greenyellow which houses 77 genes. The modules HG is *SATB2* which encodes a protein that is involved in transcription regulation and chromatin remodelling (133). *SATB2* is a transcription factor that regulates neocortical circuitry and organisation (133). *SATB2* has been shown to cause *SATB2*-associated syndrome and developmental delays (133,134). Figure 3.6.26 displays that the pink module houses only 12 genes. *CHRNA5* is the HG for this module which is a nicotinic acetylcholine receptor, three other members of the family are in this module, altered cholinergic neural transmission has been shown to increase susceptibility to cognitive deficits, and in the Chinese Han population, it has been linked to early-onset and more severe symptoms of schizophrenia (160,189). Figure 3.6.27 shows the red module and has 19 genes. *INA* is the HG in this module which is found in developing neuroblasts and in cerebellar granule in the adult CNS and *INA* maintains the morphogenesis of neurons (135). It is a prognostic marker for poor survival rates in colorectal cancer patients(190).

*RFTN2* and *SATB2* are both involved in glass syndrome that is also known as SATB2-syndrome, which is characterised by ID, craniofacial abnormalities, dental abnormalities, and behavioural problems. *CHRNA*5 has been shown to increase cognitive deficits, these and the genes involved in the *SATB2* syndrome are linked to the cognitive and negative symptoms of schizophrenia.

### 4.3.6 Recurring Hub Genes Across Developmental Stages

Of the 316 schizophrenia-associated genes 27 modules were formed and three genes repeated over two modules, *GPR52*, *INA* and *SATB2*.

*GPR52* is an orphan G- protein-coupled receptor (GPCR) which is selectively expressed in the striatum and regulates various brain functions including homeostasis, immune function, neurotransmission and metabolism (191,192). It is expressed in the striatum and nucleus accumbens which have been linked with psychiatric disorders. GPCR's are mediators of signal transduction in the CNS and have been actively investigated for their role in the development of mood disorders (193). GPCR's are the most common targets of antipsychotics and play crucial roles in controlling brain function by regulating numerous downstream signalling pathways (192). *GPR52* has been highlighted as a potential therapeutic target for schizophrenia, it is thought that *GPR52* signalling via 5'-cyclic adenosine monophosphate (cAMP) could oppose D2 signalling activity in the striatum while stimulating D1/NMDA function in the frontal cortex (194,195). The role of *GPR52* in the dopaminergic system may aggravate the symptoms of schizophrenia (193).

*SATB2* is a transcription factor that regulates neocortical organisation and circuitry (133). *SATB2* is required for the projection of upper-layer neurons and it can regulate other genes by mediating chromatin loop formation and can modify higher-order chromatin structure (133). It can control the expression of genes that are involved in pluripotency and self-renewal (196). During CNS development the SATB2 protein is expressed in the superficial cortical layers and determines neuron projection. In adult CNS it is expressed in pyramidal neurons of all cortical layers and regulates long-term memory and synaptic plasticity which is linked to cognition (197). *SATB2* facilitates callosal projection by repressing the *BCL11B* gene whose protein is required for subcortical projection neuron identity and postnatal development of the hippocampus (133). This can lead to altered cognition which is seen in schizophrenia. Loss of *BCL11B* leads to weakened hippocampal memory and learning (133). De novo structural or

single-nucleotide variants in *SATB2* is linked to *SATB2*-associated syndrome which is characterised by intellectual disability, developmental delays, abnormal craniofacial features and behavioural issues (133,197).

*INA* (α-Internexin) codes a class IV neuronal intermediate filament protein which maintains neuronal morphogenesis and provides strength to the cell (135). This protein is a structural component of the cytoskeleton and is involved in neurogenesis (136). INA regulates the expression of other neurofilaments during brain development (198). It is widely accepted that *INA* is involved in neuronal development, but its function remains unknown. *INA* is the main component of neuronal IF inclusion disease which causes rare frontal dementia and behavioural and personality changes and has been linked to brain tumours (135). The INA protein is believed to be involved in tumour initiation and progression and is one of the most overexpressed proteins in gliomas (174,190).

*INA* and *SATB2* are repeated in Developmental Stage Three and Developmental Stage Five and *GPR52* is repeated in Developmental Stage Two and Four. *GPR52* is thought to aggravate schizophrenia's positive symptoms, and *SATB2* and *INA* mutations can cause symptoms that look like schizophrenia's negative and cognitive symptoms. Our results suggest that *INA*, *SATB2* and *GPR52* represent candidate genes for future evaluation of their potential as a therapeutic target for schizophrenia.

Pardiñas et al. study applied Summary-data-based Mendelian Randomisation (SMR) analysis to the schizophrenia-associated gene with dorsal prefrontal cortex expression quantitative trait locus (eQTL) using Common Mind Consortium. This analysis aimed to uncover variants that could be causally linked to expression changes in specific genes. They applied a threshold of 0.05 which highlighted colocalised signals due to a single casual variant. From this, they discovered 22 candidate variants at 19 loci with an FDR P <0.05. *ALMS1* the HG in the green Module in Developmental Stage Two and *TRANK1* the HG in the brown Module of Developmental Stage Three identified in our study were also identified in the SMR analysis. Thus, our results suggesting these genes are HGs

adds further evidence that they would be good candidates for further research as they may regulate several schizophrenia-related genes in their respective modules.

## 4.4 Gene Ontologies

On each of the modules calculated by WGCNA, anRichment was run. anRichment is run to biologically interpret the modules. P-values were calculated and GO's which were lower than the threshold of 0.05 after Bonferroni correction was applied were retained. As anRichment produced many significant GO's, GO parent terms were excluded to focus on more specific pathways. The complete ontologies calculated from anRichment in R including the genes involved can be found in the appendix from Table 3 to Table 27.

Across the developmental stages, anRichment has highlighted the immune system and inflammation, specifically concerning macrophage proliferation (GO:00120041). Inflammation in the CNS is facilitated by astrocytes, microglial cells, proinflammatory cytokines, invading immune cells which includes macrophages, monocytes, and T or B lymphocytes. For appropriate function, a well-regulated inflammatory response is essential, but uncontrolled inflammation caused by infectious agents, genetics or physical trauma can be detrimental (199). The macrophages of the brain are called microglia and they play a crucial role in the innate immunity of the CNS and represent up to 10% of total brain cells, but their cell density depends on the area of the brain (200,201). Microglial cells are derived from the yolk sac progenitors during embryogenesis and migrate throughout the CNS, they are maintained through adulthood by self-renewal and rapid cell turnover (201,202). Microglia are involved in the synaptic organisation, phagocytosis of apoptotic cells during development, maintenance of neuronal excitability, trophic neuronal support in the developing brain and brain protection and repair (202). In post-mortem cortical tissue of patients with schizophrenia, the synapse density is reduced. This excessive pruning reflects abnormalities in synaptic structures and microglia like cells

(203). A lot of this synaptic pruning occurs during adolescence, this is when schizophrenia symptoms occur (203). *In utero* MIA is a risk factor for neurodevelopmental disorders like schizophrenia. O'Loughlin et al. administered lipopolysaccharide to mice on embryonic day 12 to induce MIA. This induced a pro-inflammatory cytokine profile which continued in the amygdala to early adulthood. These alterations in the foetal brain elicited by MIA can lead to alterations to microglia (54). Pre and perinatal activation of the immune system can increase the immune system's sensitivity throughout life (18). Diverse immune alteration has been observed in those with schizophrenia and autoimmune disorders and severe infections are linked to schizophrenia risk (21,204).

Cellular response to diacyl lipopeptide stimulus (GO:0071726) showed up in the top three gene ontologies in at least one module per Developmental Stage apart from Developmental Stage Four.

Cellular response to catecholamine stimulus was highlighted by anRichment in at least one module per Developmental Stage (GO:0071870). Catecholamines, which are neurotransmitters in the CNS and peripheral nervous system, include dopamine, norepinephrine and adrenaline (127). Catecholamine signalling underlies the mesocorticolimbic system and affects executive function and cognition (206). Catecholamine signalling pathways are pharmacological therapy targets for patients with neuropsychiatric disorders because of their relationship with affective, executive and cognitive functions (206). Catecholamines have versatile functions as slow-acting neurotransmitters in synaptic neurotransmission and controlling the effects of fast-acting neurotransmitters (205). Dopamine has been linked with schizophrenia for many years and dopamine receptor antagonists continue to be the leading therapy for schizophrenia (5). Dopamine neurotransmission is altered in many neural pathways in schizophrenia (207), these alterations include hyperactive dopaminergic transmission in the striatum, hippocampus and mesolimbic areas and hypoactive transmission in the PFC of patients which schizophrenia (207). Dopamine displays regulatory effects on an immune response

which depends on dopamine concentration, sub-type of receptors, time of exposure, type of immune cell and immune cell activation (31). This has been shown to affect cognitive functions (207). Immune cells in particular T-cells, microglial and peripheral monocytes collaborate with the CNS and have cognitive and behavioural function which are seen to be altered in schizophrenia (31,207). Dopamine has been seen to influence the activity of these immune cells since they express dopamine receptors (207). Changes in dopamine concentration and/or receptors in T cells are thought to be the cause of abnormal immune functions in people with schizophrenia and Parkinson's (208). Low levels of dopamine neurotransmission, as well as serotonin and glutamatergic neurotransmission, are seen in people with schizophrenia. These are connected to low levels of neuroinflammation, which has been hypothesised to be the reason for CNS volume loss and low levels of microglial activation in schizophrenia patients in neuroimaging studies (18). Renalase is thought to metabolise dopamine. In a study conducted by Catak et al. which used thirty-three schizophrenia patients it was found that the levels of renalase in these patients was significantly lower than the control group. This could be a potential biomarker for schizophrenia (209).

Regulation of neurotransmitter secretion (GO:0046928) was enriched in Developmental Stage One and Three and Neurotransmitter receptor activity (GO:0030594) in Developmental Stage Four. Multiple neurotransmitters have been implicated in being involved in schizophrenia including Dopamine, Serotonin, GABA and NMDA (37).

In Developmental Stage One, oestrogen 16-alpha-hydroxylase activity (GO:0101020) was highlighted as being enriched in the turquoise module. Oestrogen is produced in the brain in areas including the hippocampus, cortex, amygdala, hypothalamus and cerebellum (210). Because oestrogen can have a powerful effect on numerous areas of the brain it can affect mood, cognition and behaviour (211). It plays a critical role in influencing dopamine, serotonin, glutamate and GABA neurotransmitters which are key in schizophrenia (210).

Although oestrogen is involved in brain development the exact role of oestrogen 16-alpha-hydroxylase activity in schizophrenia is less obvious.

The immune system, inflammation, catecholamines and neurotransmitter dysfunctions have been identified as being important for each developmental stage. One of the important findings from the study performed by Pardiñas et al. was the significance of the SNP which was found in the major histocompatibility complex (xMHC) region which is linked to the adaptive immune system. Although SNPs associated with the MHC region was included in the gene list which was provided by Pardinas's study, ABA's BrainSpan resource did not include gene expression data for it and thus were not included in our downstream analysis. Our findings support the role of the immune system in Schizophrenia over each of the Developmental Stages.

## 4.5 Limitations of the study

There were several limitations of this study. Firstly, ABA's BrainSpan resource developed in 2014 uses healthy brains for each developmental stage. ABA's BrainSpan contains gene expression data across 16 brain regions from 8 post-conception to 40 years, covering the complete development process (79). The atlas contains next-generation RNA sequencing data which has collected 579 tissue samples from thirty neurologically unremarkable brains over five developmental stages (80). A limitation to the BrainSpan resource is that the brain is divided into just 16 regions and that the brain sample size is small. In addition, ~80% of participants had transcriptomes missing from at least one brain region which means you're not getting complete pictures of the brain (79). ABA uses only neurotypical brains, we applied similar methods which had proven to be successful in studies performed by McCarthy et al. and Negi et al. (82,83). An additional gene expression database is the genotype tissue expression (GTEx) project. This database was created to enable the study of human gene regulation and variation of gene expression in multiple tissues (212). The GTEx consortium collected 14,787 transcriptomes from 948 patients including 13 brain regions (79). The largest limitation of

using GTEx is that it does not have developing brain data which is why ABA was used in this study.

Brain tissue is comprised of diverse basic cell types, if the expression at tissue levels changes it may be due to alterations in the proportion of basic cell types (213). Single-cell sequencing (scRNASeq) allows for the dissection of gene expression at single-cell resolution, using scRNASeq can lead to findings in cell expression alterations and dynamics (214). This revolutionary tool if applied to schizophrenia could uncover the uniqueness of each brain cell at microscopic resolution (215). PsychENCODE is a resource that was built to elucidate the underlying molecular mechanisms of psychiatric disorders. The resource includes the integration of data from ENCODE, GTEx, Roadmap and relevant single-cell studies. PsychENCODE's main success has been the multi-omics approach of non-coding elements and transcriptome in neurotypical developing brains and adults with psychiatric disorders (213). Unfortunately, PsychENCODE does not have a developmental database and thus was not used in this current study.

The schizophrenia-associated gene set which was used in this study was taken from a study performed by Pardiñas et al. (66). Pardiñas et al. used these results as a training set to create risk profile scores to identify SNPs at high confidence (66). This study is the largest schizophrenia GWAS to date with 40,675 cases. The study size could be larger and could produce more reproducible SNP's. The SNPs identified were mapped to the closest loci and if there were no overlapping genes it was mapped to the closest gene within a 500 KB distance (66). As with all GWAS, the best way to discover more and yield more accurate results is to increase the sample size of the study. The Pardiñas study mapped to 479 genes, of these genes only 316 genes were available on ABA's BrainSpan transcriptome atlas. Missing a large portion of the Pardiñas gene set is a limitation of the current study and some SNPs did not map to any genes available in ABA.

K-means as a method has several limitations, firstly because the user has to decide on the number of clusters k before beginning the analysis. Secondly, k-means has a bias of creating modules of a similar size which may not accurately represent the group (90). Lastly, k-means centroids are immensely affected by outliers and can give the outliers disproportionate importance (89,90). K-means was used in this analysis to look for underlying patterns within the genetic data per stage. When the Pardiñas gene set was put through k-means the intercluster variation explained most of the total variance in the data which is not typical for good clustering.

An additional limitation of this study is that there was no clinical data that could be applied to the WGCNA data. Calculating gene trait significance also allows for the gene expression to be linked to biologically relevant traits. Studies which had clinical data were able to determine the GS threshold by combining it with module membership. Those with the highest GS can be considered HG for a module. In this study, MM was calculated by determining the node distance from the central ME. The MM calculated measured by Pearson correlation between ME and gene expression should only contain genes with the highest correlation but one study concluded that 25% be a better fit in other modules (216). Usually, WGCNA analysis uses much more data, more genes could mean more than one HG for a module and can give a better insight into the critical underlying pathways within a module.

## 4.6 Future Directions

To get a more comprehensive understanding of the brain changes which result in severe psychiatric diseases it is important to have a clear understanding of mechanisms that occur in normal brain development. When there is a comprehensive understanding of normal brain development it will be easier to observe the changes that occur in neuropsychiatric conditions like schizophrenia. At present, it is difficult to obtain full affected brains at any stage and as there are no biomarkers it is difficult to predict who will develop schizophrenia until it

eventually manifests. This would mean prenatal, child and early adolescence brains which are obtained could not be confirmed that they would develop schizophrenia. In schizophrenia, it is important to understand the brains mechanisms and what goes awry in the early stages. To create a developmental resource researcher would have to use brains from people who are high risk (perhaps based on the polygenic risk scores for schizophrenia) or those who have close relatives with schizophrenia.

To combat these limitations a longitudinal study where participants who are more high risk to develop schizophrenia than the general population could be studied from birth to adulthood. This type of analysis would use blood samples and MRIs but a limitation for this kind of study is the inability to study arguably the most important stage during development *in utero,* where crucial decisions involving cell fate and distinctive development of the brain occur. Thus, the incorporation of genetic, epigenome and gene expression data and machine learning approaches will allow for a better understanding of abnormal brain development.

Gene Ontology databases are ever-growing with continual studies uncovering molecular functions, cellular locations, and biological processes. With more studies utilising WGS there will be a more absolute understanding of the genome and what it does and continuously expand the scope of GO. Using GO enrichment analysis like in this study could identify biological differences between controls and schizophrenia patients which could provide pharmaceutical companies with novel therapeutic targets.

Functional work on the HG's of interest at each of the stages could be performed, looking at risk variants. These genes could be studied in knock out mice. It would be particularly interesting to study *ALMS1* and *TRANK1* which were highlighted as being interesting in this study and Pardiñas et al. Clustered regularly interspaced short palindromic repeat (CRISPR-Cas9) is a genome-editing tool that can induce double-strand breaks at target regions and can alleviate a gene's function (217,218). As *ALMS1* and *TRANK1* were highlighted as being important in both this study and Pardiñas study these would be great candidate genes for further downstream analysing including CRISPR-Cas9.

In the future, a method similar to this could be applied to other neuropsychiatric disorders including ASD, MD and BPD. Studying conditions like these over time could assist in more accurate prediction of whom will develop, how severe the symptoms could be, and which treatments could be effective as well as identify candidate genes for downstream analysis. This type of analysis could be easily adapted when newer GWAS produce more comprehensive gene lists.

## 4.7 Conclusion

Schizophrenia is still a long way from being fully understood. From the schizophrenia-associated genes which were highlighted by Pardiñas and available in ABA's BrainSpan resource, 316 genes were available. Once GO enrichment was applied to the modules produced by WGCNA, it was clear that macrophage proliferation and catecholamine dysfunction were important mechanisms underlying schizophrenia in each developmental stage. The importance of the immune system and catecholamine's most notably dopamine has been highlighted in multiple previous studies. There is an interaction between the immune system and dopamine and evidence for this is co-morbidities of schizophrenia and autoimmune diseases. Researching them together may give additional insight into their interdependency.

To more accurately pinpoint central ontologies for each stage a study like this must be done comparing controls and patients with schizophrenia. Studying them and looking at immune and catecholamine processes specifically throughout development could produce novel therapeutics with better efficacy and less severe side effects.

# 5.0 Bibliography


1. Ripke S, Neale BM, Corvin A, Walters JTR, Farh KH, Holmans PA, et al. Biological Insights From 108 Schizophrenia-Associated Genetic Loci. Nature. 2014;511(7510):421–7. Available from: http://www.nature.com/authors/editorial_policies/license.html#terms
2. Stilo SA, Murray RM. Non-Genetic Factors in Schizophrenia. Curr Psychiatry Rep. 2019;21(100).
3. Pugliese V, Bruni A, Carbone EA, Calabrò G, Cerminara G, Sampogna G, et al. Maternal stress, prenatal medical illnesses and obstetric complications: Risk factors for schizophrenia spectrum disorder, bipolar disorder and major depressive disorder. Psychiatry Res. 2019;271:23–30.
4. Haller CS, Padmanabhan JL, Lizano P, Torous J, Keshavan M. Recent advances in understanding schizophrenia. F1000Prime Rep. 2014;6(57):1–11.
5. Amanda Krogmann, Luisa Peters, Laura von Hardenberg, Katja Bödeker, Viktor B. Nöhles and CUC. Keeping up with the therapeutic advances in schizophrenia: a review of novel and emerging pharmacological entities Amanda. CNS Spectr. 2019;24:41–68.
6. Carbon M, Correll CU. Thinking and acting beyond the positive: The role of the cognitive and negative symptoms in schizophrenia. CNS Spectr. 2014;19(S1):35–53.
7. Gobira PH, Ropke J, Aguiar DC, Crippa JAS, Moreira FA. Animal models for predicting the efficacy and side effects of antipsychotic drugs. Rev Bras Psiquiatr. 2013;35:132–9.
8. Owen MJ, Sawa A, Mortensen PB. Schizophrenia. Lancet. 2016;388(10039):86–97.
9. Avramopoulos D. Recent Advances in the Genetics of Schizophrenia. Mol Neuropsychiatry [Internet]. 2018 [cited 2019 Sep 3];4:35–51. Available from: www.karger.com/mnp
10. Insel TR. Rethinking schizophrenia. Nature. 2010. p. 187–93.
11. Stępnicki P, Kondej M, Kaczor AA. Current concepts and treatments of schizophrenia. Molecules. 2018;23(2087).
12. Strous RD, Shoenfeld Y. Schizophrenia, autoimmunity and immune system dysregulation: A comprehensive model updated and revisited. J Autoimmun. 2006;27:71–80.
13. Ota VK, Moretti PN, Santoro ML, Talarico F, Spindola LM, Xavier G, et al. Gene expression over the course of schizophrenia: from clinical high-risk for psychosis to chronic stages. npj Schizophr. 2019;5(5).
14. Gejman P V., Sanders AR, Duan J. The role of genetics in the etiology of schizophrenia. Psychiatr Clin North Am. 2010;33(1):35–66.
15. Lally J, MacCabe JH. Antipsychotic medication in schizophrenia: A review. Br Med Bull. 2015;114:169–79.
16. Muller N, J. Schwarz M. The Role of Immune System in Schizophrenia. Curr Immunol Rev. 2010;6(3):213–20.
17. Van Kesteren CFMG, Gremmels H, De Witte LD, Hol EM, Van Gool AR, Falkai PG, et al. Immune involvement in the pathogenesis of schizophrenia: A meta-analysis on postmortem brain studies. Transl Psychiatry. 2017;7(3). Available from: http://dx.doi.org/10.1038/tp.2017.4
18. Müller N. Inflammation in schizophrenia: Pathogenetic aspects and therapeutic considerations. Schizophr Bull. 2018;44(5):973–82.
19. Müller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. Frontiers in Neuroscience. 2015.
20. Horváth S, Mirnics K. Immune system disturbances in schizophrenia. Biol Psychiatry. 2014;75(4):316–23. Available from: http://dx.doi.org/10.1016/j.biopsych.2013.06.010
21. Benros ME, Nielsen PR, Nordentoft M, Eaton WW, Dalton SO, Mortensen PB. Autoimmune diseases and severe infections as risk factors for schizophrenia: A 30-year population-based register study. Am J Psychiatry. 2011;168(12):1303–10.
22. Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R, Jones PB. Inflammation and immunity in schizophrenia: Implications for pathophysiology and treatment. The Lancet Psychiatry. 2015;2(3):258–70.

23. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B, Brian J. Miller, Peter Buckley, Wesley Seabolt, Andrew Mellor and BK. Meta-Analysis of Cytokine Alterations in Schizophrenia: Clinical Status and Antipsychotic Effects Brian. Biol Psychiatry. 2011;70(7):663–71.
24. Song XQ, Lv LX, Li WQ, Hao YH, Zhao JP. The Interaction of Nuclear Factor-Kappa B and Cytokines Is Associated with Schizophrenia. Biol Psychiatry. 2009;65:481–8.
25. Dinan TG, Borre YE, Cryan JF. Genomics of schizophrenia: Time to consider the gut microbiome? Mol Psychiatry. 2014;19:1252–7.
26. Zheng P, Zeng B, Liu M, Chen J, Pan J, Han Y, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. Sci Adv. 2019;5.
27. Kelly JR, Minuto C, Cryan JF, Clarke G, Dinan TG. The role of the gut microbiome in the development of schizophrenia. Schizophr Res. 2020;
28. Magdalon J, Mansur F, Teles e Silva AL, de Goes VA, Reiner O, Sertié AL. Complement System in Brain Architecture and Neurodevelopmental Disorders. Front Neurosci. 2020;14(23).
29. Owen MJ, O'donovan MC. Schizophrenia and the neurodevelopmental continuum:evidence from genomics. World Psychiatry. 2017;16:227–35.
30. Gupta S, Kulhara P. What is schizophrenia: A neurodevelopmental or neurodegenerative disorder or a combination of both A critical analysis. Indian J Psychiatry. 2010;52(1):21–7.
31. S.M Matt P. G. Where is Dopamine and How Do Immune Cells See It?: Dopamine-Mediated Immune Cell Function in Health and Disease. J Neuroimmune Pharmacol. 2020;15(1):114–64.
32. Howes O, McCutcheon R, Stone J. Glutamate and dopamine in schizophrenia: An update for the 21st century. J Psychopharmacol. 2015;29(2):97–115.
33. Kesby JP, Eyles DW, McGrath JJ, Scott JG. Dopamine, psychosis and schizophrenia: The widening gap between basic and clinical neuroscience. Transl Psychiatry. 2018;8(30).
34. Murray RM, Bhavsar V, Tripoli G, Howes O. 30 Years on: How the Neurodevelopmental Hypothesis of Schizophrenia Morphed into the Developmental Risk Factor Model of Psychosis. Schizophr Bull. 2017;43(6):1190–6.
35. Frank TVM and MJ. An Integrative Perspective on the Role of Dopamine in Schizophrenia. Biol Psychiatry. 2017;176(1):52–66.
36. Demjaha A, Egerton A, Murray RM, Kapur S, Howes OD, Stone JM, et al. Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function. Biol Psychiatry. 2014;75(5).
37. Yang AC, Tsai SJ. New targets for schizophrenia treatment beyond the dopamine hypothesis. Int J Mol Sci. 2017;18(1689).
38. Brisch R, Saniotis A, Wolf R, Bielau H, Bernstein HG, Steiner J, et al. The role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: Old fashioned, but still in vogue. Front Psychiatry. 2014;5(47).
39. Uno Y, Coyle JT. Glutamate hypothesis in schizophrenia. Psychiatry Clin Neurosci. 2019;73:204–15.
40. Stahl SM. Beyond the dopamine hypothesis of schizophrenia to three neural networks of psychosis: Dopamine, serotonin, and glutamate. CNS Spectr. 2018;23(3):187–91.
41. Bustillo JR, Chen H, Jones T, Lemke N, Abbott C, Qualls C, et al. Increased glutamine in patients undergoing long-term treatment for schizophrenia: A proton magnetic resonance spectroscopy study at 3 T. JAMA Psychiatry. 2014;71(3):265–72.
42. Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. In: Brain Research Reviews. 2000. p. 302–12.
43. Sánchez-Blázquez P, Rodríguez-Muñoz M, Garzón J. The cannabinoid receptor 1 associates with NMDA receptors to produce glutamatergic hypofunction: Implications in psychosis and schizophrenia. Front Pharmacol. 2014;4(169).
44. Marsman A, Van Den Heuvel MP, Klomp DWJ, Kahn RS, Luijten PR, Hulshoff Pol HE. Glutamate in schizophrenia: A focused review and meta-analysis of 1H-MRS studies. Schizophr Bull. 2013;39(1):120–9.
45. Egerton A, Modinos G, Ferrera D, McGuire P. Neuroimaging studies of GABA in schizophrenia: A

systematic review with meta-analysis. Transl Psychiatry. 2017;7:1–10.
46. Cohen SM, Tsien RW, Goff DC, Halassa MM. The impact of NMDA receptor hypofunction on GABAergic neurons in the pathophysiology of schizophrenia. Schizophr Res. 2015;167(1–3):98–107.
47. Glausier JR, Lewis DA. GABA and schizophrenia: Where we stand and where we need to go. Schizophr Res. 2017;181:2–3.
48. Birnbaum R, Weinberger DR. Genetic insights into the neurodevelopmental origins of schizophrenia. Nat Rev Neurosci. 2017;18:727–40.
49. Quednow BB, Geyer MA, Halberstadt AL. Serotonin and Schizophrenia. In: Handbook of Behavioral Neuroscience. 2010. p. 585–620.
50. Abi-Dargham A. Alterations of Serotonin Transmission in Schizophrenia. Int Rev Neurobiol. 2007;78:133–64.
51. Sumiyoshi T, Kunugi H, Nakagome K. Serotonin and dopamine receptors in motivational and cognitive disturbances of schizophrenia. Front Neurosci. 2014;8(395).
52. Van Os J, Kenis G, Rutten BPF. The environment and schizophrenia. Nature. 2010;468(7321):203–12. Available from: http://dx.doi.org/10.1038/nature09563
53. Janoutová J, Janáčková P, Šerý O, Zeman T, Ambroz P, Kovalová M, et al. Epidemiology and risk factors of schizophrenia. Neuroendocrine Letters. 2016;37(1):26994378–370116. Available from: www.nel.edu
54. O'Loughlin E, Pakan JMP, Yilmazer-Hanke D, McDermott KW. Acute in utero exposure to lipopolysaccharide induces inflammation in the pre- and postnatal brain and alters the glial cytoarchitecture in the developing amygdala. J Neuroinflammation. 2017;14(1):1–12.
55. Hollins SL, Cairns MJ. MicroRNA: Small RNA mediators of the brains genomic response to environmental stress. Prog Neurobiology. 2016;143:61–81. Available from: http://dx.doi.org/10.1016/j.pneurobio.2016.06.005
56. Brown AS. The environment and susceptibility to schizophrenia. Prog Neurobiol. 2011;93(1):23–58.
57. Shenton ME, Dickey CC, Frumin M, Mccarley RW. A review of MRI findings in schizophrenia. Schizophr Res . 2001;49(1):1–52. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2812015/
58. Dietsche B, Kircher T, Falkenberg I. Structural brain changes in schizophrenia at different stages of the illness: A selective review of longitudinal magnetic resonance imaging studies. Aust N Z J Psychiatry. 2017;1–9.
59. Haijma S V., Van Haren N, Cahn W, Koolschijn PCMP, Hulshoff Pol HE, Kahn RS. Brain volumes in schizophrenia: A meta-analysis in over 18 000 subjects. Schizophr Bull. 2013;39(5):1129–38.
60. Fusar-Poli P, Smieskova R, Kempton MJ, Ho BC, Andreasen NC, Borgwardt S. Progressive brain changes in schizophrenia related to antipsychotic treatment? A meta-analysis of longitudinal MRI studies. Neurosci Biobehav Rev. 2013;37(8):1680–91.
61. Scheepers FE, De Wied CCG, Pol HEH, Van De Flier W, Van Der Linden JA, Kahn RS. The effect of clozapine on caudate nucleus volume in schizophrenic patients previously treated with typical antipsychotics. Neuropsychopharmacology. 2001;24(1):47–54.
62. Hilker R, Helenius D, Fagerlund B, Skytthe A, Christensen K, Werge TM, et al. Heritability of Schizophrenia and Schizophrenia Spectrum Based on the Nationwide Danish Twin Register. Biol Psychiatry. 2018;83(6):492–8. Available from: http://dx.doi.org/10.1016/j.biopsych.2017.08.017
63. Schwab SG, Wildenauer DB. Genetics of psychiatric disorders in the GWAS era: An update on schizophrenia. Eur Arch Psychiatry Clin Neurosci. 2013;263:147–54.
64. Henriksen MG, Nordgaard J, Jansson LB. Genetics of schizophrenia: Overview of methods, findings and limitations. Front Hum Neurosci. 2017;11(322).
65. Duncan EL, Brown MA. Genome-Wide Association Studies. In: Genetics of Bone Biology and Skeletal Disease: Second Edition. 2017.
66. Antonio F. Pardiñas1, Peter Holmans1, Andrew J. Pocklington1, Valentina Escott- Price1, Stephan Ripke2, 3, Noa Carrera1, Sophie E. Legge1, Sophie Bishop1, Darren Cameron1, Marian L. Hamshere1, Jun Han1, Leon Hubbard1, Amy Lynham1, Kiran Mantripragada1 ER. Common schizophrenia alleles are enriched in mutation intolerant genes and in region under strong

background selection. Nature Genetics. 2018;50(3):381–9. Available from: file:///C:/Users/Carla Carolina/Desktop/Artigos para acrescentar na qualificação/The impact of birth weight on cardiovascular disease risk in the.pdf
67. Skene NG, Bryois J, Bakken TE, Breen G, James J, Gaspar HA, et al. Genetic identification of brain cell types underlying schizophrenia. 2019;50(6):825–33.
68. Bush WS, Moore JH. Chapter 11: Genome-Wide Association Studies. PLoS Comput Biol. 2012;8(12):1–10.
69. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, et al. 10 Years of GWAS Discovery: Biology, Function, and Translation. American Journal of Human Genetics. 2017. p. 5–22.
70. van Mierlo HC, Schot A, Boks MPM, de Witte LD. The association between schizophrenia and the immune system: Review of the evidence from unbiased 'omic-studies.' Schizophrenia Research. 2020;217:114–23. Available from: https://doi.org/10.1016/j.schres.2019.05.028
71. Aguet F, Brown AA, Castel SE, Davis JR, He Y, Jo B, et al. Genetic effects on gene expression across human tissues. Nature. 2017;550(7675):204–13.
72. Mantione KJ, Kream RM, Kuzelova H, Ptacek R, Raboch J, Samuel JM, et al. Comparing bioinformatic gene expression profiling methods: microarray and RNA-Seq. Med Sci Monit Basic Res. 2014;20:138–41.
73. Ball S, Gilbert TL, Overly CC. The Human Brain Online: An Open Resource for Advancing Brain Research. PLoS Biol. 2012;10(12).
74. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, Miller JA, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. Nature. 2012 Sep 20;489(7416):391–9. Available from: http://www.nature.com/articles/nature11405
75. Sunkin SM, Ng L, Lau C, Dolbeare T, Gilbert TL, Thompson CL, et al. Allen Brain Atlas: An integrated spatio-temporal portal for exploring the central nervous system. Nucleic Acids Res. 2013;41(database issue).
76. Arnatkevičiūtė A, Fulcher BD, Fornito A. A practical guide to linking brain-wide gene expression and neuroimaging data. Neuroimage. 2019;189:353–67.
77. Miller JA, Ding SL, Sunkin SM, Smith KA, Ng L, Szafer A, et al. Transcriptional landscape of the prenatal human brain. Nature. 2014;508:199–206.
78. Vasung L, Abaci Turk E, Ferradal SL, Sutin J, Stout JN, Ahtam B, et al. Exploring early human brain development with structural and physiological neuroimaging. Neuroimage. 2019;187:226–54.
79. Pei G, Wang YY, Simon LM, Dai Y, Zhao Z, Jia P. Gene expression imputation and cell-type deconvolution in human brain with spatiotemporal precision and its implications for brain-related disorders. Genome Res. 2021;1(1):146–58.
80. Mahfouz A, Ziats MN, Rennert OM, Lelieveldt BPF, Reinders MJT. Shared Pathways Among Autism Candidate Genes Determined by Co-expression Network Analysis of the Developing Human Brain Transcriptome. J Mol Neurosci. 2015;57:580–94.
81. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, Miller JA, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. Nature. 2012;489:391–9.
82. Negi SK, Guda C. Global gene expression profiling of healthy human brain and its application in studying neurological disorders OPEN. Nat Reports. 2017 Dec 18 ;7(897):1–12. Available from: www.nature.com/scientificreports/
83. Mccarthy MJ, Liang S, Spadoni AD, Kelsoe JR, Simmons AN. Whole Brain Expression of Bipolar Disorder Associated Genes: Structural and Genetic Analyses. PLoS One . 2014 ;9(6):1–13. Available from: www.plosone.org
84. Rodriguez MZ, Comin CH, Casanova D, Bruno OM, Amancio DR, Costa L da F, et al. Clustering algorithms: A comparative approach. PLoS One. 2019;14(1).
85. Jayanthi K, Mahesh C. A Study on machine learning methods and applications in genetics and genomics. Int J Eng Technol. 2018;7(1.7):201–4.
86. Saelens W, Cannoodt R, Saeys Y. A comprehensive evaluation of module detection methods for gene expression data. Nat Commun. 2018;9(1090):1–12.
87. Gibbons FD, Roth FP. Judging the quality of gene expression-based clustering methods using gene annotation. Genome Res. 2002;12:1574–81.

88. Jain AK. Data clustering: 50 years beyond K-means. Pattern Recognit Lett. 2010;31:651–66.
89. Shi N, Liu X, Guan Y. Research on k-means clustering algorithm: An improved k-means clustering algorithm. In: 3rd International Symposium on Intelligent Information Technology and Security Informatics, IITSI 2010. IEEE; 2010. p. 63–7.
90. Morissette L, Chartier S. The k-means clustering technique: General considerations and implementation in Mathematica. Tutor Quant Methods Psychol. 2013;9(1):15–24.
91. Charrad M, Ghazzali N, Boiteau V, Niknafs A. Nbclust: An R package for determining the relevant number of clusters in a data set. J Stat Softw. 2014;61(6):1–36.
92. Kassambara A, Mundt F. Factoextra: extract and visualize the results of multivariate data analyses [Computer sofware, package]. R package version. 2017.
93. van Dam S, Võsa U, van der Graaf A, Franke L, de Magalhães JP. Gene co-expression analysis for functional classification and gene-disease predictions. Brief Bioinform. 2018;19(4):575–92.
94. Li J, Zhou D, Qiu W, Shi Y, Yang JJ, Chen S, et al. Application of Weighted Gene Co-expression Network Analysis for Data from Paired Design. Sci Rep. 2018;8(622).
95. Langfelder P, Horvath S. WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9(559).
96. Liu Y, Gu HY, Zhu J, Niu YM, Zhang C, Guo GL. Identification of Hub Genes and Key Pathways Associated With Bipolar Disorder Based on Weighted Gene Co-expression Network Analysis. Front Physiol. 2019;10:1–9.
97. Costantini G, Epskamp S, Borsboom D, Perugini M, Mõttus R, Waldorp LJ, et al. State of the aRt personality research: A tutorial on network analysis of personality data in R. J Res Pers. 2015;54:13–29.
98. Yu D, Kim M, Xiao G, Hwang TH. Review of Biological Network Data and Its Applications. Genomics Inform. 2013;11(4):200–10.
99. Sun K, Gonçalves JP, Larminie C, Pržulj N. Predicting disease associations via biological network analysis. BMC Bioinformatics. 2014;15(304):1–13.
100. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software Environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498–504.
101. Cline MS, Smoot M, Cerami E, Kuchinsky A, Landys N, Workman C, et al. Integration of biological networks and gene expression data using cytoscape. Nat Protoc. 2007;2(10):2336–82.
102. Gang Su1, John H. Morris2, Barry Demchak3 and GDB. BIOLOGICAL NETWORK EXPLORATION WITH CYTOSCAPE 3. Curr Protoc Bioinforma. 2014;74(8).
103. Assenov Y, Ramírez F, Schelhorn SESE, Lengauer T, Albrecht M. Computing topological parameters of biological networks. Bioinformatics. 2008;24(2):282–4.
104. Kohl M, Wiese S, Warscheid B. Cytoscape: software for visualization and analysis of biological networks. Methods Mol Biol. 2011;
105. Carbon S, Dietze H, Lewis SE, Mungall CJ, Munoz-Torres MC, Basu S, et al. Expansion of the gene ontology knowledgebase and resources: The gene ontology consortium. Nucleic Acids Res. 2017;45(Database issue):331-D338.
106. Blake JA, Christie KR, Dolan ME, Drabkin HJ, Hill DP, Ni L, et al. Gene ontology consortium: Going forward. Nucleic Acids Res. 2015;43(D1):1049–56.
107. Langfelder P. anRichment: Collections and annotation data for use with anRichmentMethods. 2020.
108. Carlson M. org.Hs.eg.db: Genome wide annotation for Human. 2019.
109. Kassambara A, Mundt F, Kassambara, A.; Mundt F. Factoextra: extract and visualize the results of multivariate data analyses. URL http//www.sthda.com/english/rpkgs/factoextra BugReports. 2017;
110. Barabási AL, Oltvai ZN. Network biology: Understanding the cell's functional organization. Nat Rev Genet. 2004;5:101–13.
111. Pavlopoulos GA, Secrier M, Moschopoulos CN, Soldatos TG, Kossida S, Aerts J, et al. Using graph theory to analyze biological networks. BioData Min. 2011;4(10).
112. Jacob R, Harikrishnan KP, Misra R, Ambika G. Measure for degree heterogeneity in complex networks and its application to recurrence network analysis. R Soc Open Sci. 2017;4(1).
113. Dong J, Horvath S. Understanding network concepts in modules. BMC Syst Biol. 2007;1(24).

114. Zawerton, Ash; Mignot, Cyril; Sigafoos, Ashley; Blackburn, Patrick R.; Haseeb, Abdul; McWalter, Kirsty; Ichikawa, Shoji; Nava, Caroline; Keren, Boris; Charles, Perrine; Marey, Isabelle; Tabet, Anne-Claude; Levy, Jonathan; Perrin, Laurence; Hartmann, Andre C. Widening of the genetic and clinical spectrum of Lamb-Shaffer syndrome, a neurodevelopmental disorder due to SOX5 haploinsufficiency. Genet Med. 2019;0(0).
115. Manfred Schartl1, 2, 3, Susanne Schories1, Yuko Wakamatsu1, Yusuke Nagao4, Hisashi Hashimoto4, Chloé Bertin5, Brigitte Mourot5, Cornelia Schmidt1, Dagmar Wilhelm6, Lazaro Centanin7 YG and AH. Sox5 is involved in germ-cell regulation and sex determination in medaka following co-option of nested transposable elements. BMC Biol. 2018;16(16).
116. Tae-Gul Lee1, 2 , Eun-Hui Jeong1, Seo Yun Kim1, Hye-Ryoun Kim1 HK, Kim C-H. Fhit, a tumor suppressor protein, induces autophagy via 14-3-3τ in non-small cell lung cancer cells. Oncotarget. 2017;8(19):31923–37.
117. Garneau AP, Slimani S, Tremblay LE, Fiola MJ, Marcoux AA, Isenring P. K+-Cl− cotransporter 1 (KCC1): a housekeeping membrane protein that plays key supplemental roles in hematopoietic and cancer cells. J Hematol Oncol [Internet]. 2019 Dec 11;12(1):74. Available from: https://jhoonline.biomedcentral.com/articles/10.1186/s13045-019-0766-x
118. Fiona C. Brown, 1 Ashlee J. Conway, 1 Loretta Cerruti, 1 Janelle E. Collinge, 2 Catriona McLean, 3 James S. Wiley, 4 Ben T. Kile, 2 Stephen M. Jane, 1, 3, 5,* and David J. Curtis1iona C. Brown, 1 Ashlee J. Conway, 1 Loretta Cerruti, 1 Janelle E. Collinge, and DJC. Activation of the erythroid K-Cl cotransporter Kcc1 enhances sickle cell disease pathology in a humanized mouse model. Blood. 2015;126(26):2863–70.
119. Kourosh Parham, M.D., Ph.D.1, George A. Kuchel, M.D.2, Janet E. McElhaney, M.D.4, and Laura Haynes PD. A relationship between blood levels of otolin-1 and vitamin D. Otol Neurotol. 2018;39(4):269–73.
120. Murat Doğan , Mustafa Şahin YK. Otolin-1, as a Potential Marker for Inner Ear Trauma after Mastoidectomy. J Int Adv Otol. 2019;15(2):200–3.
121. Yanyan Liu1 2 · Chuang Tong2 · Jun Cao2 · Maoming Xiong1. NEMP1 Promotes Tamoxifen Resistance in Breast Cancer Cells. Biochem Genet. 2019;57(6):813–26.
122. Yonit Tsatskis1*, Robyn Rosenfeld1, 2*, Joel D. Pearson1*, Curtis Boswell2, 3*, Yi Qu1, Kyunga Kim1, 4, Lacramioara Fabian5, Ariz Mohammad6, Xian Wang7, 8, Michael I. Robson9, Karen Krchma10, Jun Wu10, João Gonçalves1, Didier Hodzic11, Shu Wu1, Daniel Pot HM. The NEMP family supports metazoan fertility and nuclear envelope stiffness. Sci Adv Genet. 2020;6.
123. Hiroshi Mamada, Noriyuki Takahashi MT. Involvement of an inner nuclear membrane protein, Nemp1, in Xenopus neural development through an interaction with the chromatin protein BAF. Dev Biol. 2009;327:497–507.
124. Marta Codina-Solà, Benjamín Rodríguez-Santiago, Aïda Homs, Javier Santoyo, Maria Rigau, Gemma Aznar-Laín, Miguel del Campo, Blanca Gener, Elisabeth Gabau, María Pilar Botella, Armand Gutiérrez-Arumí1, Guillermo Antiñolo, Luis Alberto Pérez-Jurado and IC. Integrated analysis of whole-exome sequencing and transcriptome profiling in males with autism spectrum disorders. Mol Autism. 2015;6(21).
125. Mitsuru Ebihara, Hisako Ohba, Mika Kikuchi TY. Structural characterization and promoter analysis of human potassium channel Kv8.1 (KCNV1) gene. Gene. 2004;325:89–96.
126. Takashi Hashimoto 1, Shohei Maekawa SM. IgLON cell adhesion mol- ecules regulate synaptogenesis in hippocampal neurons. Cell Biochem Functopm. 2009;7:496–8.
127. Lan Wen a, Lei Liu b, Linyan Tong a, Jinfang Li a, Keming Zhang a, Qinbin Zhang a CL a. NDRG4 prevents cerebral ischemia/reperfusion injury by inhibiting neuronal apoptosis. Genes Dis. 2019;6:448–54.
128. Mitsuo Wada, Kayo Yukawa, Hiroyuki Ogasawara, ..., Takeshi Ohta, Eunyoung Lee TM. GPR52 accelerates fatty acid biosynthesis in a ligand-dependent manner in hepatocytes and in response to excessive fat intake in mice. iScience. 2021;24(102260).
129. Hidetoshi Komatsu1*., Minoru Maruyama2., Shuuhei Yao3, Tokuyuki Shinohara4, Kensuke Sakuma2, Sachiko Imaichi4, Tomoko Chikatsu2, Kanako Kuniyeda5, Foo Kok Siu6, Lam Sock Peng6, Katherine Zhuo7, Lay Sock Mun7, Tan Min Han7, Yoshio Matsumoto3, Tadatoshi Has MM.

Anatomical Transcriptome of G Protein-Coupled Receptors Leads to the Identification of a Novel Therapeutic Candidate GPR52 for Psychiatric Disorders. PLoS One. 2014;9(2).
130. Lorena Benedetti1, 2 , Matteo Cereda1, 2 , LeeAnn Monteverde1, 2 , Nikita Desai1 2 and, Ciccarelli FD. Synthetic lethal interaction between the tumour suppressor STAG2 and its paralog STAG1. Oncotarget. 2017;8(23):37619–32.
131. Petra van derPetra van der Lelij1,* , Joseph A Newman2,*, Simone Lieb3,*, Julian Jude1,*, Vittorio Katis2, Thomas Hoffmann1, Matthias Hinterndorfer1, Gerd Bader3, Norbert Kraut3, Mark A Pearson3, Jan-Michael Peters1, 4, Johannes Zuber1, 4, Opher Gileadi2, MP. STAG1 vulnerabilities for exploiting cohesin synthetic lethality in STAG2-deficient cancers. Life Sci Alliance. 2020;3(7).
132. Daphné Lehalle, 1, 2 Anne-Laure Mosca-Boidron, 3, 4 Amber Begtrup, 5 Odile Boute-Benejean, 6 Perrine Charles, 7 Megan T Cho, 5 Amanda Clarkson, 8 Orrin Devinsky, 9 Yannis Duffourd, 4 Laurence Duplomb-Jego, 3, 4 Bénédicte Gérard, 10 Aurélia Jacquette, 7 Pa 4 Laurence Faivre1. STAG1 mutations cause a novel cohesinopathy characterised by unspecific syndromic intellectual disability. BMJ. 2017;54(7):479–88.
133. Whitton L, Apostolova G, Rieder D, Dechant G, Rea S, Donohoe G, et al. Genes regulated by SATB2 during neurodevelopment contribute to schizophrenia and educational attainment. PLoS Genet. 2018;14(7).
134. Qian Y, Liu J, Yang Y, Chen M, Jin C, Chen P, et al. Paternal low-level mosaicism-caused SATB2-associated syndrome. Front Genet. 2019;
135. Schult, D., Ho¨lsken MB, Schlaffer, S.-M. Siegel, S. Buslei, I. Kreitschmann-Andermahr , R. Fahlbusch R. Expression pattern of neuronal intermediate filament a-internexin in anterior pituitary gland and related tumors. Pituitary. 2015;18:465–473.
136. Yingjie Li, Liangliang Bai, Huichuan Yu, Du Cai, Xiaolin Wang, Baoyuan Huang, Shaoyong Peng, Meijin Huang, Guangwen Cao, Andrew M. Kaz, William M. Grady, Jianping Wang, and Yingjie Li, Liangliang Bai1, Huichuan Yu, Du and YLL. Epigenetic Inactivation of α-Internexin Accelerates Microtubule Polymerization in Colorectal Cancer. Cancer Res. 2020;80(23):5203–15.
137. Whelan R, Clair DS, Mustard CJ, Hallford P, Wei J. Study of novel autoantibodies in schizophrenia. Schizophr Bull. 2018;44(6):1341–9.
138. Xiaoyu Xu1, 2,† Zhao Zeng1,† Li Huo1,† Hong Liu1, 3 Yan Yu1 Ling Zhang1 Jiannong Cen1 Huiying Qiu1, 3 Xiaowen Tang1, 3 Chengcheng Fu1, 3 Yue Han1, 2, 3 Miao Miao1, 3 Zhengming Jin1, 3 Changgeng Ruan1, 2, 3 Depei Wu1, 2, 3 Suning Chen1, 2, 3 Qinrong Wang1 3, Lingzhi Yan1 3. High expression of myocyte enhancer factor 2C predicts poor prognosis for adult acute myeloid leukaemia with normal karyotype. BJH Corresp. 2019;189(1):23–7.
139. Adam J Harrington 1, Catherine M Bridges 2, Stefano Berto 3, Kayla Blankenship 1, Jennifer Y Cho 2, Ahlem Assali 1, Benjamin M Siemsen 4, Hannah W Moore 5, Evgeny Tsvetkov 1, Acadia Thielking 1, Genevieve Konopka 3, David B Everman 5, Michael D Scofield 4 CWC 6. MEF2C Hypofunction in Neuronal and Neuroimmune Populations Produces MEF2C Haploinsufficiency Syndrome-like Behaviors in Mic. Biol Psychiatry. 2020;15(88):488–99.
140. Adam J Harrington1 2†, , Aram Raissi2† , Kacey Rajkovich3 SB, Jaswinder Kumar2 4, Molinaro3 G, Raduazzo2 J, Guo2 Y, et al. MEF2C regulates cortical inhibitory and excitatory synapses and behaviors relevant to neurodevelopmental disorders. Elife. 2016;5.
141. Melanie Richter1, Nadeem Murtaza2, 3 Robin Scharrenberg1 Sean H. White2, 3 Ole Johanns1 Susan Walker4, 5 , Ryan K. C. Yuen 4, 5 Birgit Schwanke1 Bianca Bedürftig1 Melad Henis1, 6 Sarah Scharf7 Vanessa Kraus7 , Ronja Dörk7 Jakob Hellmann7 Zsuzsa Lindenmaie 3 Froylan Calderon de Anda. Altered TAOK2 activity causes autism-related neurodevelopmental and cognitive abnormalities through RhoA signaling. Mol Psychiatry. 2019;24:1329–50.
142. Chih-Yeu Fang 1, Tsung-Ching Lai 2, 3, Michael Hsiao 1, 4 and Y-CC. The Diverse Roles of TAO Kinases in Health and Diseases. J Mol Sci. 2020;27(7463).
143. Yang Shao1, 2, Jing Kong1† HX, , Xiaoli Wu1, YuePeng Cao1 , Weijian Li 1 , Jing Han4 , Dake Li 1 KX and JW. OPCML Methylation and the Risk of Ovarian Cancer: A Meta and Bioinformatics Analysis. Front Cell Dev Biol. 2021;9(570908).
144. Zhengrong Zhang, Maoqing Ye, Qiongwei Li, Rena Li, Jun Li DZ. The Schizophrenia Susceptibility Gene OPCML Regulates Spine Maturation and Cognitive Behaviors through Eph-Cofilin Signaling.

Cell Rep. 2019;29:49–61.
145. Jane Antony1, 2, 3, Elisa Zanini1 , Zoe Kelly1, Tuan Zea Tan2, Evdoxia Karali1, Mohammad Alomary1, Youngrock Jung1, Katherine Nixon1, Paula Cunnea1, Christina Fotopoulou1, Andrew Paterson1, Sushmita Roy-Nawathe1, Gordon B Mills4, Ruby Yun-Ju Huang2, 5, Je & CR. The tumour suppressor OPCML promotes AXL inactivation by the phosphatase PTPRG in ovarian cancer. EMBO J. 2081;19(e45670).
146. Vougiouklakis1 T, Ryuji Hamamoto1 Y, Vassiliki N&, Saloura* 1. The NSD family of protein methyltransferases in human cancer. Epigenomics. 2015;7(5).
147. Li LYLYQLC. Downregulation of NSD3 (WHSC1L1) inhibits cell proliferation and migration via ERK1/2 deactivation and decreasing CAPG expression in colorectal cancer cells. Onco Targets Ther. 2019;12:3933–43.
148. Chunmei Wang, 1, 2 Qinlan Wang, 3 Xiaoqing Xu, 1 Bin Xie, 3 Yong Zhao, 4 Nan Li, 2 and Xuetao Cao1, 2 3. The methyltransferase NSD3 promotes antiviral innate immunity via direct lysine methylation of IRF3. J Exp Med. 2017;214(12):3597–610.
149. Vassiliki Saloura1 , Theodore Vougiouklakis1, Makda Zewde1, Kiyotani1 Kazuma, Jae-Hyun Park1, Guimin Gao2, Theodore Karrison2, Mark Lingen3 , Yusuke Nakamura1, 4 RH. WHSC1L1 drives cell cycle progression through transcriptional regulation of CDC6 and CDK2 in squamous cell carcinoma of the head and neck. Oncotarget. 2016;7(27):42527–38.
150. Paternoster L, Soblet J, Aeby A, De Tiège X, Goldman S, Yue WW, et al. Novel homozygous variant of carbonic anhydrase 8 gene expanding the phenotype of cerebellar ataxia, mental retardation, and disequilibrium syndrome subtype 3. Am J Med Genet Part A. 2020;97(3):516–20.
151. Ma HL, Yu SJ, Chen J, Ding XF, Chen G, Liang Y, et al. CA8 promotes RCC proliferation and migration though its expression level is lower in tumor compared to adjacent normal tissue. Biomed Pharmacother. 2020;121(109578).
152. Zhuang GZ, Keeler B, Grant J, Bianchi L, Fu ES, Zhang YP, et al. Carbonic anhydrase-8 regulates inflammatory pain by inhibiting the ITPR1-cytosolic free calcium pathway. PLoS One. 2015;10(3).
153. Tom Hearn. ALMS1 and Alström syndrome: a recessive form of metabolic, neurosensory and cardiac deficits. J Mol Med. 2019;97:1–17.
154. Shao-Yan Zhang, Chao Xuan, Yi Wang, Shao-Qiang Zhang, Hui Li, Guo-Wei He and Q-WT. Association between ALMS 1 variants and early-onset coronary artery disease: a case–control study in Chinese population. Biosci Rep. 2020;40(BSR20193637).
155. Hemang M. Parikh1 2*, , Targ Elgzyri2, Amra Alibegovic3, Natalie Hiscock4, Ola Ekström2, Karl-Fredrik Eriksson2, Allan Vaag3, Leif C. Groop2, 5, Kristoffer Ström2 6† and Ola Hansson. Relationship between insulin sensitivity and gene expression in human skeletal muscle. BMC Endocr Disord. 2021;21(32).
156. Jakub Tomasik, PhD1; Santiago G. Lago, PhD1; Javier Vázquez-Bourgon, MD P. Association of Insulin Resistance With Schizophrenia Polygenic Risk Score and Response to Antipsychotic Treatment. JAMA Psychiatry. 2019;76(8).
157. Zaoa X, *, Jin Chengb *, Congle Shena , Guiwen Guana, Xiaoyu Fenga, Jun Zoua, Jing Zhanga TL, Huiling Zhenga, Ting Zhanga, Jie Wanga, Jia Liua, Deyao Lia, Fengmin Lu, Fuping You and XC. NFATc3 inhibits hepatocarcinogenesis and HBV replication via positively regulating RIG-I-mediated interferon transcription. Oncoimmunology. 2021;10(1).
158. Katia Urso, Andrés Fernández, Patricia Velasco, Javier Cotrina, Belén de Andrés, Pilar Sánchez-Gómez , Aurelio Hernández-Laín, Sonsoles Hortelano JMR& EC. NFATc3 controls tumour growth by regulating proliferation and migration of human astroglioma cells. Nat Sci Reports. 2019;9(9361).
159. Sridevi Venkatesan EKL. Chrna5 is essential for a rapid and protected response to optogenetic release of endogenous acetylcholine in prefrontal cortex. bioXriv. 2020;40(38):7255–68.
160. Thaka'a K. Al-Omoush1, Karem H. Alzoubi, Omar F. Khabour, Fawzi M. Alsheyab, Ahmed Abu-siniyeh, Nour A. Al-Sawalha, Fadia A. Mayyas, Caroline O. Cobb TE. The CHRNA5 Polymorphism (rs16969968) and its Association with Waterpipe Smoking Addiction among Jordanians. Arab J Basix Appl Sci. 2020;27(1):450–5.
161. Myriam Srour, Noriaki Shimokawa, Fadi F. Hamdan, Christina Nassif, Chantal Poulin, Lihadh Al Gazali, Jill A. Rosenfeld, Noriyuki Koibuchi, Guy A. Rouleau, Aisha Al Shamsi and JLM.

Dysfunction of the Cerebral Glucose Transporter SLC45A1 in Individuals with Intellectual Disability and Epilepsy. CellPress. 2017;100(5):824–30.
162. Thyme SB, Pieper LM, Li EH, Pandey S, Wang Y, Morris NS, et al. Phenotypic Landscape of Schizophrenia-Associated Genes Defines Candidates and Their Shared Functions. Cell [Internet]. 2019;177(2):478–91. Available from: https://doi.org/10.1016/j.cell.2019.01.048
163. Sant'Anna Feltrin A, Tahira AC, Simões SN, Brentani H, Martins DC. Assessment of complementarity of WGCNA and NERI results for identification of modules associated to schizophrenia spectrum disorders. PLoS One [Internet]. 2019 [cited 2019 Jul 3];14(1):1–22. Available from: https://doi.org/10.1371/journal.pone.0210431
164. Lai T, Jabaudon D, Molyneaux BJ, Azim E, Arlotta P, Menezes JRL, et al. SOX5 Controls the Sequential Generation of Distinct Corticofugal Neuron Subtypes. Neuron. 2008;57:232–47.
165. Sun C, Ban Y, Wang KAI, Sun Y, Zhao Z. SOX5 promotes breast cancer proliferation and invasion by transactivation of EZH2. Oncol Lett. 2019;17(3):1–9.
166. You J, Zhao Q, Fan X, Wang J. SOX5 promotes cell invasion and metastasis via activation of twist-mediated epithelial– mesenchymal transition in gastric cancer. Onco Targets Ther. 2019;12:2465–76.
167. Fu Y, Shan X, Song W, Xu K, Jiao C, Zhang Q. Correlations of breast cancer FHIT gene with the incidence and prognosis of breast cancer. JBUON. 2019;24(9):40–7.
168. Sullivan CR, Funk AJ, Shan D, Haroutunian V, McCullumsmith RE. Decreased chloride channel expression in the dorsolateral prefrontal cortex in schizophrenia. PLoS One. 2015;10(3).
169. Lewis DA, Narendran R. In Vivo Measurement of GABA Transmission in Healthy Subjects and Schizophrenia Patients W. Am J Psychiatry. 2016;172(11):1148–59.
170. Silverstein SM, Rosen R. Cognition Schizophrenia and the eye. Schizophr Res. 2015;2(Cognition 2):46–55.
171. Almonte MT, Capellàn P, Yap TE, Cordeiro MF. Retinal correlates of psychiatric disorders. Ther Adv Chronic Dis. 2020;11:1–21.
172. Zhang L, Li X, Zhou R, Xing G. Possible role of potassium channel , big K in etiology of Schizophrenia. Med Hypotheses. 2006;67:41–3.
173. Yamamoto H, Kokame K, Okuda T, Nakajo Y, Yanamoto H, Miyata T. NDRG4 Protein-deficient Mice Exhibit Spatial Learning Deficits and Vulnerabilities to Cerebral Ischemia * □. J Biol Chem. 2011;286(29):26158–65.
174. F. Ducray, MD E. Crinie`re, MSc A. Idbaih, MD, PhD K. Mokhtari, MD Y. Marie, MSc S. Paris, MSc S. Navarro, MD F. Laigle-Donadey, MD C. Dehais, MD J. Thillet, PhD K. Hoang-Xuan, MD, PhD, J.-Y. Delattre, MD M. Sanson, MD P. alpha-Internexin expression identifies 1p19q codeleted gliomas. Neurology. 2009;72(2):156–61.
175. Jiang X, Detera-Wadleigh SD, Akula N, Mallon BS, Hou L, Xiao T, et al. Sodium valproate rescues expression of TRANK1 in iPSC-derived neural cells that carry a genetic variant associated with serious mental illness. Mol Psychiatry [Internet]. 2019;24(4):613–24. Available from: http://dx.doi.org/10.1038/s41380-018-0207-1
176. Li W, Cai X, Li HJ, Song M, Zhang CY, Yang Y, et al. Independent replications and integrative analyses confirm TRANK1 as a susceptibility gene for bipolar disorder. Neuropsychopharmacology. 2020;0:1–10.
177. Mossink B, Negwer M, Schubert D, Nadif Kasri N. The emerging role of chromatin remodelers in neurodevelopmental disorders: a developmental perspective [Internet]. Vol. 78, Cellular and Molecular Life Sciences. Springer International Publishing; 2021. 2517–2563 p. Available from: https://doi.org/10.1007/s00018-020-03714-5
178. Ruderfer DM, Ripke S, McQuillin A, Boocock J, Stahl EA, Pavlides JMW, et al. Genomic dissection of bipolar disorder and schizophrenia including 28 subphenotypes. bioRxiv. 2017;173(7):1705–15.
179. Laskaris LE, Di Biase MA, Everall I, Chana G, Christopoulos A, Skafidas E, et al. Microglial activation and progressive brain changes in schizophrenia. Br J Pharmacol. 2016;173(4):666–80.
180. Li M, Santpere G, Kawasawa YI, Evgrafov O V., Gulden FO, Pochareddy S, et al. Integrative functional genomic analysis of human brain development and neuropsychiatric risks. Science (80- ). 2018;362(6420).
181. Tangliang Li1, Yue Shi2, Pei Wang1, Luis Miguel Guachalla3, Baofa Sun2, Tjard Joerss1, Yu-Sheng

Chen2, Marco Groth1, Anja Krueger1, Matthias Platzer1, Yun-Gui Yang2, Karl Lenhard Rudolph1, 3 & Zhao-Qi Wang1 4, Abstract. Smg6Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay. Embo J. 2015;34(12).
182. Tao H, Zhou X, Xie Q, Ma Z, Sun F, Cui L, et al. SRR intronic variation inhibits expression of its neighbouring SMG6 gene and protects against temporal lobe epilepsy. J Cell Mol Med. 2018;22(3):1883–93.
183. Chi TH, Hsieh BY, Liang PS, Han TH, Hsieh M. Expression and Functional Study of Single Mutations of Carbonic Anhydrase 8 in Neuronal Cells. Cell Mol Neurobiol [Internet]. 2020;(0123456789). Available from: https://doi.org/10.1007/s10571-020-00907-1
184. Citton V, Favaro A, Bettini V, Gabrieli J, Milan G, Greggio NA, et al. Brain involvement in Alström syndrome. Ophanet J Rare Dis. 2013;8(24).
185. Wang X, Christian KM, Song H, Ming G. Synaptic dysfunction in complex psychiatric disorders : from genetics to mechanisms. Genome Med. 2018;10(9):9–11.
186. Bennett MR. Synapse formation and regression in the cortex during adolescence and in schizophrenia. Med J Aust. 2009;190(4):4–6.
187. Minami T. Calcineurin-NFAT activation and DSCR-1 auto-inhibitory loop : how is homoeostasis regulated ? J Biochem. 2014;155(4):217–26.
188. Kipanyula MJ, Kimaro WH, Etet PFS. The Emerging Roles of the Calcineurin-Nuclear Factor of Activated T-Lymphocytes Pathway in Nervous System Functions and Diseases. J Aging Res. 2016;5081021.
189. Wei Han, Tianxiao Zhang, Tong Ni, Li Zhu, Dan Liu, Gang Chen, Huali Lin, Teng Chen FG. Relationship of common variants in CHRNA5 with early-onset schizophrenia and executive function. Schizophr Res. 2019;206:407–12.
190. Yuhong Wang, Yuanjia Chen, Xiaoxing Li, Wanming Hu, Yu Zhang, Luohai Chen MC and JC. Loss of expression and prognosis value of alpha-internexin in gastroenteropancreatic neuroendocrine neoplasm. BMC Cancer. 2018;18(691).
191. Xi Lin, Mingyue Li, Niandong Wang, Yiran Wu, Zhipu Luo, Shimeng Guo, Gye-Won Han, Shaobai Li, Yang Yue1, Xiaohu Wei Xin Xie, Yong Chen, Suwen Zhao, Jian Wu ML& FX. Structural basis of ligand recognition and self-activation of orphan GPR52. Nature. 2020;579(7797):152–7.
192. Hidetoshi Komatsu MF and YH. Potential Utility of Biased GPCR Signaling for Treatment of Psychiatric Disorders. Int J Mol Sci. 2019;20(3207).
193. Orlandi C, Watkins LR. Orphan g protein coupled receptors in affective disorders. Genes (Basel). 2020;11(6):1–32.
194. Pingyuan Wang, Daniel E. Felsing, Haiying Chen, Sonja J. Stutz, Ryan E. Murphy, Kathryn A. Cunningham JAA and J. Discovery of Potent and Brain-Penetrant GPR52 Agonist that Suppresses Psychostimulant Behavior. J Med Chem. 2020;22:13951–13972.
195. Nishiyama K, Suzuki H, Harasawa T, Suzuki N, Kurimoto E, Kawai T, et al. FTBMT, a novel and selective GPR52 agonist, demonstrates antipsychotic-like and procognitive effects in rodents, revealing a potential therapeutic agent for schizophrenia. J Pharmacol Exp Ther. 2017;363(2):253–64.
196. Roy SK, Shrivastava A, Srivastav S, Shankar S, Srivastava RK. SATB2 is a novel biomarker and therapeutic target for cancer. J Cell Mol Med. 2020;24(19):11064–9.
197. Gary Donohoe DWMICLW, Georg Dechant GA. Genes encoding SATB2-interacting proteins in adult cerebral cortex contribute to human cognitive ability. PLoS Genet. 2019;15(2).
198. Lu X, Chen X, Huang L, Zhu C, Gu Y, Ye S. Anti- a -Internexin Autoantibody from Neuropsychiatric Lupus Induce Cognitive Damage via Inhibiting Axonal Elongation and Promote Neuron Apoptosis. PLoS One. 2010;5(6).
199. Müller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. Front Neurosci. 2015;9(372).
200. Monji A, Kato TA, Mizoguchi Y, Horikawa H, Seki Y, Kasai M, et al. Neuroinflammation in schizophrenia especially focused on the role of microglia. Prog Neuro-Psychopharmacology Biol Psychiatry [Internet]. 2013;42:115–21. Available from: http://dx.doi.org/10.1016/j.pnpbp.2011.12.002

201. Pepe G, De Maglie M, Minoli L, Villa A, Maggi A, Vegeto E. Selective proliferative response of microglia to alternative polarization signals. J Neuroinflammation. 2017;14(236).
202. Bachiller S, Jiménez-Ferrer I, Paulus A, Yang Y, Swanberg M, Deierborg T, et al. Microglia in neurological diseases: A road map to brain-disease dependent-inflammatory response. Front Cell Neurosci. 2018;12(488).
203. Sellgren CM, Gracias J, Watmuff B, Biag JD, Thanos JM, Whittredge PB, et al. Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. Nat Neurosci [Internet]. 2019;22(3):374–85. Available from: http://dx.doi.org/10.1038/s41593-018-0334-7
204. Miller BJ, Goldsmith DR. Towards an Immunophenotype of Schizophrenia: Progress, Potential Mechanisms, and Future Directions. Neuropsychopharmacology. 2017;42:299–317.
205. Nagatsu T. The catecholamine system in health and disease - Relation to tyrosine 3-monooxygenase and other catecholamine-synthesizing enzymes. Proc Japan Acad Ser B Phys Biol Sci. 2006;82:388–415.
206. Fujita H, Carlson ES. Catecholamine signaling that modulates cerebellar operations in cognition. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2021.
207. Vidal PM, Pacheco R. The Cross-Talk Between the Dopaminergic and the Immune System Involved in Schizophrenia. Front Pharmacol. 2020;11(394).
208. Sarkar C, Basu B, Chakroborty D, Dasgupta PS, Basu S. The immunoregulatory role of dopamine: An update. Brain Behav Immun [Internet]. 2010;24(4):525–8. Available from: http://dx.doi.org/10.1016/j.bbi.2009.10.015
209. Catak Z, Kocdemir E, Ugur K, Yardim M, Sahin I, Kaya H, et al. A novel biomarker renalase and its relationship with its substrates in schizophrenia. J Med Biochem. 2019;38(3):299–305.
210. Crider A, Pillai A. Estrogen signaling as a therapeutic target in neurodevelopmental disorders. J Pharmacol Exp Ther. 2017;360(1):48–58.
211. Gogos A, Sbisa AM, Sun J, Gibbons A, Udawela M, Dean B. A Role for Estrogen in Schizophrenia: Clinical and Preclinical Findings. Int J Endocrinol. 2015;2015(615356).
212. Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, et al. The Genotype-Tissue Expression (GTEx) project. Nat Genet. 2013;45(6):580–5.
213. Wang D, Liu S, Warrell J, Won H, Shi X, Navarro FCP, et al. Comprehensive functional genomic resource and integrative model for the human brain. Science (80- ). 2018;362(6420).
214. Chen G, Ning B, Shi T. Single-cell RNA-seq technologies and related computational data analysis. Front Genet. 2019;10(317).
215. Hedlund E, Deng Q. Single-cell RNA sequencing : Technical advancements and biological applications. Mol Aspects Med [Internet]. 2018;59:36–46. Available from: https://doi.org/10.1016/j.mam.2017.07.003
216. Botía JA, Vandrovcova J, Forabosco P, Guelfi S, D'Sa K, Hardy J, et al. An additional k-means clustering step improves the biological features of WGCNA gene co-expression networks. BMC Syst Biol. 2017;
217. Barrangou R, Doudna JA. Applications of CRISPR technologies in research and beyond. Nat Biotechnol. 2016;34(9):933–41.
218. Chen CH, Xiao T, Xu H, Jiang P, Meyer CA, Li W, et al. Improved design and analysis of CRISPR knockout screens. Bioinformatics. 2018;34(23):4095–101.

# 6.0 Appendix

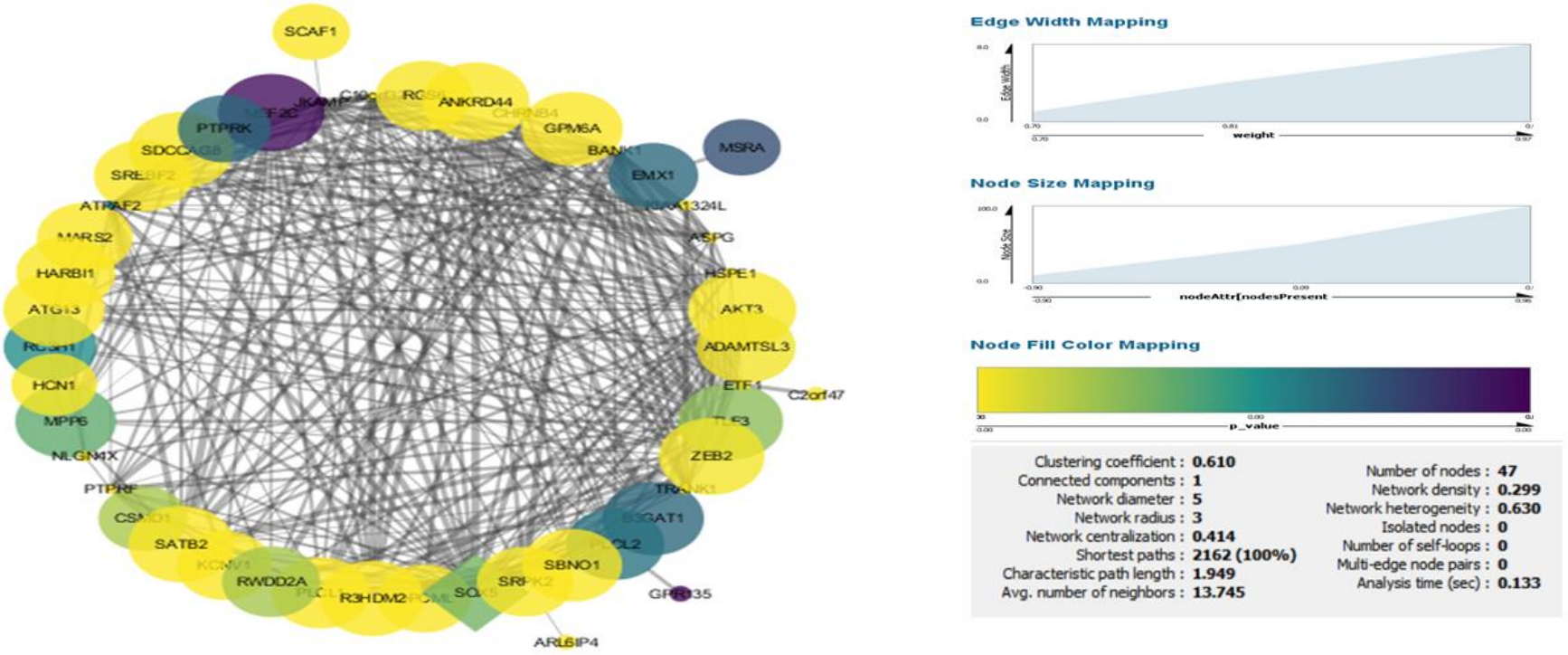



Figure 6.1.1 Black Module for developmental Stage One where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

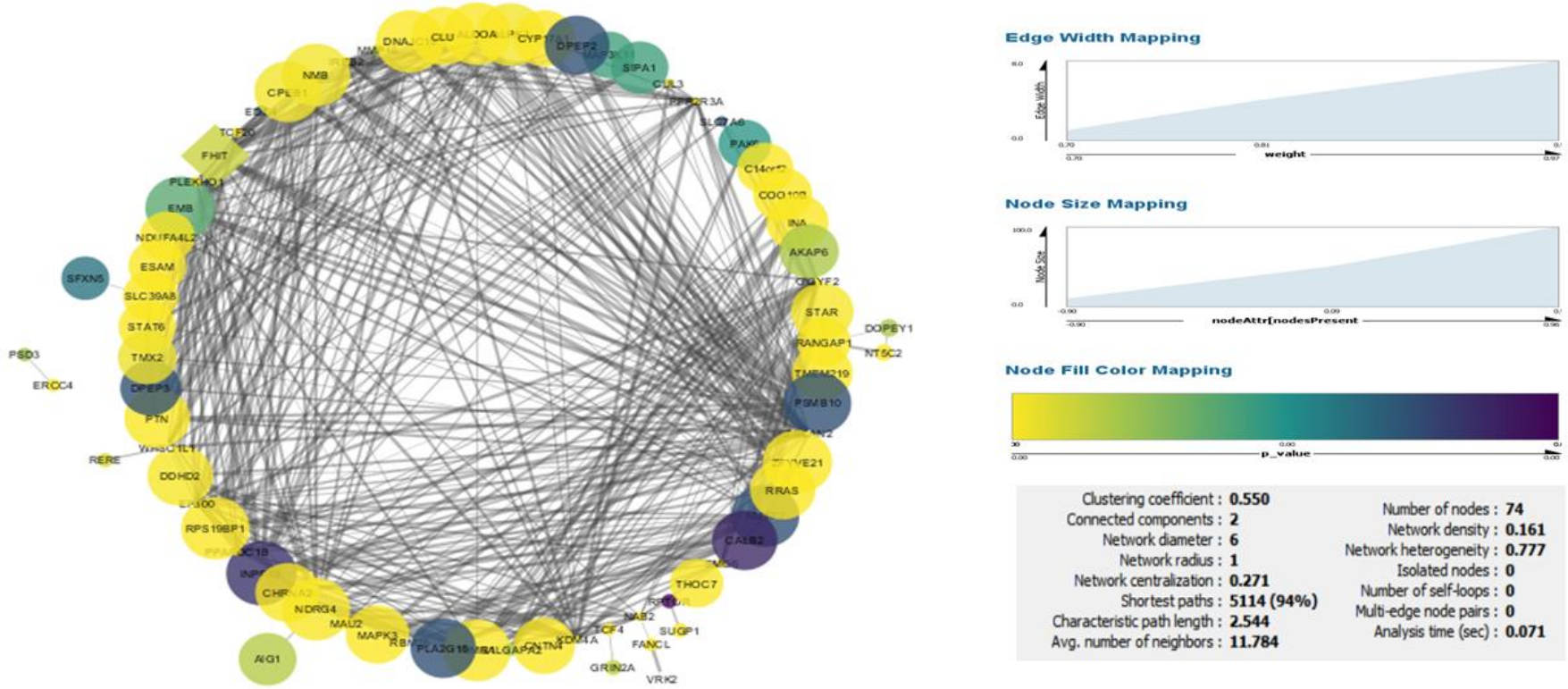



Figure 6.1.2 Blue Module for developmental Stage One where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

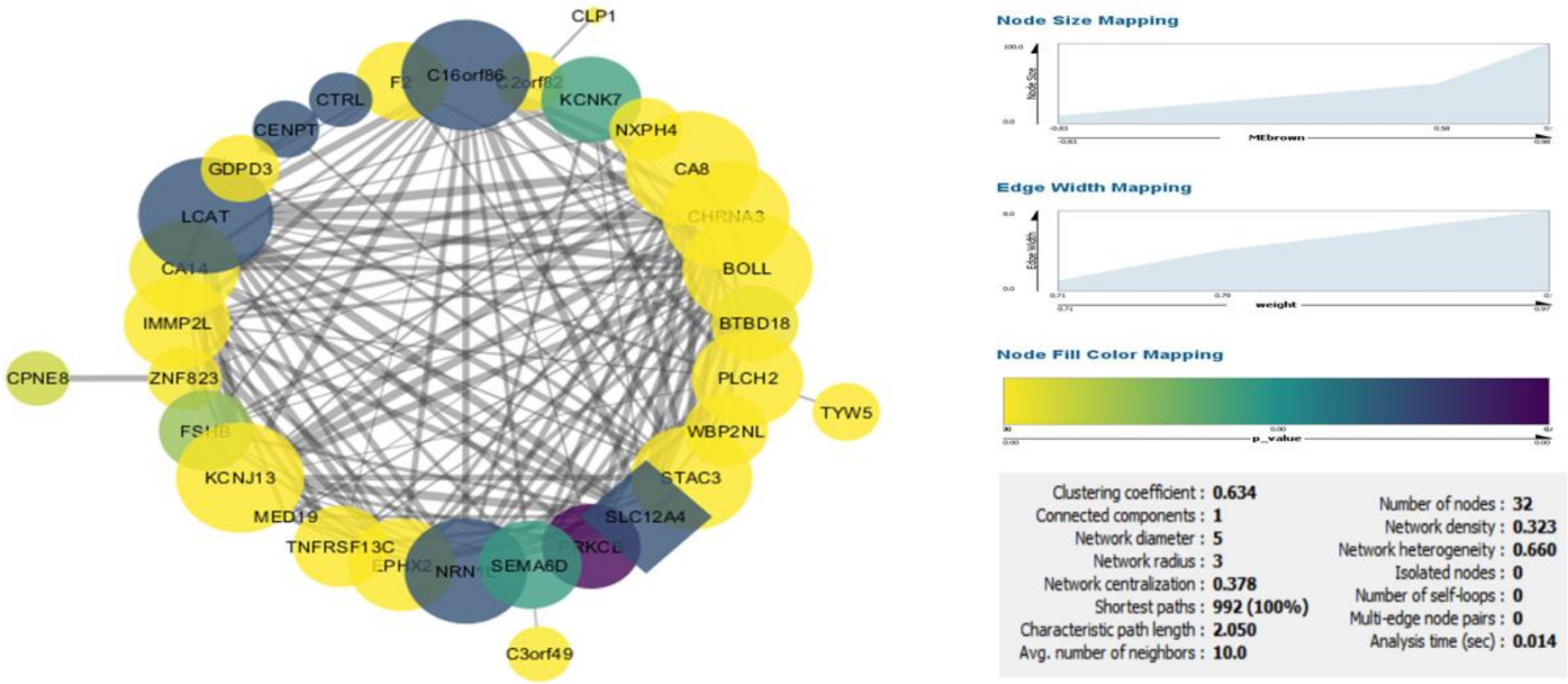



Figure 6.1.3 Brown Module for developmental Stage One where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

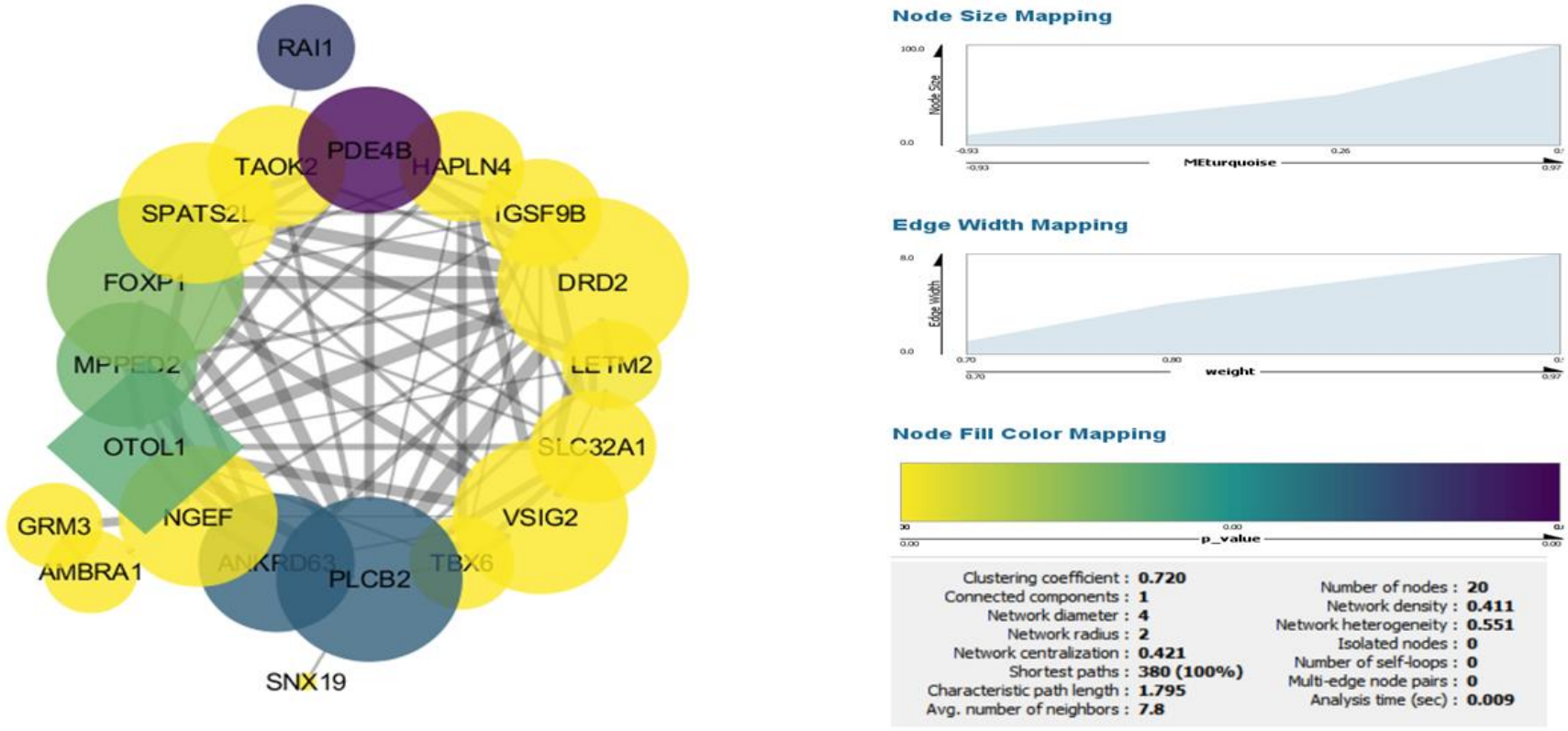



Figure 6.1.4 Pink Module for developmental Stage One where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

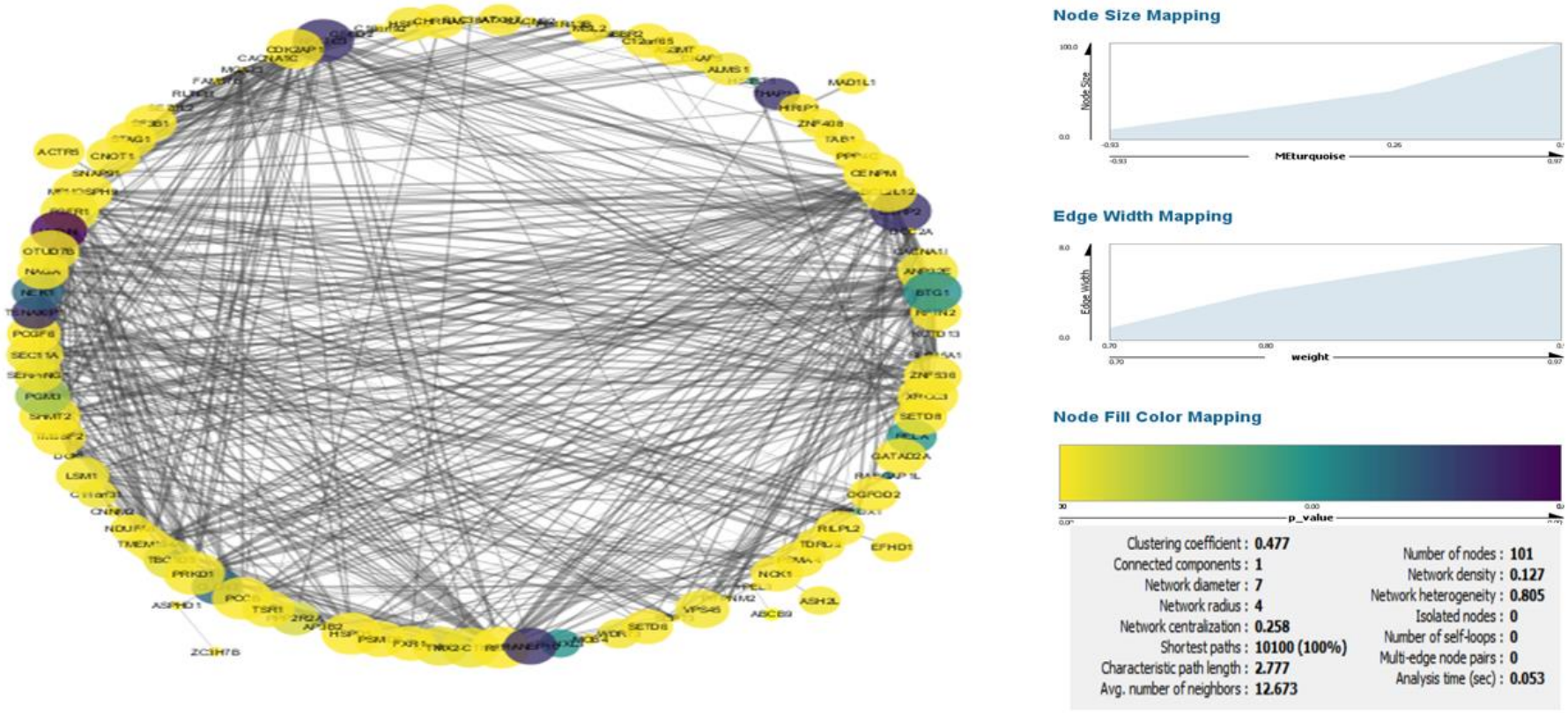



Figure 6.1.5 Turquoise Module for developmental Stage One where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

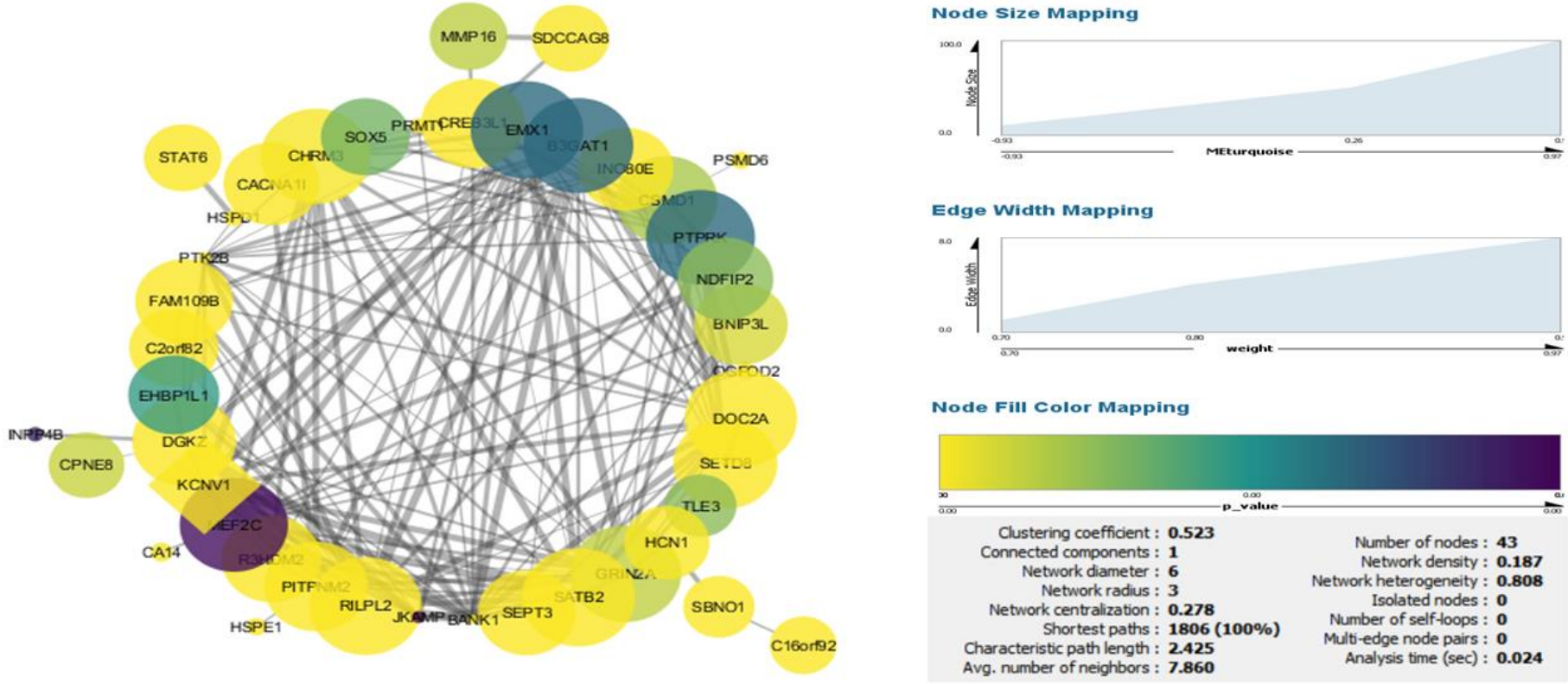
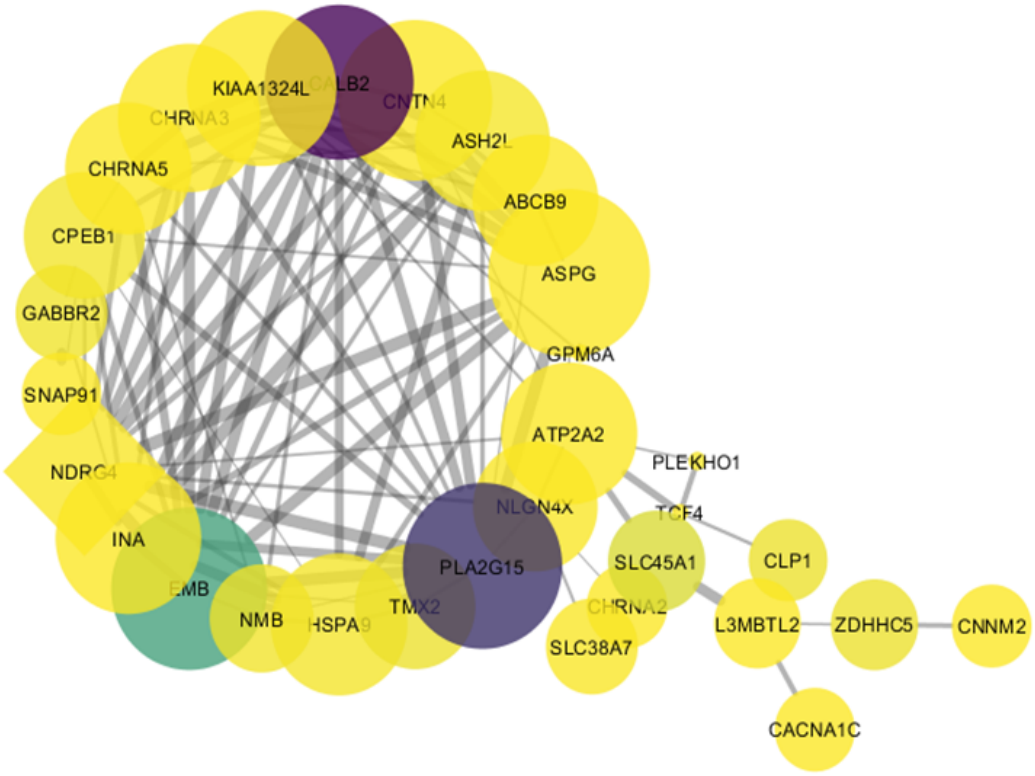
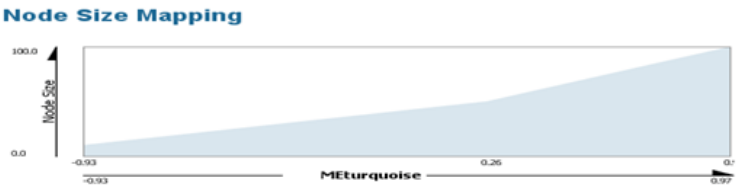
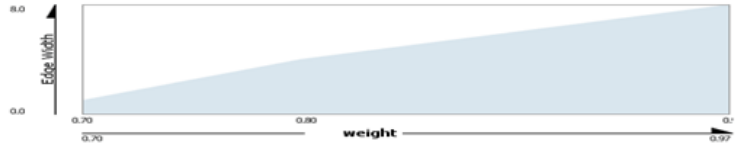
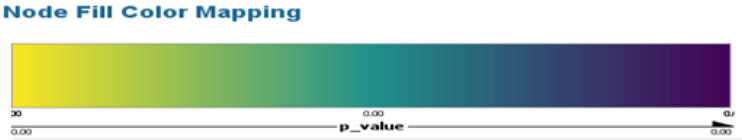



Figure 6.1.6 Blue Module for developmental Stage Two where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.




| | | | |
|---|---|---|---|
| Clustering coefficient : | **0.528** | Number of nodes : | **31** |
| Connected components : | **1** | Network density : | **0.217** |
| Network diameter : | **7** | Network heterogeneity : | **0.742** |
| Network radius : | **4** | Isolated nodes : | **0** |
| Network centralization : | **0.338** | Number of self-loops : | **0** |
| Shortest paths : | **930 (100%)** | Multi-edge node pairs : | **0** |
| Characteristic path length : | **2.781** | Analysis time (sec) : | **0.011** |
| Avg. number of neighbors : | **6.516** | | |

Figure 6.1.7 Brown Module for developmental Stage Two where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

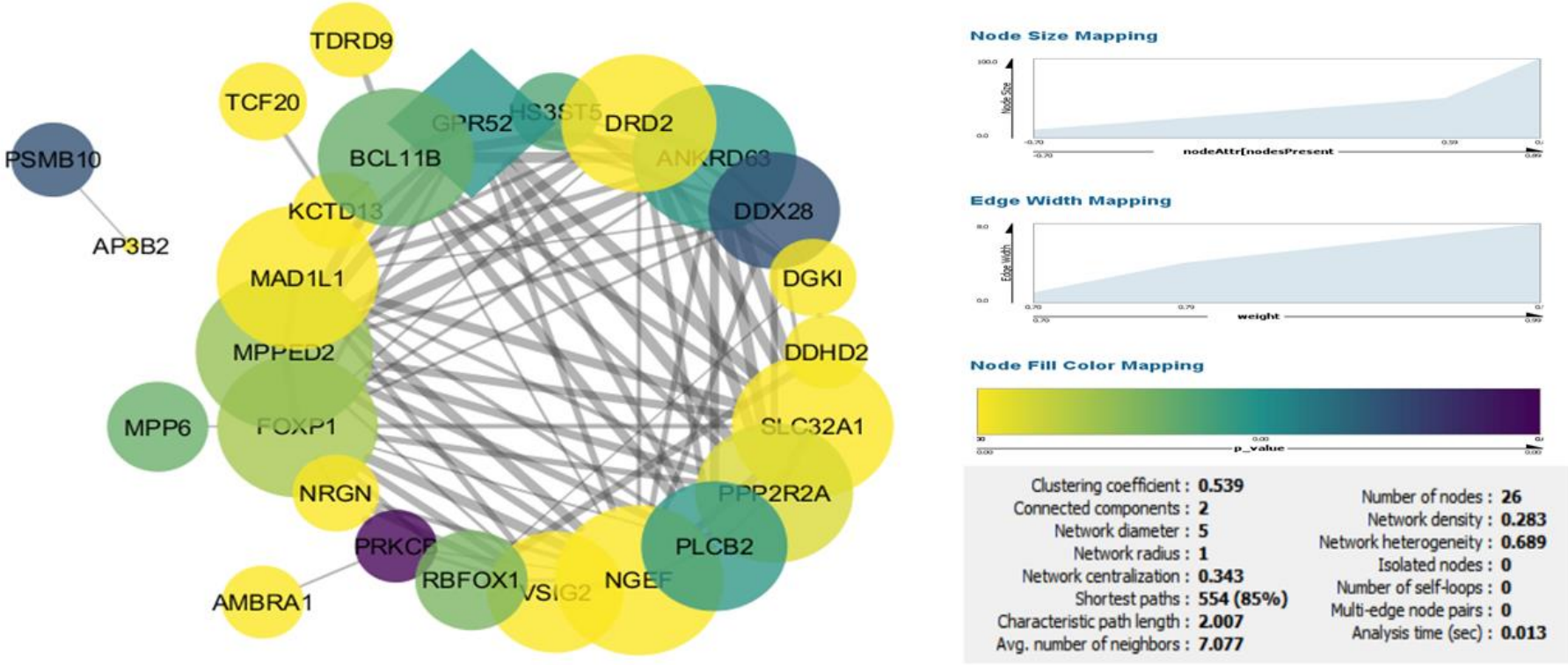



Figure 6.1.8 Green Module for developmental Stage Two where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

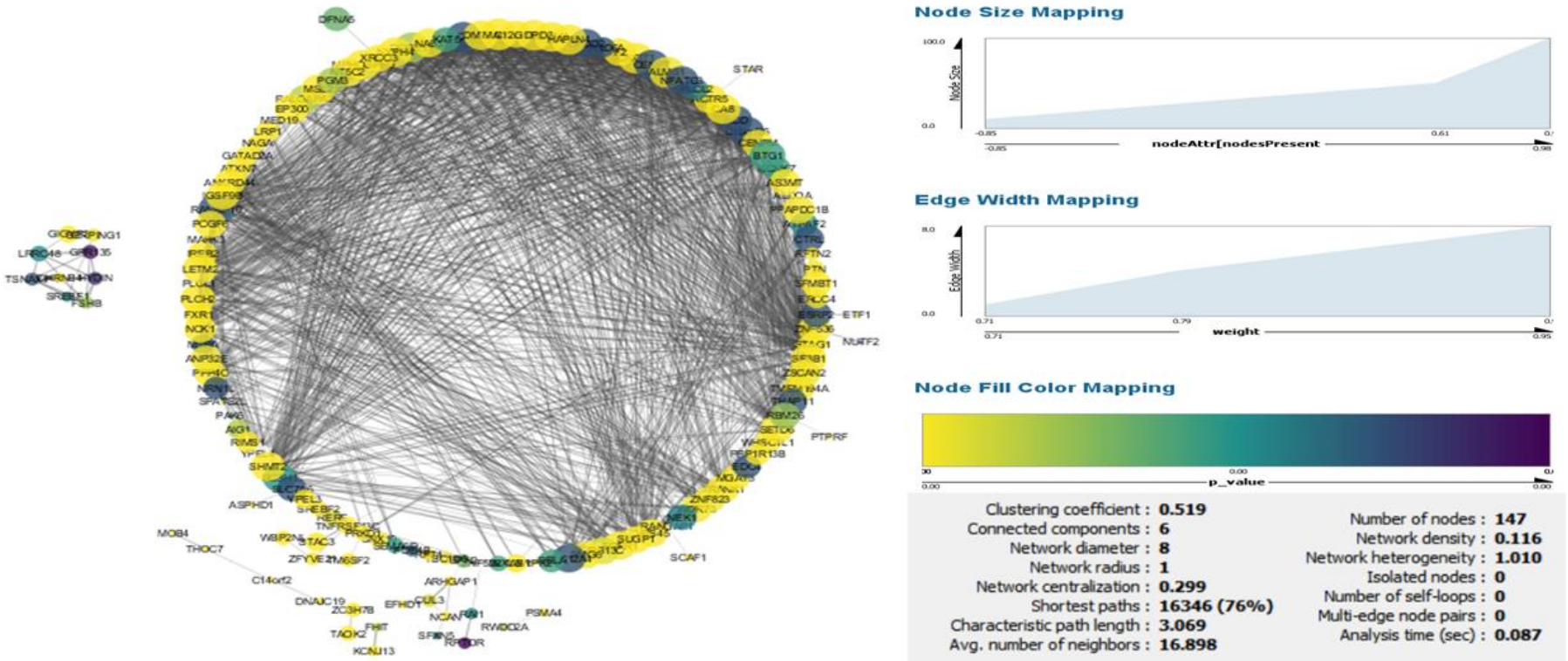



Figure 6.1.9 Turquoise Module for developmental Stage Two where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

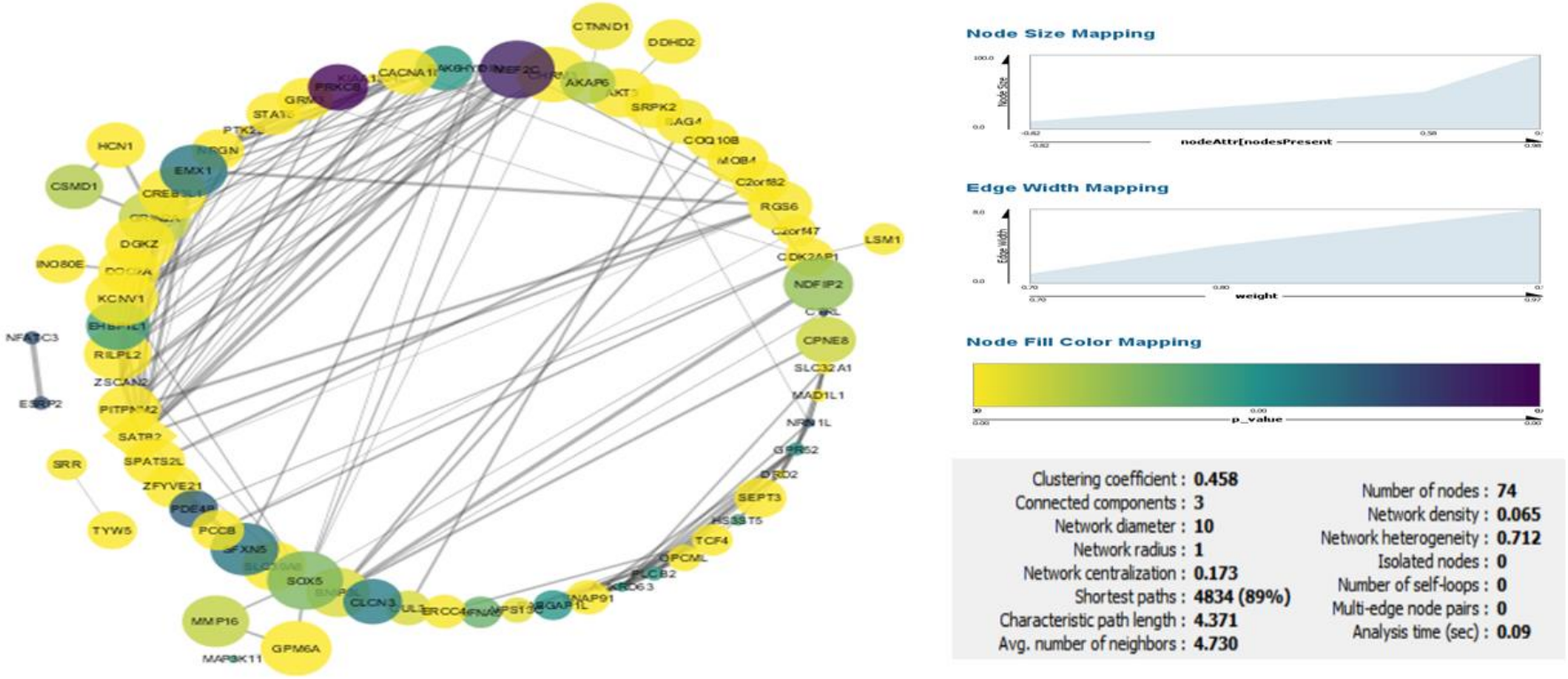



Figure 3.6.10 Brown Module for developmental Stage Three where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

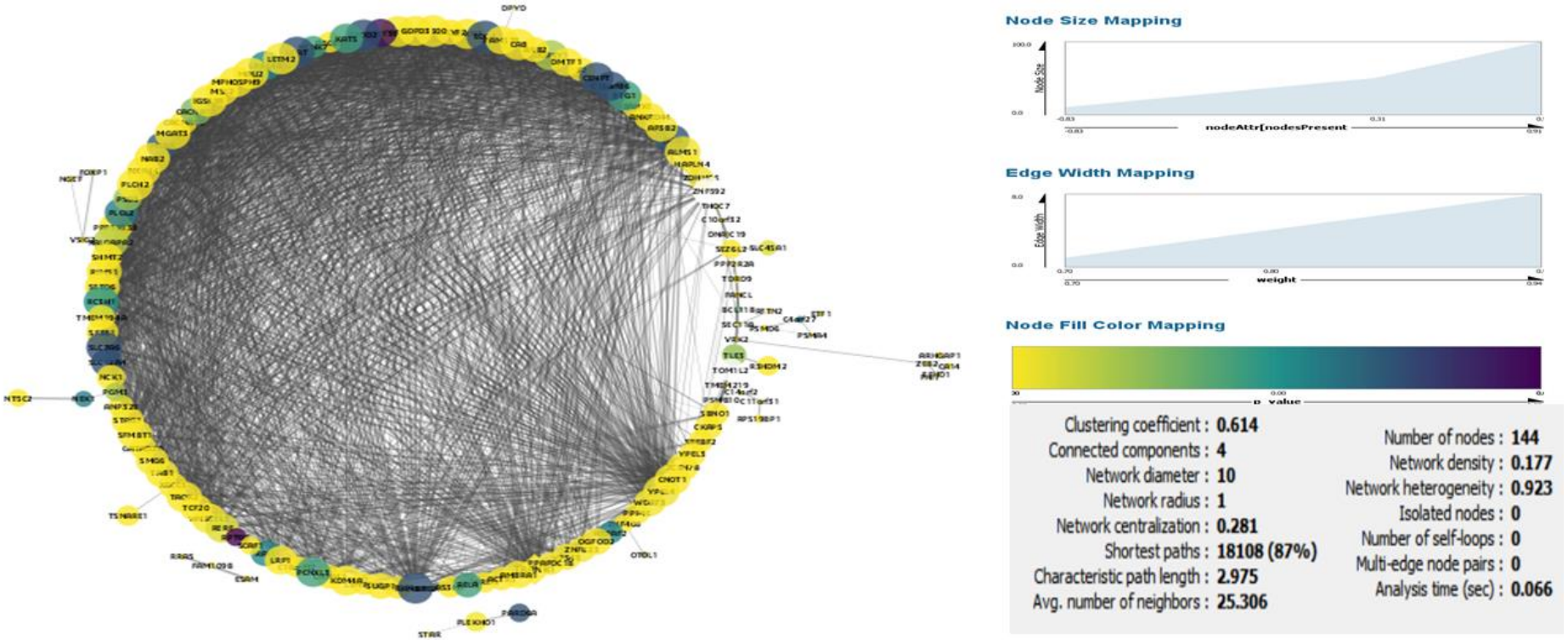



Figure 6.1.11 Turquoise Module for developmental Stage Three where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

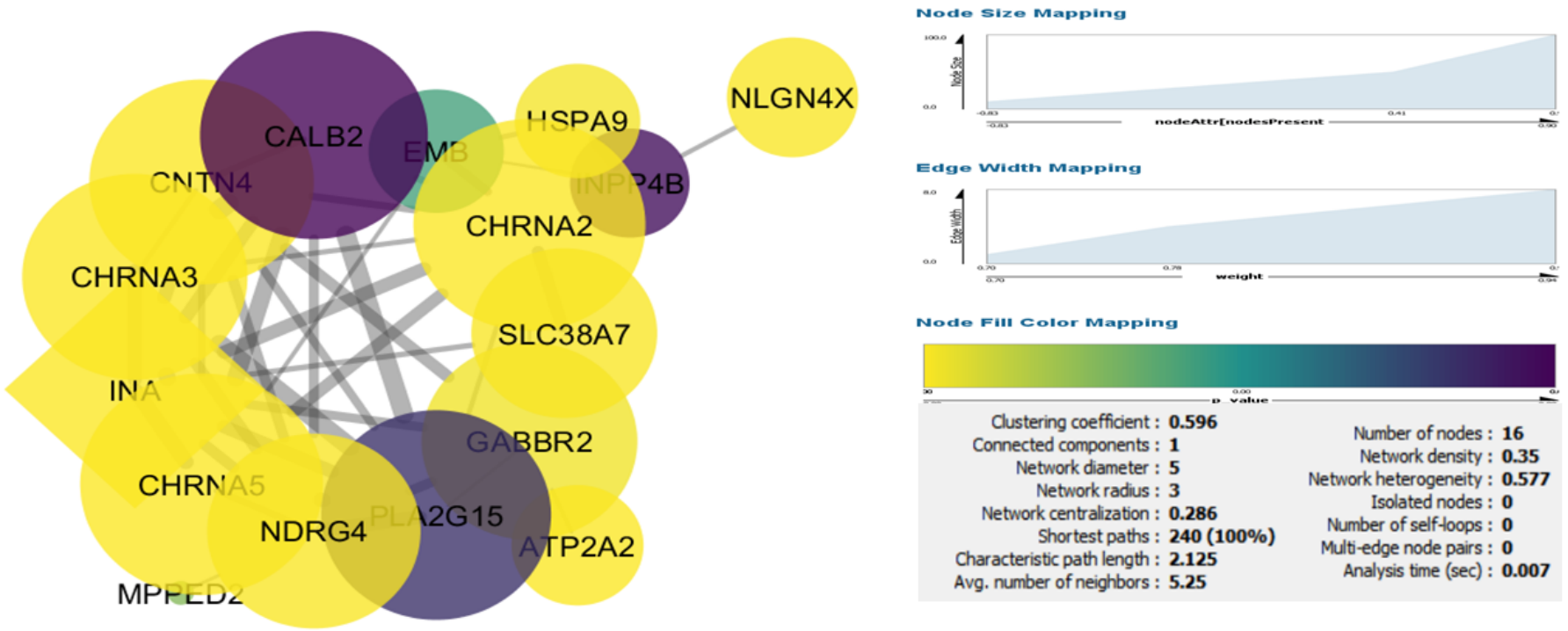



Figure 6.1.12 Yellow Module for developmental Stage Three where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

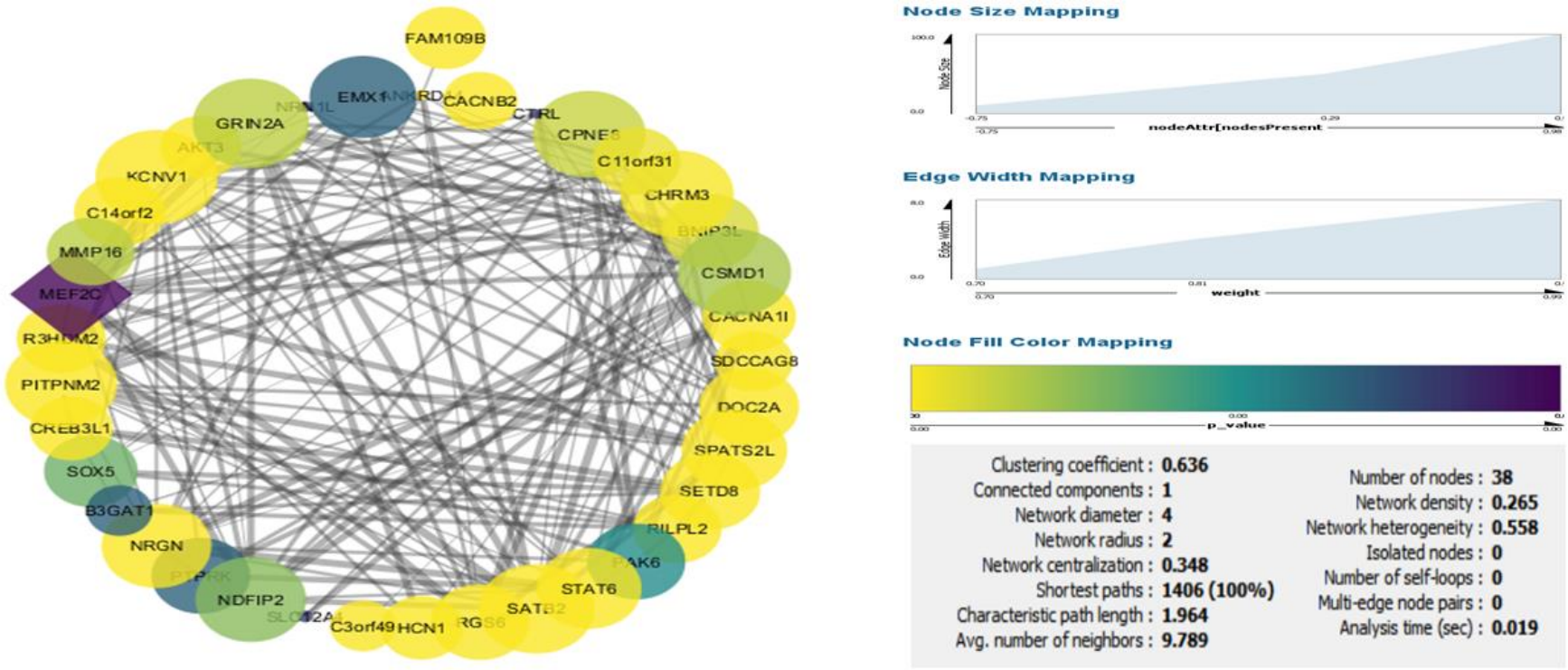



Figure 6.1.13 Blue Module for developmental Stage Four where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

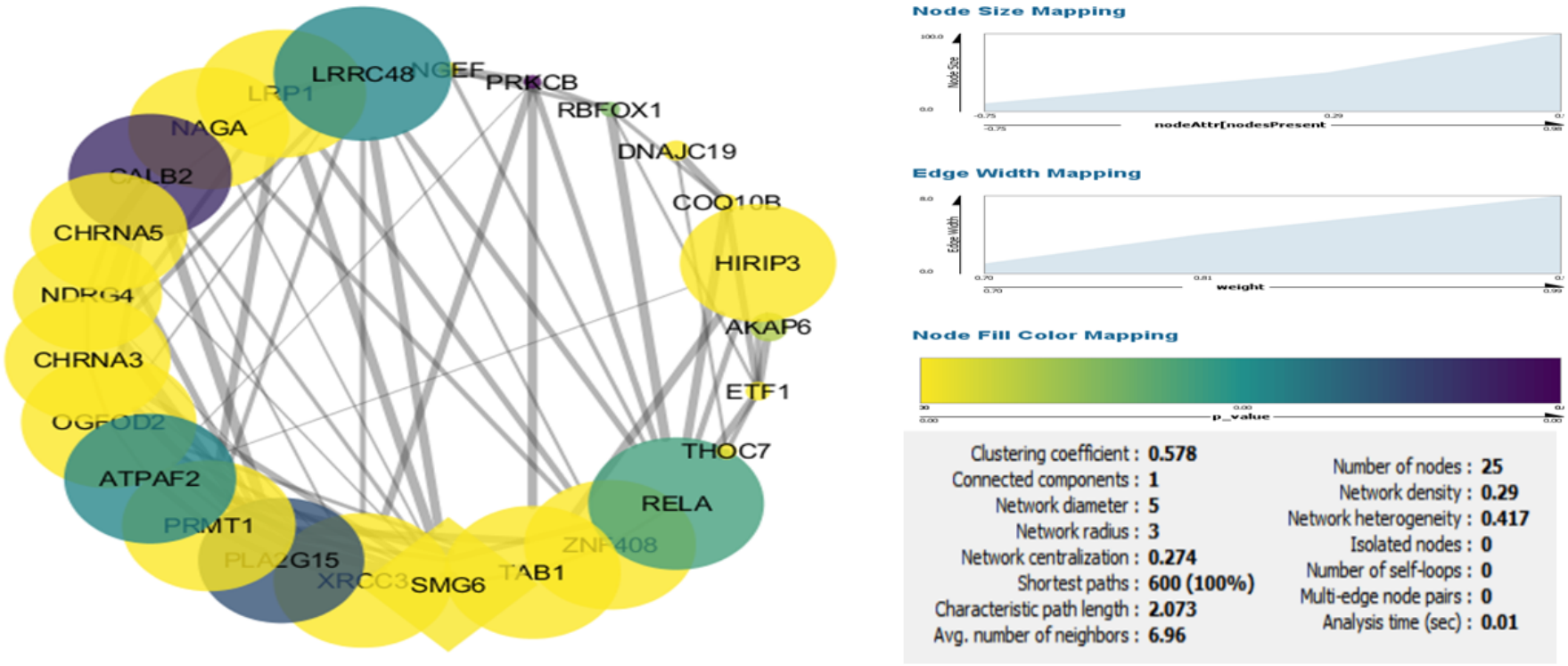



Figure 6.1.14 Brown Module for developmental Stage Four where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

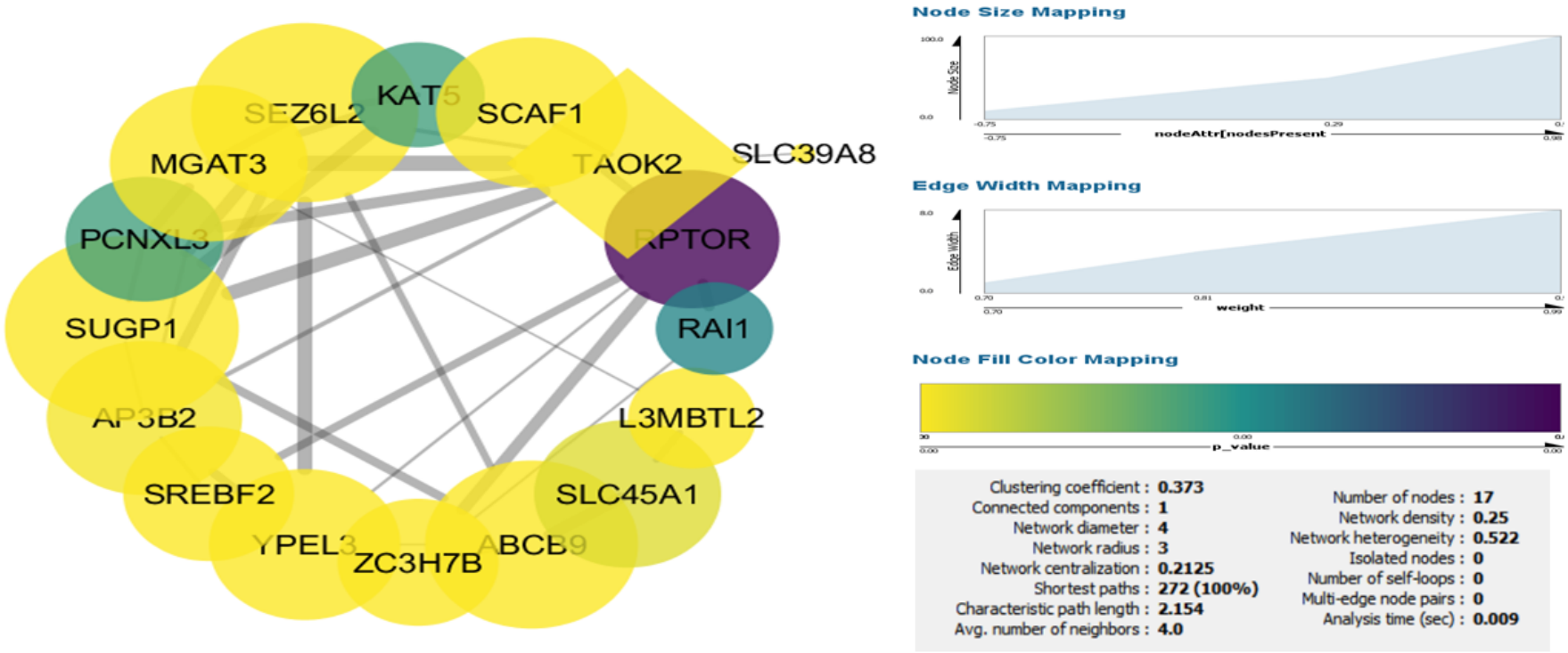



Figure 6.1.15 Green Module for developmental Stage Four where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

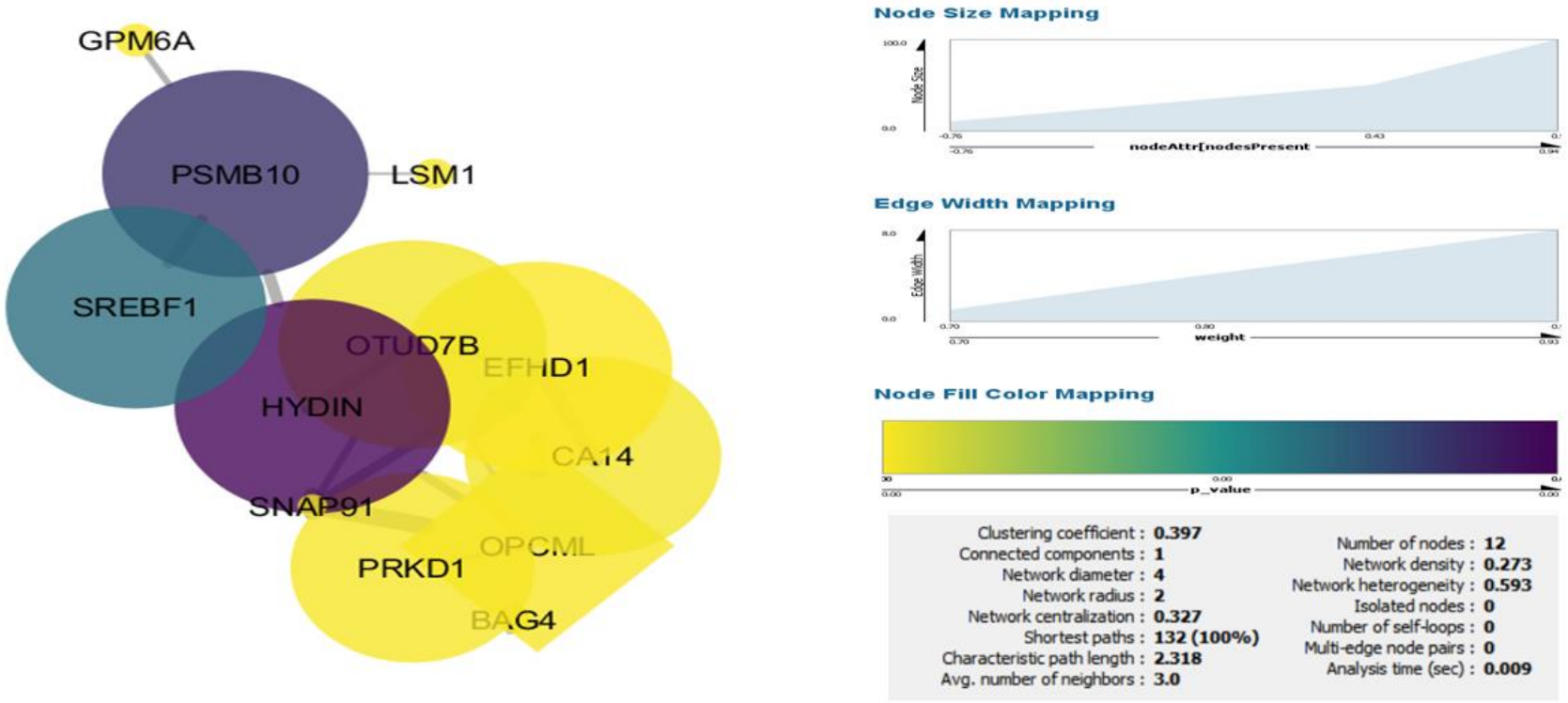



Figure 6.1.16 Magenta Module for developmental Stage Four where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

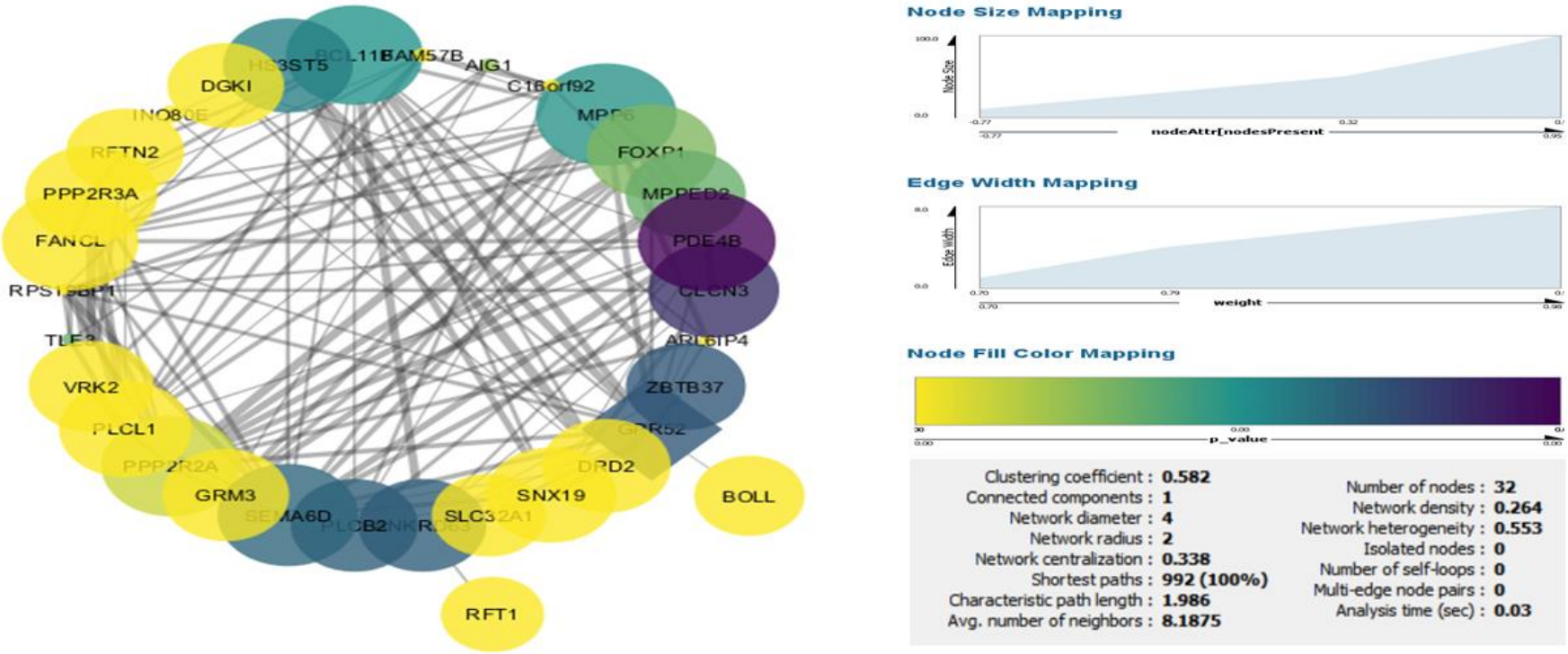



Figure 6.1.17 Purple Module for developmental Stage Four where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

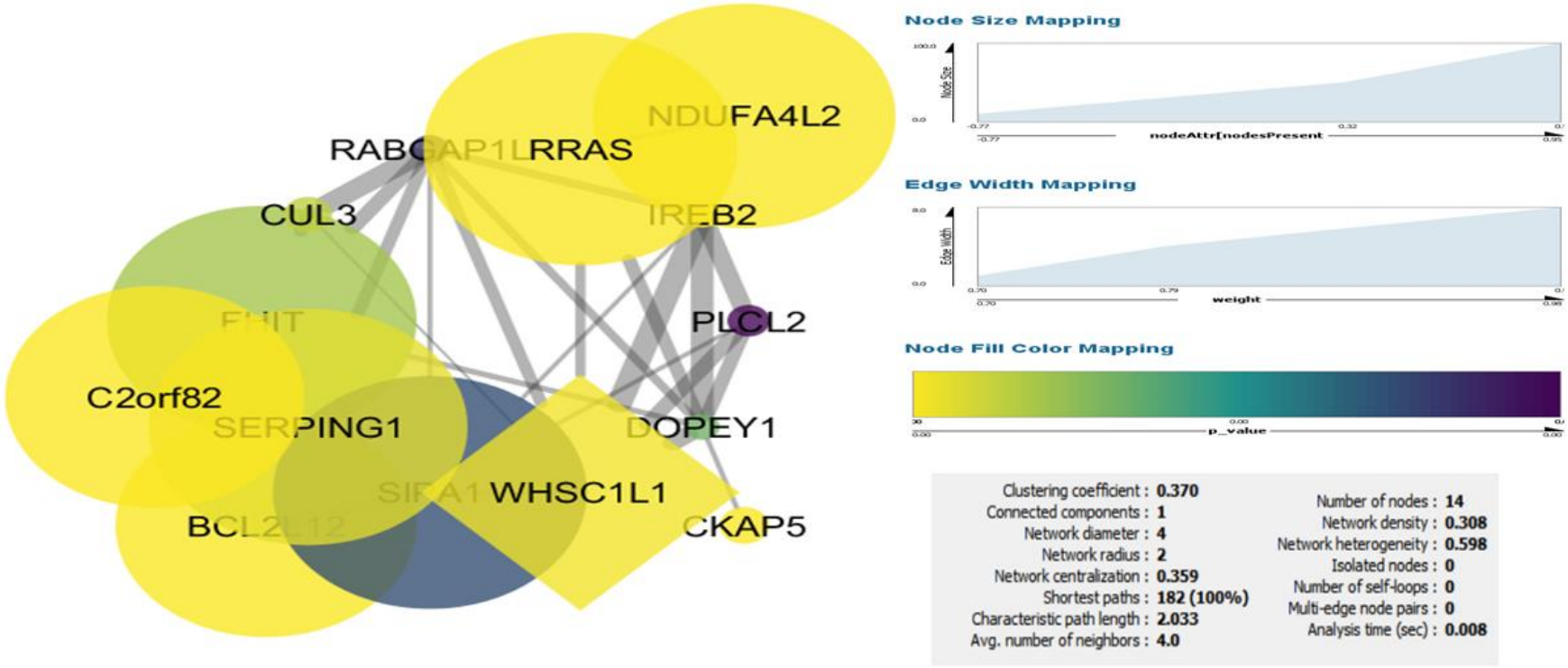



Figure 6.1.18 Red Module for developmental Stage Four where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

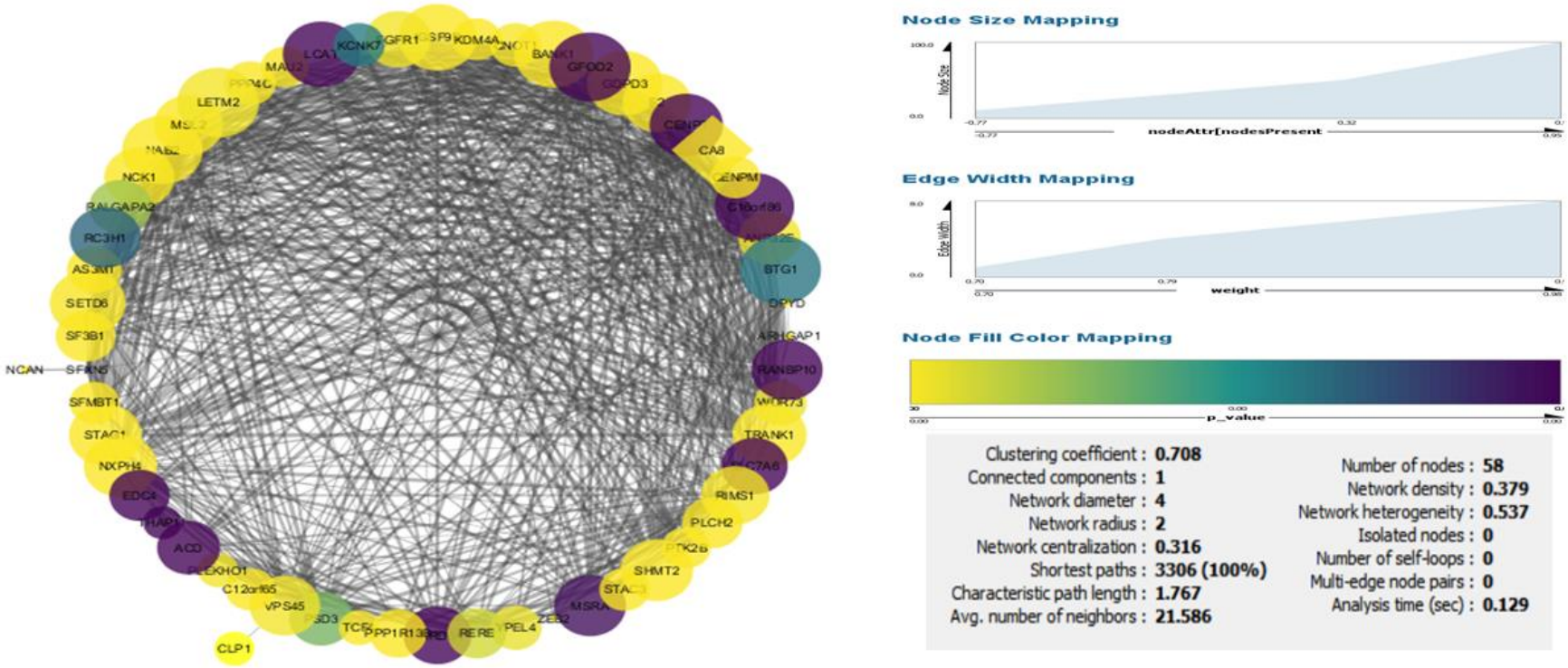
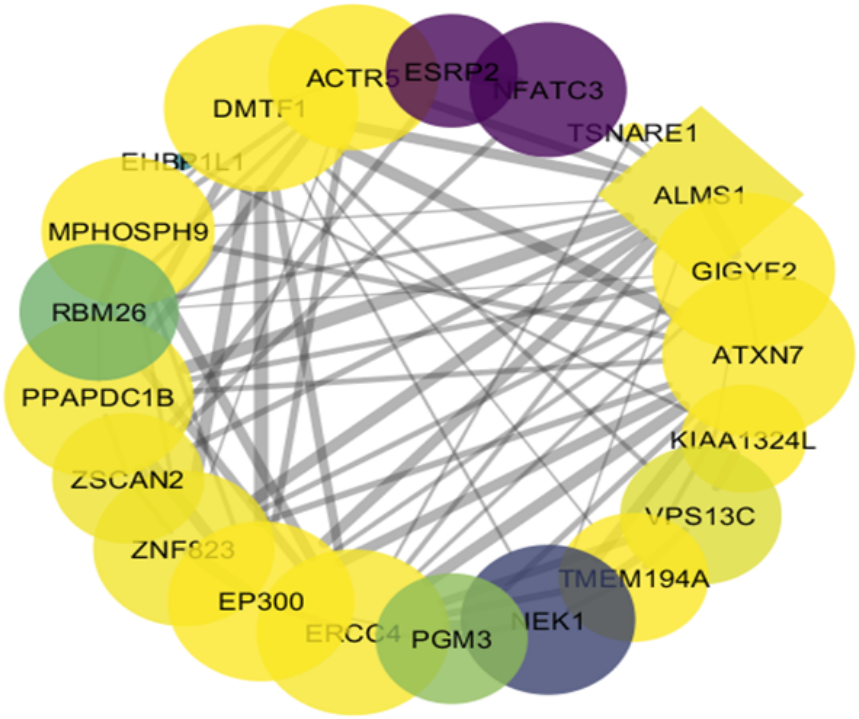



Figure 6.1.19 Turquoise Module for developmental Stage Four where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.



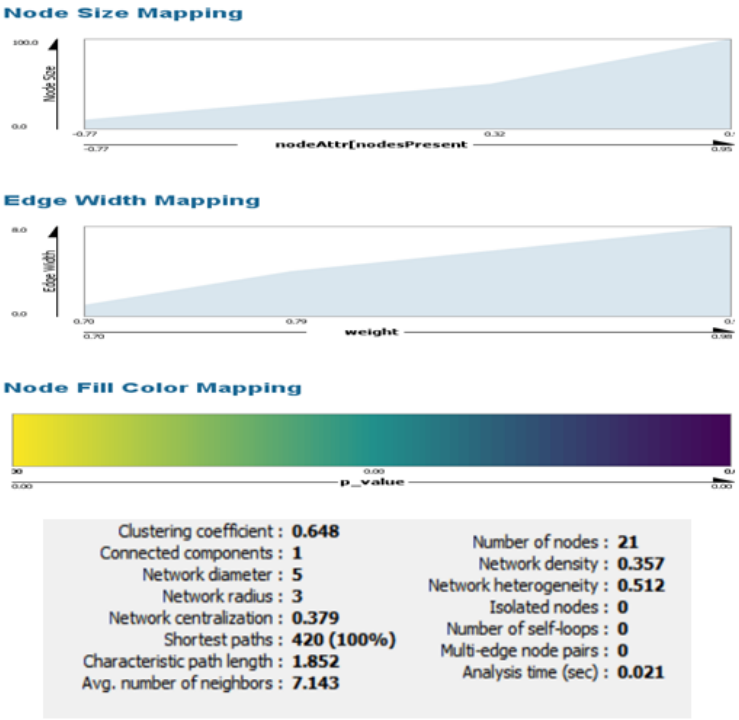




Figure 6.1.20 Yellow Module for developmental Stage Four where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

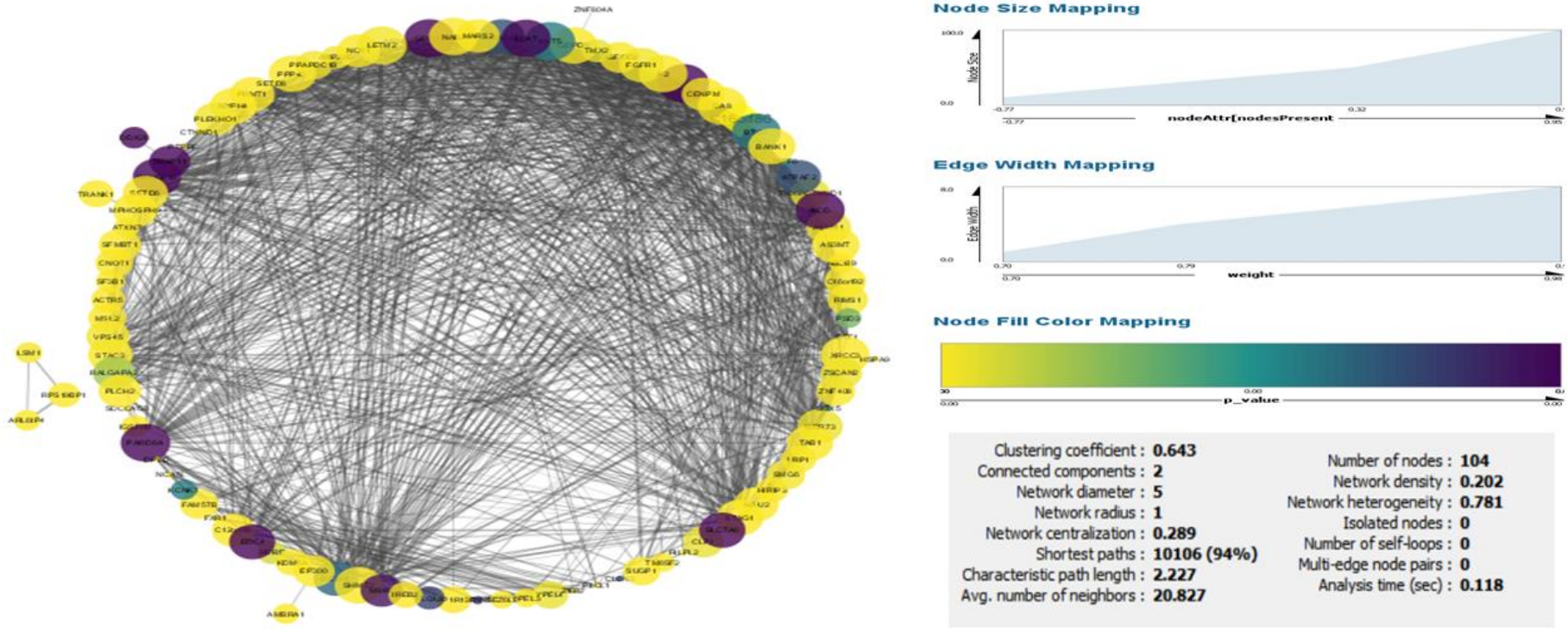



Figure 6.1.21 Black Module for developmental Stage Five where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

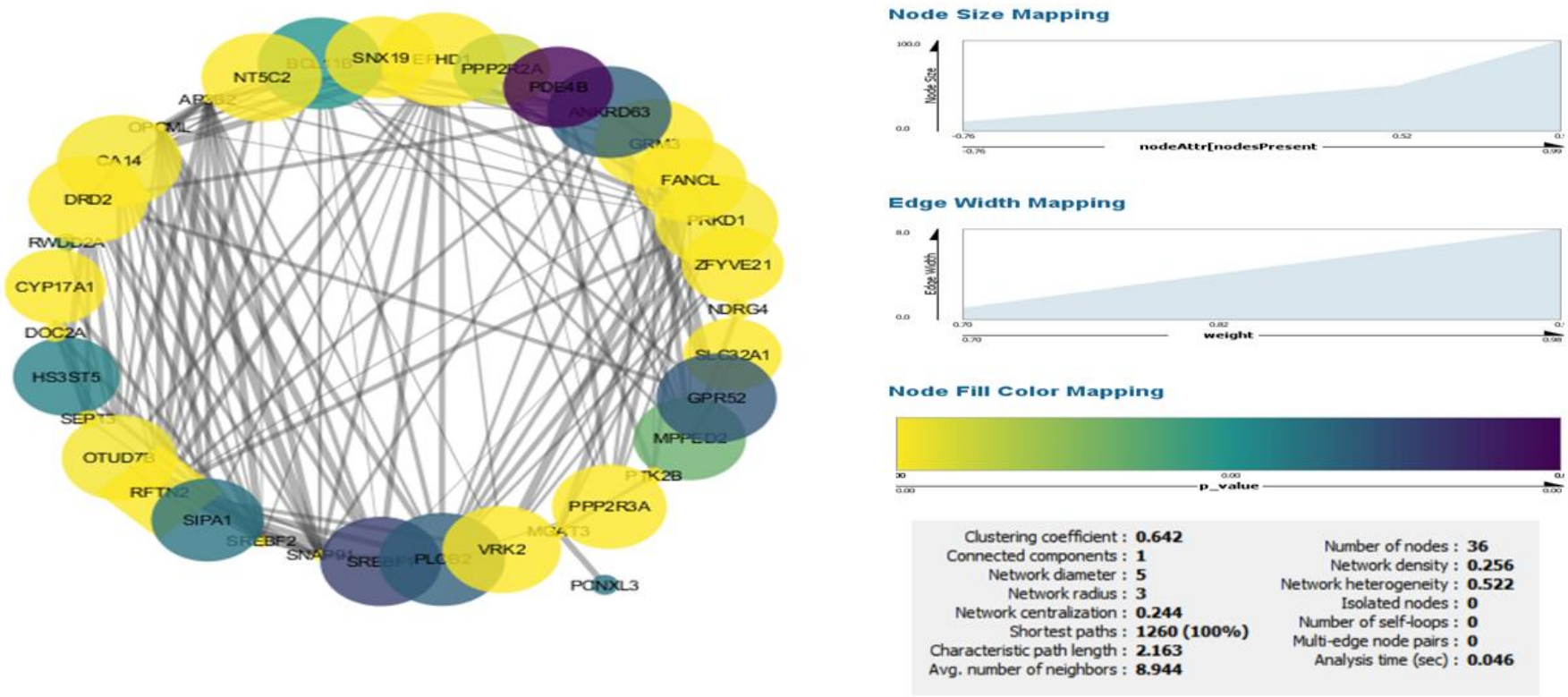



Figure 6.1.22 Brown Module for developmental Stage Five where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

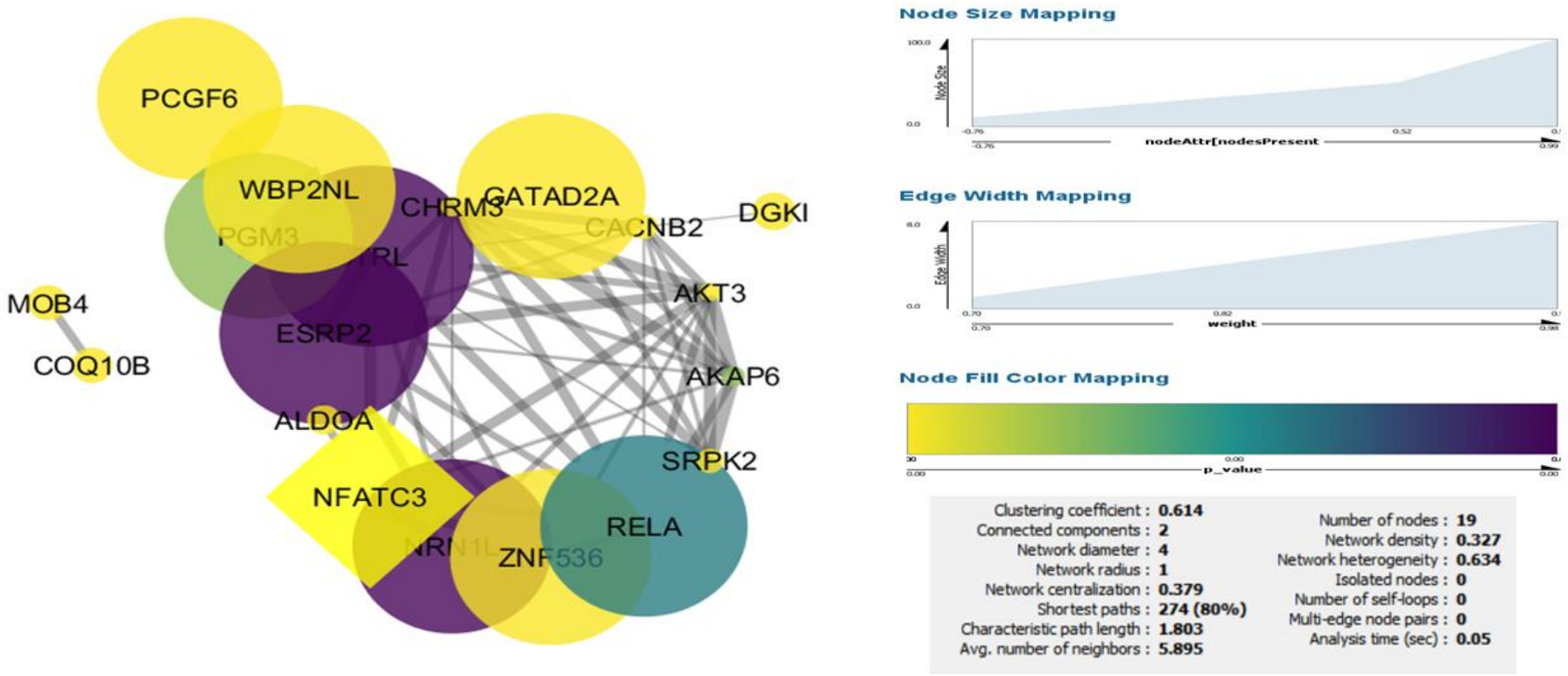



Figure 6.1.23 Green Module for developmental Stage Five where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

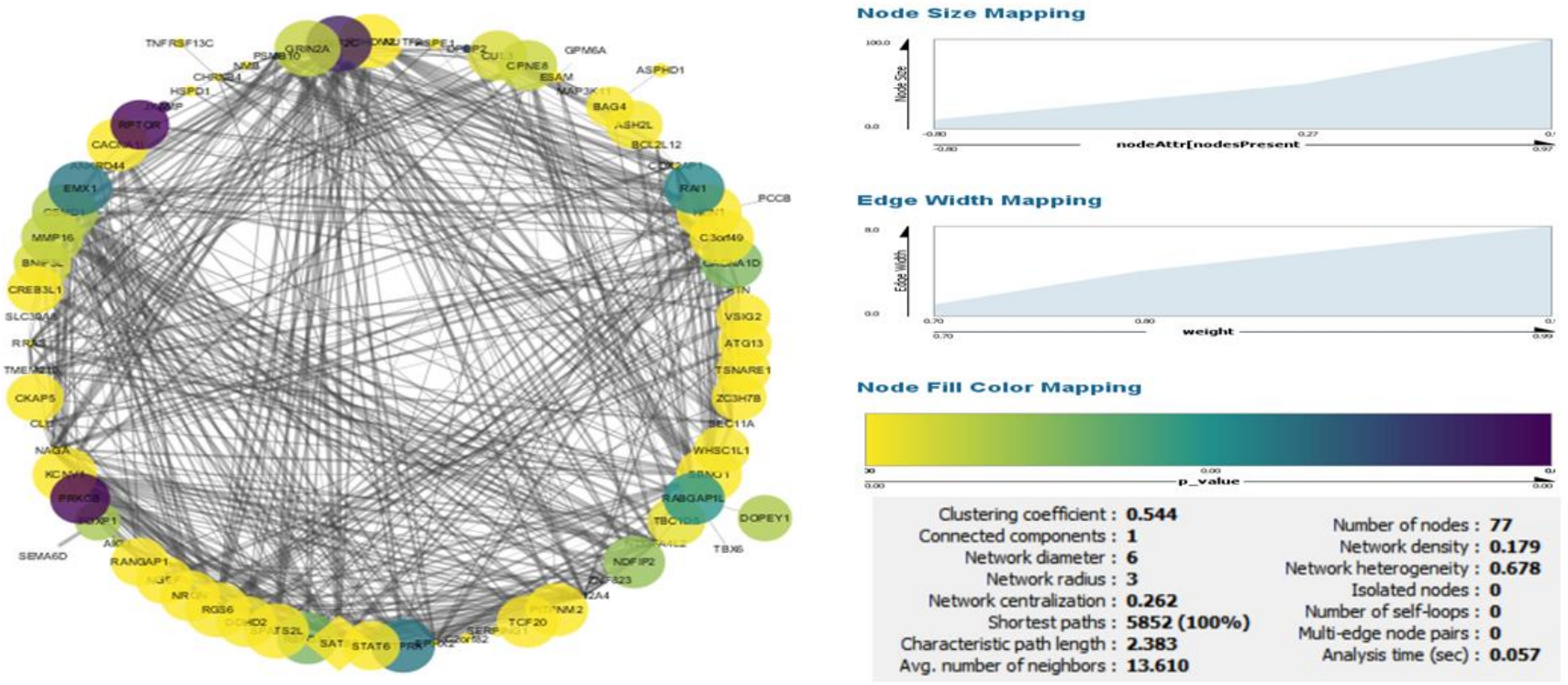



Figure 6.1.24 Greenyellow Module for developmental Stage Five where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

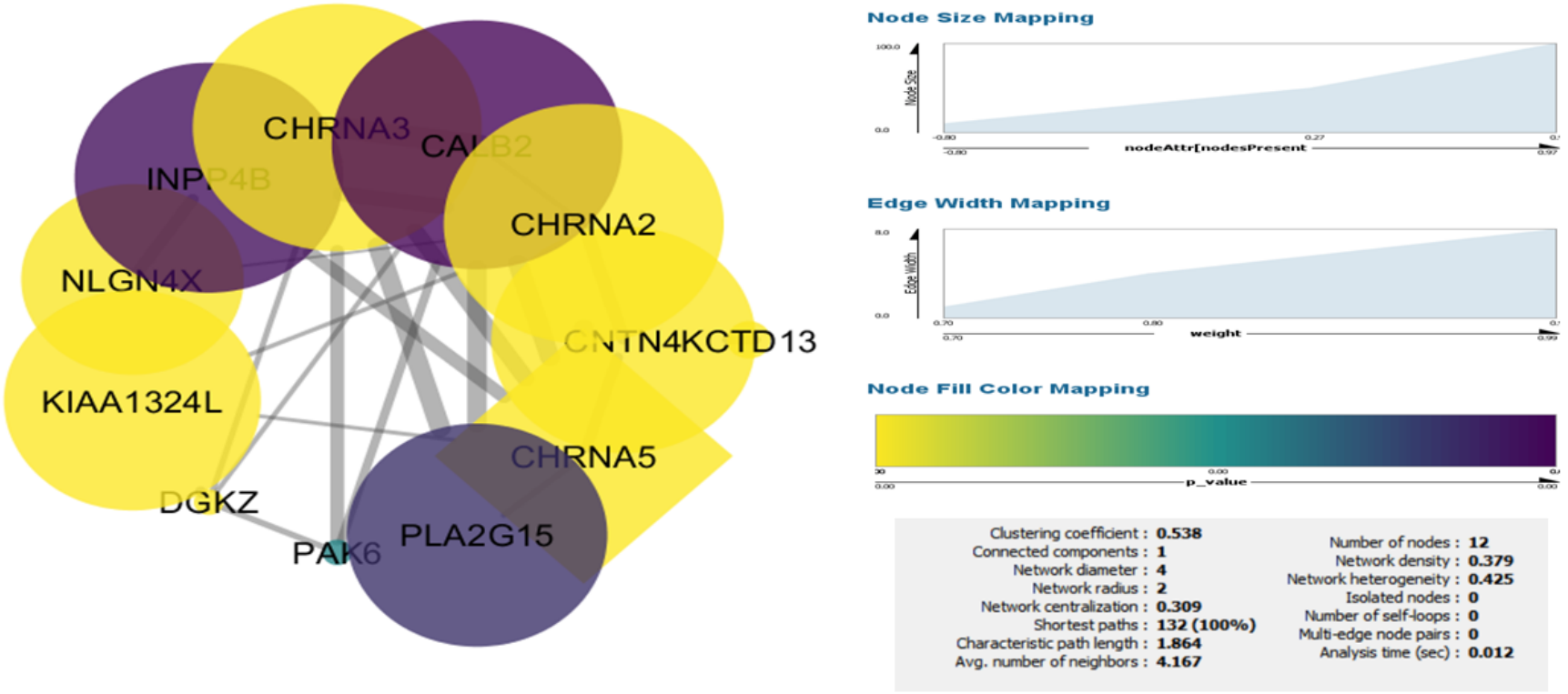



Figure 6.1.25 Pink Module for developmental Stage Five where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

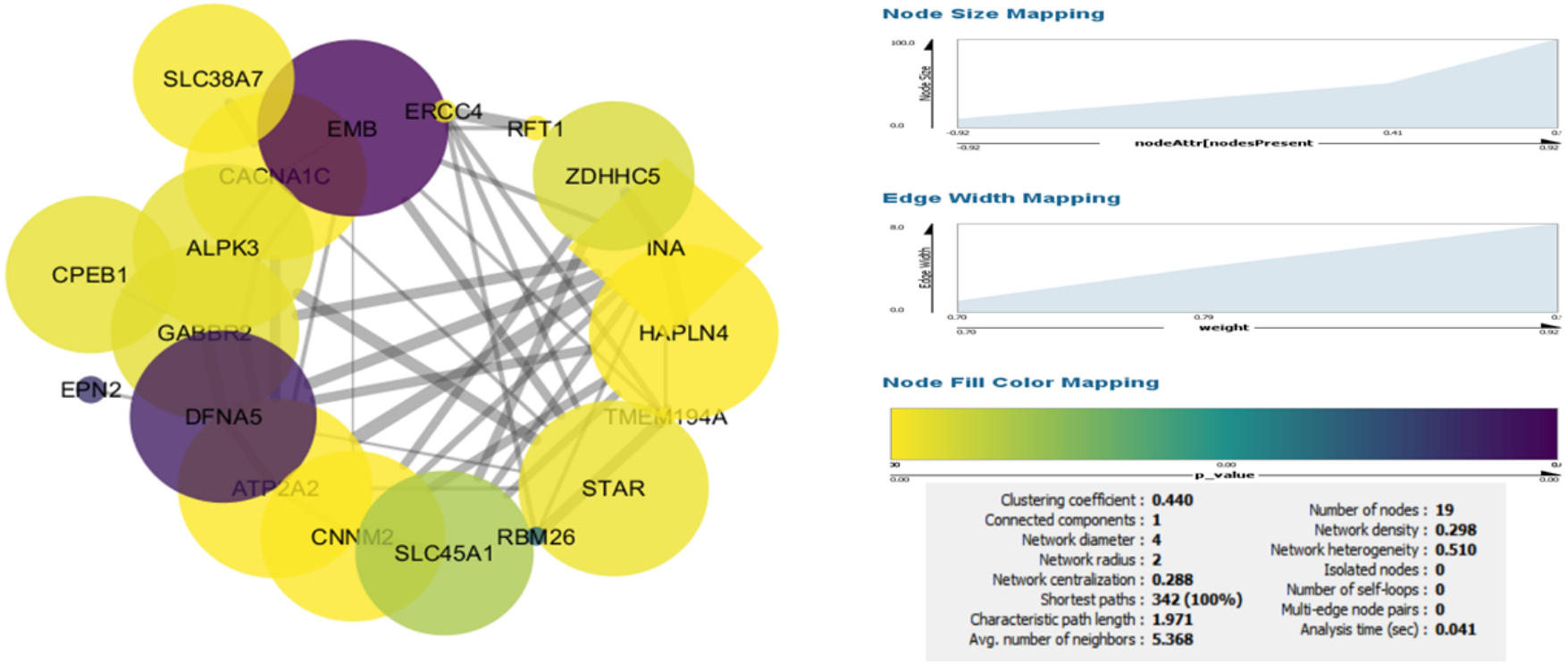



Figure 6.1.26 Red Module for developmental Stage Five where the size of the node is measured using module membership, p-values are based on p-values for SNP inclusion which was determined by Pardiñas et al. and shown by node fill colour and, edge width is measured on weight. The diamond shaped node is the HG identified by WGCNA. The weight of the edges was filtered to only include 0.8 and above.

**Table 6.1: Schizophrenia-associated gene set from the 145 loci identified by Pardiñas et al. which are available in ABA's BrainSpan resource.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ABCB9 | BTBD18 | CNOT1 | ERCC4 | IGSF9B | MPPED2 | PITPNM2 | RELA | SLC39A8 | TNFRSF13C |
| ACD | BTG1 | CNTN4 | ESAM | IMMP2L | MSL2 | PLA2G15 | RERE | SLC45A1 | TOM1L2 |
| ACTR5 | C10orf32 | COQ10B | ESRP2 | INA | MSRA | PLCB2 | RFT1 | SLC7A6 | TRANK1 |
| ADAMTSL3 | C11orf31 | CPEB1 | ETF1 | INO80E | NAB2 | PLCH2 | RFTN2 | SMG6 | TSNARE1 |
| AIG1 | C12orf65 | CPNE8 | F2 | INPP4B | NAGA | PLCL1 | RGS6 | SNAP91 | TSNAXIP1 |
| AKAP6 | C14orf2 | CREB3L1 | FAM109B | IREB2 | NCAN | PLCL2 | RILPL2 | SNX19 | TSR1 |
| AKT3 | C16orf86 | CSMD1 | FAM57B | JKAMP | NCK1 | PLEKHO1 | RIMS1 | SOX5 | TYW5 |
| ALDOA | C16orf92 | CTNND1 | FANCL | KAT5 | NDFIP2 | PPAPDC1B | RLTPR | SPATS2L | VPS13C |
| ALMS1 | C2orf47 | CTRL | FGFR1 | KCNJ13 | NDRG4 | PPP1R13B | RPS19BP1 | SREBF1 | VPS45 |
| ALPK3 | C2orf82 | CUL3 | FHIT | KCNK7 | NDUFA4L2 | PPP2R2A | RPTOR | SREBF2 | VRK2 |
| AMBRA1 | C3orf49 | CYP17A1 | FOXP1 | KCNV1 | NDUFA6 | PPP2R3A | RRAS | SRPK2 | VSIG2 |
| ANKRD44 | C4orf27 | DDHD2 | FSHB | KCTD13 | NEK1 | PPP4C | RWDD2A | SRR | WBP2NL |
| ANKRD63 | CA14 | DDX28 | FXR1 | KDM4A | NFATC3 | PRKCB | SATB2 | STAC3 | WDR73 |
| ANP32E | CA8 | DFNA5 | GABBR2 | KIAA1324L | NGEF | PRKD1 | SBNO1 | STAG1 | WHSC1L1 |
| AP3B2 | CACNA1C | DGKI | GATAD2A | L3MBTL2 | NLGN4X | PRMT1 | SCAF1 | STAR | XRCC3 |
| ARHGAP1 | CACNA1D | DGKZ | GDPD3 | LCAT | NMB | PSD3 | SDCCAG8 | STAT6 | YPEL3 |
| ARL6IP4 | CACNA1I | DMTF1 | GFOD2 | LETM2 | NRGN | PSMA4 | SEC11A | SUGP1 | YPEL4 |
| AS3MT | CACNB2 | DNAJC19 | GIGYF2 | LRP1 | NRN1L | PSMB10 | SEMA6D | TAB1 | ZBTB37 |
| ASH2L | CALB2 | DOC2A | GPM6A | LRRC48 | NT5C2 | PSMD6 | Sep-03 | TAOK2 | ZC3H7B |
| ASPG | CDK2AP1 | DOPEY1 | GPR135 | LSM1 | NUTF2 | PTK2B | SERPINC1 | TBC1D5 | ZDHHC5 |
| ASPHD1 | CENPM | DPEP2 | GPR52 | MAD1L1 | NXPH4 | PTN | SERPING1 | TBX6 | ZEB2 |
| ATG13 | CENPT | DPEP3 | GRIN2A | MAP3K11 | OGFOD2 | PTPRF | SETD6 | TCF20 | ZFYVE21 |
| ATP2A2 | CHRM3 | DPYD | GRM3 | MAPK3 | OPCML | PTPRK | SETD8 | TCF4 | ZNF408 |
| ATPAF2 | CHRNA2 | DRD2 | HAPLN4 | MARS2 | OTOL1 | R3HDM2 | SEZ6L2 | TDRD9 | ZNF536 |
| ATXN7 | CHRNA3 | EDC4 | HARBI1 | MAU2 | OTUD7B | RABGAP1L | SF3B1 | THAP11 | ZNF592 |
| B3GAT1 | CHRNA5 | EFHD1 | HCN1 | MED19 | PAK6 | RAI1 | SFMBT1 | THOC7 | ZNF804A |
| BAG4 | CHRNB4 | EHBP1L1 | HIRIP3 | MEF2C | PARD6A | RALGAPA2 | SFXN5 | TLE3 | ZNF823 |
| BANK1 | CKAP5 | EMB | HS3ST5 | MGAT3 | PCCB | RANBP10 | SHMT2 | TM6SF2 | ZSCAN2 |
| BCL11B | CLCN3 | EMX1 | HSPA9 | MMP16 | PCGF6 | RANGAP1 | SIPA1 | TMEM194A | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BCL2L12 | CLP1 | EP300 | HSPD1 | MOB4 | PCNXL3 | RBFOX1 | SLC12A4 | TMEM219 | |
| BNIP3L | CLU | EPHX2 | HSPE1 | MPHOSPH9 | PDE4B | RBM26 | SLC32A1 | TMX2 | |
| BOLL | CNNM2 | EPN2 | HYDIN | MPP6 | PGM3 | RC3H1 | SLC38A7 | TMX2-CTNND1 | |

**Table 6.2: Cluster assignments for each schizophrenia-associated gene over the five stages using the kmeans function available in R.**

| Gene Name | Developmental Stage One | Developmental Stage Two | Developmental Stage Three | Developmental Stage Four | Developmental Stage Five |
|---|---|---|---|---|---|
| ABCB1 | 3 | 2 | 2 | 2 | 2 |
| ABCB9 | 1 | 2 | 1 | 2 | 1 |
| ABCD2 | 2 | 1 | 2 | 2 | 2 |
| ACO2 | 3 | 2 | 2 | 2 | 2 |
| ACP2 | 3 | 2 | 1 | 2 | 1 |
| ACTR1A | 2 | 1 | 1 | 2 | 1 |
| ACTR5 | 3 | 2 | 1 | 2 | 2 |
| ADAMTSL3 | 2 | 2 | 2 | 2 | 2 |
| ADAMTSL4 | 3 | 2 | 1 | 2 | 2 |
| AIG1 | 3 | 2 | 1 | 2 | 2 |
| AKAP6 | 3 | 1 | 2 | 1 | 1 |
| AKT3 | 2 | 1 | 2 | 1 | 1 |
| ALAS1 | 2 | 1 | 1 | 2 | 2 |
| ALDOA | 3 | 1 | 2 | 2 | 1 |
| ALMS1 | 3 | 2 | 1 | 2 | 2 |
| ANAPC7 | 3 | 2 | 1 | 2 | 2 |
| ANKRD44 | 2 | 2 | 1 | 2 | 2 |
| ANKRD45 | 2 | 1 | 1 | 2 | 2 |
| ANKRD63 | 3 | 2 | 1 | 2 | 2 |
| APOPT1 | 3 | 1 | 2 | 1 | 2 |

| | | | | | |
|---|---|---|---|---|---|
| ARL5B | 2 | 1 | 2 | 1 | 1 |
| ARTN | 3 | 2 | 1 | 2 | 2 |
| AS3MT | 3 | 2 | 1 | 2 | 2 |
| ATF4 | 1 | 1 | 1 | 1 | 2 |
| ATP13A1 | 1 | 2 | 1 | 2 | 1 |
| ATPAF2 | 3 | 2 | 1 | 2 | 2 |
| ATXN7 | 2 | 2 | 1 | 2 | 1 |
| B9D1 | 3 | 1 | 2 | 2 | 2 |
| BAG4 | 2 | 1 | 2 | 1 | 1 |
| BANK1 | 3 | 2 | 1 | 2 | 2 |
| BCL11B | 2 | 2 | 1 | 2 | 2 |
| BCL2L12 | 3 | 2 | 1 | 2 | 2 |
| BNIP3L | 2 | 1 | 2 | 1 | 1 |
| BRD8 | 3 | 2 | 1 | 2 | 1 |
| BTBD18 | 1 | 2 | 1 | 2 | 1 |
| C2orf47 | 3 | 1 | 2 | 1 | 2 |
| C2orf82 | 1 | 1 | 2 | 1 | 2 |
| CA8 | 3 | 2 | 1 | 2 | 2 |
| CACNA1C | 1 | 2 | 1 | 2 | 1 |
| CACNA1D | 2 | 2 | 1 | 1 | 1 |
| CACNA1I | 1 | 1 | 2 | 1 | 1 |
| CACNB2 | 1 | 1 | 2 | 1 | 1 |
| CALB2 | 3 | 2 | 1 | 2 | 2 |
| CENPM | 3 | 2 | 1 | 2 | 2 |
| CENPT | 1 | 2 | 1 | 2 | 2 |
| CEP170 | 2 | 1 | 1 | 2 | 1 |
| CHRNA2 | 3 | 2 | 1 | 2 | 2 |
| CHRNA3 | 3 | 2 | 1 | 2 | 2 |
| CLCN3 | 3 | 2 | 2 | 2 | 1 |
| CLDN23 | 2 | 2 | 2 | 2 | 2 |
| CNOT1 | 3 | 2 | 1 | 2 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| CNTN4 | 3 | 2 | 1 | 2 | 2 |
| CSMD1 | 2 | 1 | 2 | 1 | 1 |
| CUL3 | 2 | 1 | 2 | 1 | 1 |
| DFNA5 | 2 | 2 | 2 | 1 | 1 |
| DGKI | 1 | 2 | 1 | 2 | 1 |
| DNAJC19 | 3 | 1 | 2 | 1 | 2 |
| DOPEY1 | 2 | 2 | 1 | 2 | 1 |
| DPYD | 1 | 1 | 2 | 2 | 2 |
| DRD2 | 3 | 2 | 1 | 2 | 2 |
| EMB | 3 | 2 | 1 | 2 | 1 |
| EMX1 | 1 | 1 | 2 | 1 | 1 |
| ESAM | 3 | 2 | 2 | 2 | 2 |
| FANCL | 1 | 1 | 1 | 2 | 2 |
| FHIT | 3 | 2 | 1 | 2 | 2 |
| FOXP1 | 3 | 1 | 2 | 1 | 1 |
| FTSJ2 | 3 | 1 | 2 | 1 | 1 |
| GABBR2 | 1 | 1 | 1 | 1 | 1 |
| GPM6A | 2 | 1 | 2 | 1 | 2 |
| GRIA1 | 1 | 2 | 1 | 2 | 2 |
| GRIN2A | 1 | 1 | 2 | 1 | 1 |
| GRM3 | 1 | 2 | 2 | 2 | 2 |
| HCN1 | 1 | 1 | 2 | 1 | 1 |
| IGSF9B | 3 | 2 | 1 | 2 | 1 |
| IL20RB | 3 | 2 | 1 | 2 | 2 |
| IMMP2L | 3 | 1 | 2 | 2 | 2 |
| INHBC | 3 | 2 | 1 | 2 | 2 |
| INPP4B | 3 | 2 | 1 | 2 | 2 |
| ME1 | 3 | 2 | 2 | 1 | 2 |
| MGAT3 | 1 | 2 | 1 | 2 | 1 |
| MMP16 | 2 | 2 | 2 | 1 | 1 |
| NDFIP2 | 2 | 1 | 2 | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| NLGN4X | 3 | 2 | 1 | 2 | 2 |
| OPCML | 2 | 1 | 1 | 1 | 1 |
| PDE4B | 3 | 2 | 2 | 2 | 2 |
| PSD3 | 1 | 1 | 1 | 2 | 1 |
| PTPRK | 1 | 1 | 2 | 1 | 1 |
| RBFOX1 | 2 | 1 | 2 | 1 | 1 |
| RERE | 2 | 2 | 1 | 2 | 1 |
| RGS6 | 1 | 1 | 2 | 1 | 1 |
| RIMS1 | 3 | 2 | 1 | 2 | 2 |
| RPTOR | 1 | 2 | 2 | 1 | 1 |
| SATB2 | 2 | 1 | 2 | 1 | 1 |
| SEMA6D | 3 | 2 | 2 | 2 | 1 |
| SNX19 | 2 | 2 | 2 | 2 | 2 |
| SPATS2L | 3 | 1 | 2 | 1 | 1 |
| TBC1D5 | 3 | 2 | 2 | 2 | 1 |
| TCF4 | 1 | 2 | 2 | 2 | 1 |
| TRANK1 | 3 | 2 | 1 | 2 | 1 |
| TSNARE1 | 1 | 2 | 2 | 1 | 1 |
| ZEB2 | 2 | 2 | 1 | 2 | 2 |
| ZNF440 | 2 | 2 | 1 | 2 | 2 |
| ZNF536 | 3 | 2 | 1 | 2 | 2 |
| ZNF804A | 3 | 2 | 1 | 2 | 1 |

**Table 6.3: Gene Ontologies identified for the Black module in developmental stage One using the anRichment function as part of WGCNA in R using the default settings.**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage | BP | Black | positive regulation of macrophage | 2.92E-15 | ARHGAP1, CYP17A1, FSHB, GRM3, HSPE1, KCNJ13, MMP16, NRGN, STAT6, FXR1, AP3B2, CUL3, INPP4B, DGKI, GPR52, KDM4A, PSMD6, RABGAP1L, MPHOSPH9, TAB1, SDCCAG8, |

| | | | | | | |
|---|---|---|---|---|---|---|
| | proliferation. | | | proliferation | | SMG6, SATB2, PSD3, ABCB9, KCNV1, FOXP1, B3GAT1, TMX2, MSL2, NDUFA4L2, AS3MT, SUGP1, DPEP2, CPEB1, MAIP1, EFHD1, YPEL3, IMMP2L, C12orf65, OTOL1, LETM2, PHETA2, YPEL4, STAC3 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Black | binding | 1.88E-10 | ARHGAP1, MPPED2, CYP17A1, FSHB, HSPE1, MMP16, NRGN, STAT6, FXR1, CUL3, INPP4B, DGKI, KDM4A, PSMD6, RABGAP1L, TAB1, SDCCAG8, SMG6, SATB2, PSD3, ABCB9, FOXP1, B3GAT1, BANK1, MSL2, SUGP1, DPEP2, CPEB1, MAIP1, EFHD1, YPEL3, C12orf65, OTOL1, LETM2, PHETA2, YPEL4, STAC3 |
| GO:0043231 | Organised structure of distinctive morphology and function, bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Black | intracellular membrane-bounded organelle | 6.74E-10 | CYP17A1, HSPE1, MMP16, NRGN, STAT6, FXR1, AP3B2, CUL3, DGKI, KDM4A, PSMD6, RABGAP1L, MPHOSPH9, TAB1, SMG6, SATB2, ABCB9, FOXP1, B3GAT1, MSL2, NDUFA4L2, SUGP1, CPEB1, MAIP1, EFHD1, YPEL3, IMMP2L, C12orf65, LETM2, PHETA2, YPEL4, STAC3 |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Black | cytoplasm | 5.00E-09 | ARHGAP1, CYP17A1, FSHB, HSPE1, MMP16, NRGN, STAT6, FXR1, AP3B2, CUL3, INPP4B, DGKI, KDM4A, PSMD6, RABGAP1L, MPHOSPH9, TAB1, SDCCAG8, SMG6, ABCB9, B3GAT1, NDUFA4L2, AS3MT, CPEB1, MAIP1, EFHD1, IMMP2L, C12orf65, LETM2, PHETA2, STAC3 |
| GO:0016020 | A lipid bilayer along with all the proteins and protein complexes embedded in it an attached to it. | CC | Black | membrane | 2.01E-08 | ARHGAP1, CYP17A1, GRM3, HSPE1, KCNJ13, MMP16, NRGN, STAT6, FXR1, AP3B2, CUL3, DGKI, GPR52, MPHOSPH9, TAB1, PSD3, ABCB9, KCNV1, B3GAT1, TMX2, NDUFA4L2, DPEP2, CPEB1, MAIP1, EFHD1, IMMP2L, LETM2, STAC3 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Black | intracellular organelle lumen | 9.92E-08 | ARHGAP1, CYP17A1, FSHB, GRM3, HSPE1, KCNJ13, MMP16, NRGN, STAT6, FXR1, CUL3, INPP4B, DGKI, GPR52, KDM4A, PSMD6, RABGAP1L, TAB1, SDCCAG8, SMG6, SATB2, PSD3, KCNV1, FOXP1, TMX2, BANK1, CPEB1, MAIP1, EFHD1, YPEL3, STAC3 |

**Table 6.4: Gene Ontologies for the Blue module in developmental stage One using the anRichment function of WGCNA on the schizophrenia-associated genes.**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Blue | positive regulation of macrophage proliferation | 1.75E-29 | More than 50 overlapping genes |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Blue | intracellular organelle lumen | 2.74E-14 | More than 50 overlapping genes |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl) benzene-1,2-diol] and derivatives formed by substitution. | BP | Blue | cellular response to catecholamine stimulus | 4.03E-13 | CLCN3, DRD2, EP300, EPHX2, ETF1, PTK2B, GPM6A, LRP1, NEK1, PPP2R2A, PRKCB, RELA, SIPA1, TCF4, HIRIP3, ASH2L, INA, AKAP6, BAG4, SNAP91, AKT3, CLP1, SF3B1, VPS13C, SRR, BCL11B, ACD, SETD6, PCGF6, WDR73, ATPAF2, DNAJC19, RILPL2, KCTD13, INO80E, HCN1, BTBD18 |
| GO:0005737 | All the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Blue | cytoplasm | 1.15E-11 | SERPING1, CALB2, CLCN3, DRD2, EP300, EPHX2, ETF1, PTK2B, LRP1, MAP3K11, NEK1, PCCB, PGM3, PLCL1, PPP2R2A, PRKCB, PSMB10, RANGAP1, RELA, SIPA1, STAR, VRK2, DGKZ, INA, AKAP6, BAG4, ATP5MPL, SNAP91, AKT3, RAI1, CLP1, DOP1A, VPS13C, SRR, BOLL, SETD6, DRC3, TLCD3B, WDR73, ATPAF2, SFXN5, BORCS7, DNAJC19, RILPL2, HS3ST5 |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) because of a diacylated bacterial lipopeptide stimulus. | BP | Blue | cellular response to diacyl bacterial lipopeptide | 1.70E-11 | SERPING1, DRD2, EP300, EPHX2, ETF1, PTK2B, LRP1, MAP3K11, NEK1, PCCB, PGM3, PLCL1, PPP2R2A, PRKCB, PSMB10, RANGAP1, RELA, RRAS, STAR, TCF4, VRK2, DGKZ, ASH2L, BAG4, AKT3, RAI1, CLP1, ZC3H7B, SF3B1, AIG1, ADAMTSL3, ALPK3, SCAF1, SRR, BCL11B, ACD, BOLL, ZNF408, SETD6, TLCD3B, PCGF6, MED19, HS3ST5, KCTD13, INO80E, BTBD18 |
| GO:0016020 | A lipid bilayer along with all the proteins and protein complexes embedded in it an attached to it. | CC | Blue | membrane | 2.79E-11 | CALB2, CLCN3, DRD2, PTK2B, GPM6A, LRP1, MAP3K11, PLCL1, PRKCB, RANGAP1, RRAS, SIPA1, STAR, VRK2, DGKZ, INA, AKAP6, BAG4, |

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| | | | | | | ATP5MPL, SNAP91, AKT3, DOP1A, SEZ6L2, SLC45A1, AIG1, CNNM2, VPS13C, SLC38A7, SRR, TLCD3B, WDR73, SFXN5, BORCS7, TMEM219, RFTN2, DNAJC19, CPNE8, C16orf92, RILPL2, HS3ST5, HCN1 |
| GO:0097178 | The aggregation, arrangement and bonding together of a set of components to form a ruffle, a projection at the leading edge of a crawling cell; the protrusions are supported by a microfilament meshwork. The formation of ruffles (also called membrane ruffling) is thought to be controlled by a group of enzymes known as Rho GTPases, specifically RhoA, Rac1 and cdc42. | BP | Blue | ruffle assembly | 1.53E-10 | CLCN3, DRD2, EP300, ETF1, PTK2B, LRP1, MAP3K11, NEK1, PCCB, PRKCB, RANGAP1, RELA, RRAS, STAR, TCF4, VRK2, DGKZ, BAG4, AKT3, CLP1, ZC3H7B, SF3B1, CNNM2, ALPK3, SCAF1, SRR, BCL11B, ACD, BOLL, ZNF408, PCGF6, HS3ST5, HCN1 |
| GO:0048519 | Any process that stops, prevents, or reduces the frequency, rate or extent of a biological process. Biological processes are regulated by many means; examples include the control of gene expression, protein modification or interaction with a protein or substrate molecule. | BP | Blue | negative regulation of biological process | 1.73E-10 | SERPING1, DRD2, EP300, ETF1, PTK2B, LRP1, PLCL1, PPP2R2A, PRKCB, PSMB10, RANGAP1, RELA, RRAS, SIPA1, STAR, AKAP6, BAG4, AKT3, RAI1, CLP1, ZC3H7B, VPS13C, BCL11B, ACD, BOLL, SETD6, TLCD3B, PCGF6, WDR73, HS3ST5, KCTD13, BTBD18 |
| GO:0005634 | A membrane-bounded organelle of eukaryotic cells in which chromosomes are housed and replicated. In most cells, the nucleus contains all of the cell's chromosomes except the organellar chromosomes and is the site of RNA synthesis and processing. In some species, or in specialized cell types, RNA metabolism or DNA replication may be absent. | CC | Blue | nucleus | 5.27E-10 | CALB2, EP300, PTK2B, LRP1, NEK1, PPP2R2A, PRKCB, PSMB10, RANGAP1, RELA, SIPA1, TCF4, VRK2, HIRIP3, DGKZ, ASH2L, INA, AKAP6, BAG4, AKT3, RAI1, CLP1, ZC3H7B, SF3B1, ALPK3, SCAF1, BCL11B, ACD, ZNF408, SETD6, PCGF6, ATPAF2, MED19, KCTD13, INO80E, BTBD18 |
| GO:0006996 | A process that is carried out at the cellular level which results in the | BP | Blue | organelle organization | 6.74E-10 | CLCN3, EP300, EPHX2, PTK2B, LRP1, NEK1, PPP2R2A, PRKCB, RELA, SIPA1, HIRIP3, ASH2L, |

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| | assembly, arrangement of constituent parts, or disassembly of an organelle within a cell. An organelle is an organized structure of distinctive morphology and function. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton. Excludes the plasma membrane. | | | | | INA, BAG4, SNAP91, AKT3, VPS13C, ACD, SETD6, PCGF6, WDR73, DNAJC19, RILPL2, KCTD13, INO80E, BTBD18 |
| GO:0060255 | Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass. | BP | Blue | regulation of macromolecule metabolic process | 1.47E-09 | SERPING1, DRD2, EP300, EPHX2, ETF1, PTK2B, LRP1, MAP3K11, NEK1, PLCL1, PPP2R2A, PRKCB, PSMB10, RELA, RRAS, STAR, TCF4, VRK2, ASH2L, BAG4, RAI1, CLP1, ZC3H7B, SF3B1, BCL11B, ACD, BOLL, ZNF408, SETD6, PCGF6, MED19, KCTD13, BTBD18 |
| GO:1901561 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a benomyl stimulus. | BP | Blue | response to benomyl | 3.08E-09 | CLCN3, EP300, ETF1, PTK2B, LRP1, MAP3K11, NEK1, PCCB, PRKCB, RANGAP1, RELA, RRAS, TCF4, VRK2, DGKZ, BAG4, AKT3, CLP1, ZC3H7B, SF3B1, CNNM2, ALPK3, SCAF1, SRR, BCL11B, ACD, BOLL, ZNF408, PCGF6, HS3ST5, HCN1 |
| GO:0080154 | Any process that modulates the rate, frequency or extent of fertilization. Fertilization is the union of gametes of opposite sexes during the process of sexual reproduction to form a zygote. It involves the fusion of the gametic nuclei (karyogamy) and cytoplasm (plasmogamy). | BP | Blue | regulation of fertilization | 1.07E-08 | SERPING1, DRD2, EP300, EPHX2, ETF1, PTK2B, LRP1, MAP3K11, NEK1, PLCL1, PPP2R2A, PRKCB, PSMB10, RELA, RRAS, STAR, TCF4, VRK2, DGKZ, ASH2L, BAG4, RAI1, BCL11B, ACD, BOLL, ZNF408, SETD6, PCGF6, MED19, BTBD18 |
| GO:0043168 | Interacting selectively and non-covalently with anions, charged atoms or groups of atoms with a net negative charge. | MF | Blue | anion binding | 1.45E-08 | CLCN3, PTK2B, LRP1, MAP3K11, NEK1, PCCB, PLCL1, PRKCB, RELA, RRAS, VRK2, DGKZ, SNAP91, AKT3, CLP1, CNNM2, ALPK3, SRR, CPNE8, HS3ST5, HCN1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| GO:0006464 | The covalent alteration of one or more amino acids occurring in proteins, peptides and nascent polypeptides (co-translational, post-translational modifications) occurring at the level of an individual cell. Includes the modification of charged tRNAs that are destined to occur in a protein (pre-translation modification). | BP | Blue | cellular protein modification process | 1.59E-08 | DRD2, EP300, ETF1, PTK2B, LRP1, MAP3K11, NEK1, PGM3, PLCL1, PPP2R2A, PRKCB, PSMB10, RANGAP1, RELA, RRAS, VRK2, ASH2L, BAG4, AKT3, ALPK3, SETD6, PCGF6, HS3ST5, KCTD13, INO80E |
| GO:1901666 | Any process that activates or increases the frequency, rate or extent of NAD+ ADP-ribosyltransferase activity. | BP | Blue | positive regulation of NAD+ ADP-ribosyltransferase activity | 2.84E-08 | SERPING1, DRD2, EP300, ETF1, PTK2B, LRP1, MAP3K11, NEK1, PCCB, PGM3, PLCL1, PPP2R2A, PRKCB, PSMB10, RANGAP1, RELA, RRAS, VRK2, ASH2L, BAG4, AKT3, ADAMTSL3, ALPK3, SRR, BOLL, SETD6, TLCD3B, PCGF6, HS3ST5, KCTD13, INO80E |
| GO:0032502 | A biological process whose specific outcome is the progression of an integrated living unit: an anatomical structure (which may be a subcellular structure, cell, tissue, or organ), or organism over time from an initial condition to a later condition. | BP | Blue | developmental process | 5.31E-08 | SERPING1, CLCN3, DRD2, EP300, PTK2B, GPM6A, LRP1, PGM3, PRKCB, PSMB10, RELA, RRAS, STAR, TCF4, ASH2L, INA, AKAP6, AKT3, RAI1, CLP1, ALPK3, SRR, BCL11B, BOLL, SETD6, TLCD3B, DNAJC19, RILPL2, HCN1, BTBD18 |
| GO:1901679 | The directed movement of nucleotide across a membrane. | BP | Blue | nucleotide transmembrane transport | 6.96E-08 | DRD2, EP300, EPHX2, ETF1, PTK2B, PGM3, PLCL1, PRKCB, PSMB10, RELA, STAR, TCF4, DGKZ, ASH2L, RAI1, CLP1, SCAF1, SRR, BCL11B, ACD, BOLL, ZNF408, SETD6, TLCD3B, PCGF6, MED19, HS3ST5, KCTD13, BTBD18 |
| GO:0044249 | The chemical reactions and pathways resulting in the formation of substances, carried out by individual cells. | BP | Blue | cellular biosynthetic process | 2.45E-07 | DRD2, EP300, EPHX2, ETF1, PTK2B, PGM3, PRKCB, PSMB10, RELA, STAR, TCF4, DGKZ, ASH2L, RAI1, CLP1, SCAF1, SRR, BCL11B, ACD, BOLL, ZNF408, SETD6, TLCD3B, PCGF6, MED19, HS3ST5, KCTD13, BTBD18 |
| GO:0006950 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene | BP | Blue | response to stress | 2.85E-07 | SERPING1, DRD2, EP300, EPHX2, PTK2B, LRP1, MAP3K11, NEK1, PRKCB, PSMB10, RANGAP1, RELA, SIPA1, STAR, VRK2, DGKZ, ASH2L, BAG4, AKT3, VPS13C, ACD, SETD6, INO80E |

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| | expression, etc.) as a result of a disturbance in organismal or cellular homeostasis, usually, but not necessarily, exogenous (e.g., temperature, humidity, ionizing radiation). | | | | | |
| GO:0031325 | Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways by which individual cells transform chemical substances. | BP | Blue | positive regulation of cellular metabolic process | 2.96E-07 | DRD2, EP300, PTK2B, LRP1, MAP3K11, NEK1, PRKCB, RELA, STAR, TCF4, DGKZ, ASH2L, BAG4, RAI1, VPS13C, BCL11B, ACD, BOLL, MED19, KCTD13, BTBD18 |
| GO:0070016 | Interacting selectively and non-covalently with the armadillo repeat domain of a protein, an approximately 40 amino acid long tandemly repeated sequence motif first identified in the Drosophila segment polarity protein armadillo. Arm-repeat proteins are involved in various processes, including intracellular signalling and cytoskeletal regulation. | MF | Blue | armadillo repeat domain binding | 2.96E-07 | SERPING1, CALB2, CLCN3, DRD2, EP300, EPHX2, PTK2B, GPM6A, LRP1, PLCL1, PRKCB, PSMB10, RANGAP1, STAR, DGKZ, AKAP6, BAG4, AKT3, CNNM2, VPS13C, ACD, BOLL, HCN1 |
| GO:0032559 | Interacting selectively and non-covalently with an adenyl ribonucleotide, any compound consisting of adenosine esterified with (ortho)phosphate or an oligophosphate at any hydroxyl group on the ribose moiety. | MF | Blue | adenyl ribonucleotide binding | 3.98E-07 | CLCN3, PTK2B, MAP3K11, NEK1, PCCB, PRKCB, VRK2, DGKZ, AKT3, CLP1, CNNM2, ALPK3, SRR, HS3ST5, HCN1 |

**Table 6.5: Gene Ontology Brown Module for developmental stage One using the anRichment function as part of WGCNA in R using the default settings**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 2.10E-15 | SERPINC1, ATP2A2, CACNB2, CHRM3, CHRNA2, CTRL, IREB2, NAB2, NAGA, NDUFA6, SREBF2, SRPK2, DOC2A, CACNA1I, GABBR2, ZNF536, R3HDM2, IGSF9B, DDHD2, MOB4, PARD6A, SFMBT1, ZSCAN2, SBNO1, ZNF823, RPTOR, RBM26, COQ10B, GFOD2, L3MBTL2, CREB3L1, WBP2NL, KIAA1324L, EHBP1L1, KMT5A, SNX19 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Brown | binding | 3.36E-10 | SERPINC1, ATP2A2, CACNB2, CHRM3, CHRNA2, IREB2, NAB2, NAGA, SREBF2, SRPK2, DOC2A, CACNA1I, GABBR2, ZNF536, R3HDM2, IGSF9B, DDHD2, MOB4, PARD6A, SFMBT1, ZSCAN2, ZNF823, RPTOR, RBM26, COQ10B, L3MBTL2, CREB3L1, WBP2NL, KMT5A, SNX19 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Brown | intracellular organelle lumen | 4.32E-09 | SERPINC1, ATP2A2, CACNB2, CHRM3, CHRNA2, IREB2, NAB2, SREBF2, SRPK2, DOC2A, CACNA1I, GABBR2, ZNF536, IGSF9B, DDHD2, PARD6A, SFMBT1, ZSCAN2, SBNO1, ZNF823, RPTOR, RBM26, L3MBTL2, CREB3L1, WBP2NL, KMT5A, SNX19 |
| GO:0043231 | Organised structure of distinctive morphology and function, bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Brown | intracellular membrane-bounded organelle | 2.02E-08 | SERPINC1, ATP2A2, IREB2, NAB2, NAGA, NDUFA6, SREBF2, SRPK2, DOC2A, ZNF536, R3HDM2, DDHD2, MOB4, PARD6A, SFMBT1, ZSCAN2, SBNO1, ZNF823, RPTOR, RBM26, COQ10B, L3MBTL2, CREB3L1, WBP2NL, KMT5A |

**Table 6.6: Gene Ontology for Pink Module in Developmental Stage One using the anRichment function as part of WGCNA in R using the default settings**

| GOID | DEFINITION | ONTOLOGY | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Pink | protein binding | 1.61E-08 | ERCC4, HSPA9, PPP2R3A, PPP4C, SHMT2, XRCC3, TBC1D5, NUTF2, STAG1, KAT5, LSM1, HPF1, PAK6, OTUD7B, SEMA6D, THOC7, TYW5, TOM1L2, TSNARE1 |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Pink | positive regulation of macrophage proliferation | 2.30E-07 | ERCC4, HSPA9, PPP4C, SHMT2, XRCC3, PLCH2, TBC1D5, NUTF2, STAG1, KAT5, LSM1, HPF1, TSR1, PAK6, OTUD7B, SEMA6D, THOC7, RFT1, TYW5, TOM1L2, TSNARE1 |
| GO:0008152 | The chemical reactions and pathways, including anabolism and catabolism, by which living organisms transform chemical substances. Metabolic processes typically transform small molecules, but also include macromolecular processes such as DNA repair and replication, and protein synthesis and degradation. | BP | Pink | metabolic process | 6.41E-07 | ERCC4, HSPA9, PPP2R3A, PPP4C, SHMT2, XRCC3, PLCH2, TBC1D5, NUTF2, STAG1, KAT5, LSM1, HPF1, TSR1, PAK6, OTUD7B, THOC7, TYW5 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Pink | intracellular organelle lumen | 8.50E-07 | ERCC4, HSPA9, PPP2R3A, PPP4C, SHMT2, XRCC3, PLCH2, TBC1D5, NUTF2, STAG1, KAT5, LSM1, HPF1, PAK6, OTUD7B, SEMA6D, RFT1, TOM1L2 |

**Table 6.7: Gene Ontologies for Turquoise Module in Developmental Stage One using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Turquoise | positive regulation of macrophage proliferation | 9.49E-49 | More than 50 overlapping genes |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to diacyl bacterial lipopeptide | 1.07E-17 | More than 50 overlapping genes |
| GO:0070016 | Interacting selectively and non-covalently with the armadillo repeat domain of a protein, an approximately 40 amino acid long tandemly repeated sequence motif first identified in the Drosophila segment polarity protein armadillo. Arm-repeat proteins are involved in various processes, including intracellular signalling and cytoskeletal regulation. | MF | Turquoise | armadillo repeat domain binding | 4.10E-17 | BNIP3L, CACNA1C, CACNA1D, CHRNA3, CHRNA5, CHRNB4, CLU, CTNND1, DPYD, EMX1, F2, FGFR1, GRIN2A, PRMT1, MEF2C, NMB, PDE4B, PRKD1, PSMA4, PTN, SLC12A4, SREBF1, TLE3, ALMS1, TAOK2, KCNK7, VPS45, RIMS1, PLCL2, PPP1R13B, NGEF, GIGYF2, PLEKHO1, TM6SF2, NLGN4X, SLC39A8, CSMD1, ZNF804A, TNFRSF13C, CARMIL2, CNTN4 |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl)benzene-1,2-diol] and derivatives formed by substitution. | BP | Turquoise | cellular response to catecholamine stimulus | 1.97E-14 | RERE, BNIP3L, BTG1, CHRNA3, CHRNB4, CLU, NCAN, CTNND1, EMX1, F2, FGFR1, PRMT1, MEF2C, PRKD1, PTN, PTPRK, ATXN7, SLC12A4, SREBF1, TLE3, ALMS1, TAOK2, ATG13, CKAP5, ZEB2, EPN2, RIMS1, CNOT1, PPP1R13B, MAU2, NGEF, PLEKHO1, HYDIN, NSD3, AMBRA1, NLGN4X, NDRG4, CENPM, ACTR5, CENPT, ANP32E, ESAM, ZNF804A, TDRD9, EMB, CARMIL2, CNTN4 |
| GO:0005829 | The part of the cytoplasm that does not contain organelles, but which does contain other particulate matter, such as protein complexes. | CC | Turquoise | cytosol | 8.03E-14 | BNIP3L, CLU, CTNND1, GSDME, DPYD, FGFR1, FHIT, PRMT1, MSRA, NFATC3, PDE4B, PRKD1, PSMA4, ATXN7, SREBF1, ALMS1, TAOK2, RGS6, ATG13, CKAP5, ZEB2, DMTF1, EPN2, NT5C2, RIMS1, CNOT1, PPP1R13B, EDC4, NGEF, SPATS2L, |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | GIGYF2, AMBRA1, RALGAPA2, PITPNM2, RANBP10, NDRG4, CENPM, CENPT, RPS19BP1, HARBI1, ASPG |
| GO:0048523 | Any process that stops, prevents, or reduces the frequency, rate or extent of a cellular process, any of those that are carried out at the cellular level, but are not necessarily restricted to a single cell. For example, cell communication occurs among more than one cell, but occurs at the cellular level. | BP | Turquoise | negative regulation of cellular process | 2.03E-13 | RERE, BNIP3L, BTG1, CACNA1C, CLU, CTNND1, GSDME, F2, FGFR1, FHIT, PRMT1, MEF2C, MGAT3, NFATC3, NMB, PRKD1, PSMA4, PTN, PTPRK, SOX5, SREBF1, TLE3, TAOK2, RGS6, ZEB2, EPN2, CNOT1, PLCL2, PPP1R13B, NGEF, GIGYF2, GATAD2A, AMBRA1, THAP11, NLGN4X, NDRG4, BCL2L12, TDRD9, CARMIL2, CNTN4 |
| GO:0080154 | Any process that modulates the rate, frequency or extent of fertilization. Fertilization is the union of gametes of opposite sexes during the process of sexual reproduction to form a zygote. It involves the fusion of the gametic nuclei (karyogamy) and cytoplasm (plasmogamy). | BP | Turquoise | regulation of fertilization | 8.45E-13 | RERE, BTG1, CHRNA3, CLU, GSDME, EMX1, F2, FGFR1, FHIT, GRIN2A, PRMT1, MEF2C, MGAT3, NFATC3, PRKD1, PSMA4, PTN, PTPRK, SOX5, SREBF1, TLE3, TAOK2, ZNF592, ATG13, ZEB2, DMTF1, CNOT1, PLCL2, GIGYF2, TM6SF2, NDFIP2, RBFOX1, GATAD2A, NSD3, AMBRA1, THAP11, NDRG4, ACTR5, ESRP2, CENPT, BCL2L12, ZBTB37, TNFRSF13C |
| GO:0071986 | A eukaryotically conserved protein complex; in humans, it is comprised of LAMTOR1, LAMTOR2, LAMTOR3, LAMTOR4, and LAMTOR5. The complex is anchored to lipid rafts in late endosome membranes via LAMTOR1, constitutes a guanine nucleotide exchange factor (GEF) for the Rag GTPases. | CC | Turquoise | Ragulator complex | 4.49E-12 | CACNA1C, CACNA1D, CHRNA3, CHRNA5, CHRNB4, CLU, CTNND1, GSDME, F2, FGFR1, FHIT, GRIN2A, OPCML, PDE4B, PRKD1, PTN, PTPRK, SLC12A4, RGS6, CKAP5, KCNK7, RIMS1, PPP1R13B, VSIG2, CA14, ZDHHC5, PLEKHO1, RALGAPA2, NLGN4X, SLC39A8, GPR135, NDRG4, PLPP5, ESAM, ZNF804A, TNFRSF13C, EMB, CARMIL2, CNTN4, HARBI1, SNORC |
| GO:1901666 | NA | BP | Turquoise | positive regulation of NAD+ ADP-ribosyltransferase activity | 7.48E-12 | BNIP3L, BTG1, CHRNA3, CLU, NCAN, GSDME, DPYD, F2, FGFR1, FHIT, GRIN2A, PRMT1, MEF2C, MGAT3, MSRA, PDE4B, PRKD1, PSMA4, PTN, PTPRK, ATXN7, SREBF1, TAOK2, ATG13, ZEB2, NT5C2, CNOT1, PLCL2, SEC11A, PLA2G15, ZDHHC5, GIGYF2, JKAMP, NDFIP2, NSD3, |

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| | | | | | | FANCL, NDRG4, GDPD3, ACTR5, BCL2L12, MARS2, TNFRSF13C, ASPHD1, ASPG |
| GO:0120069 | Any process that increases the frequency, rate or extent of any stomach fundus smooth muscle contraction. | BP | Turquoise | positive regulation of stomach fundus smooth muscle contraction | 3.89E-11 | RERE, CHRNA3, EMX1, FGFR1, PRMT1, MEF2C, PRKD1, PTN, PTPRK, ALMS1, TAOK2, CKAP5, ZEB2, RIMS1, NGEF, PLEKHO1, HYDIN, NDRG4, ZNF804A, EMB, CARMIL2, CNTN4 |
| GO:0005886 | The membrane surrounding a cell that separates the cell from its external environment. It consists of a phospholipid bilayer and associated proteins. | CC | Turquoise | plasma membrane | 5.94E-11 | CACNA1C, CACNA1D, CHRNA3, CHRNA5, CHRNB4, CTNND1, GSDME, F2, FGFR1, FHIT, GRIN2A, OPCML, PDE4B, PRKD1, PTN, PTPRK, SLC12A4, RGS6, CKAP5, KCNK7, RIMS1, PPP1R13B, VSIG2, CA14, ZDHHC5, PLEKHO1, RALGAPA2, NLGN4X, SLC39A8, GPR135, NDRG4, PLPP5, ESAM, ZNF804A, TNFRSF13C, EMB, CARMIL2, CNTN4, HARBI1 |
| GO:0016021 | The component of a membrane consisting of the gene products and protein complexes having at least some part of their peptide sequence embedded in the hydrophobic region of the membrane. | CC | Turquoise | integral component of membrane | 2.67E-10 | BNIP3L, CACNA1C, CACNA1D, CHRNA3, CHRNA5, CHRNB4, FGFR1, GRIN2A, MGAT3, PDE4B, PTPRK, SLC12A4, SREBF1, TAOK2, KCNK7, VPS45, NEMP1, SEC11A, VSIG2, CA14, ZDHHC5, GIGYF2, JKAMP, TM6SF2, NDFIP2, NLGN4X, SLC39A8, CSMD1, GPR135, GDPD3, PLPP5, ESAM, TNFRSF13C, EMB, ASPHD1, SNORC, PCNX3 |
| GO:0043232 | Organised structure of distinctive morphology and function, not bounded by a lipid bilayer membrane and occurring within the cell. Includes ribosomes, the cytoskeleton and chromosomes. | CC | Turquoise | intracellular non-membrane-bounded organelle | 3.17E-10 | CACNA1C, CACNA1D, CLU, EMX1, FGFR1, FHIT, MEF2C, MSRA, PDE4B, PSMA4, ATXN7, ALMS1, TAOK2, CKAP5, ZEB2, RIMS1, CNOT1, MAU2, EDC4, SPATS2L, GIGYF2, ARL6IP4, HYDIN, GATAD2A, NSD3, AMBRA1, CENPM, ACTR5, CENPT, ANP32E, RPS19BP1, ZNF804A, TDRD9, CARMIL2, HARBI1 |
| GO:0120060 | Any process that modulates the frequency, rate or extent of any gastric emptying process, the process in which the liquid and liquid-suspended solid contents of the | BP | Turquoise | regulation of gastric emptying | 4.35E-10 | CACNA1C, CHRNA3, CHRNA5, CHRNB4, CLU, CTNND1, GRIN2A, NMB, PDE4B, PTPRK, ALMS1, TAOK2, NGEF, ZDHHC5, GIGYF2, PLEKHO1, HYDIN, AMBRA1, |

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| | stomach exit through the pylorus into the duodenum. | | | | | NLGN4X, NDRG4, ZNF804A, EMB, CARMIL2, CNTN4 |
| GO:0070052 | Interacting selectively and non-covalently with a type V collagen trimer. | MF | Turquoise | collagen V binding | 4.49E-10 | RERE, BNIP3L, BTG1, CLU, NCAN, EMX1, F2, FGFR1, FHIT, PRMT1, MEF2C, MSRA, NFATC3, PSMA4, ATXN7, SREBF1, TLE3, TAOK2, CKAP5, ZEB2, DMTF1, PPP1R13B, MAU2, EDC4, PLA2G15, SPATS2L, ARL6IP4, GATAD2A, NSD3, FANCL, THAP11, CENPM, ACTR5, ESRP2, CENPT, ANP32E, RPS19BP1, MARS2 |
| GO:0044267 | The chemical reactions and pathways involving a specific protein, rather than of proteins in general, occurring at the level of an individual cell. Includes cellular protein modification. | BP | Turquoise | cellular protein metabolic process | 8.60E-10 | BNIP3L, BTG1, CHRNA3, CLU, GSDME, F2, FGFR1, FHIT, GRIN2A, PRMT1, MEF2C, MGAT3, MSRA, PRKD1, PSMA4, PTN, PTPRK, ATXN7, SREBF1, TAOK2, ATG13, ZEB2, CNOT1, PLCL2, SEC11A, ZDHHC5, GIGYF2, JKAMP, NDFIP2, NSD3, FANCL, NDRG4, ACTR5, BCL2L12, MARS2, ASPHD1 |
| GO:0010468 | Any process that modulates the frequency, rate or extent of gene expression. Gene expression is the process in which a gene's coding sequence is converted into a mature gene product or products (proteins or RNA). This includes the production of an RNA transcript as well as any processing to produce a mature RNA product or an mRNA or circRNA (for protein-coding genes) and the translation of that mRNA or circRNA into protein. Protein maturation is included when required to form an active form of a product from an inactive precursor form. | BP | Turquoise | regulation of gene expression | 9.68E-10 | RERE, BTG1, CLU, EMX1, F2, FGFR1, PRMT1, MEF2C, NFATC3, PDE4B, PRKD1, PSMA4, PTPRK, SOX5, SREBF1, TLE3, ZNF592, ZEB2, DMTF1, RIMS1, CNOT1, EDC4, GIGYF2, NDFIP2, RBFOX1, GATAD2A, NSD3, THAP11, ACTR5, ESRP2, CENPT, BCL2L12, ZBTB37, ZNF804A, TNFRSF13C, TDRD9 |
| GO:0031175 | The process whose specific outcome is the progression of a neuron projection over time, from its formation to the mature structure. A neuron projection is any process extending from a neural cell, such | BP | Turquoise | neuron projection development | 2.13E-09 | RERE, CHRNA3, EMX1, FGFR1, PRMT1, MEF2C, PRKD1, PTN, PTPRK, TAOK2, ZEB2, RIMS1, NGEF, NDRG4, ZNF804A, EMB, CNTN4 |

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| | as axons or dendrites (collectively called neurites). | | | | | |
| GO:1901555 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a paclitaxel stimulus. | BP | Turquoise | response to paclitaxel | 5.31E-09 | RERE, BTG1, CLU, DPYD, EMX1, FGFR1, FHIT, GRIN2A, PRMT1, MEF2C, NFATC3, PDE4B, PRKD1, PSMA4, PTPRK, SOX5, SREBF1, TLE3, ZNF592, ZEB2, DMTF1, NT5C2, CNOT1, EDC4, GIGYF2, ARL6IP4, RBFOX1, GATAD2A, NSD3, FANCL, THAP11, ACTR5, ESRP2, CENPT, BCL2L12, ZBTB37, MARS2, TDRD9, HARBI1 |
| GO:0009653 | The process in which anatomical structures are generated and organized. Morphogenesis pertains to the creation of form. | BP | Turquoise | anatomical structure morphogenesis | 1.15E-08 | RERE, BTG1, CACNA1C, CHRNA3, CLU, EMX1, F2, FGFR1, MEF2C, PRKD1, PSMA4, PTN, TLE3, TAOK2, ZEB2, EPN2, RIMS1, NGEF, PLEKHO1, NDRG4, ESRP2, TNFRSF13C, EMB, CARMIL2, CNTN4 |
| GO:1901679 | The directed movement of nucleotide across a membrane. | BP | Turquoise | nucleotide transmembrane transport | 1.22E-08 | RERE, BTG1, CLU, NCAN, DPYD, EMX1, FGFR1, PRMT1, MEF2C, MGAT3, NFATC3, PDE4B, PRKD1, PSMA4, PTPRK, SOX5, SREBF1, TLE3, ZNF592, ZEB2, DMTF1, NT5C2, CNOT1, PLCL2, ZDHHC5, GIGYF2, GATAD2A, NSD3, THAP11, PITPNM2, ACTR5, CENPT, BCL2L12, ZBTB37, MARS2, TNFRSF13C, ASPG |
| GO:0043005 | A prolongation or process extending from a nerve cell, e.g. an axon or dendrite. | CC | Turquoise | neuron projection | 1.83E-08 | CACNA1C, CHRNA3, CHRNA5, CHRNB4, CLU, CTNND1, GRIN2A, NMB, PDE4B, PTPRK, TAOK2, NGEF, ZDHHC5, GIGYF2, NLGN4X, ZNF804A, EMB, CNTN4 |
| GO:0035556 | The process in which a signal is passed on to downstream components within the cell, which become activated themselves to further propagate the signal and finally trigger a change in the function or state of the cell. | BP | Turquoise | intracellular signal transduction | 2.69E-08 | CA8, CACNA1C, CLU, GSDME, F2, FGFR1, FHIT, GRIN2A, PRMT1, MEF2C, NFATC3, PRKD1, PSMA4, TAOK2, RGS6, ZEB2, CNOT1, PLCL2, PPP1R13B, NGEF, NDFIP2, RALGAPA2, PITPNM2, NDRG4, BCL2L12 |
| GO:0007417 | The process whose specific outcome is the progression of the central nervous system over time, from its formation to the mature structure. The central nervous system is | BP | Turquoise | central nervous system development | 2.88E-08 | RERE, CLU, NCAN, CTNND1, EMX1, F2, FGFR1, GRIN2A, PTN, ZEB2, GIGYF2, HYDIN, NLGN4X, NDRG4, CNTN4, HAPLN4 |

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| | the core nervous system that serves an integrating and coordinating function. In vertebrates it consists of the brain and spinal cord. In those invertebrates with a central nervous system it typically consists of a brain, cerebral ganglia and a nerve cord. | | | | | |
| GO:0044249 | The chemical reactions and pathways resulting in the formation of substances, carried out by individual cells. | BP | Turquoise | cellular biosynthetic process | 3.33E-08 | RERE, BTG1, CLU, NCAN, DPYD, EMX1, FGFR1, PRMT1, MEF2C, MGAT3, NFATC3, PDE4B, PRKD1, PSMA4, PTPRK, SOX5, SREBF1, TLE3, ZNF592, ZEB2, DMTF1, NT5C2, CNOT1, ZDHHC5, GIGYF2, GATAD2A, NSD3, THAP11, PITPNM2, ACTR5, CENPT, BCL2L12, ZBTB37, MARS2, TNFRSF13C, ASPG |
| GO:0050793 | Any process that modulates the frequency, rate or extent of development, the biological process whose specific outcome is the progression of a multicellular organism over time from an initial condition (e.g. a zygote, or a young adult) to a later condition (e.g. a multicellular animal or an aged adult). | BP | Turquoise | regulation of developmental process | 4.70E-08 | BTG1, CHRNA3, EMX1, F2, FGFR1, PRMT1, MEF2C, NFATC3, PRKD1, PSMA4, PTN, SOX5, TAOK2, ZEB2, EPN2, RIMS1, CNOT1, NGEF, PLEKHO1, NDRG4, BCL2L12, ZNF804A, TNFRSF13C, CNTN4 |
| GO:0051252 | Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving RNA. | BP | Turquoise | regulation of RNA metabolic process | 5.30E-08 | RERE, BTG1, CLU, EMX1, FGFR1, PRMT1, MEF2C, NFATC3, PRKD1, PSMA4, PTPRK, SOX5, SREBF1, TLE3, ZNF592, ZEB2, DMTF1, CNOT1, GIGYF2, RBFOX1, GATAD2A, NSD3, THAP11, ACTR5, ESRP2, CENPT, BCL2L12, ZBTB37 |
| GO:0005654 | That part of the nuclear content other than the chromosomes or the nucleolus. | CC | Turquoise | nucleoplasm | 6.26E-08 | RERE, BNIP3L, BTG1, PRMT1, MEF2C, MSRA, NFATC3, PSMA4, ATXN7, SREBF1, TLE3, DMTF1, PPP1R13B, MAU2, EDC4, PLA2G15, ARL6IP4, GATAD2A, NSD3, FANCL, THAP11, CENPM, ACTR5, ESRP2, CENPT, ANP32E, RPS19BP1 |
| GO:0010604 | Any process that increases the frequency, rate or extent of the chemical reactions and | BP | Turquoise | positive regulation of macromolecule | 6.68E-08 | RERE, CHRNA3, CLU, GSDME, F2, FGFR1, GRIN2A, PRMT1, MEF2C, NFATC3, PDE4B, |

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| | pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass. | | | metabolic process | | PRKD1, SOX5, SREBF1, TAOK2, ATG13, ZEB2, DMTF1, RIMS1, CNOT1, GIGYF2, NDFIP2, NSD3, NDRG4, BCL2L12, ZNF804A, TNFRSF13C |
| GO:0033554 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating the organism is under stress. The stress is usually, but not necessarily, exogenous (e.g. temperature, humidity, ionizing radiation). | BP | Turquoise | cellular response to stress | 7.77E-08 | BNIP3L, CLU, PRMT1, MEF2C, MGAT3, MSRA, PRKD1, PSMA4, PTN, PTPRK, SREBF1, TAOK2, ATG13, ZEB2, CNOT1, GIGYF2, JKAMP, FANCL, AMBRA1, ACTR5, BCL2L12 |
| GO:0090317 | Any process that decreases the frequency, rate or extent of the directed movement of proteins within cells. | BP | Turquoise | negative regulation of intracellular protein transport | 8.52E-08 | RERE, BTG1, CLU, EMX1, FGFR1, PRMT1, MEF2C, NFATC3, PRKD1, PSMA4, PTPRK, SOX5, SREBF1, TLE3, ZNF592, ZEB2, DMTF1, CNOT1, EDC4, GIGYF2, ARL6IP4, RBFOX1, GATAD2A, NSD3, FANCL, THAP11, ACTR5, ESRP2, CENPT, BCL2L12, ZBTB37, MARS2, TDRD9, HARBI1 |
| GO:0031325 | Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways by which individual cells transform chemical substances. | BP | Turquoise | positive regulation of cellular metabolic process | 9.84E-08 | RERE, BNIP3L, CHRNA3, CLU, GSDME, F2, FGFR1, GRIN2A, PRMT1, MEF2C, NFATC3, PRKD1, SOX5, SREBF1, TAOK2, ATG13, ZEB2, DMTF1, CNOT1, GIGYF2, NDFIP2, NSD3, AMBRA1, NDRG4, BCL2L12, TNFRSF13C |
| GO:0051128 | Any process that modulates the frequency, rate or extent of a process involved in the formation, arrangement of constituent parts, or disassembly of cell structures, including the plasma membrane and any external encapsulating structures such as the cell wall and cell envelope. | BP | Turquoise | regulation of cellular component organization | 1.16E-07 | BNIP3L, BTG1, CHRNA3, CLU, F2, FGFR1, MEF2C, PRKD1, PTN, SREBF1, ALMS1, TAOK2, ATG13, ZEB2, RIMS1, CNOT1, PPP1R13B, NGEF, NSD3, NDRG4, ZNF804A, CARMIL2 |
| GO:0006811 | The directed movement of charged atoms or small charged molecules into, out of or within a cell, or between cells, by means | BP | Turquoise | ion transport | 1.49E-07 | CACNA1C, CACNA1D, CHRNA3, CHRNA5, CHRNB4, F2, FGFR1, GRIN2A, MEF2C, NMB, PDE4B, SLC12A4, KCNK7, RIMS1, |

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| | of some agent such as a transporter or pore. | | | | | CA14, NDFIP2, PITPNM2, SLC39A8, EMB |
| GO:0009966 | Any process that modulates the frequency, rate or extent of signal transduction. | BP | Turquoise | regulation of signal transduction | 1.94E-07 | CLU, CTNND1, GSDME, F2, FGFR1, GRIN2A, PRMT1, MEF2C, PRKD1, PSMA4, TLE3, TAOK2, RGS6, ZEB2, EPN2, RIMS1, CNOT1, PLCL2, PPP1R13B, NGEF, NDFIP2, RALGAPA2, NLGN4X, NDRG4, BCL2L12 |
| GO:0006928 | The directed, self-propelled movement of a cell or subcellular component without the involvement of an external agent such as a transporter or a pore. | BP | Turquoise | movement of cell or subcellular component | 2.51E-07 | RERE, BTG1, CACNA1C, CACNA1D, F2, FGFR1, MEF2C, MGAT3, PDE4B, PRKD1, PTN, PTPRK, TAOK2, ZEB2, PLEKHO1, HYDIN, NDRG4, ESAM, EMB, CARMIL2, CNTN4 |
| GO:0050804 | Any process that modulates the frequency or amplitude of synaptic transmission, the process of communication from a neuron to a target (neuron, muscle, or secretory cell) across a synapse. Amplitude, in this case, refers to the change in postsynaptic membrane potential due to a single instance of synaptic transmission. | BP | Turquoise | modulation of chemical synaptic transmission | 2.71E-07 | CACNA1D, CHRNA3, CHRNA5, CHRNB4, GRIN2A, MEF2C, PTN, RIMS1, PLCL2, NLGN4X, CNTN4 |
| GO:0099192 | A synapse formed by a cerebellar Golgi cell synapsing on to a cerebellar granule cell. | CC | Turquoise | cerebellar Golgi cell to granule cell synapse | 2.76E-07 | CACNA1D, CHRNA3, CHRNA5, CHRNB4, GRIN2A, MEF2C, PTN, RIMS1, PLCL2, NLGN4X, CNTN4 |
| GO:0043412 | The covalent alteration of one or more monomeric units in a polypeptide, polynucleotide, polysaccharide, or other biological macromolecule, resulting in a change in its properties. | BP | Turquoise | macromolecule modification | 3.04E-07 | BTG1, CHRNA3, CLU, GSDME, F2, FGFR1, PRMT1, MEF2C, MGAT3, MSRA, PRKD1, PSMA4, PTN, PTPRK, ATXN7, SREBF1, TAOK2, ATG13, ZEB2, PLCL2, ZDHHC5, NDFIP2, GATAD2A, NSD3, FANCL, NDRG4, ACTR5, TDRD9, ASPHD1 |
| GO:0070025 | Interacting selectively and non-covalently with carbon monoxide (CO). | MF | Turquoise | carbon monoxide binding | 3.11E-07 | CACNA1C, CACNA1D, CHRNA3, CLU, F2, FGFR1, GRIN2A, MEF2C, PDE4B, PRKD1, PTN, TAOK2, RGS6, ATG13, ZEB2, RIMS1, PLCL2, MAU2, NGEF, NDFIP2, AMBRA1, RALGAPA2, ANP32E, BCL2L12 |
| GO:0060079 | A process that leads to a temporary increase in postsynaptic potential due to the flow of positively charged ions into the | BP | Turquoise | excitatory postsynaptic potential | 3.77E-07 | CHRNA3, CHRNA5, CHRNB4, GRIN2A, MEF2C, RIMS1, NLGN4X |

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| | postsynaptic cell. The flow of ions that causes an EPSP is an excitatory postsynaptic current (EPSC) and makes it easier for the neuron to fire an action potential. | | | | | |
| GO:0009059 | The chemical reactions and pathways resulting in the formation of a macromolecule, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass. | BP | Turquoise | macromolecule biosynthetic process | 4.21E-07 | RERE, BTG1, CLU, NCAN, EMX1, FGFR1, PRMT1, MEF2C, MGAT3, NFATC3, PRKD1, PSMA4, PTPRK, SOX5, SREBF1, TLE3, ZNF592, ZEB2, DMTF1, CNOT1, ZDHHC5, GIGYF2, GATAD2A, NSD3, THAP11, ACTR5, CENPT, BCL2L12, ZBTB37, MARS2, TNFRSF13C |

**Table 6.8: Gene Ontologies for Blue Module in Developmental Stage Two using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Blue | positive regulation of macrophage proliferation | 9.38E-19 | CLU, NCAN, CTNND1, CYP17A1, EPHX2, FGFR1, GRM3, HSPD1, HSPE1, MMP16, NAGA, NDUFA6, OPCML, PCCB, STAT6, TBX6, TLE3, INPP4B, DGKI, GABBR2, KDM4A, MPHOSPH9, VPS45, SATB2, PSD3, ZDHHC5, FOXP1, B3GAT1, LSM1, RBFOX1, RALGAPA2, SUGP1, NDRG4, ZFYVE21, GDPD3, COQ10B, DRC3, YPEL3, L3MBTL2, PLPP5, C12orf65, RPS19BP1, TNFRSF13C, RFTN2, LETM2, C16orf92, WBP2NL, RILPL2, YPEL4, MED19 |
| GO:0043231 | Organised structure of distinctive morphology and function, bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Blue | intracellular membrane-bounded organelle | 2.90E-12 | CLU, NCAN, CTNND1, CYP17A1, EPHX2, FGFR1, HSPD1, HSPE1, MMP16, NAGA, NDUFA6, PCCB, STAT6, TBX6, TLE3, DGKI, KDM4A, MPHOSPH9, VPS45, SATB2, FOXP1, B3GAT1, LSM1, RBFOX1, RALGAPA2, SUGP1, NDRG4, COQ10B, YPEL3, L3MBTL2, C12orf65, RPS19BP1, LETM2, WBP2NL, YPEL4, MED19 |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Blue | cytoplasm | 1.74E-10 | CLU, NCAN, CTNND1, CYP17A1, EPHX2, FGFR1, HSPD1, HSPE1, MMP16, NAGA, NDUFA6, PCCB, STAT6, INPP4B, DGKI, GABBR2, KDM4A, MPHOSPH9, VPS45, B3GAT1, LSM1, RBFOX1, RALGAPA2, NDRG4, ZFYVE21, GDPD3, COQ10B, DRC3, PLPP5, C12orf65, RPS19BP1, LETM2, WBP2NL, RILPL2 |
| GO:0044237 | The chemical reactions and pathways by which individual cells transform chemical substances. | BP | Blue | cellular metabolic process | 6.66E-09 | CLU, NCAN, CYP17A1, EPHX2, FGFR1, HSPD1, HSPE1, NAGA, NDUFA6, PCCB, STAT6, TBX6, TLE3, INPP4B, DGKI, KDM4A, SATB2, ZDHHC5, FOXP1, B3GAT1, LSM1, RBFOX1, SUGP1, NDRG4, GDPD3, COQ10B, L3MBTL2, PLPP5, C12orf65, TNFRSF13C, WBP2NL, MED19 |
| GO:0044238 | The chemical reactions and pathways involving those compounds which are formed as a part of the normal anabolic and catabolic processes. These | BP | Blue | primary metabolic process | 2.27E-08 | CLU, NCAN, CYP17A1, EPHX2, FGFR1, HSPD1, HSPE1, MMP16, NAGA, PCCB, STAT6, TBX6, TLE3, INPP4B, DGKI, KDM4A, SATB2, ZDHHC5, FOXP1, B3GAT1, LSM1, RBFOX1, SUGP1, NDRG4, GDPD3, L3MBTL2, PLPP5, C12orf65, TNFRSF13C, WBP2NL, MED19 |

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| | processes take place in most, if not all, cells of the organism. | | | | | |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Blue | cellular response to diacyl bacterial lipopeptide | 3.35 E-08 | CLU, NCAN, CYP17A1, EPHX2, FGFR1, HSPD1, HSPE1, MMP16, NAGA, PCCB, STAT6, TBX6, TLE3, INPP4B, DGKI, KDM4A, SATB2, ZDHHC5, FOXP1, B3GAT1, LSM1, RBFOX1, SUGP1, NDRG4, GDPD3, COQ10B, L3MBTL2, PLPP5, C12orf65, TNFRSF13C, WBP2NL, MED19 |
| GO:0016020 | A lipid bilayer along with all the proteins and protein complexes embedded in it an attached to it. | CC | Blue | membrane | 1.24 E-07 | CLU, CTNND1, CYP17A1, FGFR1, GRM3, HSPD1, HSPE1, MMP16, NDUFA6, OPCML, STAT6, DGKI, GABBR2, MPHOSPH9, VPS45, PSD3, ZDHHC5, B3GAT1, RALGAPA2, NDRG4, GDPD3, COQ10B, PLPP5, TNFRSF13C, RFTN2, LETM2, C16orf92, RILPL2 |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Blue | protein binding | 1.24 E-07 | MPPED2, CLU, CTNND1, EPHX2, FGFR1, HSPD1, HSPE1, NAGA, PCCB, STAT6, TBX6, TLE3, INPP4B, DGKI, GABBR2, KDM4A, VPS45, SATB2, PSD3, FOXP1, LSM1, RBFOX1, RALGAPA2, SUGP1, NDRG4, ZFYVE21, L3MBTL2, RPS19BP1, WBP2NL, RILPL2, MED19 |
| GO:0043233 | The internal volume enclosed by the membranes of a particular organelle; includes the volume enclosed by a single organelle membrane, e.g. endoplasmic reticulum lumen, or the volume enclosed by the innermost of the two lipid bilayers of an organelle envelope, e.g. nuclear lumen. | CC | Blue | organelle lumen | 2.00 E-07 | CLU, NCAN, EPHX2, FGFR1, HSPD1, HSPE1, MMP16, PCCB, STAT6, TBX6, TLE3, DGKI, KDM4A, SATB2, FOXP1, SUGP1, YPEL3, L3MBTL2, C12orf65, RPS19BP1, YPEL4, MED19 |
| GO:0070052 | Interacting selectively and non-covalently with a type V collagen trimer. | MF | Blue | collagen V binding | 2.00 E-07 | CLU, NCAN, EPHX2, FGFR1, HSPD1, HSPE1, MMP16, PCCB, STAT6, TBX6, TLE3, DGKI, KDM4A, SATB2, FOXP1, SUGP1, YPEL3, L3MBTL2, C12orf65, RPS19BP1, YPEL4, MED19 |

**Table 6.9: Gene Ontologies for Brown Module in Developmental Stage Two using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 7.94E-15 | BTG1, CACNA1C, CHRNA5, ETF1, F2, PTK2B, KCNJ13, NEK1, NRGN, MAPK3, TCF4, FXR1, ATP5MPL, KCNK7, NXPH4, IGSF9B, SMG6, PPP1R13B, MAU2, NGEF, AMBRA1, TSNAXIP1, NDUFA4L2, ADAMTSL3, AS3MT, SRR, DPEP3, BOLL, ZNF408, SETD6, CENPT, EFHD1, WDR73, CREB3L1, TMEM219, SNORC, PCNX3 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Brown | intracellular organelle lumen | 2.07E-09 | BTG1, CACNA1C, CHRNA5, ETF1, F2, PTK2B, KCNJ13, NEK1, NRGN, MAPK3, TCF4, FXR1, KCNK7, NXPH4, IGSF9B, SMG6, PPP1R13B, MAU2, NGEF, AMBRA1, BOLL, ZNF408, SETD6, CENPT, EFHD1, WDR73, CREB3L1, TMEM219 |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Brown | cytoplasm | 9.37E-09 | BTG1, CACNA1C, ETF1, F2, PTK2B, NEK1, NRGN, MAPK3, FXR1, ATP5MPL, SMG6, PPP1R13B, NGEF, AMBRA1, TSNAXIP1, NDUFA4L2, AS3MT, SRR, DPEP3, BOLL, SETD6, CENPT, EFHD1, WDR73, CREB3L1, SNORC |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Brown | protein binding | 2.41E-08 | BTG1, CACNA1C, CHRNA5, ETF1, F2, PTK2B, NEK1, NRGN, MAPK3, TCF4, FXR1, NXPH4, IGSF9B, SMG6, PPP1R13B, MAU2, NGEF, AMBRA1, ADAMTSL3, SRR, BOLL, ZNF408, SETD6, CENPT, CREB3L1, TMEM219 |
| GO:0016043 | A process that results in the assembly, arrangement of constituent parts, or disassembly of a cellular component. | BP | Brown | cellular component organization | 4.64E-08 | BTG1, ETF1, F2, PTK2B, NEK1, MAPK3, TCF4, IGSF9B, SMG6, PPP1R13B, MAU2, NGEF, AMBRA1, SRR, DPEP3, SETD6, CENPT, EFHD1, WDR73, CREB3L1 |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2- | BP | Brown | cellular response to catecholamine stimulus | 7.59E-08 | BTG1, ETF1, F2, PTK2B, NEK1, MAPK3, TCF4, IGSF9B, SMG6, PPP1R13B, MAU2, NGEF, AMBRA1, SRR, DPEP3, SETD6, CENPT, EFHD1, WDR73, CREB3L1 |

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| | aminoethyl)benzene-1,2-diol] and derivatives formed by substitution. | | | | | |
| GO:0016020 | A lipid bilayer along with all the proteins and protein complexes embedded in it an attached to it. | CC | Brown | membrane | 1.22E-07 | CACNA1C, CHRNA5, F2, PTK2B, KCNJ13, NRGN, MAPK3, FXR1, ATP5MPL, KCNK7, IGSF9B, PPP1R13B, NGEF, AMBRA1, NDUFA4L2, SRR, DPEP3, EFHD1, WDR73, CREB3L1, TMEM219, SNORC, PCNX3 |

**Table 6.10: Gene Ontology for Green Module Stage Two using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0003674 | A molecular process that can be carried out by the action of a single macromolecular machine, usually via direct physical interactions with other molecular entities. Function in this sense denotes an action, or activity, that a gene product (or a complex) performs. These actions are described from two distinct but related perspectives: (1) biochemical activity, and (2) role as a component in a larger system/process. | MF | Green | molecular function | 2.31E-12 | BNIP3L, CACNB2, CLCN3, ERCC4, PRMT1, HSPA9, NCK1, PPP2R3A, PPP4C, PTPRK, PSMD6, NUTF2, KAT5, CLP1, NEMP1, SF3B1, SEC11A, ZSCAN2, TSR1, ACTR5, THOC7, IMMP2L, RFT1, TYW5, TSNARE1, ASPHD1, HAPLN4 |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Green | positive regulation of macrophage proliferation | 7.16E-10 | BNIP3L, CACNB2, CLCN3, ERCC4, PRMT1, HSPA9, NCK1, PPP4C, PTPRK, PSMD6, NUTF2, KAT5, CLP1, NEMP1, SF3B1, SEC11A, ZSCAN2, TSR1, ACTR5, THOC7, IMMP2L, RFT1, TYW5, TSNARE1, ASPHD1, HAPLN4 |
| GO:1902644 | NA | BP | Green | tertiary alcohol metabolic process | 2.55E-09 | ERCC4, PRMT1, NCK1, PPP2R3A, PPP4C, PSMD6, KAT5, CLP1, SF3B1, SEC11A, ACTR5 |
| GO:0032991 | A stable assembly of two or more macromolecules, i.e. proteins, nucleic acids, carbohydrates or lipids, in which at least one component is a protein and the constituent parts function together. | CC | Green | protein-containing complex | 7.70E-09 | CACNB2, ERCC4, PRMT1, NCK1, PPP2R3A, PPP4C, PSMD6, NUTF2, KAT5, CLP1, SF3B1, SEC11A, TSR1, ACTR5, THOC7, IMMP2L, TSNARE1 |
| GO:0043170 | The chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass. | BP | Green | macromolecule metabolic process | 4.82E-08 | BNIP3L, ERCC4, PRMT1, NCK1, PPP2R3A, PPP4C, PTPRK, PSMD6, NUTF2, KAT5, CLP1, SF3B1, SEC11A, ZSCAN2, TSR1, ACTR5, THOC7, IMMP2L, TYW5, ASPHD1 |
| GO:0044237 | The chemical reactions and pathways by | BP | Green | cellular | 1.56E- | BNIP3L, ERCC4, PRMT1, HSPA9, |

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| | which individual cells transform chemical substances. | | | metabolic process | 07 | NCK1, PPP2R3A, PPP4C, PTPRK, PSMD6, KAT5, CLP1, SF3B1, SEC11A, ZSCAN2, TSR1, ACTR5, THOC7, IMMP2L, TYW5, ASPHD1 |
| GO:0033554 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating the organism is under stress. The stress is usually, but not necessarily, exogenous (e.g. temperature, humidity, ionizing radiation). | BP | Green | cellular response to stress | 1.69E-07 | BNIP3L, ERCC4, PRMT1, HSPA9, NCK1, PPP4C, PTPRK, PSMD6, KAT5, ACTR5, IMMP2L |
| GO:0043227 | Organised structure of distinctive morphology and function, bounded by a single or double lipid bilayer membrane. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Green | membrane-bounded organelle | 1.88E-07 | BNIP3L, CLCN3, ERCC4, PRMT1, HSPA9, NCK1, PPP4C, PTPRK, PSMD6, NUTF2, KAT5, CLP1, NEMP1, SF3B1, SEC11A, ZSCAN2, TSR1, ACTR5, THOC7, IMMP2L, RFT1 |
| GO:0005622 | The living contents of a cell; the matter contained within (but not including) the plasma membrane, usually taken to exclude large vacuoles and masses of secretory or ingested material. In eukaryotes it includes the nucleus and cytoplasm. | CC | Green | intracellular | 2.20E-07 | BNIP3L, CLCN3, ERCC4, PRMT1, HSPA9, NCK1, PPP2R3A, PPP4C, PTPRK, PSMD6, NUTF2, KAT5, CLP1, NEMP1, SF3B1, ZSCAN2, TSR1, ACTR5, THOC7, IMMP2L, TYW5, TSNARE1 |

**Table 6.11: Gene Ontology for Turquoise Module Stage Two using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Turquoise | positive regulation of macrophage proliferation | 1.10E-69 | More than 50 overlapping genes |
| GO:0070016 | Interacting selectively and non-covalently with the armadillo repeat domain of a protein, an approximately 40 amino acid long tandemly repeated sequence motif first identified in the Drosophila segment polarity protein armadillo. Arm-repeat proteins are involved in various processes, including intracellular signalling and cytoskeletal regulation. | MF | Turquoise | armadillo repeat domain binding | 3.94E-28 | More than 50 overlapping genes |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to diacyl bacterial lipopeptide | 4.57E-25 | More than 50 overlapping genes |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl) benzene-1,2-diol] and derivatives formed by substitution. | BP | Turquoise | cellular response to catecholamine stimulus | 8.10E-24 | More than 50 overlapping genes |
| GO:0005829 | The part of the cytoplasm that | CC | Turquoise | cytosol | 1.76E-20 | More than 50 overlapping genes |

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| | does not contain organelles, but which does contain other particulate matter, such as protein complexes. | | | | | |
| GO:0080154 | Any process that modulates the rate, frequency or extent of fertilization. Fertilization is the union of gametes of opposite sexes during the process of sexual reproduction to form a zygote. It involves the fusion of the gametic nuclei (karyogamy) and cytoplasm (plasmogamy). | BP | Turquoise | regulation of fertilization | 5.50E-19 | More than 50 overlapping genes |
| GO:1901679 | The directed movement of nucleotide across a membrane. | BP | Turquoise | nucleotide transmembrane transport | 1.48E-16 | More than 50 overlapping genes |
| GO:1901666 | Any process that activates or increases the frequency, rate or extent of NAD+ ADP-ribosyltransferase activity. | BP | Turquoise | positive regulation of NAD+ ADP-ribosyltransferase activity | 1.48E-16 | More than 50 overlapping genes |
| GO:0070052 | Interacting selectively and non-covalently with a type V collagen trimer. | MF | Turquoise | collagen V binding | 3.74E-15 | More than 50 overlapping genes |
| GO:0120060 | Any process that modulates the frequency, rate or extent of any gastric emptying process, the process in which the liquid and liquid-suspended solid contents of the stomach exit through the pylorus into the duodenum. | BP | Turquoise | regulation of gastric emptying | 5.54E-15 | ATP2A2, CALB2, CHRM3, CHRNA3, CHRNB4, DRD2, GPM6A, GRIN2A, LRP1, NMB, PDE4B, PRKCB, PTPRF, RANGAP1, STAR, ALMS1, AP3B2, DOC2A, CUL3, TAOK2, SDCCAG8, GIGYF2, PARD6A, PLEKHO1, HYDIN, NLGN4X, RPTOR, CPEB1, BCL11B, ZNF804A, EMB, SLC32A1, CARMIL2, CNTN4, HCN1 |
| GO:0006996 | A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly | BP | Turquoise | organelle organization | 6.90E-15 | ALDOA, RERE, ATP2A2, EP300, LRP1, MEF2C, PPP2R2A, PRKCB, PRKD1, RELA, ATXN7, SIPA1, SREBF1, SREBF2, SRPK2, ALMS1, MAD1L1, CUL3, HIRIP3, ASH2L, |

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| | of an organelle within a cell. An organelle is an organized structure of distinctive morphology and function. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton. Excludes the plasma membrane. | | | | | INA, TAOK2, BAG4, CKAP5, ZEB2, AKT3, STAG1, SDCCAG8, CNOT1, DDHD2, PARD6A, SFMBT1, HYDIN, VPS13C, NSD3, MSL2, PAK6, CENPM, MAIP1, ANP32E, PCGF6, DNAJC19, CARMIL2, TOM1L2, SNX19, BTBD18 |
| GO:1901555 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a paclitaxel stimulus. | BP | Turquoise | response to paclitaxel | 1.74E-14 | More than 50 overlapping genes |
| GO:0099551 | Cell-cell signalling between presynapse and postsynapse, via the vesicular release and reception of neuropeptide molecules, that modulates the synaptic transmission properties of the synapse. | BP | Turquoise | trans-synaptic signalling by neuropeptide, modulating synaptic transmission | 8.65E-14 | ATP2A2, CACNA1D, CALB2, CHRM3, CHRNA3, CHRNB4, DRD2, GRIN2A, MEF2C, PLCL1, PRKCB, PTN, RELA, SLC12A4, STAR, DOC2A, RIMS1, PLCL2, NLGN4X, SLC32A1, CNTN4, STAC3 |
| GO:0046872 | Interacting selectively and non-covalently with any metal ion. | MF | Turquoise | metal ion binding | 9.32E-14 | RERE, ATP2A2, CA8, CACNA1D, CALB2, DPYD, EP300, GRIN2A, IREB2, LRP1, PDE4B, PLCB2, PRKCB, PRKD1, SHMT2, SRPK2, DOC2A, ASH2L, ZNF592, PLCH2, ZNF536, ZEB2, RAI1, NT5C2, RIMS1, DDHD2, ZC3H7B, CA14, MOB4, GATAD2A, NSD3, MSL2, ZNF823, OTUD7B, THAP11, PITPNM2, RBM26, DPEP2, CPEB1, BCL11B, PCGF6, ZBTB37, ZNF804A, OTOL1, STAC3, HARBI1 |
| GO:0097178 | The aggregation, arrangement and | BP | | ruffle assembly | 1.29E-13 | More than 50 overlapping genes |

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| | bonding together of a set of components to form a ruffle, a projection at the leading edge of a crawling cell; the protrusions are supported by a microfilament meshwork. The formation of ruffles (also called membrane ruffling) is thought to be controlled by a group of enzymes known as Rho GTPases, specifically RhoA, Rac1 and cdc42. | | Turquoise | | | |
| GO:0098935 | The directed movement of organelles or molecules along microtubules in dendrites. | BP | Turquoise | dendritic transport | 4.88E-13 | ATP2A2, CACNA1D, CALB2, CHRM3, CHRNA3, CHRNB4, DRD2, GRIN2A, MEF2C, PLCL1, PRKCB, PTN, SLC12A4, STAR, DOC2A, RIMS1, PLCL2, NLGN4X, SLC32A1, CNTN4, STAC3 |
| GO:0099552 | Cell-cell signalling between presynapse and postsynapse, via the release and reception of lipid molecules, that modulates the synaptic transmission properties of the synapse. | BP | Turquoise | trans-synaptic signalling by lipid, modulating synaptic transmission | 6.04E-13 | ATP2A2, CACNA1D, CALB2, CHRM3, CHRNA3, CHRNB4, DRD2, GRIN2A, MEF2C, PLCL1, PRKCB, PTN, SLC12A4, STAR, DOC2A, RIMS1, PLCL2, NLGN4X, SLC32A1, CNTN4, STAC3 |
| GO:0070025 | Interacting selectively and non-covalently with carbon monoxide (CO). | MF | Turquoise | carbon monoxide binding | 7.03E-13 | ARHGAP1, SERPINC1, SERPING1, CACNA1D, CHRNA3, DRD2, EP300, FSHB, GRIN2A, LRP1, MEF2C, NAB2, PDE4B, PLCB2, PLCL1, PPP2R2A, PRKCB, PRKD1, PTN, PTPRF, RANGAP1, RELA, SIPA1, TAOK2, BAG4, RGS6, TBC1D5, ZEB2, RABGAP1L, TAB1, RIMS1, PLCL2, PAK6, RPTOR, RBM26, ANP32E, DNAJC19, STAC3 |
| GO:2000225 | Any process that stops, prevents, or reduces the frequency, rate or | BP | | negative regulation of testosterone biosynthetic | 1.12E-12 | RERE, DRD2, EMX1, EP300, FSHB, IREB2, MEF2C, NAB2, NFATC3, PRKCB, PRKD1, |

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| | extent of testosterone biosynthetic process. | | Turquoise | process | | PSMA4, PSMB10, RELA, SHMT2, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, ZEB2, DMTF1, STAG1, TAB1, RAI1, CNOT1, GIGYF2, SFMBT1, GATAD2A, NSD3, BANK1, ZNF823, PAK6, OTUD7B, THAP11, RPTOR, CPEB1, BCL11B, PCGF6, ZBTB37, BTBD18 |
| GO:1901561 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a benomyl stimulus. | BP | Turquoise | response to benomyl | 1.16E-12 | More than 50 overlapping genes |
| GO:0005886 | The membrane surrounding a cell that separates the cell from its external environment. It consists of a phospholipid bilayer and associated proteins. | CC | Turquoise | plasma membrane | 2.53E-12 | More than 50 overlapping genes |
| GO:0005654 | That part of the nuclear content other than the chromosomes or the nucleolus. | CC | Turquoise | nucleoplasm | 3.26E-12 | RERE, ATP2A2, EP300, MEF2C, MSRA, NFATC3, PPP2R2A, PRKCB, PSMA4, PSMB10, RANGAP1, RELA, ATXN7, SREBF1, SREBF2, SRPK2, CDK2AP1, CUL3, ASH2L, INA, DMTF1, STAG1, TAB1, RAI1, EDC4, PLA2G15, ARL6IP4, SFMBT1, GATAD2A, NSD3, MSL2, THAP11, RPTOR, CPEB1, CENPM, ESRP2, ANP32E, PCGF6, ATPAF2, STAC3 |
| GO:0035556 | The process in which a signal is passed on to downstream components within the cell, which become activated themselves to further propagate the signal and finally trigger a change in the | BP | | intracellular signal transduction | 5.12E-12 | ARHGAP1, ATP2A2, CA8, GSDME, DRD2, EP300, FHIT, GRIN2A, LRP1, MEF2C, NFATC3, PLCB2, PLCL1, PRKCB, PRKD1, PSMA4, PSMB10, RELA, SIPA1, SRPK2, CUL3, TAOK2, BAG4, RGS6, PLCH2, ZEB2, AKT3, TAB1, CNOT1, PLCL2, |

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| | function or state of the cell. | | Turquoise | | | BANK1, PAK6, OTUD7B, RPTOR, PITPNM2, STAC3 |
| GO:0071986 | A eukaryotically conserved protein complex; in humans, it is comprised of LAMTOR1, LAMTOR2, LAMTOR3, LAMTOR4, and LAMTOR5. The complex is anchored to lipid rafts in late endosome membranes via LAMTOR1, constitutes a guanine nucleotide exchange factor (GEF) for the Rag GTPases. | CC | Turquoise | Ragulator complex | 5.36E-12 | More than 50 overlapping genes |
| GO:1901558 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a metformin stimulus. | BP | Turquoise | response to metformin | 6.72E-12 | ALDOA, RERE, DRD2, EMX1, EP300, FSHB, IREB2, MEF2C, NAB2, NFATC3, PDE4B, PRKCB, PRKD1, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, STAR, CUL3, ASH2L, ZNF592, ZNF536, ZEB2, DMTF1, STAG1, TAB1, RAI1, NT5C2, CNOT1, SFMBT1, GATAD2A, NSD3, ZNF823, PAK6, OTUD7B, THAP11, RPTOR, SCAF1, BCL11B, PCGF6, ZBTB37, BTBD18 |
| GO:0043232 | Organised structure of distinctive morphology and function, not bounded by a lipid bilayer membrane and occurring within the cell. Includes ribosomes, the cytoskeleton and chromosomes. | CC | Turquoise | intracellular non-membrane-bounded organelle | 8.10E-12 | ALDOA, CACNA1D, CALB2, EMX1, EP300, FHIT, MEF2C, MSRA, PDE4B, PSMA4, RANGAP1, RELA, ATXN7, SHMT2, SRPK2, ALMS1, MAD1L1, DOC2A, CUL3, HIRIP3, ASH2L, INA, TAOK2, CKAP5, ZEB2, STAG1, SDCCAG8, RIMS1, CNOT1, DDHD2, EDC4, SPATS2L, GIGYF2, PARD6A, ARL6IP4, HYDIN, GATAD2A, NSD3, PAK6, RPTOR, CPEB1, |

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| | | | | | | CENPM, ANP32E, ZNF804A, CARMIL2, HARBI1 |
| GO:0120069 | Any process that increases the frequency, rate or extent of any stomach fundus smooth muscle contraction. | BP | Turquoise | positive regulation of stomach fundus smooth muscle contraction | 2.94E-11 | RERE, CHRNA3, DRD2, EMX1, EP300, GPM6A, LRP1, MEF2C, PRKD1, PTN, PTPRF, ALMS1, TAOK2, BAG4, CKAP5, ZEB2, SDCCAG8, RIMS1, PLEKHO1, HYDIN, BCL11B, SEMA6D, ZNF804A, EMB, CARMIL2, CNTN4 |
| GO:0090317 | Any process that decreases the frequency, rate or extent of the directed movement of proteins within cells. | BP | Turquoise | negative regulation of intracellular protein transport | 3.44E-11 | RERE, DRD2, EMX1, EP300, FSHB, MEF2C, NAB2, NFATC3, PPP2R2A, PRKCB, PRKD1, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, SRPK2, CUL3, ASH2L, ZNF592, ZNF536, ZEB2, DMTF1, STAG1, TAB1, RAI1, CNOT1, ZC3H7B, EDC4, GIGYF2, ARL6IP4, SFMBT1, GATAD2A, NSD3, ZNF823, PAK6, OTUD7B, THAP11, RPTOR, SCAF1, RBM26, CPEB1, BCL11B, ESRP2, PCGF6, ZBTB37, HARBI1, BTBD18 |
| GO:0097707 | A programmed cell death characterized morphologically by the presence of smaller than normal mitochondria with condensed mitochondrial membrane densities, reduction or vanishing of mitochondria crista, and outer mitochondrial membrane rupture. Activation of mitochondrial voltage-dependent anion channels and mitogen-activated protein kinases, | BP | Turquoise | ferroptosis | 4.67E-11 | RERE, DRD2, EMX1, EP300, FSHB, MEF2C, NAB2, NFATC3, PRKCB, PRKD1, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, ZEB2, DMTF1, STAG1, TAB1, RAI1, CNOT1, SFMBT1, GATAD2A, NSD3, ZNF823, PAK6, OTUD7B, THAP11, RPTOR, SCAF1, BCL11B, PCGF6, ZBTB37, BTBD18 |

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| | upregulation of endoplasmic reticulum stress, and inhibition of cystine/glutamate antiporter are involved in the induction of ferroptosis. This process is characterized by the accumulation of lipid peroxidation products and lethal reactive oxygen species (ROS) derived from iron metabolism. Glutathione peroxidase 4 (GPX4), heat shock protein beta-1, and nuclear factor erythroid 2-related factor 2 function as negative regulators of ferroptosis by limiting ROS production and reducing cellular iron uptake, respectively. In contrast, NADPH oxidase and p53 act as positive regulators of ferroptosis by promotion of ROS production and inhibition of expression of SLC7A11 (a specific light-chain subunit of the cystine/glutamate antiporter), respectively. Misregulated ferroptosis has been implicated in multiple physiological and pathological processes. | | | | | |
| GO:0099192 | A synapse formed by a cerebellar Golgi cell synapsing on to a cerebellar granule cell. | CC | Turquoise | cerebellar Golgi cell to granule cell synapse | 4.68E-11 | ATP2A2, CACNA1D, CALB2, CHRNA3, CHRNB4, DRD2, GRIN2A, MEF2C, PLCL1, PRKCB, PTN, STAR, RIMS1, PLCL2, NLGN4X, CNTN4 |
| GO:1903593 | Any process that modulates the frequency, rate or extent of histamine secretion by mast cell. | BP | | regulation of histamine secretion by mast cell | 6.09E-11 | RERE, DRD2, EMX1, EP300, FSHB, MEF2C, NAB2, NFATC3, PRKCB, PRKD1, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, ZEB2, DMTF1, STAG1, TAB1, RAI1, |

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| | | | Turquoise | | | CNOT1, SFMBT1, GATAD2A, NSD3, ZNF823, PAK6, OTUD7B, THAP11, RPTOR, BCL11B, PCGF6, ZBTB37, BTBD18 |
| GO:2001234 | Any process that stops, prevents, or reduces the frequency, rate or extent of apoptotic signalling pathway. | BP | Turquoise | negative regulation of apoptotic signalling pathway | 6.32E-11 | RERE, DRD2, EMX1, EP300, FSHB, MEF2C, NAB2, NFATC3, PRKCB, PRKD1, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, ZEB2, DMTF1, STAG1, TAB1, RAI1, CNOT1, SFMBT1, GATAD2A, NSD3, ZNF823, PAK6, OTUD7B, THAP11, RPTOR, BCL11B, PCGF6, ZBTB37, BTBD18 |
| GO:0030054 | A cellular component that forms a specialized region of connection between two or more cells or between a cell and the extracellular matrix. At a cell junction, anchoring proteins extend through the plasma membrane to link cytoskeletal proteins in one cell to cytoskeletal proteins in neighbouring cells or to proteins in the extracellular matrix. | CC | Turquoise | cell junction | 8.62E-11 | ATP2A2, CACNA1D, CALB2, CHRM3, CHRNA3, CHRNB4, DRD2, GPM6A, GRIN2A, LRP1, MEF2C, PDE4B, PPP2R2A, PRKCB, PTN, RELA, DOC2A, INA, SDCCAG8, RIMS1, PARD6A, PAK6, NLGN4X, CPEB1, ESAM, ZNF804A, EMB, SLC32A1 |
| GO:0033554 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating the organism is | BP | | cellular response to stress | 1.64E-10 | ATP2A2, EP300, LRP1, MEF2C, MGAT3, MSRA, PRKD1, PSMA4, PSMB10, PTN, PTPRF, RELA, SIPA1, SREBF1, SREBF2, CUL3, ASH2L, TAOK2, BAG4, ZEB2, AKT3, TAB1, CNOT1, GIGYF2, JKAMP, VPS13C, PAK6, RPTOR, CPEB1 |

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| | under stress. The stress is usually, but not necessarily, exogenous (e.g. temperature, humidity, ionizing radiation). | | Turquoise | | | |
| GO:0006357 | Any process that modulates the frequency, rate or extent of transcription mediated by RNA polymerase II. | BP | Turquoise | regulation of transcription by RNA polymerase II | 2.45E-10 | RERE, DRD2, EMX1, EP300, FSHB, MEF2C, NFATC3, PRKCB, PRKD1, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, ZEB2, DMTF1, STAG1, RAI1, CNOT1, GATAD2A, ZNF823, OTUD7B, THAP11, BCL11B, PCGF6, ZBTB37, BTBD18 |
| GO:0051172 | Any process that stops, prevents, or reduces the frequency, rate or extent of the chemical reactions and pathways involving nitrogen or nitrogenous compounds. | BP | Turquoise | negative regulation of nitrogen compound metabolic process | 3.20E-10 | SERPINC1, RERE, SERPING1, DRD2, EP300, FHIT, GRIN2A, IREB2, MEF2C, MGAT3, NAB2, NFATC3, PTN, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF536, ZEB2, CNOT1, GIGYF2, PARD6A, SFMBT1, GATAD2A, BANK1, OTUD7B, THAP11, RPTOR, CPEB1, PCGF6 |
| GO:0030425 | A neuron projection that has a short, tapering, morphology. Dendrites receive and integrate signals from other neurons or from sensory stimuli, and conduct nerve impulses towards the axon or the cell body. In most neurons, the impulse is conveyed from dendrites to axon via the cell body, but in some types of unipolar neuron, the impulse does not travel via the cell body. | CC | Turquoise | dendrite | 3.31E-10 | CALB2, CHRM3, CHRNA3, DRD2, GPM6A, GRIN2A, LRP1, PDE4B, RANGAP1, TAOK2, GIGYF2, NLGN4X, RPTOR, CPEB1, ZNF804A, SLC32A1, HCN1 |
| GO:0097473 | Any apoptotic process in a retinal | BP | | retinal rod cell apoptotic | 3.48E-10 | CALB2, CHRM3, CHRNA3, DRD2, GPM6A, |

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| | rod cell, one of the two photoreceptor cell types of the vertebrate retina. | | Turquoise | process | | GRIN2A, LRP1, PDE4B, RANGAP1, TAOK2, GIGYF2, NLGN4X, RPTOR, CPEB1, ZNF804A, SLC32A1, HCN1 |
| GO:0071516 | The initial formation of a stable single-strand DNA lesion that triggers programmed gene conversion at the mating-type locus, thereby restricting mating-type interconversion to one of the two sister chromatids during DNA replication. | BP | Turquoise | establishment of imprinting at mating-type locus | 6.63E-10 | CHRM3, CHRNA3, DRD2, EP300, FSHB, LRP1, MEF2C, PDE4B, PRKCB, PTN, RANGAP1, RELA, SHMT2, SOX5, SREBF1, STAR, BAG4, TAB1, CNOT1, PARD6A, RPTOR, CPEB1, ESRP2, HCN1 |
| GO:0019899 | Interacting selectively and non-covalently with any enzyme. | MF | Turquoise | enzyme binding | 8.13E-10 | ARHGAP1, SERPINC1, ATP2A2, FHIT, LRP1, MEF2C, PPP2R2A, PRKCB, PTN, RANGAP1, RELA, SLC12A4, SREBF1, CDK2AP1, CUL3, TAOK2, BAG4, TBC1D5, RABGAP1L, TAB1, RIMS1, PARD6A, JKAMP, BANK1, PAK6, RPTOR, PITPNM2, SCAF1, TOM1L2 |
| GO:0070901 | The posttranscriptional addition of methyl groups to specific residues in a mitochondrial tRNA molecule. | BP | Turquoise | mitochondrial tRNA methylation | 1.75E-09 | ATP2A2, CHRM3, CHRNA3, GSDME, DRD2, EP300, FSHB, LRP1, MEF2C, MGAT3, MSRA, PDE4B, PRKCB, PRKD1, PSMA4, PSMB10, PTN, RANGAP1, RELA, SHMT2, SIPA1, SOX5, SREBF1, STAR, CUL3, INA, BAG4, TAB1, CNOT1, PARD6A, RPTOR, CPEB1, ESRP2, CPNE8, HCN1 |
| GO:2000134 | Any cell cycle regulatory process that prevents the commitment of a cell from G1 to S phase of the mitotic cell cycle. | BP | | negative regulation of G1/S transition of mitotic cell cycle | 2.39E-09 | CHRNA3, DRD2, EMX1, EP300, FSHB, LRP1, MEF2C, NFATC3, PRKCB, PRKD1, PSMA4, PSMB10, PTN, PTPRF, RELA, SOX5, STAR, ASH2L, ZNF536, ZEB2, AKT3, RIMS1, PARD6A, SFMBT1, BCL11B, SEMA6D, ZNF804A, CNTN4 |

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| | | | Turquoise | | | |
| GO:0016477 | The controlled self-propelled movement of a cell from one site to a destination guided by molecular cues. Cell migration is a central process in the development and maintenance of multicellular organisms. | BP | Turquoise | cell migration | 4.23E-09 | RERE, DRD2, FSHB, GPM6A, LRP1, MEF2C, MGAT3, PDE4B, PRKD1, PTN, PTPRF, CUL3, SLC7A6, TAOK2, BAG4, ZEB2, AKT3, SDCCAG8, PLEKHO1, PAK6, BCL11B, SEMA6D, ESAM, CARMIL2 |
| GO:0016021 | The component of a membrane consisting of the gene products and protein complexes having at least some part of their peptide sequence embedded in the hydrophobic region of the membrane. | CC | Turquoise | integral component of membrane | 4.24E-09 | ATP2A2, CACNA1D, CHRM3, CHRNA3, CHRNB4, DRD2, GPM6A, GRIN2A, LRP1, MGAT3, PDE4B, PTPRF, SLC12A4, SREBF1, SREBF2, CACNA1I, SLC7A6, GPR52, TAOK2, ABCB9, VSIG2, CA14, GIGYF2, SEZ6L2, SLC45A1, TMX2, AIG1, JKAMP, TM6SF2, CNNM2, NLGN4X, SLC39A8, CSMD1, GPR135, SEMA6D, TLCD3B, ESAM, SFXN5, DNAJC19, EMB, SLC32A1, KIAA1324L, STAC3, HCN1 |
| GO:0050767 | Any process that modulates the frequency, rate or extent of neurogenesis, the generation of cells in the nervous system. | BP | Turquoise | regulation of neurogenesis | 5.08E-09 | CHRNA3, DRD2, EMX1, EP300, LRP1, MEF2C, PRKD1, PTN, PTPRF, RELA, STAR, ZNF536, ZEB2, RIMS1, BCL11B, SEMA6D, ZNF804A, CNTN4 |
| GO:0071727 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a triacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to triacyl bacterial lipopeptide | 7.84E-09 | ARHGAP1, CACNA1D, CHRNA3, DRD2, LRP1, PRKCB, RANGAP1, SREBF1, SREBF2, AP3B2, SLC7A6, TAOK2, BAG4, TBC1D5, CKAP5, RABGAP1L, RIMS1, DOP1A, ABCB9, PARD6A, VPS13C, BANK1, RBM26, MAIP1, SFXN5, DNAJC19, SLC32A1, TOM1L2, SNX19 |
| GO:0031175 | The process whose specific outcome is the progression of a | BP | | neuron projection development | 9.95E-09 | RERE, CHRNA3, DRD2, EMX1, EP300, GPM6A, LRP1, MEF2C, PRKD1, PTN, |

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| | neuron projection over time, from its formation to the mature structure. A neuron projection is any process extending from a neural cell, such as axons or dendrites (collectively called neurites). | | Turquoise | | | PTPRF, TAOK2, ZEB2, RIMS1, BCL11B, SEMA6D, ZNF804A, EMB, CNTN4 |
| GO:1901890 | Any process that activates or increases the frequency, rate or extent of cell junction assembly. | BP | Turquoise | positive regulation of cell junction assembly | 1.58E-08 | CHRM3, CHRNA3, DRD2, GRIN2A, LRP1, MEF2C, PDE4B, PPP2R2A, PRKCB, PTN, RANGAP1, RELA, SHMT2, SREBF1, SREBF2, STAR, JKAMP, RPTOR, CPEB1, HCN1 |
| GO:0051254 | Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways involving RNA. | BP | Turquoise | positive regulation of RNA metabolic process | 1.58E-08 | RERE, DRD2, EP300, FSHB, MEF2C, NFATC3, PRKCB, PRKD1, RELA, SOX5, SREBF1, SREBF2, ASH2L, ZEB2, DMTF1, STAG1, RAI1, CNOT1, GIGYF2, NSD3, RPTOR, CPEB1, BCL11B, BTBD18 |
| GO:0101020 | Catalysis of the reaction: oestrogen + donor-H2 + O2 = 16-alpha-hydroxyestrogen + H2O. | MF | Turquoise | oestrogen 16-alpha-hydroxylase activity | 1.71E-08 | ATP2A2, CACNA1D, CHRNA3, CHRNB4, DRD2, MEF2C, PRKCB, DOC2A, RIMS1, SLC32A1 |
| GO:0070852 | A neuron projection that is found in unipolar neurons and corresponds to the region between the cell body and the point at which the single projection branches. | CC | Turquoise | cell body fibre | 2.40E-08 | ATP2A2, CACNA1D, DRD2, GPM6A, GRIN2A, PDE4B, PLCB2, PRKCB, CACNA1I, PLCH2, CNNM2, SLC39A8, MAIP1, STAC3 |
| GO:0098631 | The binding by a cell-adhesion protein on the cell surface to an extracellular matrix component, to mediate adhesion of the cell to the external substrate or to another cell. | MF | | cell adhesion mediator activity | 2.43E-08 | ARHGAP1, ATP2A2, CHRNB4, DRD2, LRP1, MGAT3, PRKD1, SLC12A4, SREBF1, SREBF2, AP3B2, DOC2A, CUL3, TBC1D5, TAB1, DOP1A, ABCB9, MOB4, TM6SF2, VPS13C, RPTOR, GPR135, TLCD3B, BORCS7, SLC32A1, SNX19 |

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| | | | Turquoise | | | |
| GO:0071329 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a sucrose stimulus. | BP | Turquoise | cellular response to sucrose stimulus | 2.52E-08 | CHRM3, CHRNA3, GSDME, DRD2, EP300, FSHB, LRP1, MEF2C, PDE4B, PRKCB, PRKD1, PSMA4, PSMB10, PTN, RANGAP1, RELA, SHMT2, SOX5, SREBF1, STAR, CUL3, INA, BAG4, TAB1, CNOT1, PARD6A, RPTOR, CPEB1, ESRP2, HCN1 |
| GO:0072528 | The chemical reactions and pathways resulting in the formation of a pyrimidine-containing compound, i.e. any compound that contains pyrimidine or a formal derivative thereof. | BP | Turquoise | pyrimidine-containing compound biosynthetic process | 2.86E-08 | ATP2A2, CACNA1D, DRD2, GPM6A, GRIN2A, PDE4B, PLCB2, PRKCB, CACNA1I, PLCH2, CNNM2, SLC39A8, MAIP1, STAC3 |
| GO:0044248 | The chemical reactions and pathways resulting in the breakdown of substances, carried out by individual cells. | BP | Turquoise | cellular catabolic process | 2.95E-08 | DPYD, DRD2, EP300, FHIT, LRP1, MGAT3, PDE4B, PPP2R2A, PRKD1, PSMA4, PSMB10, SHMT2, SREBF1, SREBF2, CUL3, TBC1D5, NT5C2, CNOT1, DDHD2, EDC4, PLA2G15, GIGYF2, AIG1, JKAMP, VPS13C, OTUD7B, RPTOR |
| GO:0098808 | Interacting selectively and non-covalently with a 7-methylguanosine (m7G) group or derivative located at the 5' end of an mRNA molecule. | MF | Turquoise | mRNA cap binding | 3.23E-08 | CACNA1D, CALB2, CHRM3, DRD2, GPM6A, GRIN2A, PDE4B, PRKCB, PTN, DOC2A, RIMS1, NLGN4X, ZNF804A, SLC32A1 |
| GO:0006468 | The process of introducing a phosphate group on to a protein. | BP | | protein phosphorylation | 5.64E-08 | CHRNA3, GSDME, DRD2, LRP1, MEF2C, NAB2, PLCL1, PRKCB, PRKD1, PSMA4, PSMB10, SRPK2, CDK2AP1, CUL3, TAOK2, BAG4, ZEB2, AKT3, TAB1, PLCL2, PARD6A, BANK1, PAK6, RPTOR, ALPK3 |

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| | | | Turquoise | | | |
| GO:0007420 | The process whose specific outcome is the progression of the brain over time, from its formation to the mature structure. Brain development begins with patterning events in the neural tube and ends with the mature structure that is the centre of thought and emotion. The brain is responsible for the coordination and control of bodily activities and the interpretation of information from the senses (sight, hearing, smell, etc.). | BP | Turquoise | brain development | 6.79E-08 | RERE, DRD2, EMX1, GRIN2A, LRP1, PTN, STAR, INA, ZEB2, AKT3, HYDIN, NLGN4X, BCL11B, SEMA6D, SLC32A1, CNTN4 |
| GO:1901678 | The directed movement of an iron coordination entity into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. | BP | Turquoise | iron coordination entity transport | 6.97E-08 | ALDOA, CTRL, DPYD, FHIT, GRIN2A, LRP1, MGAT3, PDE4B, PLCB2, PPP2R2A, PSMA4, PSMB10, RELA, SHMT2, CUL3, PLCH2, NT5C2, CNOT1, DDHD2, EDC4, PLA2G15, GIGYF2, AIG1, JKAMP, OTUD7B, ASPG |
| GO:0031399 | Any process that modulates the frequency, rate or extent of the covalent alteration of one or more amino acid residues within a protein. | BP | Turquoise | regulation of protein modification process | 8.87E-08 | CHRNA3, GSDME, DRD2, EP300, LRP1, NAB2, PLCL1, PPP2R2A, PRKD1, PTN, RELA, SREBF1, CDK2AP1, CUL3, TAOK2, BAG4, ZEB2, TAB1, PLCL2, PARD6A, NSD3, BANK1, PAK6, RPTOR |
| GO:0140244 | Any process that regulates translation occurring at the presynapse. | BP | Turquoise | regulation of translation at presynapse | 1.07E-07 | RERE, EMX1, EP300, MEF2C, NAB2, NFATC3, PRKCB, RELA, SOX5, SREBF1, SREBF2, ZNF592, ZNF536, ZEB2, DMTF1, STAG1, RAI1, SFMBT1, GATAD2A, NSD3, ZNF823, THAP11, BCL11B, PCGF6, ZBTB37 |
| GO:0010558 | Any process that decreases the | BP | | negative regulation of | 1.21E-07 | RERE, EP300, IREB2, MEF2C, NAB2, |

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| | rate, frequency or extent of the chemical reactions and pathways resulting in the formation of a macromolecule, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass. | | Turquoise | macromolecule biosynthetic process | | NFATC3, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF536, ZEB2, CNOT1, GIGYF2, SFMBT1, GATAD2A, BANK1, OTUD7B, THAP11, CPEB1, PCGF6 |
| GO:0043168 | Interacting selectively and non-covalently with anions, charged atoms or groups of atoms with a net negative charge. | MF | Turquoise | anion binding | 1.21E-07 | SERPINC1, ATP2A2, GSDME, DPYD, LRP1, PDE4B, PLCL1, PRKCB, PRKD1, PTN, PTPRF, RELA, SHMT2, SRPK2, DOC2A, TAOK2, AKT3, PLCL2, ABCB9, PLA2G15, CNNM2, PAK6, NLGN4X, ALPK3, PITPNM2, CPNE8, CARMIL2, HCN1, SNX19 |
| GO:0055065 | Any process involved in the maintenance of an internal steady state of metal ions within an organism or cell. | BP | Turquoise | metal ion homeostasis | 1.27E-07 | ATP2A2, CACNA1D, CALB2, DRD2, GRIN2A, IREB2, LRP1, NMB, PLCB2, PRKCB, SLC12A4, PLCH2, CNNM2, SLC39A8, MAIP1 |
| GO:0004620 | Catalysis of the hydrolysis of a glycerophospholipid. | MF | Turquoise | phospholipase activity | 1.37E-07 | CHRM3, PLCB2, PLCL1, PLCH2, PLCL2, DDHD2, PLA2G15, ASPG |
| GO:0010647 | Any process that increases the frequency, rate or extent of cell communication. Cell communication is the process that mediates interactions between a cell and its surroundings. Encompasses interactions such as signalling or attachment between one cell and another cell, between a cell and an extracellular matrix, or between a cell and any other | BP | | positive regulation of cell communication | 1.41E-07 | ARHGAP1, CACNA1D, CALB2, CHRNB4, GSDME, DRD2, EP300, GRIN2A, LRP1, NMB, PRKCB, PRKD1, PSMA4, PSMB10, PTN, RELA, TAOK2, BAG4, ZEB2, AKT3, TAB1, RIMS1, BANK1, RPTOR |

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| | aspect of its environment. | | Turquoise | | | |
| GO:0120071 | Any process that modulates the frequency, rate or extent of any pyloric antrum smooth muscle contraction. | BP | Turquoise | regulation of pyloric antrum smooth muscle contraction | 1.44E-07 | RERE, CHRNA3, DRD2, EMX1, GPM6A, LRP1, PTN, TAOK2, ZEB2, RIMS1, PLEKHO1, BCL11B, SEMA6D, EMB, CNTN4 |
| GO:0023056 | Any process that activates, maintains or increases the frequency, rate or extent of a signalling process. | BP | Turquoise | positive regulation of signalling | 1.51E-07 | ARHGAP1, CACNA1D, CALB2, CHRNB4, GSDME, DRD2, EP300, GRIN2A, LRP1, NMB, PRKCB, PRKD1, PSMA4, PSMB10, PTN, RELA, TAOK2, BAG4, ZEB2, AKT3, TAB1, RIMS1, BANK1, RPTOR |
| GO:0048858 | The process in which the anatomical structures of a cell projection are generated and organized. | BP | Turquoise | cell projection morphogenesis | 1.55E-07 | RERE, CHRNA3, DRD2, EMX1, GPM6A, LRP1, PTN, TAOK2, ZEB2, RIMS1, PLEKHO1, BCL11B, SEMA6D, EMB, CNTN4 |
| GO:0046928 | Any process that modulates the frequency, rate or extent of the regulated release of a neurotransmitter from a cell. | BP | Turquoise | regulation of neurotransmitter secretion | 1.56E-07 | ATP2A2, CACNA1D, CHRNA3, CHRNB4, DRD2, MEF2C, PRKCB, RIMS1 |
| GO:0010628 | Any process that increases the frequency, rate or extent of gene expression. Gene expression is the process in which a gene's coding sequence is converted into a mature gene product or products (proteins or RNA). This includes the production of an RNA transcript as well as any processing to produce a mature RNA product or an mRNA or circRNA (for protein-coding genes) and the translation of that mRNA or circRNA into protein. Protein maturation is included | BP | | positive regulation of gene expression | 1.73E-07 | DRD2, EP300, FSHB, LRP1, MEF2C, NFATC3, PDE4B, PRKD1, RELA, SOX5, SREBF1, SREBF2, SRPK2, STAR, ASH2L, ZEB2, DMTF1, STAG1, RAI1, RIMS1, NSD3, RPTOR, CPEB1, BCL11B, ZNF804A, BTBD18 |

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| | when required to form an active form of a product from an inactive precursor form. | | Turquoise | | | |
| GO:2000226 | Any process that modulates the frequency, rate or extent of pancreatic A cell differentiation. | BP | Turquoise | regulation of pancreatic A cell differentiation | 2.32E-07 | EP300, IREB2, MEF2C, NAB2, NFATC3, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF536, ZEB2, CNOT1, GIGYF2, SFMBT1, GATAD2A, BANK1, OTUD7B, THAP11, CPEB1, PCGF6 |
| GO:0097733 | A specialised 9+0 non-motile cilium found in photoreceptor cells. A ciliary transition zone called 'photoreceptor connecting cilium' links the photoreceptor outer segment to the inner segment. | CC | Turquoise | photoreceptor cell cilium | 2.32E-07 | ALDOA, ARHGAP1, SERPING1, CHRNB4, DRD2, GRIN2A, LRP1, PDE4B, SIPA1, SREBF1, SREBF2, AP3B2, DOC2A, TAOK2, TBC1D5, RABGAP1L, TAB1, DOP1A, DDHD2, GIGYF2, GPR135, ANP32E, SLC32A1, CARMIL2, EHBP1L1, SNX19 |
| GO:0007166 | A series of molecular signals initiated by activation of a receptor on the surface of a cell. The pathway begins with binding of an extracellular ligand to a cell surface receptor, or for receptors that signal in the absence of a ligand, by ligand-withdrawal or the activity of a constitutively active receptor. The pathway ends with regulation of a downstream cellular process, e.g. transcription. | BP | Turquoise | cell surface receptor signalling pathway | 2.76E-07 | CHRNA3, CHRNB4, DRD2, EP300, FSHB, GRIN2A, LRP1, MEF2C, NFATC3, PDE4B, PLCB2, PRKCB, PRKD1, PSMA4, PSMB10, PTN, PTPRF, RELA, SREBF1, CUL3, BAG4, ZEB2, TAB1, RIMS1, PLCL2, GIGYF2, PARD6A, NLGN4X, ESRP2, SEMA6D |
| GO:1901894 | Any process that modulates the frequency, rate or extent of an ATPase-coupled calcium transmembrane transporter activity | BP | Turquoise | regulation of calcium-transporting ATPase activity | 3.17E-07 | CHRM3, CHRNA3, DRD2, LRP1, MEF2C, PDE4B, PRKCB, RANGAP1, RELA, SHMT2, SREBF1, STAR, RPTOR, CPEB1, HCN1 |

**Table 6.12: Gene Ontologies for Blue Module Stage Three using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Blue | positive regulation of macrophage proliferation | 2.37E-12 | BNIP3L, CLU, GRM3, HSPD1, HSPE1, MAP3K11, MMP16, NAGA, PCCB, PRKCB, STAR, STAT6, TBX6, FXR1, DGKI, GABBR2, VPS45, SATB2, FOXP1, TM6SF2, RBFOX1, ZSCAN2, TSR1, NDUFA4L2, PAK6, RALGAPA2, BCL11B, NDRG4, ZFYVE21, GDPD3, DRC3, YPEL3, C16orf92, MED19 |
| GO:0043231 | Organized structure of distinctive morphology and function, bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Blue | intracellular membrane-bounded organelle | 1.70E-10 | BNIP3L, CLU, HSPD1, HSPE1, MMP16, NAGA, PCCB, PRKCB, STAR, STAT6, TBX6, FXR1, DGKI, VPS45, SATB2, FOXP1, TM6SF2, RBFOX1, ZSCAN2, TSR1, NDUFA4L2, PAK6, RALGAPA2, BCL11B, NDRG4, YPEL3, MED19 |
| GO:0044238 | The chemical reactions and pathways involving those compounds which are formed as a part of the normal anabolic and catabolic processes. These processes take place in most, if not all, cells of the organism. | BP | Blue | primary metabolic process | 1.03E-09 | BNIP3L, CLU, HSPD1, HSPE1, MAP3K11, MMP16, NAGA, PCCB, PPP2R3A, PRKCB, STAR, STAT6, TBX6, FXR1, DGKI, SATB2, FOXP1, TM6SF2, RBFOX1, ZSCAN2, TSR1, PAK6, BCL11B, NDRG4, GDPD3, MED19 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Blue | intracellular organelle lumen | 3.05E-09 | BNIP3L, CLU, GRM3, HSPD1, HSPE1, MAP3K11, MMP16, PPP2R3A, PRKCB, STAR, STAT6, TBX6, FXR1, DGKI, GABBR2, VPS45, SATB2, FOXP1, TM6SF2, RBFOX1, ZSCAN2, PAK6, RALGAPA2, BCL11B, NDRG4, YPEL3, MED19 |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Blue | cellular response to diacyl bacterial lipopeptide | 6.83E-09 | BNIP3L, CLU, HSPD1, HSPE1, MAP3K11, MMP16, NAGA, PCCB, PPP2R3A, PRKCB, STAR, STAT6, TBX6, FXR1, DGKI, SATB2, FOXP1, TM6SF2, RBFOX1, ZSCAN2, TSR1, PAK6, BCL11B, NDRG4, GDPD3, MED19 |

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| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Blue | cytoplasm | 1.69E-08 | BNIP3L, CLU, HSPD1, HSPE1, MAP3K11, MMP16, NAGA, PCCB, PRKCB, STAR, STAT6, FXR1, DGKI, GABBR2, VPS45, TM6SF2, RBFOX1, TSR1, NDUFA4L2, PAK6, RALGAPA2, NDRG4, ZFYVE21, GDPD3, DRC3 |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Blue | protein binding | 4.22E-08 | BNIP3L, CLU, HSPD1, HSPE1, MAP3K11, NAGA, PCCB, PPP2R3A, PRKCB, STAR, STAT6, TBX6, FXR1, DGKI, GABBR2, VPS45, SATB2, FOXP1, RBFOX1, PAK6, RALGAPA2, BCL11B, NDRG4, ZFYVE21, MED19 |
| GO:0080154 | Any process that modulates the rate, frequency or extent of fertilization. Fertilization is the union of gametes of opposite sexes during the process of sexual reproduction to form a zygote. It involves the fusion of the gametic nuclei (karyogamy) and cytoplasm (plasmogamy). | BP | Blue | regulation of fertilization | 6.19E-08 | CLU, HSPD1, HSPE1, MAP3K11, PPP2R3A, PRKCB, STAR, STAT6, TBX6, FXR1, SATB2, FOXP1, TM6SF2, RBFOX1, ZSCAN2, PAK6, BCL11B, NDRG4, MED19 |
| GO:0031325 | Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways by which individual cells transform chemical substances. | BP | Blue | positive regulation of cellular metabolic process | 8.68E-08 | BNIP3L, CLU, HSPD1, HSPE1, MAP3K11, PRKCB, STAR, STAT6, TBX6, FXR1, SATB2, PAK6, BCL11B, NDRG4, MED19 |
| GO:0070052 | Interacting selectively and non-covalently with a type V collagen trimer. | MF | Blue | collagen V binding | 2.27E-07 | BNIP3L, CLU, HSPD1, HSPE1, MMP16, PCCB, PRKCB, STAR, STAT6, TBX6, FXR1, DGKI, SATB2, FOXP1, TSR1, PAK6, YPEL3, MED19 |
| GO:0006807 | The chemical reactions and pathways involving organic or inorganic compounds that contain nitrogen. | BP | Blue | nitrogen compound metabolic process | 2.46E-07 | BNIP3L, CLU, HSPD1, HSPE1, MAP3K11, MMP16, NAGA, PCCB, PPP2R3A, PRKCB, STAT6, TBX6, FXR1, SATB2, FOXP1, RBFOX1, ZSCAN2, TSR1, PAK6, BCL11B, NDRG4, GDPD3, MED19 |

**Table 6.13: Gene Ontologies for Brown Module Stage Three using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 4.06E-08 | CHRNA2, CHRNA5, ETF1, PTK2B, TCF4, ATP5MPL, PSMD6, KCNK7, IGSF9B, SMG6, MAU2, NGEF, AMBRA1, AS3MT, BOLL, ZNF408, SETD6, THOC7, TMEM219, ASPHD1 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Brown | intracellular organelle lumen | 8.00E-06 | CHRNA2, CHRNA5, ETF1, PTK2B, TCF4, PSMD6, KCNK7, IGSF9B, SMG6, MAU2, NGEF, AMBRA1, BOLL, ZNF408, SETD6, TMEM219 |
| GO:0099582 | Any neurotransmitter receptor activity that is involved in regulating the concentration of calcium in the presynaptic cytosol. | MF | Brown | neurotransmitter receptor activity involved in regulation of presynaptic cytosolic calcium ion concentration | 1.25E-05 | CHRNA2, CHRNA5, PTK2B, IGSF9B |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Brown | protein binding | 2.88E-05 | CHRNA5, ETF1, PTK2B, TCF4, PSMD6, IGSF9B, SMG6, MAU2, NGEF, AMBRA1, BOLL, ZNF408, SETD6, THOC7, TMEM219 |
| GO:0060078 | Any process that modulates the potential difference across a post-synaptic membrane. | BP | Brown | regulation of postsynaptic membrane potential | 3.17E-05 | CHRNA2, CHRNA5, PTK2B, IGSF9B |

**Table 6.14: Gene Ontology for Turquoise Stage Three using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Turquoise | positive regulation of macrophage proliferation | 5.64E-77 | More than 50 overlapping genes |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl)benzene-1,2-diol] and derivatives formed by substitution. | BP | Turquoise | cellular response to catecholamine stimulus | 4.05E-29 | More than 50 overlapping genes |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to diacyl bacterial lipopeptide | 1.01E-23 | More than 50 overlapping genes |
| GO:0070016 | Interacting selectively and non-covalently with the armadillo repeat domain of a protein, an approximately 40 amino acid long tandemly repeated sequence motif first identified in the Drosophila segment polarity protein armadillo. Arm-repeat proteins are involved in various processes, including intracellular signalling and cytoskeletal regulation. | MF | Turquoise | armadillo repeat domain binding | 2.25E-18 | More than 50 overlapping genes |
| GO:0005829 | The part of the cytoplasm that does not contain organelles, but which does contain other particulate matter, such as protein complexes. | CC | Turquoise | cytosol | 7.69E-18 | More than 50 overlapping genes |

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| GO:0070052 | Interacting selectively and non-covalently with a type V collagen trimer. | MF | Turquoise | collagen V binding | 1.91E-15 | More than 50 overlapping genes |
| GO:0033554 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating the organism is under stress. The stress is usually, but not necessarily, exogenous (e.g. temperature, humidity, ionizing radiation). | BP | Turquoise | cellular response to stress | 4.35E-14 | ATP2A2, EP300, LRP1, MEF2C, MSRA, NEK1, MAPK3, PSMA4, PSMB10, PTN, PTPRF, RELA, SIPA1, SREBF1, SREBF2, XRCC3, CUL3, ASH2L, TAOK2, BAG4, ATG13, AKT3, TAB1, CNOT1, B3GAT1, VPS13C, FANCL, RPTOR, CPEB1, ACD, ACTR5, IMMP2L, CREB3L1, INO80E |
| GO:0043232 | Organised structure of distinctive morphology and function, not bounded by a lipid bilayer membrane and occurring within the cell. Includes ribosomes, the cytoskeleton and chromosomes. | CC | Turquoise | intracellular non-membrane-bounded organelle | 5.38E-14 | CACNA1C, EMX1, EP300, FGFR1, MEF2C, MSRA, NEK1, MAPK3, PSMA4, RELA, SHMT2, SRPK2, XRCC3, ALMS1, MAD1L1, DOC2A, CUL3, HIRIP3, ASH2L, INA, TAOK2, MPHOSPH9, STAG1, SDCCAG8, RIMS1, CNOT1, DDHD2, SPATS2L, LSM1, ARL6IP4, HYDIN, GATAD2A, NSD3, DDX28, RPTOR, CPEB1, ACD, CENPM, ACTR5, CREB3L1, C12orf65, RPS19BP1, ZNF804A, TDRD9, CARMIL2, WBP2NL, RILPL2, YPEL4, HARBI1, INO80E |
| GO:0097178 | The aggregation, arrangement and bonding together of a set of components to form a ruffle, a projection at the leading edge of a crawling cell; the protrusions are supported by a microfilament meshwork. The formation of ruffles (also called membrane ruffling) is thought to be controlled by a group of enzymes known as Rho GTPases, specifically RhoA, Rac1 and cdc42. | BP | Turquoise | ruffle assembly | 7.53E-14 | More than 50 overlapping genes |
| GO:1901666 | Any process that activates or increases the frequency, rate or extent of NAD+ | BP | Turquoise | positive regulation of NAD+ ADP-ribosyltransferase | 1.35E-13 | More than 50 overlapping genes |

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| | ADP-ribosyltransferase activity. | | | activity | | |
| GO:1901679 | The directed movement of nucleotide across a membrane. | BP | Turquoise | nucleotide transmembrane transport | 1.45E-13 | More than 50 overlapping genes |
| GO:1901561 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a benomyl stimulus. | BP | Turquoise | response to benomyl | 1.71E-13 | More than 50 overlapping genes |
| GO:1901555 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a paclitaxel stimulus. | BP | Turquoise | response to paclitaxel | 1.71E-13 | More than 50 overlapping genes |
| GO:0005654 | That part of the nuclear content other than the chromosomes or the nucleolus. | CC | Turquoise | nucleoplasm | 2.85E-13 | RERE, ATP2A2, EP300, MEF2C, MSRA, NFATC3, PPP2R2A, MAPK3, PSMA4, PSMB10, RELA, SREBF1, SREBF2, SRPK2, XRCC3, CUL3, ASH2L, INA, DMTF1, NUTF2, STAG1, TAB1, RAI1, CLP1, PPP1R13B, PLA2G15, ARL6IP4, GATAD2A, NSD3, FANCL, MSL2, THAP11, RPTOR, SUGP1, CPEB1, ACD, CENPM, ACTR5, RPS19BP1, ATPAF2, STAC3, INO80E |
| GO:0080154 | Any process that modulates the rate, frequency or extent of fertilization. Fertilization is the union of gametes of opposite sexes during the process of sexual reproduction to form a zygote. It involves the fusion of the gametic nuclei (karyogamy) and cytoplasm (plasmogamy). | BP | Turquoise | regulation of fertilization | 1.39E-12 | More than 50 overlapping genes |
| GO:0010604 | Any process that increases the frequency, rate or extent of the chemical reactions and pathways involving macromolecules, any | BP | Turquoise | positive regulation of macromolecule metabolic process | 1.59E-12 | RERE, GSDME, DRD2, EP300, F2, FGFR1, FSHB, GRIN2A, LRP1, MEF2C, NAB2, NEK1, NFATC3, MAPK3, RELA, SOX5, SREBF1, SREBF2, SRPK2, CUL3, ASH2L, |

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| | molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass. | | | | | TAOK2, BAG4, ATG13, TBC1D5, DMTF1, STAG1, TAB1, RAI1, RIMS1, CNOT1, NDFIP2, NSD3, RPTOR, CPEB1, ACD, CREB3L1, ZNF804A, TNFRSF13C, WBP2NL, BTBD18 |
| GO:0003824 | Catalysis of a biochemical reaction at physiological temperatures. In biologically catalysed reactions, the reactants are known as substrates, and the catalysts are naturally occurring macromolecular substances known as enzymes. Enzymes possess specific binding sites for substrates, and are usually composed wholly or largely of protein, but RNA that has catalytic activity (ribozyme) is often also regarded as enzymatic. | MF | Turquoise | catalytic activity | 1.69E-12 | More than 50 overlapping genes |
| GO:0120060 | Any process that modulates the frequency, rate or extent of any gastric emptying process, the process in which the liquid and liquid-suspended solid contents of the stomach exit through the pylorus into the duodenum. | BP | Turquoise | regulation of gastric emptying | 2.44E-12 | ATP2A2, CACNA1C, CHRNB4, DRD2, GPM6A, GRIN2A, LRP1, NMB, NRGN, MAPK3, PTPRF, ALMS1, AP3B2, DOC2A, CUL3, TAOK2, SDCCAG8, PSD3, ZDHHC5, LSM1, PLEKHO1, HYDIN, RPTOR, CPEB1, ZNF804A, EMB, SLC32A1, CARMIL2, CNTN4, WBP2NL, RILPL2, HCN1 |
| GO:0048513 | Development of a tissue or tissues that work together to perform a specific function or functions. Development pertains to the process whose specific outcome is the progression of a structure over time, from its formation to the mature structure. Organs are commonly observed as visibly distinct structures but may also exist as loosely associated clusters of cells that work | BP | Turquoise | animal organ development | 2.56E-12 | SERPINC1, RERE, CACNA1C, GSDME, DRD2, EMX1, EP300, FGFR1, FSHB, GPM6A, GRIN2A, IREB2, LRP1, MEF2C, NAB2, NRGN, PGM3, MAPK3, PSMA4, PSMB10, PTN, RELA, SOX5, SREBF1, MAD1L1, CUL3, ASH2L, INA, AKAP6, AKT3, TAB1, CLP1, HYDIN, ALPK3, IMMP2L, DNAJC19, SLC32A1, CNTN4, STAC3, HCN1, SNORC |

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| | together to perform a specific function or functions. | | | | | |
| GO:0090317 | Any process that decreases the frequency, rate or extent of the directed movement of proteins within cells. | BP | Turquoise | negative regulation of intracellular protein transport | 5.06E-12 | More than 50 overlapping genes |
| GO:0031325 | Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways by which individual cells transform chemical substances. | BP | Turquoise | positive regulation of cellular metabolic process | 5.77E-12 | RERE, GSDME, DRD2, EP300, F2, FGFR1, FSHB, GRIN2A, LRP1, MEF2C, NAB2, NEK1, NFATC3, MAPK3, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, TAOK2, BAG4, ATG13, TBC1D5, DMTF1, STAG1, TAB1, RAI1, CNOT1, NDFIP2, VPS13C, NSD3, RPTOR, CPEB1, ACD, CREB3L1, TNFRSF13C, WBP2NL, BTBD18 |
| GO:0051173 | Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways involving nitrogen or nitrogenous compounds. | BP | Turquoise | positive regulation of nitrogen compound metabolic process | 3.82E-11 | RERE, GSDME, DRD2, EP300, F2, FGFR1, FSHB, GRIN2A, LRP1, MEF2C, NAB2, NEK1, NFATC3, MAPK3, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, TAOK2, BAG4, ATG13, DMTF1, STAG1, TAB1, RAI1, CNOT1, NDFIP2, NSD3, RPTOR, CPEB1, ACD, CREB3L1, TNFRSF13C, WBP2NL, BTBD18 |
| GO:0071986 | A eukaryotically conserved protein complex; in humans, it is comprised of LAMTOR1, LAMTOR2, LAMTOR3, LAMTOR4, and LAMTOR5. The complex is anchored to lipid rafts in late endosome membranes via LAMTOR1, constitutes a guanine nucleotide exchange factor (GEF) for the Rag GTPases. | CC | Turquoise | Ragulator complex | 4.75E-11 | SERPINC1, ATP2A2, CACNA1C, CHRNB4, CLCN3, GSDME, DRD2, F2, FGFR1, GPM6A, GRIN2A, LRP1, NRGN, MAPK3, PTN, PTPRF, CACNA1I, SLC7A6, GPR52, AKAP6, BAG4, RGS6, PLCH2, TBC1D5, SNAP91, RIMS1, PSD3, PPP1R13B, VSIG2, CA14, ZDHHC5, SEZ6L2, PLEKHO1, MPP6, SLC39A8, DPEP3, CPEB1, GPR135, ESAM, ZNF804A, TNFRSF13C, EMB, SLC32A1, CPNE8, CARMIL2, CNTN4, STAC3, HARBI1, HCN1, SNORC |
| GO:0035556 | The process in which a signal is passed on to downstream components within the cell, which become activated themselves to further propagate the | BP | Turquoise | intracellular signal transduction | 5.12E-11 | ATP2A2, CA8, CACNA1C, GSDME, DRD2, EP300, F2, FGFR1, GRIN2A, LRP1, MEF2C, NEK1, NFATC3, MAPK3, PSMA4, PSMB10, RELA, SIPA1, SRPK2, CUL3, |

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| | signal and finally trigger a change in the function or state of the cell. | | | | | TAOK2, AKAP6, BAG4, RGS6, PLCH2, AKT3, TAB1, CNOT1, PSD3, PPP1R13B, NDFIP2, OTUD7B, RPTOR, CREB3L1, STAC3 |
| GO:0046872 | Interacting selectively and non-covalently with any metal ion. | MF | Turquoise | metal ion binding | 7.72E-11 | RERE, ATP2A2, CA8, CACNA1C, DPYD, EP300, F2, GRIN2A, IREB2, LRP1, NEK1, PGM3, SHMT2, SRPK2, DOC2A, ASH2L, ZNF592, PLCH2, ZNF536, RAI1, NT5C2, RIMS1, DDHD2, ZC3H7B, CA14, MOB4, B3GAT1, GATAD2A, NSD3, FANCL, MSL2, ZNF823, OTUD7B, THAP11, DPEP2, DPEP3, CPEB1, ZBTB37, ZNF804A, YPEL4, STAC3, HARBI1 |
| GO:0005886 | The membrane surrounding a cell that separates the cell from its external environment. It consists of a phospholipid bilayer and associated proteins. | CC | Turquoise | plasma membrane | 8.47E-11 | SERPINC1, ATP2A2, CACNA1C, CHRNB4, CLCN3, GSDME, DRD2, F2, FGFR1, GPM6A, GRIN2A, LRP1, NRGN, MAPK3, PTN, PTPRF, CACNA1I, SLC7A6, GPR52, AKAP6, BAG4, RGS6, PLCH2, TBC1D5, SNAP91, RIMS1, PSD3, PPP1R13B, VSIG2, CA14, ZDHHC5, SEZ6L2, PLEKHO1, MPP6, SLC39A8, DPEP3, CPEB1, GPR135, ESAM, ZNF804A, TNFRSF13C, EMB, SLC32A1, CPNE8, CARMIL2, CNTN4, STAC3, HARBI1, HCN1 |
| GO:1901558 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a metformin stimulus. | BP | Turquoise | response to metformin | 2.46E-10 | RERE, DRD2, EMX1, EP300, FGFR1, FSHB, IREB2, MEF2C, NAB2, NFATC3, PGM3, MAPK3, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, DMTF1, STAG1, TAB1, RAI1, CLP1, NT5C2, CNOT1, GATAD2A, NSD3, ZNF823, OTUD7B, THAP11, RPTOR, SCAF1, ACD, ACTR5, ZBTB37, CREB3L1, WBP2NL, BTBD18 |
| GO:0006464 | The covalent alteration of one or more amino acids occurring in proteins, peptides and nascent polypeptides (co-translational, post-translational | BP | Turquoise | cellular protein modification process | 2.46E-10 | SERPINC1, GSDME, DRD2, EP300, F2, FGFR1, LRP1, MEF2C, MSRA, NAB2, NEK1, PGM3, PPP2R2A, MAPK3, PSMA4, PSMB10, PTN, PTPRF, RELA, SHMT2, |

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| | modifications) occurring at the level of an individual cell. Includes the modification of charged tRNAs that are destined to occur in a protein (pre-translation modification). | | | | | SREBF1, SRPK2, CUL3, ASH2L, TAOK2, BAG4, ATG13, AKT3, TAB1, ZDHHC5, B3GAT1, NDFIP2, NSD3, FANCL, MSL2, OTUD7B, RPTOR, ALPK3, ACTR5, HS3ST5, INO80E |
| GO:0050793 | Any process that modulates the frequency, rate or extent of development, the biological process whose specific outcome is the progression of a multicellular organism over time from an initial condition (e.g. a zygote, or a young adult) to a later condition (e.g. a multicellular animal or an aged adult). | BP | Turquoise | regulation of developmental process | 2.46E-10 | DRD2, EMX1, EP300, F2, FGFR1, FSHB, LRP1, MEF2C, NAB2, NFATC3, PSMA4, PSMB10, PTN, PTPRF, RELA, SOX5, ASH2L, TAOK2, AKAP6, ZNF536, AKT3, RAI1, EPN2, RIMS1, CNOT1, DDHD2, LSM1, PLEKHO1, TLCD3B, CREB3L1, ZNF804A, TNFRSF13C, CNTN4 |
| GO:0098631 | The binding by a cell-adhesion protein on the cell surface to an extracellular matrix component, to mediate adhesion of the cell to the external substrate or to another cell. | MF | Turquoise | cell adhesion mediator activity | 2.85E-10 | ATP2A2, CHRNB4, CLCN3, DRD2, LRP1, NRGN, SREBF1, SREBF2, AP3B2, DOC2A, CUL3, AKAP6, TBC1D5, MPHOSPH9, NUTF2, TAB1, EPN2, DOP1A, ABCB9, MOB4, B3GAT1, NDFIP2, VPS13C, RPTOR, GPR135, TLCD3B, BORCS7, SLC32A1, HS3ST5 |
| GO:0097707 | A programmed cell death characterized morphologically by the presence of smaller than normal mitochondria with condensed mitochondrial membrane densities, reduction or vanishing of mitochondria crista, and outer mitochondrial membrane rupture. Activation of mitochondrial voltage-dependent anion channels and mitogen-activated protein kinases, upregulation of endoplasmic reticulum stress, and inhibition of cystine/glutamate antiporter are involved in the induction of ferroptosis. This process is | BP | Turquoise | ferroptosis | 4.03E-10 | RERE, DRD2, EMX1, EP300, FGFR1, FSHB, MEF2C, NAB2, NFATC3, MAPK3, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, DMTF1, STAG1, TAB1, RAI1, CLP1, CNOT1, GATAD2A, NSD3, ZNF823, OTUD7B, THAP11, RPTOR, SCAF1, ACTR5, ZBTB37, CREB3L1, WBP2NL, BTBD18 |

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| | characterized by the accumulation of lipid peroxidation products and lethal reactive oxygen species (ROS) derived from iron metabolism. Glutathione peroxidase 4 (GPX4), heat shock protein beta-1, and nuclear factor erythroid 2-related factor 2 function as negative regulators of ferroptosis by limiting ROS production and reducing cellular iron uptake, respectively. In contrast, NADPH oxidase and p53 act as positive regulators of ferroptosis by promotion of ROS production and inhibition of expression of SLC7A11 (a specific light-chain subunit of the cystine/glutamate antiporter), respectively. Misregulated ferroptosis has been implicated in multiple physiological and pathological processes. | | | | | |
| GO:0003676 | Interacting selectively and non-covalently with any nucleic acid. | MF | Turquoise | nucleic acid binding | 5.31E-10 | RERE, EMX1, EP300, IREB2, LRP1, MEF2C, NFATC3, RELA, SOX5, SREBF1, SREBF2, SRPK2, XRCC3, BAG4, ZNF592, ZNF536, DMTF1, R3HDM2, RIMS1, CNOT1, ZC3H7B, SPATS2L, LSM1, ARL6IP4, GATAD2A, ZNF823, DDX28, OTUD7B, THAP11, SUGP1, SCAF1, CPEB1, ACD, ZBTB37, CREB3L1, C12orf65, RPS19BP1, ZNF804A, TDRD9, WBP2NL, SELENOH |
| GO:2000225 | Any process that stops, prevents, or reduces the frequency, rate or extent of testosterone biosynthetic process. | BP | Turquoise | negative regulation of testosterone biosynthetic process | 7.81E-10 | RERE, DRD2, EMX1, EP300, FGFR1, FSHB, IREB2, MEF2C, NAB2, NFATC3, MAPK3, PSMA4, PSMB10, RELA, SHMT2, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, DMTF1, STAG1, TAB1, RAI1, CNOT1, GATAD2A, NSD3, ZNF823, OTUD7B, THAP11, RPTOR, CPEB1, ACD, |

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| | | | | | | ACTR5, ZBTB37, CREB3L1, BTBD18 |
| GO:0051130 | Any process that activates or increases the frequency, rate or extent of a process involved in the formation, arrangement of constituent parts, or disassembly of cell structures, including the plasma membrane and any external encapsulating structures such as the cell wall and cell envelope. | BP | Turquoise | positive regulation of cellular component organization | 1.03E-09 | DRD2, EP300, FGFR1, GPM6A, LRP1, MAPK3, PTN, SREBF1, SREBF2, CUL3, BAG4, ATG13, TBC1D5, RIMS1, CNOT1, DDHD2, PPP1R13B, VPS13C, NSD3, ACD, ZNF804A, CARMIL2 |
| GO:0120069 | Any process that increases the frequency, rate or extent of any stomach fundus smooth muscle contraction. | BP | Turquoise | positive regulation of stomach fundus smooth muscle contraction | 2.08E-09 | RERE, DRD2, EMX1, EP300, FGFR1, GPM6A, LRP1, MEF2C, NEK1, MAPK3, PTN, PTPRF, ALMS1, TAOK2, BAG4, SDCCAG8, RIMS1, PLEKHO1, HYDIN, ZNF804A, EMB, CARMIL2, CNTN4, RILPL2 |
| GO:1903593 | Any process that modulates the frequency, rate or extent of histamine secretion by mast cell. | BP | Turquoise | regulation of histamine secretion by mast cell | 2.25E-09 | RERE, DRD2, EMX1, EP300, FGFR1, FSHB, MEF2C, NAB2, NFATC3, MAPK3, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, DMTF1, STAG1, TAB1, RAI1, CNOT1, GATAD2A, NSD3, ZNF823, OTUD7B, THAP11, RPTOR, ACTR5, ZBTB37, CREB3L1, WBP2NL, BTBD18 |
| GO:0016021 | The component of a membrane consisting of the gene products and protein complexes having at least some part of their peptide sequence embedded in the hydrophobic region of the membrane. | CC | Turquoise | integral component of membrane | 2.25E-09 | ATP2A2, CACNA1C, CHRNB4, CLCN3, DRD2, FGFR1, GPM6A, GRIN2A, LRP1, PTPRF, SREBF1, SREBF2, CACNA1I, SLC7A6, GPR52, TAOK2, AKAP6, ABCB9, VSIG2, CA14, ZDHHC5, SEZ6L2, B3GAT1, SLC45A1, TMX2, AIG1, NDFIP2, SLC39A8, GPR135, TLCD3B, IMMP2L, ESAM, CREB3L1, SFXN5, TNFRSF13C, DNAJC19, EMB, SLC32A1, TSNARE1, KIAA1324L, HS3ST5, STAC3, HCN1, SNORC, PCNX3 |
| GO:2001234 | Any process that stops, prevents or reduces the frequency, rate or extent of | BP | | negative regulation of apoptotic signalling pathway | 2.32E-09 | RERE, DRD2, EMX1, EP300, FGFR1, FSHB, MEF2C, NAB2, NFATC3, MAPK3, |

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| | apoptotic signalling pathway. | | Turquoise | | | PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, DMTF1, STAG1, TAB1, RAI1, CNOT1, GATAD2A, NSD3, ZNF823, OTUD7B, THAP11, RPTOR, ACTR5, ZBTB37, CREB3L1, WBP2NL, BTBD18 |
| GO:0071727 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) because of a triacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to triacyl bacterial lipopeptide | 2.75E-09 | CACNA1C, DRD2, LRP1, MAPK3, SREBF1, SREBF2, AP3B2, SLC7A6, TAOK2, AKAP6, BAG4, ATG13, TBC1D5, SNAP91, NUTF2, RIMS1, DOP1A, PPP1R13B, ABCB9, NDFIP2, VPS13C, ACD, MAIP1, IMMP2L, SFXN5, DNAJC19, SLC32A1, TOM1L2, RILPL2, TSNARE1 |
| GO:0048699 | The process in which nerve cells are generated. This includes the production of neuroblasts and their differentiation into neurons. | BP | Turquoise | generation of neurons | 2.92E-09 | RERE, GSDME, DRD2, EMX1, EP300, F2, FGFR1, GPM6A, LRP1, MEF2C, MAPK3, PTN, PTPRF, RELA, SOX5, TAOK2, ZNF536, SDCCAG8, RIMS1, LSM1, ZNF804A, EMB, CNTN4, HCN1 |
| GO:0033043 | Any process that modulates the frequency, rate or extent of a process involved in the formation, arrangement of constituent parts, or disassembly of an organelle. | BP | Turquoise | regulation of organelle organization | 3.22E-09 | LRP1, MEF2C, MAPK3, SREBF1, SREBF2, XRCC3, ALMS1, MAD1L1, CUL3, TAOK2, BAG4, ATG13, STAG1, SDCCAG8, CNOT1, DDHD2, PPP1R13B, VPS13C, NSD3, ACD, CARMIL2, TOM1L2 |
| GO:0007049 | The progression of biochemical and morphological phases and events that occur in a cell during successive cell replication or nuclear replication events. Canonically, the cell cycle comprises the replication and segregation of genetic material followed by the division of the cell, but in endocycles or syncytial cells nuclear replication or nuclear division may not be followed by cell division. | BP | Turquoise | cell cycle | 4.58E-09 | DRD2, EP300, FGFR1, NEK1, PPP2R2A, MAPK3, PSMA4, PSMB10, SIPA1, SRPK2, XRCC3, ALMS1, MAD1L1, CUL3, TAOK2, DMTF1, STAG1, SDCCAG8, CNOT1, PPP1R13B, RPTOR, DPEP3, TDRD9, TOM1L2, WBP2NL, BTBD18 |
| GO:0030054 | A cellular component that forms a specialized region of connection between two or more cells or between | CC | | cell junction | 4.95E-09 | ATP2A2, CACNA1C, CHRNB4, CLCN3, DRD2, GPM6A, GRIN2A, LRP1, MEF2C, NRGN, PPP2R2A, MAPK3, PTN, RELA, |

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| | a cell and the extracellular matrix. At a cell junction, anchoring proteins extend through the plasma membrane to link cytoskeletal proteins in one cell to cytoskeletal proteins in neighbouring cells or to proteins in the extracellular matrix. | | Turquoise | | | DOC2A, INA, AKAP6, SNAP91, SDCCAG8, RIMS1, PSD3, CPEB1, ESAM, ZNF804A, EMB, SLC32A1 |
| GO:0048468 | The process whose specific outcome is the progression of the cell over time, from its formation to the mature structure. Cell development does not include the steps involved in committing a cell to a specific fate. | BP | Turquoise | cell development | 5.13E-09 | RERE, ATP2A2, DRD2, EMX1, EP300, F2, FGFR1, GPM6A, LRP1, MEF2C, MAPK3, PTN, PTPRF, RELA, CUL3, TAOK2, AKAP6, ZNF536, RIMS1, LSM1, HYDIN, ALPK3, ZNF804A, EMB, CNTN4, RILPL2, STAC3, HCN1 |
| GO:0045202 | The junction between a nerve fibre of one neuron and another neuron, muscle fibre or glial cell. As the nerve fibre approaches the synapse it enlarges into a specialized structure, the presynaptic nerve ending, which contains mitochondria and synaptic vesicles. At the tip of the nerve ending is the presynaptic membrane; facing it and separated from it by a minute cleft (the synaptic cleft) is a specialized area of membrane on the receiving cell, known as the postsynaptic membrane. In response to the arrival of nerve impulses, the presynaptic nerve ending secretes molecules of neurotransmitters into the synaptic cleft. These diffuse across the cleft and transmit the signal to the postsynaptic membrane. | CC | Turquoise | synapse | 6.10E-09 | ATP2A2, CACNA1C, CHRNB4, CLCN3, DRD2, GPM6A, GRIN2A, MEF2C, NRGN, PPP2R2A, PTN, RELA, DOC2A, INA, SNAP91, RIMS1, PSD3, CPEB1, ZNF804A, EMB, SLC32A1 |
| GO:0034220 | A process in which an ion is transported across a membrane. | BP | | ion transmembrane transport | 7.66E-09 | ATP2A2, CACNA1C, CHRNB4, CLCN3, DRD2, F2, GPM6A, GRIN2A, MEF2C, CACNA1I, SLC7A6, AKAP6, PLCH2, |

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| | | | Turquoise | | | ABCB9, SLC39A8, MAIP1, SFXN5, EMB, SLC32A1, STAC3, HCN1 |
| GO:0006357 | Any process that modulates the frequency, rate or extent of transcription mediated by RNA polymerase II. | BP | Turquoise | regulation of transcription by RNA polymerase II | 1.01E-08 | RERE, DRD2, EMX1, EP300, FGFR1, FSHB, MEF2C, NFATC3, MAPK3, PSMA4, PSMB10, RELA, SOX5, SREBF1, SREBF2, CUL3, ASH2L, ZNF592, ZNF536, DMTF1, STAG1, RAI1, CNOT1, GATAD2A, ZNF823, OTUD7B, THAP11, ZBTB37, CREB3L1, BTBD18 |
| GO:0043168 | Interacting selectively and non-covalently with anions, charged atoms or groups of atoms with a net negative charge. | MF | Turquoise | anion binding | 1.03E-08 | SERPINC1, ATP2A2, CLCN3, GSDME, DPYD, F2, FGFR1, LRP1, NEK1, NRGN, MAPK3, PTN, PTPRF, RELA, SHMT2, SRPK2, XRCC3, DOC2A, TAOK2, SNAP91, AKT3, CLP1, ABCB9, PLA2G15, DDX28, ALPK3, TDRD9, CPNE8, CARMIL2, HS3ST5, HCN1 |
| GO:0060322 | The biological process whose specific outcome is the progression of a head from an initial condition to its mature state. The head is the anterior-most division of the body. | BP | Turquoise | head development | 2.76E-08 | RERE, DRD2, EMX1, EP300, FGFR1, GRIN2A, LRP1, NRGN, MAPK3, PTN, INA, AKT3, CLP1, HYDIN, IMMP2L, SLC32A1, CNTN4 |
| GO:0019899 | Interacting selectively and non-covalently with any enzyme. | MF | Turquoise | enzyme binding | 3.58E-08 | SERPINC1, ATP2A2, LRP1, MEF2C, PPP2R2A, MAPK3, PTN, RELA, SREBF1, CUL3, TAOK2, AKAP6, BAG4, ATG13, TBC1D5, SNAP91, NUTF2, TAB1, RIMS1, PSD3, FANCL, RPTOR, SCAF1, ACD, RPS19BP1, TOM1L2, RILPL2 |
| GO:0006928 | The directed, self-propelled movement of a cell or subcellular component without the involvement of an external agent such as a transporter or a pore. | BP | Turquoise | movement of cell or subcellular component | 3.63E-08 | RERE, ATP2A2, CACNA1C, DRD2, F2, FGFR1, FSHB, GPM6A, LRP1, MEF2C, MAPK3, PTN, PTPRF, AP3B2, CUL3, CACNA1I, SLC7A6, TAOK2, BAG4, AKT3, SDCCAG8, PLEKHO1, HYDIN, ESAM, EMB, CARMIL2, CNTN4 |
| GO:0010647 | Any process that increases the frequency, rate or extent of cell communication. Cell communication is the process that mediates | BP | | positive regulation of cell communication | 4.57E-08 | CHRNB4, GSDME, DRD2, EP300, F2, FGFR1, GRIN2A, LRP1, NMB, NRGN, MAPK3, PSMA4, PSMB10, PTN, RELA, TAOK2, AKAP6, BAG4, AKT3, TAB1, |

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| | interactions between a cell and its surroundings. Encompasses interactions such as signalling or attachment between one cell and another cell, between a cell and an extracellular matrix, or between a cell and any other aspect of its environment. | | Turquoise | | | EPN2, RIMS1, PPP1R13B, NDFIP2, RPTOR |
| GO:0070762 | A subcomplex of the nuclear pore complex (NPC) that spans the nuclear membrane and anchors the NPC to the nuclear envelope. In S. cerevisiae, the transmembrane ring is composed of Pom152p, Pom34p, and Ndc1p. In vertebrates, it is composed of Gp210, Ndc1, and Pom121. Components are arranged in 8-fold symmetrical 'spokes' around the central transport channel. A single 'spoke', can be isolated and is sometime referred to as the Ndc1 complex. | CC | Turquoise | nuclear pore transmembrane ring | 4.65E-08 | F2, GRIN2A, LRP1, PTN, SREBF1, SREBF2, AP3B2, TAOK2, AKAP6, BAG4, ATG13, TBC1D5, NUTF2, RIMS1, PPP1R13B, VPS13C, OTUD7B, ACD, MAIP1, IMMP2L, DNAJC19, TOM1L2, RILPL2, TSNARE1, STAC3 |
| GO:0042592 | Any biological process involved in the maintenance of an internal steady state. | BP | Turquoise | homeostatic process | 4.67E-08 | ATP2A2, CACNA1C, CLCN3, DRD2, EMX1, F2, FSHB, GRIN2A, IREB2, LCAT, LRP1, MEF2C, NMB, MAPK3, SREBF2, XRCC3, ALMS1, AKAP6, PLCH2, AKT3, TMX2, SLC39A8, ACD, MAIP1, TNFRSF13C |
| GO:0023056 | Any process that activates, maintains or increases the frequency, rate or extent of a signalling process. | BP | Turquoise | positive regulation of signalling | 4.85E-08 | CHRNB4, GSDME, DRD2, EP300, F2, FGFR1, GRIN2A, LRP1, NMB, NRGN, MAPK3, PSMA4, PSMB10, PTN, RELA, TAOK2, AKAP6, BAG4, AKT3, TAB1, EPN2, RIMS1, PPP1R13B, NDFIP2, RPTOR |
| GO:0070025 | Interacting selectively and non-covalently with carbon monoxide (CO). | MF | | carbon monoxide binding | 6.02E-08 | SERPINC1, CACNA1C, DRD2, EP300, F2, FGFR1, FSHB, GRIN2A, LRP1, MEF2C, NAB2, NEK1, PPP2R2A, MAPK3, PTN, PTPRF, RELA, SIPA1, TAOK2, AKAP6, |

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| | | | Turquoise | | | BAG4, RGS6, ATG13, TBC1D5, TAB1, RIMS1, NDFIP2, RPTOR, ACD, DNAJC19, STAC3 |
| GO:0040011 | Self-propelled movement of a cell or organism from one location to another. | BP | Turquoise | locomotion | 6.02E-08 | RERE, DRD2, EP300, F2, FGFR1, FSHB, GPM6A, GRIN2A, LRP1, MEF2C, MAPK3, PTN, PTPRF, CUL3, CACNA1I, SLC7A6, TAOK2, BAG4, AKT3, SDCCAG8, PLEKHO1, ESAM, EMB, CARMIL2, CNTN4 |
| GO:0097733 | A specialised 9+0 non-motile cilium found in photoreceptor cells. A ciliary transition zone called 'photoreceptor connecting cilium' links the photoreceptor outer segment to the inner segment. | CC | Turquoise | photoreceptor cell cilium | 8.68E-08 | CHRNB4, CLCN3, DRD2, FGFR1, GRIN2A, LRP1, NRGN, MAPK3, SIPA1, SREBF1, SREBF2, AP3B2, DOC2A, TAOK2, TBC1D5, SNAP91, TAB1, EPN2, DOP1A, DDHD2, NDFIP2, DPEP3, GPR135, SLC32A1, CARMIL2, PHETA2, EHBP1L1 |
| GO:0006796 | The chemical reactions and pathways involving the phosphate group, the anion or salt of any phosphoric acid. | BP | Turquoise | phosphate-containing compound metabolic process | 1.35E-07 | GSDME, DRD2, F2, FGFR1, LCAT, LRP1, MEF2C, NAB2, NEK1, PGM3, PPP2R2A, MAPK3, PSMA4, PSMB10, PTN, PTPRF, SHMT2, SRPK2, CUL3, INPP4B, TAOK2, BAG4, PLCH2, ATG13, AKT3, TAB1, CLP1, NT5C2, DDHD2, PLA2G15, RPTOR, ALPK3 |
| GO:0070901 | The posttranscriptional addition of methyl groups to specific residues in a mitochondrial tRNA molecule. | BP | Turquoise | mitochondrial tRNA methylation | 2.13E-07 | ATP2A2, GSDME, DRD2, EP300, FGFR1, FSHB, LRP1, MEF2C, MSRA, MAPK3, PSMA4, PSMB10, PTN, RELA, SHMT2, SIPA1, SOX5, SREBF1, CUL3, INA, AKAP6, BAG4, TAB1, EPN2, CNOT1, B3GAT1, RPTOR, CPEB1, CREB3L1, TNFRSF13C, CPNE8, HCN1 |
| GO:0030135 | Small membrane-bounded organelle formed by pinching off of a coated region of membrane. Some coats are made of clathrin, whereas others are made from other proteins. | CC | Turquoise | coated vesicle | 2.27E-07 | LRP1, NRGN, SREBF1, SREBF2, AP3B2, TBC1D5, SNAP91, EPN2, DDHD2, SLC32A1, PHETA2 |
| GO:0009056 | The chemical reactions and pathways resulting in the breakdown of substances, including the breakdown | BP | | catabolic process | 2.37E-07 | CTRL, DPYD, DRD2, EP300, GRIN2A, LRP1, PPP2R2A, MAPK3, PSMA4, PSMB10, RELA, SHMT2, SREBF1, |

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| | of carbon compounds with the liberation of energy for use by the cell or organism. | | Turquoise | | | SREBF2, CUL3, PLCH2, ATG13, TBC1D5, NT5C2, CNOT1, DDHD2, PLA2G15, LSM1, AIG1, NDFIP2, VPS13C, OTUD7B, RPTOR |
| GO:0000902 | The developmental process in which the size or shape of a cell is generated and organized. | BP | Turquoise | cell morphogenesis | 2.46E-07 | RERE, DRD2, EMX1, EP300, F2, GPM6A, LRP1, MEF2C, MAPK3, PSMB10, PTN, CUL3, TAOK2, RIMS1, PLEKHO1, EMB, CNTN4, RILPL2 |

**Table 6.15: Gene Ontology for Yellow Module Stage Three using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | GO Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Yellow | binding | 3.20E-07 | BTG1, CHRM3, CYP17A1, PRMT1, HSPA9, NCK1, PLCL1, PPP4C, TLE3, RABGAP1L, KAT5, SF3B1, GIGYF2, JKAMP, CNNM2, TYW5, LETM2 |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Yellow | positive regulation of macrophage proliferation | 3.44E-07 | BTG1, CHRM3, CYP17A1, PRMT1, HSPA9, NCK1, PLCL1, PPP4C, TLE3, RABGAP1L, KAT5, SF3B1, SEC11A, GIGYF2, JKAMP, CNNM2, TYW5, LETM2 |
| GO:0044237 | The chemical reactions and pathways by which individual cells transform chemical substances. | BP | Yellow | cellular metabolic process | 1.60E-06 | BTG1, CHRM3, CYP17A1, PRMT1, HSPA9, NCK1, PLCL1, PPP4C, TLE3, KAT5, SF3B1, SEC11A, GIGYF2, JKAMP, TYW5 |
| GO:0043227 | Organized structure of distinctive morphology and function, bounded by a single or double lipid bilayer membrane. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Yellow | membrane-bounded organelle | 1.00E-05 | BTG1, CYP17A1, PRMT1, HSPA9, NCK1, PPP4C, TLE3, RABGAP1L, KAT5, SF3B1, SEC11A, GIGYF2, JKAMP, CNNM2, LETM2 |

**Table 6.16: Gene Ontology for Blue Module Stage 4 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Blue | positive regulation of macrophage proliferation | 1.70E-10 | CHRM3, CYP17A1, GRM3, HSPD1, HSPE1, MMP16, NDUFA6, PCCB, STAR, STAT6, DGKI, VPS45, SATB2, VSIG2, MOB4, FOXP1, LSM1, RBFOX1, TSR1, PAK6, RALGAPA2, NDRG4, ZFYVE21, GDPD3, MAIP1, COQ10B, DRC3, YPEL3, C12orf65, RPS19BP1, RILPL2, YPEL4, EHBP1L1, ASPG |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Blue | cytoplasm | 4.22E-09 | CYP17A1, HSPD1, HSPE1, MMP16, NDUFA6, PCCB, STAR, STAT6, DGKI, VPS45, MOB4, LSM1, RBFOX1, TSR1, PAK6, RALGAPA2, NDRG4, ZFYVE21, GDPD3, MAIP1, COQ10B, DRC3, C12orf65, RPS19BP1, RILPL2, EHBP1L1, ASPG |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Blue | binding | 4.46E-08 | MPPED2, CHRM3, CYP17A1, HSPD1, HSPE1, MMP16, PCCB, STAR, STAT6, DGKI, VPS45, SATB2, MOB4, FOXP1, LSM1, RBFOX1, TSR1, PAK6, RALGAPA2, NDRG4, ZFYVE21, GDPD3, MAIP1, COQ10B, YPEL3, C12orf65, RPS19BP1, RILPL2, YPEL4 |
| GO:0043231 | Organized structure of distinctive morphology and function, bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Blue | intracellular membrane-bounded organelle | 1.57E-07 | CYP17A1, HSPD1, HSPE1, MMP16, NDUFA6, PCCB, STAR, STAT6, DGKI, VPS45, SATB2, MOB4, FOXP1, LSM1, RBFOX1, TSR1, PAK6, RALGAPA2, NDRG4, MAIP1, COQ10B, YPEL3, C12orf65, RPS19BP1, YPEL4 |

**Table 6.17: Gene Ontology for Brown Module Stage 4 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 1.89E-08 | BNIP3L, CA8, ERCC4, ETF1, FGFR1, SIPA1, SREBF1, ATP5MPL, GABBR2, TAB1, IGSF9B, ABCB9, HYDIN, CNNM2, GATAD2A, ZSCAN2, AMBRA1, NLGN4X, RANBP10, SETD6, CENPT, TLCD3B, ZNF804A, SFXN5, STAC3 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Brown | binding | 4.85E-07 | BNIP3L, CA8, ERCC4, ETF1, FGFR1, SIPA1, SREBF1, GABBR2, TAB1, IGSF9B, ABCB9, CNNM2, GATAD2A, ZSCAN2, AMBRA1, NLGN4X, RANBP10, SETD6, CENPT, ANKRD44, ZNF804A, STAC3 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Brown | intracellular organelle lumen | 2.09E-06 | BNIP3L, CA8, ERCC4, ETF1, FGFR1, SIPA1, SREBF1, GABBR2, TAB1, IGSF9B, CNNM2, GATAD2A, ZSCAN2, AMBRA1, NLGN4X, SETD6, CENPT, TLCD3B, ZNF804A, STAC3 |

**Table 6.18: Gene Ontology for Green Module Stage 4 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Green | positive regulation of macrophage proliferation | 3.43E-06 | CACNA1C, FSHB, MAP3K11, PTN, PTPRF, GPR52, TBC1D5, RAI1, NXPH4, EPN2, DOP1A, CA14, THAP11, ACTR5, CARMIL2, TOM1L2, PCNX3 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Green | intracellular organelle lumen | 9.16E-05 | CACNA1C, FSHB, MAP3K11, PTN, PTPRF, GPR52, TBC1D5, RAI1, NXPH4, EPN2, THAP11, ACTR5, CARMIL2, TOM1L2 |
| GO:0035373 | Interacting selectively and non-covalently with a chondroitin sulphate proteoglycan, any proteoglycan containing chondroitin sulphate as the glycosaminoglycan carbohydrate unit. | MF | Green | chondroitin sulphate proteoglycan binding | 0.000174 | PTN, PTPRF |
| GO:0050896 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus. The process begins with detection of the stimulus and ends with a change in state or activity or the cell or organism. | BP | Green | response to stimulus | 0.000336 | CACNA1C, FSHB, MAP3K11, PTN, PTPRF, GPR52, TBC1D5, NXPH4, EPN2, ACTR5, CARMIL2, TOM1L2 |

**Table 6.19: Gene Ontology for Magenta Module Stage 4 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Magenta | cytoplasm | 7.51E-05 | BTG1, PLCB2, ALMS1, FXR1, CDK2AP1, TAOK2, CLP1, TM6SF2, PLPP5, LETM2, WBP2NL |
| GO:0003674 | A molecular process that can be carried out by the action of a single macromolecular machine, usually via direct physical interactions with other molecular entities. Function in this sense denotes an action, or activity, that a gene product (or a complex) performs. These actions are described from two distinct but related perspectives: (1) biochemical activity, and (2) role as a component in a larger system/process. | MF | Magenta | molecular function | 0.000264 | BTG1, PLCB2, ALMS1, FXR1, CDK2AP1, TAOK2, CLP1, TM6SF2, BCL2L12, PLPP5, LETM2, WBP2NL |
| GO:0019222 | Any process that modulates the frequency, rate or extent of the chemical reactions and pathways within a cell or an organism. | BP | Magenta | regulation of metabolic process | 0.000537 | BTG1, ALMS1, FXR1, CDK2AP1, TAOK2, CLP1, TM6SF2, BCL2L12, WBP2NL |
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Magenta | positive regulation of macrophage proliferation | 0.000585 | BTG1, PLCB2, ALMS1, FXR1, CDK2AP1, TAOK2, CLP1, TM6SF2, BCL2L12, PLPP5, LETM2, WBP2NL |
| GO:0044238 | The chemical reactions and pathways involving those compounds which are formed as a part of the normal anabolic and catabolic processes. These processes take place in most, if not all, cells of the organism. | BP | Magenta | primary metabolic process | 0.000978 | BTG1, PLCB2, FXR1, CDK2AP1, TAOK2, CLP1, TM6SF2, BCL2L12, PLPP5, WBP2NL |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Magenta | cellular response to diacyl bacterial lipopeptide | 0.001976 | BTG1, PLCB2, FXR1, CDK2AP1, TAOK2, CLP1, TM6SF2, BCL2L12, PLPP5, WBP2NL |

**Table 6.20: Gene Ontology for Purple Module Stage 4 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Purple | positive regulation of macrophage proliferation | 4.37E-10 | CHRNA2, CHRNA3, CLU, FHIT, GRIN2A, HSPA9, NCK1, PPP4C, SLC12A4, VRK2, AP3B2, AKAP6, CKAP5, PSMD6, KAT5, PLCL2, SEC11A, B3GAT1, FANCL, SBNO1, OTUD7B, RBM26, SEMA6D, THOC7, WDR73, RFT1, TNFRSF13C, BORCS7, TYW5, ASPHD1, HAPLN4 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Purple | intracellular organelle lumen | 6.00E-08 | CHRNA2, CHRNA3, CLU, FHIT, GRIN2A, HSPA9, NCK1, PPP2R3A, PPP4C, SLC12A4, VRK2, AKAP6, CKAP5, PSMD6, KAT5, PLCL2, BANK1, FANCL, SBNO1, OTUD7B, RBM26, SEMA6D, WDR73, RFT1, TNFRSF13C |
| GO:1901666 | Any process that activates or increases the frequency, rate or extent of NAD+ ADP-ribosyltransferase activity. | BP | Purple | positive regulation of NAD+ ADP-ribosyltransferase activity | 2.40E-07 | CHRNA3, CLU, FHIT, GRIN2A, NCK1, PPP2R3A, PPP4C, VRK2, PSMD6, KAT5, PLCL2, SEC11A, B3GAT1, BANK1, FANCL, OTUD7B, TNFRSF13C, TYW5, ASPHD1 |
| GO:0044267 | The chemical reactions and pathways involving a specific protein, rather than of proteins in general, occurring at the level of an individual cell. Includes cellular protein modification. | BP | Purple | cellular protein metabolic process | 4.22E-07 | CHRNA3, CLU, FHIT, GRIN2A, NCK1, PPP2R3A, PPP4C, VRK2, PSMD6, KAT5, PLCL2, SEC11A, B3GAT1, BANK1, FANCL, OTUD7B, ASPHD1 |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Purple | protein binding | 7.41E-07 | CHRNA3, CLU, FHIT, GRIN2A, HSPA9, NCK1, PPP2R3A, PPP4C, SLC12A4, VRK2, AKAP6, CKAP5, PSMD6, KAT5, PLCL2, BANK1, FANCL, OTUD7B, RBM26, SEMA6D, THOC7, BORCS7, TYW5 |

**Table 6.21: Gene Ontology for Red Module Stage 4 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Red | positive regulation of macrophage proliferation | 3.43E-06 | CACNA1D, DRD2, GPM6A, NAGA, PGM3, PLCL1, PSMB10, HIRIP3, ATG13, ZDHHC5, NDFIP2, SLC38A7, PITPNM2, CPEB1, GFOD2, TDRD9, HS3ST5 |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Red | protein binding | 0.0004 | CACNA1D, DRD2, GPM6A, NAGA, PLCL1, PSMB10, HIRIP3, ATG13, NDFIP2, SLC38A7, PITPNM2, CPEB1, HS3ST5 |
| GO:0043412 | The covalent alteration of one or more monomeric units in a polypeptide, polynucleotide, polysaccharide, or other biological macromolecule, resulting in a change in its properties. | BP | Red | macromolecule modification | 0.000479 | DRD2, PGM3, PLCL1, PSMB10, ATG13, ZDHHC5, NDFIP2, TDRD9, HS3ST5 |
| GO:0099080 | A cellular component that consists of an indeterminate number of proteins or macromolecular complexes, organized into a regular, higher-order structure such as a polymer, sheet, network, or a fibre. | CC | Red | supramolecular complex | 0.00068 | CACNA1D, DRD2, GPM6A |

**Table 6.22: Gene Ontology for Turquoise Module Stage 4 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Turquoise | positive regulation of macrophage proliferation | 3.42E-25 | More than 50 overlapping genes |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Turquoise | intracellular organelle lumen | 2.23E-12 | SERPINC1, RERE, ATP2A2, GSDME, EMX1, IREB2, LRP1, NAB2, NEK1, NFATC3, NMB, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF2, MAD1L1, CUL3, INPP4B, CACNA1I, RGS6, ZNF592, PLCH2, ZNF536, DMTF1, AKT3, SDCCAG8, CNOT1, DDHD2, VPS13C, NSD3, ZNF823, SLC39A8, BCL11B, ZBTB37, CREB3L1, SLC32A1, CNTN4, MED19, HCN1, BTBD18 |
| GO:0005634 | A membrane-bounded organelle of eukaryotic cells in which chromosomes are housed and replicated. In most cells, the nucleus contains all of the cell's chromosomes except the organellar chromosomes and is the site of RNA synthesis and processing. In some species, or in specialized cell types, RNA metabolism or DNA replication may be absent. | CC | Turquoise | nucleus | 5.73E-11 | RERE, ATP2A2, EMX1, LRP1, NAB2, NEK1, NFATC3, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF2, MAD1L1, CUL3, RGS6, ZNF592, ZNF536, DMTF1, AKT3, R3HDM2, CNOT1, PLA2G15, SPATS2L, NSD3, ZNF823, BCL11B, CENPM, ZBTB37, CREB3L1, MED19, HARBI1, BTBD18 |
| GO:2000225 | Any process that stops, prevents, or reduces the frequency, rate or extent of testosterone biosynthetic process. | BP | Turquoise | negative regulation of testosterone biosynthetic process | 3.29E-09 | RERE, EMX1, IREB2, NAB2, NFATC3, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF2, CUL3, ZNF592, ZNF536, DMTF1, CNOT1, NSD3, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19, BTBD18 |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Turquoise | cellular response to diacyl bacterial lipopeptide | 3.32E-09 | SERPINC1, RERE, GSDME, EMX1, IREB2, LRP1, NAB2, NEK1, NFATC3, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF2, CUL3, INPP4B, SLC7A6, ZNF592, PLCH2, ZNF536, DMTF1, AKT3, CNOT1, DDHD2, PLA2G15, AIG1, NSD3, ZNF823, ADAMTSL3, DPEP2, BCL11B, ZBTB37, CREB3L1, MED19, HARBI1, BTBD18 |
| GO:0016020 | A lipid bilayer along with all the proteins and | CC | Turquoise | membrane | 5.76E- | SERPINC1, ATP2A2, GSDME, LRP1, MAPK3, |

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| | protein complexes embedded in it an attached to it. | | | | 09 | SHMT2, SREBF2, CUL3, CACNA1I, SLC7A6, RGS6, PLCH2, AKT3, MPHOSPH9, CNOT1, DDHD2, PLA2G15, SEZ6L2, SLC45A1, AIG1, MPP6, VPS13C, SLC39A8, DPEP2, ESAM, CREB3L1, EMB, SLC32A1, CPNE8, CNTN4, KIAA1324L, HARBI1, HCN1 |
| GO:0080154 | Any process that modulates the rate, frequency or extent of fertilization. Fertilization is the union of gametes of opposite sexes during the process of sexual reproduction to form a zygote. It involves the fusion of the gametic nuclei (karyogamy) and cytoplasm (plasmogamy). | BP | Turquoise | regulation of fertilization | 7.34E-09 | SERPINC1, RERE, GSDME, EMX1, IREB2, LRP1, NAB2, NEK1, NFATC3, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF2, CUL3, ZNF592, ZNF536, DMTF1, CNOT1, NSD3, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19, BTBD18 |
| GO:0006357 | Any process that modulates the frequency, rate or extent of transcription mediated by RNA polymerase II. | BP | Turquoise | regulation of transcription by RNA polymerase II | 1.03E-08 | RERE, EMX1, NFATC3, MAPK3, PSMA4, RELA, SOX5, SREBF2, CUL3, ZNF592, ZNF536, DMTF1, CNOT1, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19, BTBD18 |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Turquoise | protein binding | 1.04E-08 | SERPINC1, RERE, ATP2A2, EMX1, IREB2, LRP1, NAB2, NEK1, NFATC3, NMB, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF2, MAD1L1, CUL3, INPP4B, CACNA1I, RGS6, ZNF592, AKT3, SDCCAG8, CNOT1, AIG1, MPP6, NSD3, ADAMTSL3, BCL11B, ESAM, CREB3L1, EMB, CNTN4, MED19, HCN1 |
| GO:1903593 | Any process that modulates the frequency, rate or extent of histamine secretion by mast cell. | BP | Turquoise | regulation of histamine secretion by mast cell | 1.95E-08 | RERE, EMX1, NAB2, NFATC3, MAPK3, PSMA4, RELA, SOX5, SREBF2, CUL3, ZNF592, ZNF536, DMTF1, CNOT1, NSD3, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19, BTBD18 |
| GO:2001234 | Any process that stops, prevents or reduces the frequency, rate or extent of apoptotic signalling pathway. | BP | Turquoise | negative regulation of apoptotic signalling pathway | 1.96E-08 | RERE, EMX1, NAB2, NFATC3, MAPK3, PSMA4, RELA, SOX5, SREBF2, CUL3, ZNF592, ZNF536, DMTF1, CNOT1, NSD3, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19, BTBD18 |
| GO:0016043 | A process that results in the assembly, arrangement of constituent parts, or disassembly of a cellular component. | BP | Turquoise | cellular component organization | 2.04E-08 | RERE, ATP2A2, EMX1, LRP1, NEK1, MAPK3, RELA, SHMT2, SREBF2, MAD1L1, CUL3, SLC7A6, AKT3, SDCCAG8, CNOT1, DDHD2, MPP6, VPS13C, NSD3, BCL11B, CENPM, |

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|---|---|---|---|---|---|---|
| | | | | | | ESAM, CREB3L1, EMB, CNTN4, HCN1, BTBD18 |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl)benzene-1,2-diol] and derivatives formed by substitution. | BP | Turquoise | cellular response to catecholamine stimulus | 3.49E-08 | RERE, ATP2A2, EMX1, LRP1, NEK1, MAPK3, RELA, SHMT2, SREBF2, MAD1L1, CUL3, SLC7A6, AKT3, SDCCAG8, CNOT1, DDHD2, MPP6, VPS13C, NSD3, BCL11B, CENPM, ESAM, CREB3L1, EMB, CNTN4, HCN1, BTBD18 |
| GO:0097707 | A programmed cell death characterized morphologically by the presence of smaller than normal mitochondria with condensed mitochondrial membrane densities, reduction or vanishing of mitochondria crista, and outer mitochondrial membrane rupture. Activation of mitochondrial voltage-dependent anion channels and mitogen-activated protein kinases, upregulation of endoplasmic reticulum stress, and inhibition of cystine/glutamate antiporter are involved in the induction of ferroptosis. This process is characterized by the accumulation of lipid peroxidation products and lethal reactive oxygen species (ROS) derived from iron metabolism. Glutathione peroxidase 4 (GPX4), heat shock protein beta-1, and nuclear factor erythroid 2-related factor 2 function as negative regulators of ferroptosis by limiting ROS production and reducing cellular iron uptake, respectively. In contrast, NADPH oxidase and p53 act as positive regulators of ferroptosis by promotion of ROS production and inhibition of expression of SLC7A11 (a specific light-chain subunit of the cystine/glutamate antiporter), respectively. Misregulated ferroptosis has been implicated in multiple physiological and pathological | BP | Turquoise | ferroptosis | 3.52E-08 | RERE, EMX1, NAB2, NFATC3, MAPK3, PSMA4, RELA, SOX5, SREBF2, CUL3, ZNF592, ZNF536, DMTF1, CNOT1, NSD3, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19, BTBD18 |

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|---|---|---|---|---|---|---|
| | processes. | | | | | |
| GO:1901679 | The directed movement of nucleotide across a membrane. | BP | Turquoise | nucleotide transmembrane transport | 4.74E-08 | RERE, EMX1, IREB2, NAB2, NFATC3, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF2, CUL3, INPP4B, ZNF592, PLCH2, ZNF536, DMTF1, CNOT1, DDHD2, NSD3, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19, BTBD18 |
| GO:1901558 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a metformin stimulus. | BP | Turquoise | response to metformin | 8.94E-08 | RERE, EMX1, IREB2, NAB2, NFATC3, MAPK3, PSMA4, RELA, SOX5, SREBF2, CUL3, ZNF592, ZNF536, DMTF1, CNOT1, NSD3, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19, BTBD18 |
| GO:0140244 | Any process that regulates translation occurring at the presynapse. | BP | Turquoise | regulation of translation at presynapse | 1.50E-07 | RERE, EMX1, NAB2, NFATC3, RELA, SOX5, SREBF2, ZNF592, ZNF536, DMTF1, NSD3, ZNF823, BCL11B, ZBTB37, CREB3L1, MED19 |
| GO:0031325 | Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways by which individual cells transform chemical substances. | BP | Turquoise | positive regulation of cellular metabolic process | 2.81E-07 | RERE, GSDME, LRP1, NAB2, NEK1, NFATC3, MAPK3, RELA, SOX5, SREBF2, CUL3, DMTF1, CNOT1, VPS13C, NSD3, BCL11B, CREB3L1, MED19, BTBD18 |

**Table 6.23: Gene Ontology for Yellow Module Stage 4 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0003674 | A molecular process that can be carried out by the action of a single macromolecular machine, usually via direct physical interactions with other molecular entities. Function in this sense denotes an action, or activity, that a gene product (or a complex) performs. These actions are described from two distinct but related perspectives: (1) biochemical activity, and (2) role as a component in a larger system/process. | MF | Yellow | molecular function | 6.12E-08 | CALB2, DPYD, EP300, LCAT, MGAT3, MSRA, PPP2R2A, PRKCB, PRKD1, TBX6, DOC2A, NT5C2, RIMS1, ZC3H7B, PLEKHO1, ARL6IP4, NDUFA4L2, RPTOR, ALPK3, DPEP3, DNAJC19, KMT5A |
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Yellow | positive regulation of macrophage proliferation | 2.55E-07 | CALB2, DPYD, EP300, LCAT, MGAT3, MSRA, PPP2R2A, PRKCB, PRKD1, TBX6, DOC2A, NT5C2, RIMS1, ZC3H7B, PLEKHO1, ARL6IP4, NDUFA4L2, RPTOR, ALPK3, DPEP3, DNAJC19, KMT5A |
| GO:0043227 | Organized structure of distinctive morphology and function, bounded by a single or double lipid bilayer membrane. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. | CC | Yellow | membrane-bounded organelle | 9.65E-07 | CALB2, EP300, LCAT, MGAT3, MSRA, PPP2R2A, PRKCB, PRKD1, TBX6, DOC2A, ZC3H7B, PLEKHO1, ARL6IP4, NDUFA4L2, RPTOR, ALPK3, DPEP3, DNAJC19, KMT5A |
| GO:0043229 | Organized structure of distinctive morphology and function, occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes, and the cytoskeleton. Excludes the plasma membrane. | CC | Yellow | intracellular organelle | 1.06E-06 | CALB2, EP300, MGAT3, MSRA, PPP2R2A, PRKCB, PRKD1, TBX6, DOC2A, RIMS1, ZC3H7B, PLEKHO1, ARL6IP4, NDUFA4L2, RPTOR, ALPK3, DPEP3, DNAJC19, KMT5A |
| GO:0008152 | The chemical reactions and pathways, including anabolism and catabolism, by which living organisms transform chemical substances. Metabolic processes typically transform small molecules, but also include macromolecular processes such as DNA repair and replication, and protein synthesis and degradation. | BP | Yellow | metabolic process | 5.41E-06 | DPYD, EP300, LCAT, MGAT3, MSRA, PPP2R2A, PRKCB, PRKD1, TBX6, NT5C2, RIMS1, ZC3H7B, ARL6IP4, NDUFA4L2, RPTOR, ALPK3, DPEP3, KMT5A |

**Table 6.24: Gene Ontology for Black Module Stage 5 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Black | positive regulation of macrophage proliferation | 2.43E-38 | More than 50 overlapping genes |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Black | cellular response to diacyl bacterial lipopeptide | 3.66E-14 | More than 50 overlapping genes |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4-(2-aminoethyl)benzene-1,2-diol] and derivatives formed by substitution. | BP | Black | cellular response to catecholamine stimulus | 3.49E-13 | RERE, ATP2A2, CACNB2, CHRNA3, EMX1, EP300, FGFR1, GPM6A, LRP1, PRKCB, MAPK3, PTPRF, RELA, SHMT2, SIPA1, SREBF1, SREBF2, MAD1L1, CUL3, SLC7A6, AKAP6, CKAP5, AKT3, SDCCAG8, EPN2, CNOT1, DDHD2, MAU2, MPP6, HYDIN, VPS13C, NSD3, BCL11B, CENPM, CENPT, IMMP2L, ESAM, CREB3L1, EMB, CARMIL2, CNTN4, TSNARE1, HCN1, BTBD18 |
| GO:1901679 | The directed movement of nucleotide across a membrane. | BP | Black | nucleotide transmembrane transport | 1.02E-12 | RERE, DPYD, EMX1, EP300, FGFR1, IREB2, NAB2, NFATC3, PDE4B, PRKCB, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF1, SREBF2, CUL3, INPP4B, ZNF592, PLCH2, ZNF536, PSMD6, DMTF1, TAB1, RAI1, NT5C2, CNOT1, DDHD2, GATAD2A, NSD3, BANK1, ZNF823, CPEB1, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, MARS2, TNFRSF13C, BTBD18 |
| GO:2000225 | Any process that stops, prevents, or reduces the frequency, rate or extent of testosterone biosynthetic process. | BP | Black | negative regulation of testosterone biosynthetic process | 1.86E-12 | RERE, EMX1, EP300, FGFR1, IREB2, NAB2, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, TAB1, RAI1, CNOT1, GATAD2A, NSD3, BANK1, |

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|---|---|---|---|---|---|---|
| | | | | | | ZNF823, CPEB1, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, BTBD18 |
| GO:0070016 | Interacting selectively and non-covalently with the armadillo repeat domain of a protein, an approximately 40 amino acid long tandemly repeated sequence motif first identified in the Drosophila segment polarity protein armadillo. Arm-repeat proteins are involved in various processes, including intracellular signalling and cytoskeletal regulation. | MF | Black | armadillo repeat domain binding | 4.04E-12 | SERPINC1, ATP2A2, CACNA1C, CACNB2, CHRNA3, DPYD, EMX1, EP300, FGFR1, GPM6A, IREB2, LRP1, NMB, PDE4B, PRKCB, MAPK3, PSMA4, SHMT2, SREBF1, SREBF2, CUL3, CACNA1I, AKAP6, PLCH2, PSMD6, AKT3, TMX2, VPS13C, SLC39A8, TNFRSF13C, SLC32A1, CARMIL2, CNTN4, HCN1 |
| GO:0080154 | Any process that modulates the rate, frequency or extent of fertilization. Fertilization is the union of gametes of opposite sexes during the process of sexual reproduction to form a zygote. It involves the fusion of the gametic nuclei (karyogamy) and cytoplasm (plasmogamy). | BP | Black | regulation of fertilization | 1.59E-11 | SERPINC1, RERE, CHRNA3, GSDME, EMX1, EP300, FGFR1, IREB2, LRP1, NAB2, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, TAB1, RAI1, CNOT1, RBFOX1, GATAD2A, NSD3, BANK1, ZNF823, CPEB1, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, TNFRSF13C, BTBD18 |
| GO:1901561 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a benomyl stimulus. | BP | Black | response to benomyl | 2.72E-11 | RERE, ATP2A2, MPPED2, DPYD, EMX1, EP300, FGFR1, IREB2, LRP1, NFATC3, PDE4B, PRKCB, MAPK3, RELA, SHMT2, SOX5, SREBF1, SREBF2, ZNF592, ZNF536, DMTF1, AKT3, R3HDM2, NT5C2, CNOT1, MAU2, ABCB9, SPATS2L, ARL6IP4, RBFOX1, GATAD2A, ZNF823, ALPK3, CPEB1, BCL11B, CENPT, ZBTB37, CREB3L1, MARS2, HCN1 |
| GO:0006357 | Any process that modulates the frequency, rate or extent of transcription mediated by RNA polymerase II. | BP | Black | regulation of transcription by RNA polymerase II | 3.13E-11 | RERE, EMX1, EP300, FGFR1, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, RAI1, CNOT1, GATAD2A, ZNF823, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, BTBD18 |

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|---|---|---|---|---|---|---|
| GO:0048513 | Development of a tissue or tissues that work together to perform a specific function or functions. Development pertains to the process whose specific outcome is the progression of a structure over time, from its formation to the mature structure. Organs are commonly observed as visibly distinct structures but may also exist as loosely associated clusters of cells that work together to perform a specific function or functions. | BP | Black | animal organ development | 3.43E-11 | SERPINC1, RERE, CACNA1C, GSDME, EMX1, EP300, FGFR1, GPM6A, IREB2, LRP1, NAB2, PRKCB, MAPK3, PSMA4, RELA, SOX5, SREBF1, MAD1L1, CUL3, AKAP6, PSMD6, AKT3, TAB1, HYDIN, ALPK3, BCL11B, IMMP2L, SLC32A1, CNTN4, STAC3, HCN1 |
| GO:0097178 | The aggregation, arrangement and bonding together of a set of components to form a ruffle, a projection at the leading edge of a crawling cell; the protrusions are supported by a microfilament meshwork. The formation of ruffles (also called membrane ruffling) is thought to be controlled by a group of enzymes known as Rho GTPases, specifically RhoA, Rac1 and cdc42. | BP | Black | ruffle assembly | 4.10E-11 | RERE, ATP2A2, MPPED2, DPYD, EMX1, EP300, FGFR1, IREB2, LRP1, NFATC3, PDE4B, PRKCB, MAPK3, RELA, SHMT2, SOX5, SREBF1, SREBF2, ZNF592, ZNF536, DMTF1, AKT3, R3HDM2, NT5C2, CNOT1, MAU2, ABCB9, SPATS2L, ARL6IP4, RBFOX1, GATAD2A, ZNF823, ALPK3, CPEB1, BCL11B, CENPT, ZBTB37, CREB3L1, MARS2, HCN1 |
| GO:1901555 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a paclitaxel stimulus. | BP | Black | response to paclitaxel | 4.48E-11 | RERE, DPYD, EMX1, EP300, FGFR1, IREB2, NAB2, NFATC3, PDE4B, PRKCB, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, TAB1, RAI1, NT5C2, CNOT1, ARL6IP4, RBFOX1, GATAD2A, NSD3, FANCL, ZNF823, CPEB1, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, MARS2, HARBI1, BTBD18 |
| GO:0005829 | The part of the cytoplasm that does not contain organelles, but which does contain other particulate matter, such as protein complexes. | CC | Black | cytosol | 6.13E-11 | GSDME, DPYD, EP300, FGFR1, IREB2, NFATC3, PDE4B, PRKCB, MAPK3, PSMA4, RELA, SIPA1, SREBF1, SREBF2, MAD1L1, CUL3, INPP4B, RGS6, CKAP5, PSMD6, |

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| | | | | | | DMTF1, TAB1, SDCCAG8, EPN2, NT5C2, CNOT1, DDHD2, SPATS2L, VPS13C, RALGAPA2, RANBP10, CPEB1, CENPM, CENPT, STAC3, HARBI1 |
| GO:0009653 | The process in which anatomical structures are generated and organized. Morphogenesis pertains to the creation of form. | BP | Black | anatomical structure morphogenesis | 9.42E-11 | RERE, CACNA1C, CHRNA3, EMX1, EP300, FGFR1, GPM6A, LRP1, NAB2, PRKCB, MAPK3, PSMA4, RELA, CUL3, PSMD6, AKT3, TAB1, SDCCAG8, EPN2, DDHD2, BCL11B, CREB3L1, TNFRSF13C, EMB, CARMIL2, CNTN4, HCN1 |
| GO:1903593 | Any process that modulates the frequency, rate, or extent of histamine secretion by mast cell. | BP | Black | regulation of histamine secretion by mast cell | 1.07E-10 | RERE, EMX1, EP300, FGFR1, NAB2, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, TAB1, RAI1, CNOT1, GATAD2A, NSD3, ZNF823, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, BTBD18 |
| GO:2001234 | Any process that stops, prevents, or reduces the frequency, rate or extent of apoptotic signalling pathway. | BP | Black | negative regulation of apoptotic signalling pathway | 1.10E-10 | RERE, EMX1, EP300, FGFR1, NAB2, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, TAB1, RAI1, CNOT1, GATAD2A, NSD3, ZNF823, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, BTBD18 |
| GO:1901558 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a metformin stimulus. | BP | Black | response to metformin | 1.33E-10 | RERE, EMX1, EP300, FGFR1, IREB2, NAB2, NFATC3, PDE4B, PRKCB, MAPK3, PSMA4, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, TAB1, RAI1, NT5C2, CNOT1, GATAD2A, NSD3, ZNF823, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, BTBD18 |
| GO:0097707 | A programmed cell death characterized morphologically by the presence of smaller than normal mitochondria with condensed mitochondrial membrane densities, reduction or vanishing of | BP | Black | ferroptosis | 2.84E-10 | RERE, EMX1, EP300, FGFR1, NAB2, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, TAB1, RAI1, CNOT1, GATAD2A, NSD3, ZNF823, BCL11B, CENPT, BCL2L12, ZBTB37, |

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|---|---|---|---|---|---|---|
| | mitochondria crista, and outer mitochondrial membrane rupture. Activation of mitochondrial voltage-dependent anion channels and mitogen-activated protein kinases, upregulation of endoplasmic reticulum stress, and inhibition of cystine/glutamate antiporter are involved in the induction of ferroptosis. This process is characterized by the accumulation of lipid peroxidation products and lethal reactive oxygen species (ROS) derived from iron metabolism. Glutathione peroxidase 4 (GPX4), heat shock protein beta-1, and nuclear factor erythroid 2-related factor 2 function as negative regulators of ferroptosis by limiting ROS production and reducing cellular iron uptake, respectively. In contrast, NADPH oxidase and p53 act as positive regulators of ferroptosis by promotion of ROS production and inhibition of expression of SLC7A11 (a specific light-chain subunit of the cystine/glutamate antiporter), respectively. Misregulated ferroptosis has been implicated in multiple physiological and pathological processes. | | | | | CREB3L1, BTBD18 |
| GO:0090317 | Any process that decreases the frequency, rate or extent of the directed movement of proteins within cells. | BP | Black | negative regulation of intracellular protein transport | 9.81E-10 | RERE, EMX1, EP300, FGFR1, NAB2, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SOX5, SREBF1, SREBF2, CUL3, ZNF592, ZNF536, PSMD6, DMTF1, TAB1, RAI1, CNOT1, ARL6IP4, RBFOX1, GATAD2A, |

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|---|---|---|---|---|---|---|
| | | | | | | NSD3, FANCL, ZNF823, CPEB1, BCL11B, CENPT, BCL2L12, ZBTB37, CREB3L1, MARS2, HARBI1, BTBD18 |
| GO:0035556 | The process in which a signal is passed on to downstream components within the cell, which become activated themselves to further propagate the signal and finally trigger a change in the function or state of the cell. | BP | Black | intracellular signal transduction | 1.56E-09 | ATP2A2, CA8, CACNA1C, GSDME, EP300, FGFR1, LRP1, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SIPA1, CUL3, AKAP6, RGS6, PLCH2, PSMD6, AKT3, TAB1, CNOT1, BANK1, RALGAPA2, BCL2L12, CREB3L1, STAC3 |
| GO:0005886 | The membrane surrounding a cell that separates the cell from its external environment. It consists of a phospholipid bilayer and associated proteins. | CC | Black | plasma membrane | 4.80E-09 | SERPINC1, ATP2A2, CACNA1C, CACNB2, CHRNA3, GSDME, FGFR1, GPM6A, LRP1, PDE4B, PRKCB, MAPK3, PTPRF, CACNA1I, SLC7A6, AKAP6, GABBR2, RGS6, PLCH2, CKAP5, SEZ6L2, MPP6, RALGAPA2, SLC39A8, CPEB1, ESAM, TNFRSF13C, EMB, SLC32A1, CPNE8, CARMIL2, CNTN4, STAC3, HARBI1, HCN1 |
| GO:0033554 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating the organism is under stress. The stress is usually, but not necessarily, exogenous (e.g. temperature, humidity, ionizing radiation). | BP | Black | cellular response to stress | 4.92E-09 | ATP2A2, EP300, LRP1, MAPK3, PSMA4, PTPRF, RELA, SIPA1, SREBF1, SREBF2, CUL3, PSMD6, AKT3, TAB1, CNOT1, JKAMP, VPS13C, FANCL, CPEB1, BCL2L12, IMMP2L, CREB3L1 |
| GO:0120060 | Any process that modulates the frequency, rate or extent of any gastric emptying process, the process in which the liquid and liquid-suspended solid contents of the stomach exit through the pylorus into the duodenum. | BP | Black | regulation of gastric emptying | 6.68E-09 | ATP2A2, CACNA1C, CHRNA3, GPM6A, LRP1, NMB, PDE4B, PRKCB, MAPK3, PTPRF, AP3B2, CUL3, GABBR2, SDCCAG8, HYDIN, CPEB1, BCL11B, EMB, SLC32A1, CARMIL2, CNTN4, HCN1 |
| GO:0071986 | A eukaryotically conserved protein complex; in humans, it is comprised of LAMTOR1, LAMTOR2, | CC | Black | Ragulator complex | 8.46E-09 | SERPINC1, ATP2A2, CACNA1C, CACNB2, CHRNA3, GSDME, FGFR1, GPM6A, LRP1, PDE4B, PRKCB, MAPK3, PTPRF, CACNA1I, |

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|---|---|---|---|---|---|---|
| | LAMTOR3, LAMTOR4, and LAMTOR5. The complex is anchored to lipid rafts in late endosome membranes via LAMTOR1, constitutes a guanine nucleotide exchange factor (GEF) for the Rag GTPases. | | | | | SLC7A6, AKAP6, GABBR2, RGS6, PLCH2, CKAP5, SEZ6L2, MPP6, RALGAPA2, SLC39A8, CPEB1, ESAM, TNFRSF13C, EMB, SLC32A1, CPNE8, CARMIL2, CNTN4, STAC3, HARBI1, HCN1 |
| GO:0006928 | The directed, self-propelled movement of a cell or subcellular component without the involvement of an external agent such as a transporter or a pore. | BP | Black | movement of cell or subcellular component | 1.74E-08 | RERE, ATP2A2, CACNA1C, CACNB2, FGFR1, GPM6A, LRP1, PDE4B, MAPK3, PTPRF, AP3B2, CUL3, CACNA1I, SLC7A6, AKT3, SDCCAG8, HYDIN, BCL11B, ESAM, EMB, CARMIL2, CNTN4 |
| GO:0140244 | Any process that regulates translation occurring at the presynapse. | BP | Black | regulation of translation at presynapse | 2.89E-08 | RERE, EMX1, EP300, NAB2, NFATC3, PRKCB, RELA, SOX5, SREBF1, SREBF2, ZNF592, ZNF536, DMTF1, RAI1, GATAD2A, NSD3, ZNF823, BCL11B, CENPT, ZBTB37, CREB3L1 |
| GO:1901666 | Any process that activates or increases the frequency, rate or extent of NAD+ ADP-ribosyltransferase activity. | BP | Black | positive regulation of NAD+ ADP-ribosyltransferase activity | 3.59E-08 | SERPINC1, CHRNA3, GSDME, DPYD, EP300, FGFR1, IREB2, LRP1, NAB2, PDE4B, PRKCB, MAPK3, PSMA4, PTPRF, RELA, SHMT2, SREBF1, CUL3, SLC7A6, PSMD6, AKT3, TAB1, NT5C2, CNOT1, PLA2G15, JKAMP, NSD3, BANK1, FANCL, ADAMTSL3, ALPK3, DPEP2, CPEB1, BCL2L12, IMMP2L, MARS2, TNFRSF13C |
| GO:0070901 | The posttranscriptional addition of methyl groups to specific residues in a mitochondrial tRNA molecule. | BP | Black | mitochondrial tRNA methylation | 4.16E-08 | ATP2A2, CHRNA3, GSDME, EP300, FGFR1, LRP1, PDE4B, PRKCB, MAPK3, PSMA4, RELA, SHMT2, SIPA1, SOX5, SREBF1, CUL3, AKAP6, PSMD6, TAB1, EPN2, CNOT1, CPEB1, CREB3L1, TNFRSF13C, CPNE8, HCN1 |
| GO:0031981 | The volume enclosed by the nuclear inner membrane. | CC | Black | nuclear lumen | 6.21E-08 | RERE, ATP2A2, EMX1, EP300, FGFR1, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SREBF1, SREBF2, CUL3, CKAP5, PSMD6, DMTF1, TAB1, RAI1, MAU2, PLA2G15, SPATS2L, ARL6IP4, GATAD2A, NSD3, FANCL, CPEB1, CENPM, CENPT, CREB3L1, |

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|---|---|---|---|---|---|---|
| | | | | | | STAC3 |
| GO:0048699 | The process in which nerve cells are generated. This includes the production of neuroblasts and their differentiation into neurons. | BP | Black | generation of neurons | 8.25E-08 | RERE, CHRNA3, GSDME, EMX1, EP300, FGFR1, GPM6A, LRP1, MAPK3, PTPRF, RELA, SOX5, ZNF536, SDCCAG8, BCL11B, EMB, CNTN4, HCN1 |
| GO:0048468 | The process whose specific outcome is the progression of the cell over time, from its formation to the mature structure. Cell development does not include the steps involved in committing a cell to a specific fate. | BP | Black | cell development | 8.25E-08 | RERE, ATP2A2, CHRNA3, EMX1, EP300, FGFR1, GPM6A, LRP1, MAPK3, PTPRF, RELA, CUL3, AKAP6, ZNF536, HYDIN, ALPK3, BCL11B, EMB, CNTN4, STAC3, HCN1 |
| GO:0003824 | Catalysis of a biochemical reaction at physiological temperatures. In biologically catalysed reactions, the reactants are known as substrates, and the catalysts are naturally occurring macromolecular substances known as enzymes. Enzymes possess specific binding sites for substrates, and are usually composed wholly or largely of protein, but RNA that has catalytic activity (ribozyme) is often also regarded as enzymatic. | MF | Black | catalytic activity | 1.04E-07 | MPPED2, CA8, DPYD, EP300, FGFR1, IREB2, PDE4B, PRKCB, MAPK3, PSMA4, PTPRF, SHMT2, CUL3, INPP4B, RGS6, PLCH2, PSMD6, AKT3, TAB1, NT5C2, CNOT1, DDHD2, ABCB9, PLA2G15, AIG1, NSD3, FANCL, ADAMTSL3, ALPK3, DPEP2, IMMP2L, MARS2, HARBI1 |
| GO:0070052 | Interacting selectively and non-covalently with a type V collagen trimer. | MF | Black | collagen V binding | 1.39E-07 | SERPINC1, RERE, ATP2A2, EMX1, EP300, FGFR1, NFATC3, PRKCB, MAPK3, PSMA4, RELA, SHMT2, SREBF1, SREBF2, CUL3, CKAP5, PSMD6, DMTF1, TAB1, RAI1, MAU2, PLA2G15, SPATS2L, ARL6IP4, GATAD2A, NSD3, FANCL, CPEB1, CENPM, CENPT, CREB3L1, MARS2, STAC3 |
| GO:0034220 | A process in which an ion is transported across a membrane. | BP | Black | ion transmembrane transport | 1.41E-07 | ATP2A2, CACNA1C, CACNB2, CHRNA3, GPM6A, PDE4B, CACNA1I, SLC7A6, AKAP6, PLCH2, ABCB9, SLC39A8, EMB, SLC32A1, STAC3, HCN1 |
| GO:0071329 | Any process that results in a change | | | cellular response to sucrose | 1.50E- | CHRNA3, GSDME, EP300, FGFR1, LRP1, |

| | | | | | | |
|---|---|---|---|---|---|---|
| | in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a sucrose stimulus. | BP | Black | stimulus | 07 | PDE4B, PRKCB, MAPK3, PSMA4, RELA, SHMT2, SOX5, SREBF1, CUL3, AKAP6, PSMD6, TAB1, EPN2, CNOT1, CPEB1, CREB3L1, TNFRSF13C, HCN1 |
| GO:0006996 | A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of an organelle within a cell. An organelle is an organized structure of distinctive morphology and function. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton. Excludes the plasma membrane. | BP | Black | organelle organization | 1.64E-07 | RERE, ATP2A2, EP300, LRP1, PRKCB, MAPK3, RELA, SIPA1, SREBF1, SREBF2, MAD1L1, CUL3, CKAP5, AKT3, SDCCAG8, CNOT1, DDHD2, MAU2, HYDIN, VPS13C, NSD3, CENPM, CENPT, IMMP2L, CARMIL2, TSNARE1, BTBD18 |
| GO:0046872 | Interacting selectively and non-covalently with any metal ion. | MF | Black | metal ion binding | 1.69E-07 | RERE, ATP2A2, MPPED2, CA8, CACNA1C, DPYD, EP300, IREB2, LRP1, PDE4B, PRKCB, SHMT2, ZNF592, PLCH2, ZNF536, RAI1, NT5C2, DDHD2, GATAD2A, NSD3, FANCL, ZNF823, DPEP2, CPEB1, BCL11B, ZBTB37, STAC3, HARBI1 |
| GO:0071516 | The initial formation of a stable single-strand DNA lesion that triggers programmed gene conversion at the mating-type locus, thereby restricting mating-type interconversion to one of the two sister chromatids during DNA replication. | BP | Black | establishment of imprinting at mating-type locus | 2.85E-07 | CHRNA3, EP300, FGFR1, LRP1, PDE4B, PRKCB, MAPK3, RELA, SHMT2, SOX5, SREBF1, AKAP6, TAB1, CNOT1, CPEB1, CREB3L1, HCN1 |
| GO:0003700 | A protein or a member of a complex that interacts selectively and non-covalently with a specific DNA sequence (sometimes referred to as a motif) within the regulatory region of a gene to modulate transcription. Regulatory regions include promoters (proximal and distal) and | MF | Black | DNA-binding transcription factor activity | 4.06E-07 | RERE, EMX1, EP300, NFATC3, RELA, SOX5, SREBF1, SREBF2, ZNF592, ZNF536, DMTF1, RAI1, GATAD2A, ZNF823, BCL11B, CENPT, ZBTB37, CREB3L1 |

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| | enhancers. Genes are transcriptional units and include bacterial operons. | | | | | |
| GO:0016021 | The component of a membrane consisting of the gene products and protein complexes having at least some part of their peptide sequence embedded in the hydrophobic region of the membrane. | CC | Black | integral component of membrane | 4.11E-07 | ATP2A2, CACNA1C, CACNB2, CHRNA3, FGFR1, GPM6A, LRP1, PDE4B, PTPRF, SREBF1, SREBF2, CACNA1I, SLC7A6, AKAP6, GABBR2, ABCB9, SEZ6L2, TMX2, AIG1, JKAMP, SLC39A8, IMMP2L, ESAM, CREB3L1, TNFRSF13C, EMB, SLC32A1, TSNARE1, KIAA1324L, STAC3, HCN1 |
| GO:0051254 | Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways involving RNA. | BP | Black | positive regulation of RNA metabolic process | 4.21E-07 | RERE, EP300, NFATC3, PRKCB, MAPK3, RELA, SOX5, SREBF1, SREBF2, DMTF1, RAI1, CNOT1, NSD3, CPEB1, BCL11B, BCL2L12, CREB3L1, BTBD18 |
| GO:0070852 | A neuron projection that is found in unipolar neurons and corresponds to the region between the cell body and the point at which the single projection branches. | CC | Black | cell body fibre | 4.52E-07 | ATP2A2, CACNA1C, CACNB2, GPM6A, PDE4B, PRKCB, CACNA1I, AKAP6, PLCH2, SLC39A8, STAC3 |

**Table 6.25: Gene Ontology for Brown Module Stage 5 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Brown | positive regulation of macrophage proliferation | 1.14E-11 | BTG1, CALB2, GRIN2A, HSPA9, NCK1, PLCB2, PPP4C, PTN, TLE3, VRK2, ALMS1, ASH2L, GPR52, TAOK2, KAT5, SEC11A, CA14, B3GAT1, OTUD7B, GDPD3, ACTR5, SETD6, SEMA6D, THOC7, PLPP5, WDR73, TYW5, RFTN2, LETM2, WBP2NL, MED19, HS3ST5, HAPLN4 |
| GO:0005488 | The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule. | MF | Brown | binding | 1.07E-09 | BTG1, CALB2, GRIN2A, HSPA9, NCK1, PLCB2, PPP2R3A, PPP4C, PTN, TLE3, VRK2, ALMS1, ASH2L, TAOK2, KAT5, CA14, B3GAT1, OTUD7B, GDPD3, ACTR5, SETD6, SEMA6D, THOC7, TYW5, LETM2, WBP2NL, MED19, HS3ST5, HAPLN4 |
| GO:0044237 | The chemical reactions and pathways by which individual cells transform chemical substances. | BP | Brown | cellular metabolic process | 9.83E-09 | BTG1, GRIN2A, HSPA9, NCK1, PLCB2, PPP2R3A, PPP4C, PTN, TLE3, VRK2, ASH2L, TAOK2, KAT5, SEC11A, B3GAT1, OTUD7B, GDPD3, ACTR5, SETD6, THOC7, PLPP5, TYW5, WBP2NL, MED19, HS3ST5 |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Brown | cytoplasm | 1.07E-08 | BTG1, CALB2, GRIN2A, HSPA9, NCK1, PLCB2, PPP4C, PTN, VRK2, ALMS1, TAOK2, KAT5, B3GAT1, OTUD7B, GDPD3, ACTR5, SETD6, SEMA6D, THOC7, PLPP5, WDR73, TYW5, LETM2, WBP2NL, HS3ST5 |
| GO:0044238 | The chemical reactions and pathways involving those compounds which are formed as a part of the normal anabolic and catabolic processes. These processes take place in most, if not all, cells of the organism. | BP | Brown | primary metabolic process | 5.50E-08 | BTG1, GRIN2A, NCK1, PLCB2, PPP2R3A, PPP4C, PTN, TLE3, VRK2, ASH2L, TAOK2, KAT5, SEC11A, B3GAT1, OTUD7B, GDPD3, ACTR5, SETD6, THOC7, PLPP5, TYW5, WBP2NL, MED19, HS3ST5 |
| GO:0043226 | Organized structure of distinctive morphology and function. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton, and prokaryotic structures | CC | Brown | organelle | 1.51E-07 | BTG1, CALB2, GRIN2A, HSPA9, NCK1, PPP4C, PTN, TLE3, VRK2, ALMS1, ASH2L, TAOK2, KAT5, SEC11A, B3GAT1, OTUD7B, GDPD3, ACTR5, SETD6, SEMA6D, THOC7, WDR73, LETM2, WBP2NL, MED19, HS3ST5 |

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| | such as anammoxosomes and pirellulosomes. Excludes the plasma membrane. | | | | | |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Brown | cellular response to diacyl bacterial lipopeptide | 2.99E-07 | BTG1, GRIN2A, NCK1, PLCB2, PPP2R3A, PPP4C, PTN, TLE3, VRK2, ASH2L, TAOK2, KAT5, SEC11A, B3GAT1, OTUD7B, GDPD3, ACTR5, SETD6, THOC7, PLPP5, TYW5, WBP2NL, MED19, HS3ST5 |

**Table 6.26: Gene Ontology for Green Module Stage 5 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Green | positive regulation of macrophage proliferation | 5.52E-06 | CHRM3, CHRNB4, CYP17A1, GRM3, MEF2C, MGAT3, PRKD1, ATXN7, INA, RABGAP1L, MOB4, PARD6A, PLEKHO1, CNNM2, ANP32E, ZNF804A, ASPHD1, ASPG |
| GO:0098809 | Catalysis of the reaction: nitrite + acceptor = product(s) of nitrate reduction + reduced acceptor. | MF | Green | nitrite reductase activity | 1.04E-05 | CHRM3, CHRNB4, GRM3, MEF2C, INA, ZNF804A |
| GO:0043226 | Organized structure of distinctive morphology and function. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton, and prokaryotic structures such as anammoxosomes and pirellulosomes. Excludes the plasma membrane. | CC | Green | organelle | 1.33E-05 | CHRM3, CHRNB4, CYP17A1, GRM3, MEF2C, MGAT3, PRKD1, ATXN7, INA, RABGAP1L, MOB4, PARD6A, PLEKHO1, CNNM2, ANP32E, ZNF804A |
| GO:0005737 | All of the contents of a cell excluding the plasma membrane and nucleus but including other subcellular structures. | CC | Green | cytoplasm | 8.25E-05 | CHRNB4, CYP17A1, MEF2C, MGAT3, PRKD1, ATXN7, INA, RABGAP1L, MOB4, PARD6A, PLEKHO1, ANP32E, ZNF804A, ASPG |
| GO:0016020 | A lipid bilayer along with all the proteins and protein complexes embedded in it an attached to it. | CC | Green | membrane | 0.000105 | CHRM3, CHRNB4, CYP17A1, GRM3, MGAT3, PRKD1, INA, MOB4, PARD6A, PLEKHO1, CNNM2, ZNF804A, ASPHD1 |

**Table 6.27: Gene Ontology for Greenyellow Module Stage 5 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Greenyellow | positive regulation of macrophage proliferation | 1.22E-34 | More than 50 overlapping genes |
| GO:0071726 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a diacylated bacterial lipopeptide stimulus. | BP | Greenyellow | cellular response to diacyl bacterial lipopeptide | 3.08E-18 | More than 50 overlapping genes |
| GO:0070052 | Interacting selectively and non-covalently with a type V collagen trimer. | MF | Greenyellow | collagen V binding | 7.65E-15 | BNIP3L, SERPING1, NCAN, ERCC4, HSPD1, HSPE1, MMP16, PCCB, PSMB10, STAT6, XRCC3, FXR1, CDK2AP1, HIRIP3, DGKZ, DGKI, KDM4A, ZEB2, NUTF2, CLP1, SATB2, SF3B1, FOXP1, MSL2, TSR1, PAK6, THAP11, RPTOR, SUGP1, ACD, MAIP1, ESRP2, YPEL3, L3MBTL2, C12orf65, YPEL4, INO80E, KMT5A |
| GO:0016020 | A lipid bilayer along with all the proteins and protein complexes embedded in it an attached to it. | CC | Greenyellow | membrane | 9.83E-12 | ARHGAP1, BNIP3L, CACNA1D, CLCN3, HSPD1, HSPE1, KCNJ13, MAP3K11, MMP16, NDUFA6, OPCML, PLCL1, RRAS, STAT6, FXR1, DGKZ, DGKI, BAG4, ATG13, TBC1D5, SNAP91, NUTF2, VPS45, DOP1A, PSD3, ZDHHC5, GIGYF2, SLC45A1, NDFIP2, SLC38A7, NLGN4X, RPTOR, PITPNM2, SRR, DPEP3, NDRG4, MAIP1, COQ10B, RFT1, BORCS7, RILPL2, EHBP1L1, SNORC |
| GO:0071870 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a catecholamine stimulus. A catecholamine is any of a group of biogenic amines that includes 4-(2-aminoethyl) pyrocatechol [4- | BP | Greenyellow | cellular response to catecholamine stimulus | 1.02E-10 | BNIP3L, CLCN3, NCAN, ERCC4, HSPD1, MMP16, NDUFA6, XRCC3, HIRIP3, BAG4, KDM4A, ATG13, TBC1D5, ZEB2, SNAP91, CLP1, SATB2, SF3B1, MSL2, TSR1, PAK6, NLGN4X, RPTOR, SRR, DPEP3, NDRG4, ACD, MAIP1, L3MBTL2, C12orf65, RFT1, TDRD9, RILPL2, INO80E, KMT5A |

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| | (2-aminoethyl)benzene-1,2-diol] and derivatives formed by substitution. | | | | | |
| GO:0031323 | Any process that modulates the frequency, rate or extent of the chemical reactions and pathways by which individual cells transform chemical substances. | BP | Greenyellow | regulation of cellular metabolic process | 1.36E-10 | BNIP3L, SERPING1, ERCC4, HSPD1, HSPE1, MAP3K11, PLCL1, PSMB10, RRAS, STAT6, FXR1, CDK2AP1, DGKZ, BAG4, KDM4A, ATG13, TBC1D5, ZEB2, PLCL2, SATB2, GIGYF2, FOXP1, NDFIP2, HPF1, ZSCAN2, PAK6, THAP11, RPTOR, RBM26, NDRG4, ACD, ESRP2, L3MBTL2, KMT5A |
| GO:0060255 | Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass. | BP | Greenyellow | regulation of macromolecule metabolic process | 2.83E-10 | SERPING1, ERCC4, HSPD1, HSPE1, MAP3K11, PLCL1, PSMB10, RRAS, STAT6, FXR1, CDK2AP1, BAG4, KDM4A, ATG13, TBC1D5, ZEB2, NUTF2, CLP1, PLCL2, SATB2, SF3B1, GIGYF2, FOXP1, NDFIP2, HPF1, ZSCAN2, PAK6, THAP11, RPTOR, NDRG4, ACD, ESRP2, L3MBTL2, TDRD9, KMT5A |
| GO:1901666 | Any process that activates or increases the frequency, rate or extent of NAD+ ADP-ribosyltransferase activity. | BP | Greenyellow | positive regulation of NAD+ ADP-ribosyltransferase activity | 1.20E-09 | BNIP3L, SERPING1, NCAN, CTRL, HSPD1, HSPE1, MAP3K11, MMP16, NAGA, PCCB, PGM3, PLCL1, PSMB10, RRAS, FXR1, CDK2AP1, BAG4, KDM4A, ATG13, ZEB2, PLCL2, ZDHHC5, GIGYF2, NDFIP2, HPF1, MSL2, PAK6, RPTOR, SRR, DPEP3, NDRG4, C12orf65, INO80E, KMT5A |
| GO:0080154 | Any process that modulates the rate, frequency or extent of fertilization. Fertilization is the union of gametes of opposite sexes during the process of sexual reproduction to form a zygote. It involves the fusion of the gametic nuclei (karyogamy) and cytoplasm (plasmogamy). | BP | Greenyellow | regulation of fertilization | 9.82E-09 | SERPING1, ERCC4, HSPD1, HSPE1, MAP3K11, PLCL1, PSMB10, RRAS, STAT6, FXR1, CDK2AP1, DGKZ, BAG4, KDM4A, ATG13, ZEB2, PLCL2, SATB2, GIGYF2, FOXP1, NDFIP2, HPF1, ZSCAN2, PAK6, THAP11, RPTOR, NDRG4, ACD, ESRP2, L3MBTL2, KMT5A |
| GO:0005654 | That part of the nuclear content other than the chromosomes or the nucleolus. | CC | Greenyellow | nucleoplasm | 1.24E-08 | BNIP3L, ERCC4, PSMB10, STAT6, XRCC3, CDK2AP1, DGKZ, DGKI, KDM4A, NUTF2, CLP1, SATB2, SF3B1, FOXP1, MSL2, TSR1, THAP11, RPTOR, SUGP1, ACD, ESRP2, L3MBTL2, INO80E, KMT5A |
| GO:0005829 | The part of the cytoplasm that does not | CC | Greenyellow | cytosol | 2.46E-08 | ARHGAP1, BNIP3L, HSPD1, PCCB, PGM3, |

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| | contain organelles, but which does contain other particulate matter, such as protein complexes. | | | | | PSMB10, STAT6, XRCC3, FXR1, CDK2AP1, DGKI, BAG4, KDM4A, ATG13, TBC1D5, ZEB2, NUTF2, CLP1, DOP1A, GIGYF2, TSR1, RPTOR, PITPNM2, SRR, NDRG4, BORCS7, RILPL2, KMT5A |
| GO:0051171 | Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving nitrogen or nitrogenous compounds. | BP | Greenyellow | regulation of nitrogen compound metabolic process | 2.49E-08 | SERPING1, ERCC4, HSPD1, HSPE1, MAP3K11, PLCL1, PSMB10, RRAS, STAT6, FXR1, CDK2AP1, BAG4, KDM4A, ATG13, ZEB2, PLCL2, SATB2, GIGYF2, FOXP1, NDFIP2, HPF1, ZSCAN2, PAK6, THAP11, RPTOR, NDRG4, ACD, ESRP2, L3MBTL2, KMT5A |
| GO:0006810 | The directed movement of substances (such as macromolecules, small molecules, ions) or cellular components (such as complexes and organelles) into, out of or within a cell, or between cells, or within a multicellular organism by means of some agent such as a transporter, pore or motor protein. | BP | Greenyellow | transport | 4.37E-08 | ARHGAP1, BNIP3L, SERPING1, CACNA1D, CLCN3, HSPD1, KCNJ13, PSMB10, DGKI, BAG4, ATG13, TBC1D5, SNAP91, NUTF2, VPS45, DOP1A, FOXP1, SLC45A1, NDFIP2, SLC38A7, NLGN4X, PITPNM2, RBM26, NDRG4, ACD, MAIP1, RFT1, RILPL2 |
| GO:0048523 | Any process that stops, prevents, or reduces the frequency, rate or extent of a cellular process, any of those that are carried out at the cellular level, but are not necessarily restricted to a single cell. For example, cell communication occurs among more than one cell, but occurs at the cellular level. | BP | Greenyellow | negative regulation of cellular process | 7.30E-08 | ARHGAP1, BNIP3L, SERPING1, ERCC4, HSPD1, PSMB10, RRAS, STAT6, XRCC3, FXR1, DGKI, BAG4, KDM4A, ZEB2, PLCL2, SATB2, GIGYF2, FOXP1, THAP11, NLGN4X, RPTOR, RBM26, NDRG4, ACD, L3MBTL2, TDRD9, KMT5A |
| GO:1901561 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a benomyl stimulus. | BP | Greenyellow | response to benomyl | 7.32E-08 | CLCN3, ERCC4, HSPD1, HSPE1, MAP3K11, PCCB, RRAS, STAT6, XRCC3, FXR1, DGKZ, DGKI, BAG4, ZEB2, CLP1, SATB2, SF3B1, GIGYF2, FOXP1, ZSCAN2, TSR1, PAK6, THAP11, SUGP1, SRR, RBM26, ACD, ESRP2, C12orf65, TDRD9 |
| GO:0071725 | Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a triacylated bacterial lipopeptide stimulus. | BP | Greenyellow | response to triacyl bacterial lipopeptide | 8.59E-08 | ARHGAP1, BNIP3L, CACNA1D, HSPD1, BAG4, ATG13, TBC1D5, SNAP91, NUTF2, VPS45, DOP1A, FOXP1, SLC45A1, NDFIP2, SLC38A7, PITPNM2, RBM26, ACD, MAIP1, RFT1, RILPL2 |

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| GO:0097178 | The aggregation, arrangement and bonding together of a set of components to form a ruffle, a projection at the leading edge of a crawling cell; the protrusions are supported by a microfilament meshwork. The formation of ruffles (also called membrane ruffling) is thought to be controlled by a group of enzymes known as Rho GTPases, specifically RhoA, Rac1 and cdc42. | BP | Greenyellow | ruffle assembly | 1.01E-07 | CLCN3, ERCC4, HSPD1, HSPE1, MAP3K11, PCCB, RRAS, STAT6, XRCC3, FXR1, DGKZ, DGKI, BAG4, ZEB2, CLP1, SATB2, SF3B1, GIGYF2, FOXP1, ZSCAN2, TSR1, PAK6, THAP11, SUGP1, SRR, RBM26, ACD, ESRP2, C12orf65, TDRD9 |
| GO:0033554 | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating the organism is under stress. The stress is usually, but not necessarily, exogenous (e.g. temperature, humidity, ionizing radiation). | BP | Greenyellow | cellular response to stress | 1.37E-07 | BNIP3L, ERCC4, MAP3K11, PSMB10, STAT6, XRCC3, BAG4, ATG13, ZEB2, GIGYF2, FOXP1, HPF1, PAK6, RPTOR, ACD, YPEL3, INO80E, KMT5A |
| GO:0070025 | Interacting selectively and non-covalently with carbon monoxide (CO). | MF | Greenyellow | carbon monoxide binding | 1.57E-07 | ARHGAP1, SERPING1, CACNA1D, ERCC4, HSPD1, HSPE1, MAP3K11, MMP16, PLCL1, DGKZ, DGKI, BAG4, ATG13, TBC1D5, ZEB2, PLCL2, NDFIP2, PAK6, RPTOR, RBM26, ACD |
| GO:0051649 | Any process, occurring in a cell, that localizes a substance or cellular component. This may occur via movement, tethering or selective degradation. | BP | Greenyellow | establishment of localization in cell | 2.30E-07 | ARHGAP1, BNIP3L, SERPING1, CACNA1D, CLCN3, HSPD1, DGKI, BAG4, ATG13, TBC1D5, SNAP91, NUTF2, VPS45, DOP1A, NLGN4X, RBM26, NDRG4, ACD, MAIP1, RILPL2 |
| GO:0044267 | The chemical reactions and pathways involving a specific protein, rather than of proteins in general, occurring at the level of an individual cell. Includes cellular protein modification. | BP | Greenyellow | cellular protein metabolic process | 2.60E-07 | BNIP3L, SERPING1, HSPD1, HSPE1, MAP3K11, PGM3, PLCL1, PSMB10, RRAS, FXR1, CDK2AP1, BAG4, KDM4A, ATG13, ZEB2, PLCL2, ZDHHC5, GIGYF2, NDFIP2, HPF1, MSL2, PAK6, RPTOR, NDRG4, C12orf65, INO80E, KMT5A |
| GO:0043168 | Interacting selectively and non-covalently with anions, charged atoms or groups of atoms with a net negative charge. | MF | Greenyellow | anion binding | 2.82E-07 | CLCN3, NCAN, HSPD1, HSPE1, MAP3K11, PCCB, PLCL1, RRAS, XRCC3, DGKZ, DGKI, SNAP91, CLP1, PLCL2, TSR1, PAK6, NLGN4X, PITPNM2, SRR, TDRD9 |

**Table 6.28: Gene Ontology for Pink Module Stage 5 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate or extent of macrophage proliferation. | BP | Pink | positive regulation of macrophage proliferation | 0.000262 | EPHX2, ETF1, STAR, TCF4, ATP5MPL, IGSF9B, SMG6, NEMP1, AMBRA1, NDUFA4L2, ZNF408, C16orf92 |
| GO:0019866 | The inner, i.e. lumen-facing, lipid bilayer of an organelle envelope; usually highly selective to most ions and metabolites. | CC | Pink | organelle inner membrane | 0.000645 | STAR, ATP5MPL, NEMP1, NDUFA4L2 |
| GO:0031966 | Either of the lipid bilayers that surround the mitochondrion and form the mitochondrial envelope. | CC | Pink | mitochondrial membrane | 0.001661 | STAR, ATP5MPL, AMBRA1, NDUFA4L2 |
| GO:0003674 | A molecular process that can be carried out by the action of a single macromolecular machine, usually via direct physical interactions with other molecular entities. Function in this sense denotes an action, or activity, that a gene product (or a complex) performs. These actions are described from two distinct but related perspectives: (1) biochemical activity, and (2) role as a component in a larger system/process. | MF | Pink | molecular function | 0.002147 | EPHX2, ETF1, STAR, TCF4, ATP5MPL, IGSF9B, SMG6, NEMP1, AMBRA1, NDUFA4L2, ZNF408 |

**Table 6.29: Gene Ontology for Red Module Stage 5 using the anRichment function as part of WGCNA in R using the default settings**

| GOID | Definition | Ontology | Module | Go Process | FDR | Genes |
|---|---|---|---|---|---|---|
| GO:0120041 | Any process that activates or increases the frequency, rate, or extent of macrophage proliferation. | BP | Red | positive regulation of macrophage proliferation | 1.84E-07 | ALDOA, CHRNA5, F2, PTK2B, FHIT, MSRA, PPP2R2A, RANGAP1, KCNK7, STAG1, ZC3H7B, PPP1R13B, NGEF, TSNAXIP1, BOLL, PCGF6, ATPAF2, TMEM219, PCNX3 |
| GO:0005515 | Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules). | MF | Red | protein binding | 1.43E-05 | ALDOA, CHRNA5, F2, PTK2B, FHIT, PPP2R2A, RANGAP1, STAG1, ZC3H7B, PPP1R13B, NGEF, BOLL, PCGF6, ATPAF2, TMEM219 |
| GO:0070013 | An organelle lumen that is part of an intracellular organelle. | CC | Red | intracellular organelle lumen | 3.82E-05 | ALDOA, CHRNA5, F2, PTK2B, FHIT, PPP2R2A, RANGAP1, KCNK7, STAG1, ZC3H7B, PPP1R13B, NGEF, BOLL, PCGF6, TMEM219 |
| GO:0005829 | The part of the cytoplasm that does not contain organelles, but which does contain other particulate matter, such as protein complexes. | CC | Red | cytosol | 0.000218 | ALDOA, PTK2B, FHIT, MSRA, PPP2R2A, RANGAP1, STAG1, PPP1R13B, NGEF, ATPAF2 |