



Dysregulation of MECP2, COMT, CACNA1H, and GABRB3 genes in Autism spectrum disorder

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Abstract

Autism is a neurodevelopmental disease that expresses itself in a variety of ways, most often within the first 3 years of a person's life. Difficulties in social communication and repetitive and stereotypical behaviors characterize it. The global prevalence of autism is estimated to be about 1 in 100 children, reaching up to 1 in 36 children in some high-income countries. This research focuses on studying the genetic alteration (MECP2, COMT, CACNA1H, and GABRB3) in children with Autism Spectrum Disorder (ASD) to improve scientific understanding of the biological changes associated with the disease. This study was conducted in Al-Zahra Teaching Hospital in Al-Kut / Iraq assay 75 children aged (3-12). Fifty with autism (40 males and 10 females) and a control group of 25 healthy children (14 males and 11 females). Blood samples were collected from all participants. PCR and gene expression analysis were used to determine the levels of the target genes. Regarding gene expression, the study showed differences at a significance level of $P \leq 0.05$ in the expression of the four target genes (MECP2, COMT, CACNA1H, GABRB3) between autistic children and healthy controls (these genes are associated with various brain functions such as neuronal regulation, neurotransmitters, and neurochemical balance). The difference in the COMT gene was not significant despite its decrease in the patient group, 0.47-fold in patients compared to 1.0 in controls. At the same time, there was a significant increase in the GABRB3 gene in patients, 2.73-fold compared to 1.0 in healthy controls. Conversely, there was a significant decrease in the MECP2 and CACNA1H genes, -2.73 and 0.04-fold, respectively, in the group of patients compared to the healthy controls, 1.0. Based on changes in gene expression of the studied genes, which are related to the regulation of neural gene expression and neurotransmission regulation, it can be concluded that autism is genetically related.

Keywords: Autism Spectrum Disorder (ASD), Gene expression, MECP2, COMT, CACNA1H, GABRB3

Introduction

Autism Spectrum Disorder (ASD) is a complex and heterogeneous neurodevelopmental disorder characterized by persistent deficits in social communication, restricted interests, and repetitive behaviors. First clinically described by Kanner (1943) and Asperger (1944), ASD is now recognized as a spectrum encompassing a wide range of clinical phenotypes and severities (American Psychiatric Association, 2013). The global prevalence of ASD has increased significantly over recent decades, with estimates suggesting approximately 1 in 100 children are affected worldwide, and higher rates reported in developed countries such as the United States (Zeidan *et al.*, 2022; Maenner *et al.*, 2020). Although its etiology remains multifactorial, growing evidence supports a strong genetic contribution to ASD, with numerous genes implicated in its pathophysiology (Durand *et al.*, 2007; Lai *et al.*, 2014).

Among the candidate genes of interest, *MECP2*, *COMT*, *CACNA1H*, and *GABRB3* have emerged as critical

regulators of neuronal function and neurodevelopment. The *MECP2* gene, located on Xq28, encodes a methyl-CpG binding protein that plays a central role in epigenetic regulation by modulating gene expression in neurons; its dysregulation is linked to autism and Rett syndrome (Neul, 2012; Pejhan and Rastegar, 2021).

The *COMT* gene (catechol-O-methyltransferase), situated on chromosome 22q11, is essential for dopamine metabolism, particularly within the prefrontal cortex, and has been associated with cognitive and behavioral disturbances observed in ASD (Esmail *et al.*, 2020; Tunbridge *et al.*, 2011).

Additionally, the *CACNA1H* gene encodes the $\alpha 1H$ subunit of T-type calcium channels (Cav3.2), which are crucial for neuronal excitability and neurotransmission. Mutations in *CACNA1H* have been linked to altered calcium signaling and ASD risk (Splawski *et al.*, 2006; Weiss and Zamponi, 2020).

Furthermore, *GABRB3* encodes the $\beta 3$ subunit of the GABA-A receptor, a major inhibitory receptor in the

central nervous system. Alterations in *GABRB3* expression can disrupt inhibitory signaling and have been implicated in ASD-related sensory processing abnormalities (Chen *et al.*, 2014; Tavassoli *et al.*, 2012).

Understanding the expression patterns and functional roles of these genes in individuals with ASD is vital to elucidating the molecular mechanisms underlying the disorder and may provide novel biomarkers or therapeutic targets for early intervention.

Materials and Methods

Study sample description

The study included a total of 75 children with age rang (3-12) comprising 50 patients diagnosed with autism spectrum disorder and 25 healthy as control.

The majority of ASD cases falling between the 5 to 8 years age range. The ASD group consisted of 40 males (80%) and 10 females (20%). All patients studded were from the Al-Zahra Teaching Hospital in Al-Kut / Iraq.

Diagnosis of ASD

All ASD diagnoses were confirmed based on clinical evaluation and standardized behavioral assessments specifically the Childhood Autism Rating Scale-Second Edition (CARS-2) and used to stratify symptom severity. The diagnosis was carried out by a consultant psychiatrist.

Molecular study

Total RNA extraction

Total RNA of all samples was extracted using the TRIzol® LS Reagent following the protocol provided by the manufacturer (Simões *et al.*, 2012)

Primers design

The design process for primers was obtained by Primer3 web version 4.1.0 (online at website <http://primer3.ut.ee>) for *CACNA1H*, *COMT*, *GABRB3*, *MECP2* genes and *GAPDH* gene then checked by the University Code of Student Conduct (UCSC) programs. They were synthesized and lyophilized by Alpha DNA Ltd. (Canada). Table (1) displays all primer sequences utilized in this study's assays.

Table (1): The primers sequence that used in the study

| Primer | Sequence (5'→3' direction) | Primer size per base pair | Product size base pair | Temperature in Celsius |
|--|----------------------------|---------------------------|------------------------|------------------------|
| Calcium Voltage-Gated Channel Subunit Alpha1 H Gene (<i>CACNA1H</i>) | | | | |
| Forward | AACCTGGGCCTTCTTTTCAT | 20 | 195 | 59.94 C° |
| Reverse | CTTCATGATCCCGTTCCAGT | 20 | | 59.93 C° |
| Catechol-O-Methyltransferase Gene (<i>COMT</i>) | | | | |
| Forward | TCCTGGAATACAGGGAGGTG | 20 | 195 | 59.92 C° |
| Reverse | CGAGGTGTGCTTTGCATTTA | 20 | | 59.87 C° |
| Gamma-Aminobutyric Acid A Receptor Beta 3 Gene (<i>GABRB3</i>) | | | | |
| Forward | CTGTGGAGGGGTTTCACTGT | 20 | 206 | 60.00 C° |
| Reverse | CGCTGTGTTTGGACAAGAGA | 20 | | 60.02 C° |
| Methyl CpG Binding Protein 2 Gene (<i>MECP2</i>) | | | | |
| Forward | CCGTGACCGAGAGAGTTAGC | 20 | 192 | 60.01 C° |
| Reverse | CCAACTACTCCCACCCTGAA | 20 | | 59.96 C° |
| Glyceraldehyde 3-Phosphate Dehydrogenase Gene (<i>GAPDH</i>) | | | | |
| Forward | CCCCTTCAATTGACCTCAACTAC | 22 | 135 | 60.3 C° |
| Reverse | CGCTCCTGGAAGATGGTGA | 19 | | 61.6 C° |

CDNA synthesis

Total RNA was reversely transcribed to complementary DNA (cDNA) using Easyscript® Kit.

The procedure was carried out in a reaction volume of 20 µl according to the manufacturer's instructions.

Quantitative real time PCR

The expression levels of *CACNA1H*, *COMT*, *GABRB3*, and *MECP2* genes were estimated by Quantitative Real Time PCR (qRT-PCR). To confirm the expression of target gene, *TransStart*[®]Top Green qPCR Super Mix (SYBR Green) was used. Primers sequences for these genes were synthesized by Alpha DNA Ltd (Canada) and stored lyophilized at (-20°C). The mRNA levels of endogenous control Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) housekeeping gene were used as an internal control to be used to normalize the mRNA levels of the target genes.

Statistical analysis

Statistical analyses were conducted using SPSS 27 (2020). Data are presented as mean ± standard deviation. one-way ANOVA, independent T-test and Pearson correlation coefficient were used to assess significant differences and correlations among markers at P≤0.05.

Results

The distribution of sex and age in autism patients and healthy controls was clearing in Table (2).

Table (2): Distribution of sex and age in autism patients and healthy controls

| Autism Patients | | | Healthy Controls | | |
|---------------------|--------|------------|------------------|--------|------------|
| Distribution of Sex | | | | | |
| Sex | Number | Percentage | Sex | Number | Percentage |
| Female | 10 | 20% | Female | 11 | 44% |
| Male | 40 | 80% | Male | 14 | 56% |
| Total | 50 | 100% | Total | 25 | 100% |
| Distribution of Age | | | | | |
| Age Group | Number | Percentage | Age Group | Number | Percentage |
| >5 year | 8 | 16% | >5 year | 6 | 24 |
| 5-8 year | 29 | 58% | 5-8 year | 10 | 40 |
| <8 year | 13 | 26% | <8 year | 9 | 36 |
| Total | 50 | 100% | Total | 25 | 100% |

Table (3) presents the gene folding expression levels of *MECP2*, *COMT*, *CACNA1H*, and *GABRB3* in individuals with Autism Spectrum Disorder (ASD) compared to healthy control subjects. The table

includes the mean values and standard deviations for each gene, along with statistical significance determined by independent samples t-tests.

Table (3): *MECP2*, *COMT*, *CACNA1H* and *GABRB3* Genes folding in autism patients and healthy controls

| Gene | Group | N | Mean ± Standard Deviation | P-value |
|----------------|--------------|----|---------------------------|------------------|
| <i>MECP2</i> | ASD Patients | 50 | -2.73 ± 1.27 | 0.000 † S |
| | Control | 25 | 1.0 | |
| <i>COMT</i> | ASD Patients | 50 | 0.47 ± 0.16 | 0.202 † NS |
| | Control | 25 | 1.0 | |
| <i>CACNA1H</i> | ASD Patients | 50 | 0.04 ± 0.01 | 0.009 † S |
| | Control | 25 | 1.0 | |
| <i>GABRB3</i> | ASD Patients | 50 | 2.73 ± 1.31 | 0.000 † S |
| | Control | 25 | 1.0 | |

N: Number; †: Independent Samples T-Test; NS: Not Significant at P≤0.05; S: Significant at P≤0.05.

For the *MECP2* gene, ASD patients (n=50) exhibited a significantly down regulated expression level with a

mean ± SD of -2.73 ± 1.27 compared to the control group (n=25), which had a normalized expression value of 1.0.

The difference was statistically significant with a P-value of 0.000, indicating a strong association between MECP2 down regulation and ASD. In the case of the COMT gene, the mean expression in ASD patients was 0.47 ± 0.16 , while it remained at 1.0 in the control group. However, the P-value was 0.202, suggesting that the difference was not statistically significant (NS).

Regarding the CACNA1H gene, ASD patients had a markedly lower mean expression 0.04 ± 0.01 compared to the control group 1.0, this reduction was statistically significant, with a P-value of 0.009, indicating a potential involvement of CACNA1H gene dysregulation in ASD.

Lastly, the GABRB3 gene showed a substantial increase in expression among ASD patients 2.73 ± 1.31 , whereas the control group maintained a value of 1.0, this difference was also highly significant with a P-value of 0.000.

Table (4) illustrates the frequency distribution of gene folding for MECP2, COMT, CACNA1H, and GABRB3 according to sex in individuals diagnosed with Autism Spectrum Disorder (ASD) compared to healthy controls. The table includes the mean gene expression values along with standard deviations, Least Significant Difference (LSD), and P-values, analyzed using a one-way ANOVA test.

For the MECP2 gene, the control group (n=25) showed a normalized expression value of 1.0. In contrast, ASD males (n=40) exhibited a significantly downregulated expression level of -3.09 ± 1.47 , while ASD females (n=10) showed a less pronounced decrease at -1.29 ± 0.42 . The P-value was 0.000, indicating a statistically significant difference across groups, with different letters (A, B, C) confirming the significance of changes between the control, ASD male, and ASD female subgroups.

For the COMT gene, both ASD males and females had the same mean expression value of 0.47, with standard deviations of ± 0.19 and ± 0.21 , respectively. The control group remained at 1.0. However, the P-value was 0.666, indicating that the observed differences were not statistically significant.

In the case of the CACNA1H gene, the control group had an expression value of 1.0. ASD males showed a reduced expression at 0.13 ± 0.06 , while ASD females had a negative mean value of -0.32 ± 0.12 . Despite these differences, the P-value was 0.138, suggesting that the group differences were not statistically significant.

Regarding the GABRB3 gene, the control group had a normalized value of 1.0, whereas ASD males and females showed elevated expression levels of 2.79 ± 1.18 and 2.51 ± 1.21 , respectively. The P-value was 0.031, indicating a statistically significant difference in gene expression across the groups.

Table (4): Frequency distribution of MECP2, COMT, CACNA1H and GABRB3 genes folding according to sex

| Gene | Group | N | Mean ± Standard Deviation | LSD | P-value |
|---------|------------|----|-------------------------------|------|------------------|
| MECP2 | Control | 25 | ^A 1.0 | 0.71 | 0.000 A S |
| | ASD Male | 40 | ^B -3.09 ± 1.47 | | |
| | ASD Female | 10 | ^C -1.29 ± 0.42 | | |
| COMT | Control | 25 | ^A 1.0 | 0.77 | 0.666 A NS |
| | ASD Male | 40 | ^A 0.47 ± 0.19 | | |
| | ASD Female | 10 | ^A 0.47 ± 0.21 | | |
| CACNA1H | Control | 25 | ^A 1.0 | 0.67 | 0.138 A NS |
| | ASD Male | 40 | ^B 0.13 ± 0.06 | | |
| | ASD Female | 10 | ^C -0.32 ± 0.12 | | |
| GABRB3 | Control | 25 | ^A 1.0 | 0.86 | 0.031 A S |
| | ASD Male | 40 | ^B 2.79 ± 1.18 | | |
| | ASD Female | 10 | ^B 2.51 ± 1.21 | | |

N: Number; LSD: Least Significant Difference; A: One Way ANOVA Test; NS: Not Significant at $P \leq 0.05$; S: Significant at $P \leq 0.05$

Note: Different Letters within Gene Groups Denote to the Significant Differences at $P \leq 0.05$

The correlations between all studied parameters in autism patients were shown in table (5). The present results show significant positive correlation at $P \leq 0.05$ between *GABRB3* and *COMT* ($r=0.320$, $p=0.023$). Furthermore, the present results show significant positive correlation at $P \leq 0.01$ between *MECP2* and *GABRB3* ($r=0.396$, $p=0.004$), *CACNA1H* and *GABRB3* ($r=0.753$, $p=0.000$), *COMT* and *CACNA1H* ($r=0.414$, $p=0.003$). The rest of the results are not show significant correlation between all other parameters.

Table (5): Pearson coefficient correlation between the parameters for autism patients' group

| | | GABRB3 | COMT | CACNA1H |
|---------------|---|---------------|-------------|----------------|
| <i>MECP2</i> | R | 0.396** | 0.133 | 0.227 |
| | P | 0.004 | 0.357 | 0.113 |
| <i>GABRB3</i> | R | 1 | 0.320* | 0.753** |
| | P | | 0.023 | 0 |
| <i>COMT</i> | R | | 1 | 0.414** |
| | P | | | 0.003 |

*: Correlation is significant at the 0.05 level (2-tailed); **: Correlation is significant at the 0.01 level (2-tailed); R: Pearson Correlation; P: P-Value

Discussion

The effect of genetic polymorphisms (SNPs) on brain shape in premature infants was examined. Among the genes analyzed was the *COMT* gene, which has a known role in regulating neurotransmitters such as dopamine and has been previously associated with psychiatric and neurological disorders, including autism spectrum disorder, anxiety, and schizophrenia. A common variation in the *COMT* gene (often referred to as Val158Met or rs4680) was found to be associated with altered cerebral curvature in premature infants. This altered brain shape is believed to be an early indicator of atypical neurodevelopmental trajectories and may later contribute to an increased risk of disorders such as autism (Kim *et al.*, 2022).

Esmail *et al.*, (2020) examines the role of the *COMT* gene in regulating dopamine levels in the brain. There are several genetic variants of the gene that affect the activity of this enzyme, such as rs8192488 [C>T], rs4680 (Val158Met), and rs4818 [C>G]. The polymorphisms of the *COMT* gene have also been suggested in the pathogenesis of ASD. One of the

genetic variants, CC/MM/CG, showed a significant decrease in dopamine levels, while the CC/MM/CC variant showed a significant increase in dopamine levels.

In a study designed on Egyptian children with ASD there was an association between *COMT* Val158Met polymorphism and hyperactivity symptoms. Genetic polymorphisms can affect gene expression, depending on the type and location of the polymorphism. In the case of the Val158Met polymorphism in the *COMT* gene, the presence of this polymorphism affects gene function and, consequently, gene expression. When there are variations in genetic polymorphisms, such as Val158Met, this results in differences in the amount of the enzyme *COMT* produced and its effectiveness in breaking down dopamine. These differences in gene expression can affect dopamine levels in different brain regions (Karam *et al.*, 2013).

In research focused on how genetic factors influence the response of individuals with autism spectrum disorder to drug treatment. Variations in the *COMT* gene can lead to unexpected or heterogeneous responses to medications that affect dopamine or other neurotransmitters. Some versions of the *COMT* gene may be associated with different levels of enzyme activity, thereby affecting dopamine concentrations in the brain. This can result in variability in individual responses to treatments, such as medications that target dopamine pathways (Simpson *et al.*, 2014).

In a study aimed at understanding how genes can influence individuals' response to psychiatric medications, particularly in patients with autism spectrum disorder. The effectiveness of *COMT* depends on the genetic version an individual carries. Individuals can have an active or inactive version of the gene, which affects how dopamine is broken down. People with a low-activity version of *COMT* may show a better response to some medications due to higher levels of dopamine in the brain. Whereas, people with a high-activity version of *COMT* may have difficulty achieving the full benefits of medications that affect dopamine (de Miguel *et al.*, 2023).

The study investigated the role of rare variants in voltage-gated calcium channel (VGCC) genes in Autism Spectrum Disorder (ASD) by analyzing whole

genome sequences from 105 families. Researchers identified 53 rare damaging inherited variants in VGCC genes in 40 families, with particular focus on biallelic missense variants in the *CACNA1H* gene, which encodes the Cav3.2 T-type calcium channel. Electrophysiological analysis revealed that three out of four *CACNA1H* variants slightly altered channel function, potentially disrupting calcium signaling in neurons. These findings support the involvement of *CACNA1H* variants in ASD, highlighting their potential contribution to ASD susceptibility (Viggiano *et al.*, 2022).

A study found that *CACNA1H* knockout (KO) mice exhibited autistic-like behaviors, including impaired social novelty recognition, increased anxiety, and repetitive grooming. Although brain size and weight were unaffected, KO mice showed a significant reduction in hippocampal neuron numbers and increased dendritic spine density in the dentate gyrus, with no change in spine maturity. These findings suggest that *CACNA1H* gene deletion may contribute to autism-like phenotypes through altered hippocampal neuronal structure and connectivity (Jiao *et al.*, 2022).

Splawski *et al.* (2006) performed case-control research with 461 patients and 480 controls, all of Caucasian descent, and identified four missense loss-of-function variants (R212C, R902W, W962C, and A1874V) in the *CACNA1H* gene among six of the 461 ASD cases. These mutations were identified in the essential area of the protein and resulted in diminished currents that reduced current conductance, leading to lower channel function.

A study by DeLorey *et al.* (2011) showed that mice with a heterozygous mutation in the *GABRB3* gene—having one normal and one defective copy exhibited notable physical and sensorimotor impairments. These included lower body weight, reduced muscle strength, impaired coordination (evident in tests like the Rota rod and grip strength), and abnormal tactile responses. The findings indicate that even partial loss of *GABRB3* function can cause physiological and behavioral changes, supporting its essential role in neural circuits involved in sensory and motor functions often disrupted in ASD. Notably, this study was the first to report tactile and heat hypersensitivity in an ASD mouse model.

A study investigated the association between *GABRB3* gene variants and autism spectrum disorders (ASD) in 356 patients and 386 controls. While common SNPs showed no association with ASD, rare variants were significantly more frequent in patients. Notably, 12 rare variants in the 5' regulatory region were found only in ASD patients. Reporter gene assays showed that several of these variants increased gene expression. Family analysis indicated most variants were inherited. These findings suggest that rare regulatory and exonic *GABRB3* variants may contribute to ASD by enhancing gene expression, highlighting a potential role for *GABRB3* dysregulation in ASD pathogenesis (Chen *et al.*, 2014).

A study investigated the association between specific genetic variants and autism, focusing on GABA and serotonin receptor genes. Among the analyzed variants, the rs2081648 (T/C) polymorphism in the *GABRB3* gene showed no statistically significant difference in homozygous C genotype frequency between the autism group and the control group ($p = 0.36$). These findings suggest that the *GABRB3* gene variant is not a major risk factor for autism in the studied population. Unlike other variants, *GABRB3* rs2081648 does not appear to have a direct contribution to autism susceptibility based on the current data (Coskunpinar *et al.*, 2023).

In a pilot study, the genetic variant rs4906902 of the *GABRB3* gene and others was found to significantly increase the risk of autism spectrum disorders (ASD). The study involved 192 ASD probands, their parents, and 184 healthy controls from West Bengal. The rs4906902G allele showed a significant association with ASD, and it was also linked to deficits in ASD-related phenotypes such as "relating to people," "imitation," "emotional response," and others. The transmission bias of rs4906902G was observed in family-based analysis, indicating a potential genetic risk factor for ASD in this population (Adak *et al.*, 2021).

Warrier *et al.*, (2013) investigates the association between the *GABRB3* gene and autism spectrum conditions (ASC), particularly Asperger syndrome (AS), as well as related cognitive and behavioral traits. Analyzing 45 single nucleotide polymorphisms (SNPs) within *GABRB3*, the researchers found several SNPs significantly associated with AS and with

specific cognitive measures such as empathy, systemizing, and spatial processing. Haplotype analyses further identified significant genetic regions linked to AS. These findings support the involvement of *GABRB3* in both ASC and individual differences in associated endophenotypes, highlighting its role as a key genetic candidate in autism related traits.

In a study examining the relationship between genetic imprinting and brain development genes and their role in the development of autism spectrum disorder, the study found that *GABRB3*: exhibits differential expression (changes in activity) during brain development. It is an imprinted gene (expressed by only one parent). Functional enrichment analysis revealed that the gene is linked to biological pathways such as: GABA signaling pathway (important in the balance of neural activity), regulation of synaptic transmission as well as memory and learning (Li *et al.*, 2020).

The meta-analysis by Mahdavi *et al.* (2018) found no significant association between *GABRB3* gene polymorphisms and autism spectrum disorders (ASD). The overall odds ratio was 0.846 (95% CI: 0.595–1.201), and specific SNPs such as rs2081648 and rs1426217 also showed no significant links, suggesting that *GABRB3* variants are not associated with ASD in the studied populations.

In a study focusing on the relationship between genes located on chromosome 15q11-q13, especially *GABRG3* and autism among individuals of Han Chinese descent, several rare variants in the *GABRB3* gene were identified in autistic patients. Specific details about the rare variants found in this gene were not provided, but it was noted that six rare variants were discovered in the *GABRB3* gene in children with autism. This suggests that this gene may play a role in causing autism (Wang *et al.*, 2018).

The research investigated the correlation between genetic variation in the *GABRB3* gene and tactile sensitivity in individuals. Two assessments of tactile sensitivity were employed: a parent report and a behavioral measure. The research identified three SNPs (rs11636966, rs8023959, and rs2162241) that were marginally correlated with both symptoms, providing internal confirmation of the findings. Furthermore, parent-reported scores exhibited a nominal association with six SNPs, but the behavioral

assessment of tactile sensitivity showed a nominal association with ten SNPs, three of which retained significance after Bonferroni correction. This work is the first human investigation establishing a correlation between *GABRB3* variation and tactile sensitivity, corroborating findings from animal models that suggest *GABRB3*'s involvement in abnormal sensory sensitivity in autism spectrum disorders (Tavassoli *et al.*, 2012; Masih *et al.*, 2025).

MECP2 is a crucial regulator of cerebral function throughout development and into maturity. Mutations that result in loss of function in the *MECP2* gene are responsible for Rett syndrome. Moreover, mutations in *MECP2* or diminished expression have been implicated with many additional neurological and neuropsychiatric illnesses, including X-linked intellectual disability, Angelman syndrome, autism spectrum disorder, severe newborn encephalopathy, and Huntington's disease (D'Mello III, 2021).

The study investigated the impact of maternal separation (MS) stress on *MECP2* gene expression in the hippocampus and evaluated the potential protective effects of Umbelliprenin (UMB). Results showed that MS significantly reduced *MECP2* expression, which is associated with autism-like behaviors. Treatment with UMB restored *MECP2* expression levels in the hippocampus, suggesting its neuroprotective role. The findings imply that UMB may alleviate MS-induced behavioral deficits partly by upregulating *MECP2*, highlighting its potential therapeutic value in conditions linked to *MECP2* dysregulation, such as autism spectrum disorder (Karimi *et al.*, 2024).

In a recent study conducted on mice to study some genes that cause autism, including *MECP2*, researchers used a technique called mRNA boosters. This involves adding a poly (A) tail to the 3' end of specifically targeted mRNAs. This technology, which term mRNA boosters, lends itself to uses on haploinsufficiency disorders, where reduced gene expression manifests in a disease state. Gene expression of the studied genes, including *MECP2*, increased in both animal models and human cell cultures (Torkzaban *et al.*, 2025).

Artificial autism models were created in mice by injecting them with ethyl formic acid, and then they were treated with both Neuquinon (NQ) and its

improved version, Liposomal Neuquinon (LNQ), as the improved version can improve the absorption of the drug, and help it cross the blood-brain barrier and reach the brain. After that, some neurotransmitters and gene expression of some important proteins in the brain were studied, including *MECP2*, as its levels increased after using the treatment compared to the autism model (Mahmoud *et al.*, 2024).

The measuring the expression of a common protein *MECP2* in the prefrontal cortex of autistic individuals, which coincides with abnormal changes in the methylation of the promoter region of the *MECP2* gene, a significant decrease in *MECP2* protein was found in most cases, 79% of the total autism samples, compared to the control group, while a significant increase in the methylation status of the gene was found (Nagarajan *et al.*, 2006).

Pejhan and Rastegar (2021) demonstrated that neurons with appropriate *MECP2* levels have typical neuronal architecture, but neurons with diminished *MECP2* levels are linked to autism. Conversely, neurons exhibiting *MECP2* loss or gain of function are impacted in Rett syndrome and *MECP2* duplication syndrome, respectively.

A study demonstrates that hippocampal *MECP2* knockdown in rats induces behavioral abnormalities resembling core symptoms of autism spectrum disorder (ASD), offering a novel model for exploring therapeutic strategies. Building on prior associations between *MECP2* dysfunction and ASD, researchers evaluated the effects of ketamine, an NMDA receptor antagonist, on ASD-like behaviors. Using both *MECP2* knockdown and valproic acid (VPA)-induced rat models, they analyzed gene expression and behavioral outcomes. Remarkably, a single dose of ketamine significantly reversed social impairments in *MECP2* knockdown rats, suggesting its potential as a rapid-acting therapeutic agent for ASD. This model provides new insights into ASD pathophysiology and treatment (Choi *et al.*, 2022).

Therefore, present study demonstrates significant alterations in the expression of *MECP2*, *CACNA1H*, and *GABRB3* genes among children with autism spectrum disorder, reinforcing their involvement in the molecular mechanisms underlying ASD. The upregulation of *GABRB3* and the downregulation of *MECP2* and *CACNA1H* suggest a dysregulation of

inhibitory signaling and neuronal excitability, which may contribute to ASD-related phenotypes. Although *COMT* expression did not differ significantly, its positive correlations with other genes warrant further investigation. These findings support the potential use of these genes, particularly *GABRB3*, as promising molecular biomarkers for early detection and targeted therapeutic approaches in ASD.

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