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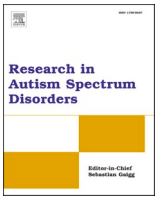

# Genetic networks suggest Asperger's syndrome as a distinct subtype of autism spectrum disorders.

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ABSTRACT

*Background:* The Diagnostic and Statistical Manual of Mental Disorders (DSM-V) issued new diagnostic criteria for autism spectrum disorders (ASD) which resulted in missing the diagnosis of some cases of Asperger's syndrome (AS). This negatively affected the support received by those affected. In this study, we explored if AS could be biologically stratified from the broader spectrum through a gene co-expression network preservation analysis.

*Methods:* We analysed the GEO microarray data of 24 individuals with Asperger's syndrome and 72 individuals with autism. Then, we used a weighted gene co-expression network (WGCNA) pipeline to construct gene co-expression networks. We explored whether these modules share the same co-expression patterns between autism and Asperger's syndrome using network preservation analysis.

*Results:* Our results showed that all co-expression modules of autism are preserved into the Asperger's syndrome. However, three modules of Asperger's syndrome out of 30 modules were not preserved in autism.

Gene enrichment analysis revealed that these modules were involved in chromatin remodelling, immune and neuroinflammatory response, synaptic and neuronal development. Brain enrichment analysis showed significant downregulation of neurodevelopment genes in different brain regions associated with impaired social recognition in Asperger's syndrome.

*Conclusions:* The identified genetic and molecular profiles suggest that Asperger's syndrome, despite sharing numerous similarities with autism, possesses a distinct genetic profile that makes it a distinct subtype of autism. This distinction could have significant implications for the management and treatment strategies tailored to individuals with Asperger's syndrome.

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## 1. Introduction

Autism spectrum disorders are among the most common neurodevelopmental disorders that are typically diagnosed at a young age. The World Health Organization (WHO) has estimated that it has a global prevalence of 0.76 %−2.67 % (Zeidan et al., 2022). Similar to other psychiatric diseases, autism spectrum disorders are diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders (DSM), a manual produced by the American Psychiatric Association to aid clinicians in diagnosing mental illnesses (Regier et al., 2009).

The latest version of the DSM focuses on creating dimensions for different mental illnesses rather than categorizing them which reflected the spectrum nature of many psychiatric conditions (Williams & First, 2013). DSM-5 removed the four categories of autism: pervasive developmental disorder not otherwise specified (PDD-NOS), Asperger's disorder, and autistic disorder, and used new criteria for diagnosing autism. DSM-5 excluded Rett's disorder and childhood disintegrative disorder from the Autism category (Williams & First, 2013). Moreover, the DSM-5 introduced sensory symptoms as one of the criteria for the diagnosis of repetitive and restricted behaviors (Williams & First, 2013). The DSM-5 also added a fifth and new diagnostic category called social communication disorder (SCD), for patients with problems in the social domain only without restrictive and repetitive behavior (Williams & First, 2013).

DSM-5 updates led to the development of a higher threshold for diagnosis of ASD, and it resulted in a more accurate estimation of ASD cases (Kulage et al., 2020). Moreover, DSM-5 had high specificity for autistic spectrum disorders, but a very low sensitivity towards cases of Asperger's syndrome (McPartland et al., 2012). This means that the new classification will miss cases with Asperger's syndrome. The study showed that these changes are mainly due to changes in the diagnostic rubric and not, as many anticipated, due to changes in terminologies (Kulage et al., 2020). A recent study assessed the impact of this new classification on persons with Asperger's syndrome and their families (Smith & Jones, 2020). The study reported that some individuals with Asperger's syndrome agreed with the new classification as it provided a sense of belonging for all patients on the autism spectrum. The study noticed that this group of patients was comfortable with being identified as autistic. Other persons with AS stated that the new classification affected the identity of persons who were diagnosed with Asperger's syndrome (Smith & Jones, 2020). These patients were afraid of being stereotyped as disabled persons with, for instance, limited career opportunities (Smith & Jones, 2020). In conditions where the disease can be presents with spectrum of clinical picture, molecular subtyping was suggested as a way to decipher the heterogeneity of the disease and tailoring the treatment plans for each subtype (Hyman, 2000).

At the genetic level, specific single nucleotide polymorphisms (SNPs) in the aryl-hydrocarbon receptor nuclear translocator 2 (*ARNT2*) gene have been identified as a characteristic of individuals with Asperger's syndrome (Salyakina et al., 2010a; b). Moreover, structural MRI meta-analysis showed distinct grey matter morphometry in AS compared to other types of autism (Yu et al., 2011). Furthermore, Asperger's syndrome shares genetic loci associated with high risk of autism spectrum disorders as well as specific SNPs that are unique genetic risk factor with Asperger's syndrome (Salyakina et al., 2010a; b). A group of genes were associated with high risk of autistic traits which were found to be present in different degrees in different subtypes of autism. These genes were coding for proteins involved in different signaling pathways (Hu & Bi, 2020). Asperger's syndrome had distinct and different genetic variants compared to other types of autism spectrum disorders (Li et al., 2019). However, the study also demonstrated that AS shares common genetic variants responsible for transcription regulation and chromatin remodeling with other subtypes of autism spectrum disorder. Interestingly, a small proportion of AS patients did not share this common genetic signature. However, the study supported the DSM-5 classification as it reflects the shared genetic signature (Li et al., 2019). Notwithstanding, this conclusion did not consider how this genetic difference is reflected in the phenotype, thus, the management of patients. For instance, a study reviewed the current literature and reported that Asperger syndrome is a distinct type of autism (Edelson, 2022). The overlap between Asperger syndrome and autism highlights their similarities, but there are also distinguishable characteristics in Asperger syndrome. This supports the view of Asperger syndrome as a subtype of ASD. Currently, ASD diagnosis in DSM-V relies solely on observable behaviors, with no established biological criteria (Edelson, 2022). Thus, identifying biomarkers to which could link behavioral profiles with biomarkers to subtype Asperger syndrome. The study also reported a higher incidence of motor coordination disorders in individuals with Asperger's syndrome compared to other subtypes (Edelson, 2022). Furthermore, there is a higher significant risk of schizophrenia, and major depressive disorder in Asperger's syndrome compared to other subtypes of autism (Edelson, 2022).

The type of management is different in Asperger's syndrome compared to other ASD individuals, highlighting the need for the characterization of this subtype in DSM-5 (Jordan, 2005). Children with AS often suffered from social isolation and bullying at an early age due to difficulties in social interactions (Mirkovic & Gérardin, 2019). Many of these individuals still need support and social skills training to improve interpersonal relationships and understand social situations (Mirkovic & Gérardin, 2019). However, the type of management is different in Asperger's syndrome than other ASD individuals underscoring the need for the characterization of this subtype in DSM-5 (Baghdadli et al., 2013; Mirkovic & Gérardin, 2019). All of this might suggest the need for independent subtype of Asperger's syndrome but still under the umbrella of autism spectrum disorder.

Despite the currently available body of literature, there has been little investigation into molecular pathogenesis underlying DSM-5 new classification. In our study, we investigated the preservation of gene co-expression networks of autism in Asperger's syndrome. By expanding our understanding of these distinctions, we can pave the way for more targeted and personalised approaches to diagnosis and management in the future.

## 2. Methods

### 2.1. Participants

The GEO dataset (GSE18123) describes the whole blood gene expression of autistic patients using the Affymetrix array. It comprises two independent datasets (P1 and P2), P1 includes 66 autism spectrum disorders and 33 control groups, while the second dataset has 104 autism spectrum patients and 82 control groups (Kong et al., 2012). We excluded the control cases and PDD-NOS as we focused on autism and Asperger's syndrome.

### 2.2. Statistical analysis

For each dataset, a different platform of Affymetrix microarray profiling was used. Affymetrix array U133p2 was used for the first dataset and the GeneST platform for the second dataset. The data was exported to R language and environment for statistical computing using the GEOquery package (Davis & Meltzer, 2007; Team, 2023).

We processed each dataset individually using the Oligo package (Carvalho & Irizarry, 2010). The steps included background correction, quantile normalization, batch effect removal using the SVA package, filtering, and annotation (Leek et al., 2012). After combining P1 and P2, the expression data was inspected for any duplicates and if there are duplicate genes, the mean of the expression value was calculated. After processing the data, overlapped genes between two datasets were used to build an expression dataset for network preservation analysis (Langfelder et al., 2011).

### 2.3. Construction of a weighted gene correlation network

Weighted gene co-expression networks were constructed using the WGCNA package for Autism and Asperger's syndrome groups (Langfelder & Horvath, 2008). To identify outlier microarray samples, average linkage agglomerative hierarchical clustering was performed to detect and exclude outlier samples. For the whole dataset, the network involved the construction of an adjacency matrix from Pearson correlation. The adjacencies were then transformed into topological overlap matrix (TOM) followed by calculation of corresponding dissimilarity (1–TOM). Hierarchical clustering was performed using dissimilarity (1–TOM) as the distance measure. Subsequently, a dynamic tree cut algorithm with a module size of 50 and a minimum cut height of 0.995. The gene module is a collection of genes with high topological overlap similarity. The module eigengene is the first principal component of gene expression. Random subsampling approach is used to validate the constructed modules. To identify genes that construct a module, module membership was calculated as the association between a gene and module eigengene of a given module (MM). The hub gene is defined as the gene with MM and high intramodular connectivity (Langfelder & Horvath, 2008). The gray module consists of genes that do not show correlation with each other in specific diseases, and it is excluded from the downstream analysis. Therefore, one of the recommendations from the authors of WGCNA is that differential gene expression analysis should not be performed prior to constructing the weighted gene co-expression network (Langfelder & Horvath, 2008).

### 2.4. Network preservation analysis

In this study, Network preservation analysis was conducted to assess how characteristic gene co-expression networks of autism are preserved in Asperger's syndrome providing insights into the shared genetic expression patterns (Langfelder & Horvath, 2008; Langfelder et al., 2011). Furthermore, the analysis can identify the least preserved networks which may suggest a distinct molecular mechanism underlying each condition and contribute to the characteristic phenotype.

Fischer's exact test was used to assess the genetic overlap between each module in the two groups. Network preservation analysis was used to assess whether the gene networks identified in Asperger's syndrome can be observed in autism group and vice versa.

Using 1000 permutations, the network preservation function was used to analyse the preservation as explained in Langfelder et al. (Langfelder et al., 2011). To measure how strongly the gene network preservation between two conditions, $Z_{summary}$ scores are used to assess the network density and connectivity. Density-based statistics measure whether the strength of module connections in the test network remains highly connected as the reference network. Network connectivity-based statistics assess the similarity of connection patterns between the test and reference networks (Langfelder et al., 2011). Network connectivity-based statistics is assessing the similarity of connection patterns between the test and reference networks. Z summary is calculated as follows:

$$Zsummary = \frac{Zdensity + Zconnectivity}{2}$$

Since $Z_{summary}$ scores can depend on the module size, median rank statistics is also used to assess the preservation independently of the module size. A module is considered non-preserved and unique to the group if Z summary is less than 10 and median rank is more than 8. Preservation and overlap analysis were corrected using Bonferonni correction and it is considered significant if less than 0.001 (Langfelder et al., 2011). In preservation analysis, the p-value determines whether the observed preservation pattern is statistically significant or happened due to random chance (Langfelder et al., 2011).(Wu et al., 2021).

### 2.5. Enrichment analysis

We performed gene enrichment analysis for genes of significant modules. A gene ontology analysis (GO) was used to identify characteristic cellular components, biological processes and molecular functions (Wu et al., 2021; Xie et al., 2021). Brain enrichment analysis was performed using the BrainimageR package that uses Allen Brain Atlas data to detect enrichment in developing human brain (Linker et al., 2019). Genemania web application was used to visualize the hub gene networks (Warde-Farley et al., 2010). Pathway enrichment analysis was performed using ReactomePA package (Yu & He, 2016).

## 3. Results

### 3.1. Patient characteristics

Ninety-six patients were split into two groups: Asperger's syndrome (n = 24), and autism (n = 72). Four patients were excluded from the analysis: three individuals with autism had Landau Kleffner Syndrome, 16p13.1 amplification and deletion 11p11.12, and one case of Asperger's syndrome has maternally inherited 3p duplication. Individuals with Asperger's syndrome had significantly higher rate of psychiatric disorders, and ADHD Table 1.

### 3.2. Gene preservation analysis in autism and Asperger's syndrome

Soft threshold power beta values of 15 and 18 achieved a scale-free topology of 0.9 for the construction of the co-expression networks in autism and Asperger's syndrome, respectively. There were 19 modules identified in autism and 30 modules in Asperger's syndrome.

Fisher's exact test was used to assess the overlap of genes in each module of Asperger's syndrome with those in the autism modules (Fig. 1). The largest overlap was observed between the tan module of Asperger's syndrome and the black module of autism, with both modules sharing 1016 genes ($p < 0.001$).

According to the results of the preservation analysis, all gene modules of autism were preserved in Asperger's syndrome. However, three modules in Asperger's syndrome were not preserved in autism and are unique to Asperger's syndrome (Supplementary Table 1). These three unique modules are dark turquoise (Z summary = 6 and median rank = 8, *adjusted* $p < 0.001$), light yellow (Z summary = 7 and median rank = 6, *adjusted* $p < 0.001$), and salmon (Z summary = 8 and median rank = 8, *adjusted* $p < 0.001$).

### 3.3. Transcriptomic profiles of non-preserved modules

For the salmon module, gene enrichment analysis showed significant enrichment for different molecular functions like DNA-binding transcription, histone binding, protein serine/threonine kinase activities Fig. 2. For biologic processes, the module was enriched for different related processes like histone modification and methylation, however, it was also enriched for metabolic process and T cell differentiation. The genes are predominantly found in the nuclear specks and SWI/SNF superfamily type complex Fig. 2.

Brain enrichment analysis showed that some genes were significantly and highly enriched in the frontal polar cortex and lateral ganglionic eminence in developing human brain Fig. 3A. Another set of genes was negatively enriched in frontal cortex, motor cortex, lateral dentate nucleus Fig. 3A.

Disease enrichment analysis showed that these genes were associated with intellectual disability, delayed speech development and language delay Supplementary Figure 1 A.

The second module is light yellow module which is associated with immunity regulation including cytokines production and inflammatory response. It is also involved in neutrophil regulation and programmed cell death. Genes were enriched in different

**Table 1**
showing the characteristics of individuals included in the study.

| | ASPERGER'S DISORDER | AUTISM | *p-value* |
|---|---|---|---|
| n | 24 | 72 | |
| Male (%) | 22 ( 91.7) | 62 (86.1) | 0.722 |
| Age (months, median [IQR]) | 121.50 [96.00, 168.25] | 84.00 [60.00, 132.00] | 0.005 |
| Race (%) | | | 0.396 |
| Asian | 0 ( 0.0) | 4 ( 5.6) | |
| Caucasian | 23 ( 95.8) | 62 (86.1) | |
| Mixed | 1 ( 4.2) | 2 ( 2.8) | |
| Unknown | 0 ( 0.0) | 4 ( 5.6) | |
| developmental/speech disorder | 7 ( 29.2) | 33 (45.8) | 0.232 |
| Mental retardation | 1 ( 4.2) | 15 (20.8) | 0.114 |
| Seizures | 2 ( 8.3) | 6 ( 8.3) | > 0.9 |
| Psychiatric disorder | 15 ( 62.5) | 11 (15.3) | < 0.001 |
| ADHD | 11 ( 45.8) | 2 ( 2.8) | < 0.001 |
| GIT | 5 ( 20.8) | 13 (18.1) | > 0.9 |
| Seizure | 1 ( 4.2) | 6 ( 8.3) | 0.821 |

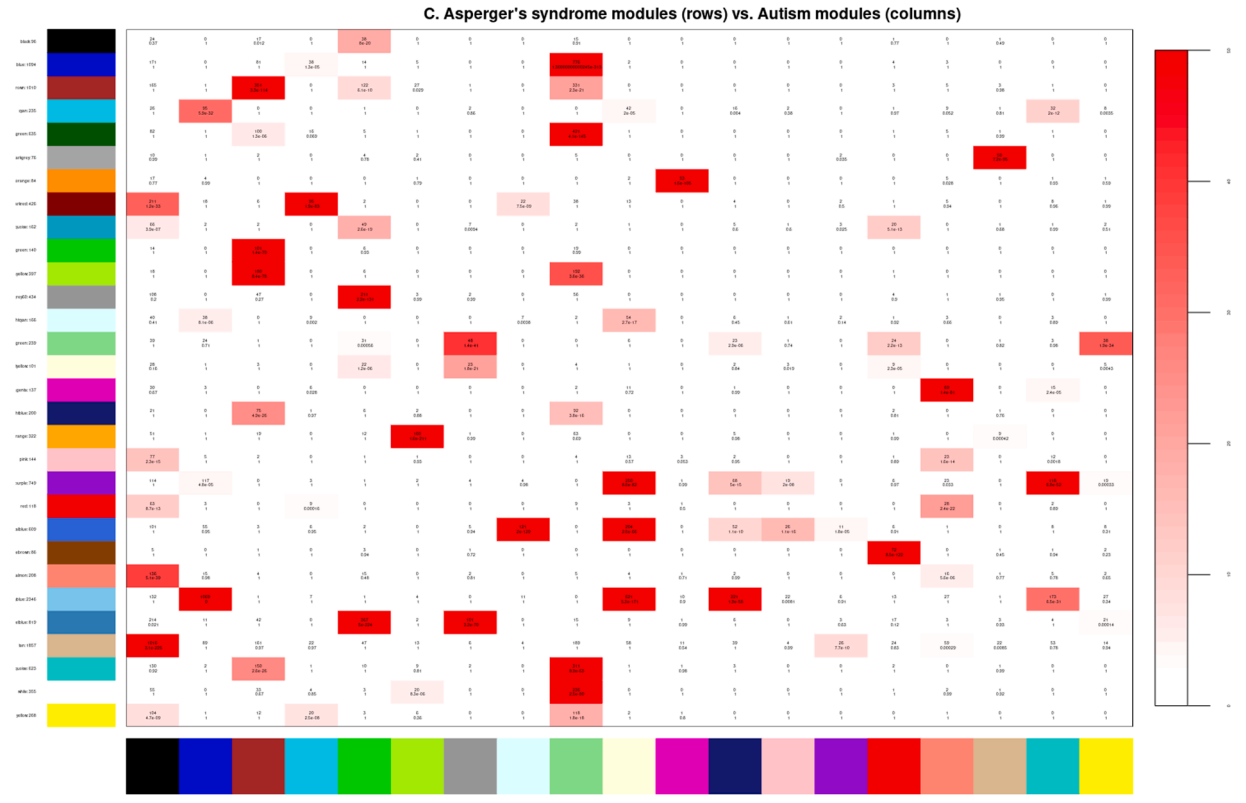


**Fig. 1.** showing the number of overlapping genes between each module from the autism network (columns) and the Asperger's syndrome network (rows), along with the p-value from the hypergeometric test to determine the significance of the overlap.

granules including secretory granule membrane and ficolin-1- rich granule Fig. 4. Brain enrichment analysis showed significant positive enrichment in caudal hippocampal proper, dorsomedial extra striate cortex and superolateral temporal cortex and significant negative enrichment in dorsolateral frontal cortex and ventral parietal cortex Fig. 3B. Disease enrichment analysis revealed that the module is enriched with genes associated with infection and malignancy Supplementary Figure 1B. The hub gene for this module is Glycerol Kinase 3 (GK3) which is involved in glycerol metabolism Supplementary Figure 2B.

The third module is dark Turquoise, unlike other modules, the genes were only significantly enriched for development of neuron projection development (*p-value = 0.0002*). Brain enrichment analysis showed that most of genes in these modules are negatively enriched in the brain specifically frontal polar cortex, choroid plexus of the fourth ventricle and dorsal lateral geniculate nucleus Fig. 3C. Ras and Rab interactor 2 (RIN2) is the hub gene and it is involved in membrane trafficking and cellular transport Supplementary Figure 2 C.

## 4. Discussion

This study was set-out to re-examine the DSM-5 classification of autism spectrum disorders through gene co-expression network preservation analysis. This study focused on Asperger's syndrome because some cases of Asperger's were missed after the new DSM-5 classification. Our results showed that gene co-expression patterns in autism were preserved into Asperger's syndrome. For Asperger's syndrome, most of gene co-expression patterns were similar to autism except for three gene co-expression modules.

The first module "salmon" was enriched with genes that were significantly involved in negative regulation of catabolic processes and T cell differentiation, histone modifications and SWI/SNF pathway. The top hub genes in the module includes mediator of RNA polymerase II transcription (MED12) whose mutations was associated with autistic traits development (Graham & Schwartz, 2013). Another hub gene is ubiquitin specific peptidase 9 (USP9X) which was associated with developmental delay, delayed speech, autistic and behavioural problems and gastrointestinal disturbances (Wang et al., 2020). Chromodomain Helicase DNA Binding Proteins (CHD), Myc binding protein 2, Dynein Cytoplasmic 1 Heavy Chain 1 (DYNC1H) are other genes that were associated with high risk of autistic traits (Wilkinson et al., 2015; Su et al., 2022; AlAbdi et al., 2023).

One of remarkable finding of enrichment analysis is SWI/SNF superfamily type complex or BRM associated factors in humans which is involved in chromatin remodelling. This complex was associated with what is called syndromic autism spectrum disorders. Syndromic ASD is defined as a type of ASD associated with somatic abnormalities and/or neurobehavioral symptoms (Fernandez & Scherer, 2017). The complex is related to the activity-dependent neuroprotective protein (ADNP). ADNP mutation was identified as

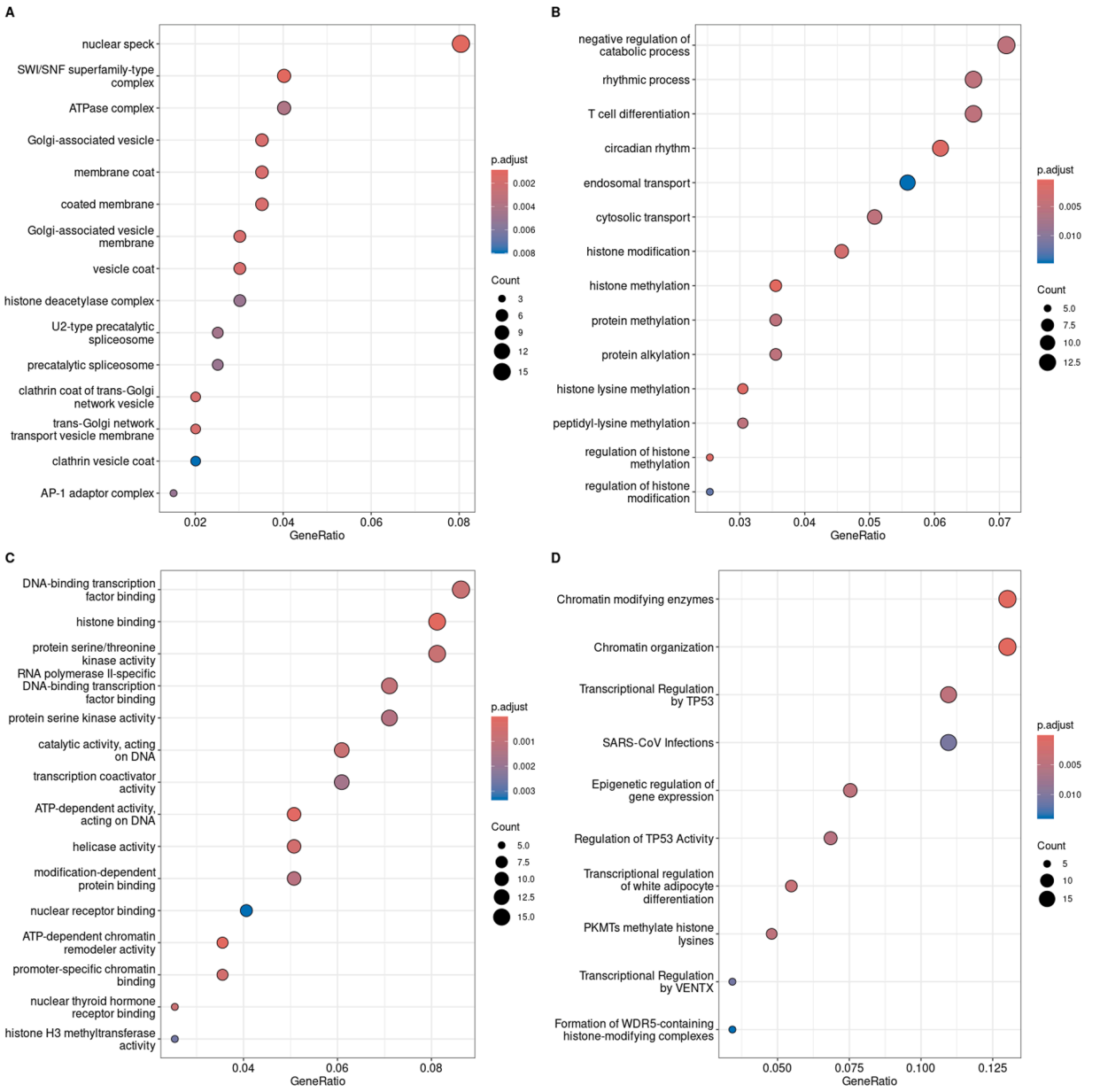


**Fig. 2.** The gene and pathway enrichment analysis results of the Salmon module; A) Cellular components, B) Biologic processes, C) Molecular functions, D) Reactome pathway analysis.

one of the risk factors for development of autistic traits (Vandeweyer et al., 2014). The BAF complexes with ADNP is important for neurodevelopment as it has important role in dendritic growth and axonal development (Lessard et al., 2007). Another chromatin remodelling protein that was associated with SWI/SNF superfamily and syndromic ASD is AT-rich interactive domain-containing protein 1B (ARID1B) (Santen & Clayton-Smith, 2014; Yu et al., 2015). The mutations in these genes were associated with short stature, malformed ear, coarse facies and small hypoplastic 5th fingers but data on how much this was evidenced in children with Asperger's syndrome is lacking (Santen & Clayton-Smith, 2014; Yu et al., 2015). Syndromic ASD is also caused by mutation in CHD4 which is the hub genes for the salmon module. CHD4 encodes an ATP-driven chromatin remodeler, which is a fundamental part of the nucleosome remodeling and histone deacetylation (NuRD) complex (Lai & Wade, 2011). It acts mainly as transcription repressor and is one of main proteins involved in cortical development (Basta & Rauchman, 2015). *CHD4* is involved in multisystemic neuro-developmental disorder characterized by global developmental delay, dysmorphic features and various congenital anomalies (Weiss et al., 2020).

Histone modifications abnormalities are another risk factor for the development of autistic traits (Yoon et al., 2020). For instance, one of genes associated with the risk of autistic traits is *CHD8* which binds with B-catenin for chromatin modifications and acts as

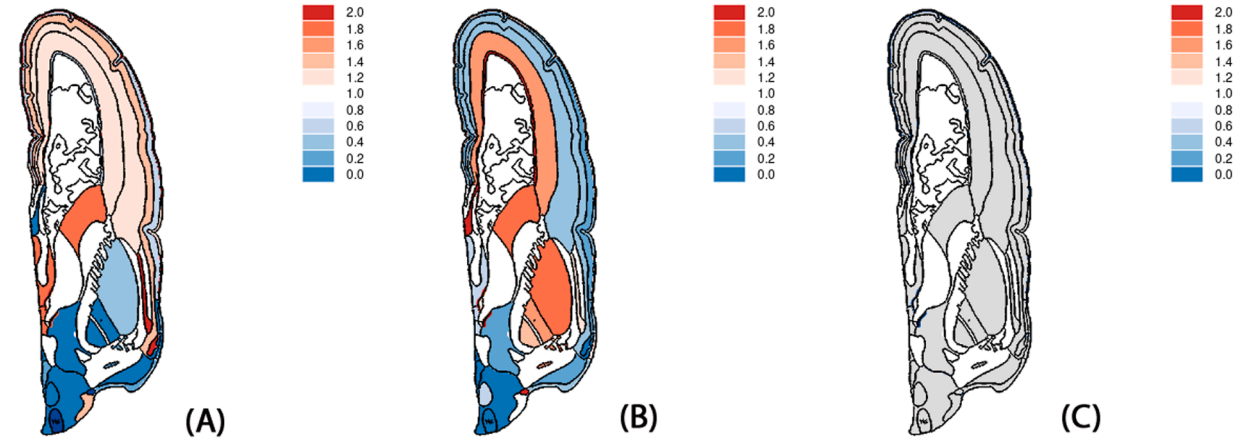


**Fig. 3.** Results of the brain region enrichment analysis in each module, A) Salmon, B) Light yellow, C) Dark Turquoise. The color bar indicates the levels of enrichment, with values below one representing downregulation.

regulator of *Wnt* signaling (Cotney et al., 2015). The mutation of CHD8 was reported to be determinant for specific subtype of ASD (Bernier et al., 2014). This subtype was characterized by developmental delay and gastrointestinal complaints (Bernier et al., 2014). In our study, some individuals with Asperger's syndrome reported having gastrointestinal manifestations and anomalies. It was also reported that *CHD8* regulated other autism risk genes such as histone lysine methyltransferase (*ASH1L)* whose mutation was associated with autistic like traits in ASD (Qin et al., 2021; Yan et al., 2022). Another gene is *SPAST* gene that is involved in cellular trafficking and cellular transport, and its mutations was found in autism with family history of Asperger's syndrome *(*Matthews et al., 2017*)*.

For the negative regulation of catabolic process, Ormstad et al. showed that increased tryptophan and decreased synthesis of 5 hydroxy tryptophan is the hall mark for Asperger's syndrome compared to other subtypes of autism (Ormstad et al., 2018). Another study that was performed on Asperger's syndrome and autistic children revealed there is a defect in the proteolysis of casomorphins (*CM*) that led to higher levels of urine CM-7 (Sokolov et al., 2014). Interestingly, the severity of clinical symptoms was positively correlated with the level of urinary *CM-7* (Sokolov et al., 2014). This pattern was not reported in other subtypes of autism spectrum disorders.

Another catabolic process is proteasome-mediated ubiquitin-dependent protein catabolic process, positive regulation of proteolysis and *NF-kappaB* signalling whose genes were enriched in this module. Moreover, Asperger's syndrome and autism patients had altered proteolysis of mitochondrial *IMMP2L* that was linked to social behavioural changes (Ma et al., 2023).

Another non-preserved module showed enrichment for positive regulation of cytokine production, inflammatory response, neutrophil degranulation, and programmed cell death. Three genes were also involved in cysteine-type endopeptidase activity in apoptotic process. Glycerol kinase is the hub gene in this module. A study showed that autism was associated with abnormalities in polyunsaturated fatty acid metabolism (Das, 2013), however, no specific study investigated glycerol kinase 3 in Asperger's syndrome and Autism. Another hub gene is ankyrin repeat domain 17 which was associated with developmental delay specifically speech delay and was one of novel markers in autism-associated features (Chopra et al., 2021). Another hub gene is BID (BH3 Interacting Domain Death Agonist) which is member of Bcl-2 protein family (Hegde et al., 1998). This gene interacts with caspases in the apoptotic pathway and was considered as one of the therapeutic targets in different psychiatric diseases (Malkesman et al., 2012). This might explain why Asperger's syndrome had a higher rate of psychiatric disorders compared to autism.

Moreover, caspase family was implied as one of the main proteins involved in pathogenesis of autism spectrum disorders which was evident by high plasma *caspase 7* and *caspase 3* level and apoptotic cell markers in male ASD (El-Ansary & Al-Ayadhi, 2012; Malkesman et al., 2012; Ayaydın et al., 2020). In our study, Caspase genes patterns in Asperger's syndrome were among the non-preserved gene networks in individuals with autism spectrum disorders. However, no studies have investigated how Asperger's syndrome is different to autism when it comes to programmed cell death.

In terms of immune response, one of the reported features in Asperger's syndrome was high inflammatory response as evidenced by rhinitis, bronchial asthma, and atopy (Magalhães et al., 2009). The study found high levels of IgE and eosinophils which were not reported in other subtypes of autism (Magalhães et al., 2009). In general, immune dysregulation caused by cytokine imbalance is one of the characteristic features in autism spectrum disorders. The reported imbalance was high levels of *IL-1, IL-8, TNF and INF-ɣ* and increased T-helper 2 inflammatory response (Heidari et al., 2022).

One of interesting enrichments was gene enrichment in secretory granules specifically *ficolin-1* rich granule which is important for activating immune response through complement pathways. It was reported there was high oxidative stress and impaired bioenergetic signals which resulted in impaired granulocytes functions in children with autism (Napoli et al., 2014). The *ficolin 1* is synthesized by monocytes and neutrophil and is important part of neutrophil secretory granules (Rørvig et al., 2009). This also coincides with the enrichment analysis, in which neutrophil degranulation and cytokine release are the top hits. It was also found that neutrophil release high levels of *IL-12* and *IFN-γ*. The neuronal cell death is reported in autistic children as evidenced by high cytokines level including

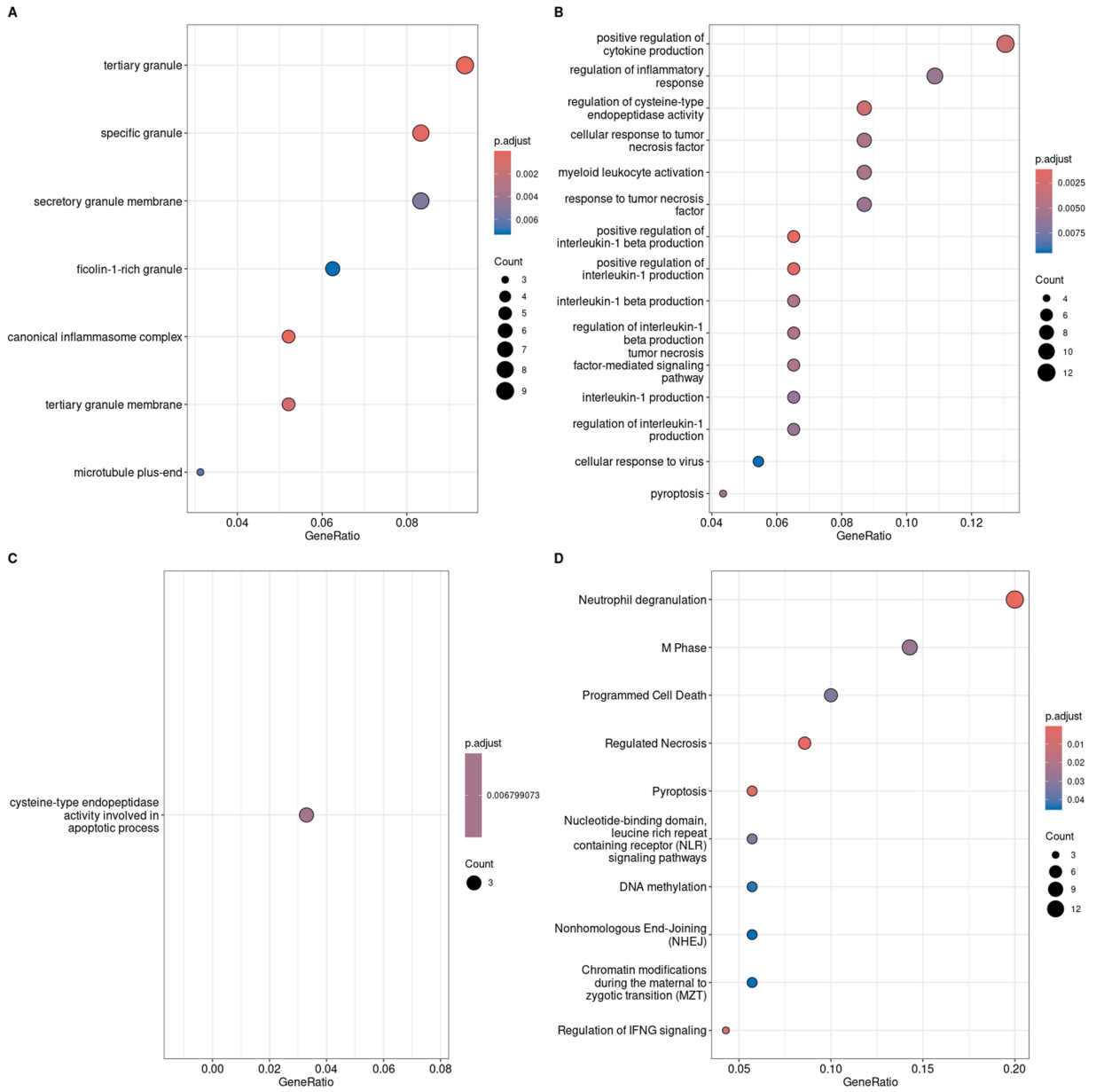


**Fig. 4.** The gene and pathway enrichment analysis results of the light yellow module; A) Cellular components, B) Biologic processes, C) Molecular functions, D) Reactome pathway analysis.

*Transforming growth factor-β* which is highly expressed in ischemia induced brain injury and damaged neurons (Buisson et al., 2003; El-Ansary & Al-Ayadhi, 2012).

The results of two previous modules aligned with results of a study that investigated sex specific differences in lipid metabolism and inflammation profiles (Steeb et al., 2014). They found that females with AS had significant modification in proteins related to lipid transport and metabolism pathways, whereas male with AS had significant changes in inflammation signalling (Steeb et al., 2014).

The dark turquoise module was only enriched with genes associated with neuronal projection development. A study found that Asperger's syndrome has abnormalities in white matter of frontal, parietal, temporal, and occipital lobes and, more specifically, the cingulum, the body of the corpus callosum, and the right cerebellar region (McAlonan et al., 2002). The study reported that these abnormalities resulted from variations in genes encoding neuronal growth (McAlonan et al., 2002). Synaptic proteins such as *neuroligins 3* and 4 (*NLGN4, NLGN4*), *neurexin 1* (*NRXN1*), *contactin associated protein-like* 2 (*CNTNAP2*), and *SHANK3* are involved in the development of the synapses in autism, thus, any impairments of these proteins caused morphometric changes in the brain (Walsh et al., 2008). In addition, this is also reflected on the pathogenesis of Asperger's syndrome as the brain enrichment analysis showed genes are negatively enriched in the lateral geniculate nucleus. Moreover, impaired facial emotional recognition in Asperger's

syndrome is due to impairment of neural signals in lateral geniculate nucleus (Kätsyri et al., 2008). The sensorimotor gating in individuals with Asperger's syndrome is associated with less gray matter volume in fronto-striatal areas which is mainly due to alterations of brain development (McAlonan et al., 2002). It was also reported abnormal functional connectivity in medial temporal lobe was associated with impaired facial recognition and social cognition (Welchew et al., 2005).

Most of our results are discussed in the context of autism studies, as most research has not focused on examining the reported molecular and genetic differences between autism and Asperger's syndrome. However, our results showed that the non-preserved modules still share the same molecular mechanisms with autism. Some studies considered that these similarities should support the current DSM-5 classification as it combined all subtypes in one category (Li et al., 2019). However, this point can be argued because the management of Asperger's syndrome is different from autism (Jordan, 2005; Southall & Gast, 2011). Moreover, many studies have reported that understanding the distinct molecular network pattern of different psychiatric diseases improves the practices of disease management (Hyman, 2000; Grennan et al., 2014). Hence, our results suggest that Asperger's syndrome can be established as a distinct subtype under the umbrella of autism.

In terms of comorbidities, our study found that compared to other subtypes of autism spectrum disorders, children with Asperger's syndrome have higher incidence of ADHD and other psychiatric diseases. This was in line with another research study that showed not only that it had higher incidence of these diseases in patients with Asperger's syndrome but also similar gene expression pattern (González-Peñas et al., 2020).

## 5. Conclusion

In this study, we re-examined the DSM-5 classification of autism spectrum disorders (ASD) through gene co-expression network preservation analysis, with a specific focus on Asperger's syndrome. Our findings reveal that gene co-expression patterns in autism are preserved in Asperger's syndrome, however, Asperger's syndrome has three unique gene co-expression modules that are not present in autism. These modules had gene networks that are implied in the development of autistic traits and neurobehavioral comorbidities. Hence, Asperger's syndrome should be considered a unique entity within the autism spectrum, characterized by specific genetic and molecular patterns. This could improve our understanding and clinical practices, ensuring that individuals receive the most appropriate and effective care based on their unique genetic makeup and associated comorbidities.

## Limitation

Our results should be interpreted cautiously due to the small sample size in each group. There is a significant lack of studies that compared the molecular and biologic signature in autism to Asperger's syndrome. Thus, more studies are needed to explore these differences.

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None.

## CRediT authorship contribution statement

**Sadiq Naveed:** Writing – review & editing, Writing – original draft. **Sherief Ghozy:** Writing – review & editing, Writing – original draft. **Adam A. Dmytriw:** Writing – review & editing, Writing – original draft. **Sara Morsy:** Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

None.

## Data Availability

The data is available in GEO database (GSE18123) and we used WGCNA pipeline available through this link (https://horvath.genetics.ucla.edu/html/CoexpressionNetwork/Rpackages/WGCNA/).

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rasd.2024.102484.

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