



Altered Global mRNA Expressions of Pain and Aggression Related Genes in the Blood of Children with Autism Spectrum Disorders

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Abstract

Autism spectrum disorder (ASD) is characterized by repetitive stereotypic behaviors, restricted interests, social withdrawal, and communication deficits. Aggression and insensitivity to pain are largely unexplained in these cases. We analyzed nine mRNA expressions of the candidate genes related to aggression and insensitivity to pain in the peripheral blood of patients with ASD. Whole blood samples were obtained from 40 autistic patients (33 boys, 7 girls) and 50 age- and sex-matched controls (37 boys and 13 girls) to isolate RNA. Gene expression was assessed by quantitative Real-Time PCR (qRT-PCR) in the Erciyes University Genome and Stem Cell Center (GENKOK). All of the gene expressions except *CRHR1* and *SLC6A4* were found to be statistically different between the ASD patients and controls. Gene expression also differed according to gender. Alterations in the mRNA expression patterns of the *HTR1E*, *OPRL1*, *OPRM1*, *TACR1*, *PRKG1*, *SCN9A* and *DRD4* genes provide further evidence for a relevant effect of the respective candidate genes on the pathophysiology of ASD. Future studies may determine the sensitivity of these candidate markers in larger samples including further neuropsychiatric diagnosis.

Keywords Autism spectrum disorders · Gene expression · Aggression · Insensitivity to pain

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Introduction

Autism spectrum disorder (ASD) is manifested by common social interaction and communication abnormalities and a severe degree of limited interest and excessive repetitive behaviors (Sener et al. 2016). Approximately 50% of patients have intellectual disability, and comorbidity with neurodevelopmental and psychiatric disorders is also frequent (Woodbury-Smith and Scherer 2018). The prevalence of ASD is approximately 1/68, according to the results of recent studies (Rose et al. 2015). It is well established that ASD is more prevalent in males than in females (Waye and Cheng 2018). With the availability of new sequencing methodologies, major advances have been made in our understanding of the genetic background of autism (Woodbury-Smith and Scherer 2018). Autistic children may excessively react to stimulations from one or several senses (taste, touch, hearing, sight, etc.) or may be unresponsive. The prevalence of insensitivity to pain in autism is approximately 25–40% (Moore 2015). Children with ASD have an abnormal sensitivity to painful stimuli, and hypersensitive responses to pain have been described in individuals with ASD (Riquelme et al. 2018). Both experimental and case studies imply that individuals with ASD can show insensitivity to pain (Allely 2013; Ross-Russell and Sloan 2005; Mieres et al. 2011; Elwin et al. 2012; Tordjman et al. 2009). It has been considered that the stereotypic behaviors and self-mutilating behavior often observed in individuals with ASD are also related to the insensitivity to pain (Smith and Matson 2010; Furniss and Biswas 2012). Insensitivity to pain which is a part of an unusual response is the subject of our research, but the studies regarding the perception of pain and its forms of expression in individuals with ASD are limited. However, this relationship has not yet been fully understood (MacClean et al. 2010). Aggression is a complex social behavior that involves a similarly complex neurochemical background (Katsouni et al. 2009). The available data indicated that aggression is a common problem reported in individuals with ASD and intellectual disability (ID) (Farmer and Aman 2010; Benson and Brooks 2008; Holden and Gitlesen 2006). Although it is hypothesized that ASD is related to aggression, very few systematic studies have been conducted thus far (Farmer and Aman 2010).

This study was based on the commonly accepted terms of ASD, such as “pain” or “insensitivity to pain” and “aggression” to select genes. Our previous preliminary study supported this hypothesis in ASD (Sener et al. 2017). *OPRL1* and *OPRM1* (opiate receptors that are associated with pain management) and *TACR1* genes are related to pain (Provavi 2015; Steinhoff et al. 2014; Becker et al. 2014). *SCN9A* also plays a role in pain mechanisms, especially in the development of inflammatory pain (Meijer et al. 2014; Spratt et al. 2015). *PRKGI* has a major role in nociception (Zhao et al. 2013). Serotonin (5-HT) as a neurotransmitter plays a major role in

neural plasticity as a neurotransmitter (Kepser and Homberg 2015). It has been determined in recent years that 5-HT acts as a neurotrophic factor and not only influences brain functioning but also influences brain development (Miceli et al. 2013). Some studies have found that autistic children have elevated whole blood serotonin levels (hyperserotonemia), which is considered to be the most commonly observed findings and well-replicated change in ASD patients (Coutinho et al. 2007). *HTR1E* (5-hydroxytryptamine (serotonin) receptor 1 E) is a gene that was studied in the groups with attention deficit hyperactivity disorder (ADHD), and it was found to be correlated with low serotonergic activity and aggressive behavior in the ADHD group (Turecki et al. 2003; Oades et al. 2008). There is a relationship between autism and serotonin (5-hydroxytryptamine; 5-HT); this relationship has been previously shown by several studies (Longo et al. 2009; Koishi et al. 2006). The *SLC6A4* gene (*SERT* or *5-HTT*) modulates serotonergic neurotransmission by the active reuptake of 5-HT from the synaptic cleft into presynaptic nerves (Huang and Santangelo 2008). This gene is thought to have contributed to the clinical picture of ASD (Ro et al. 2013; Valencia et al. 2012). The *DRD4* gene encodes the D4 subtype of the dopamine receptor and the dopaminergic system is implicated in certain human behaviors, such as aggression (Gizer et al. 2009). In addition, the polymorphisms of these genes have been investigated in anxiety disorders and ASD (Gadow et al. 2010). The mRNA expression of the *DRD4* gene was studied in the ADHD and ASD groups, and further studies including other neuropsychiatric diseases have been suggested to reveal *DRD4* as a biomarker (Taurines et al. 2011). The *CRHR1* gene encodes a G protein coupled receptor that binds neuropeptides of the corticotropin releasing hormone family and regulates the hypothalamic-pituitary-adrenal (HPA) pathway. Diseases associated with *CRHR1* include depressive and anxiety disorders (Bortoluzzi et al. 2015; Ressler et al. 2010).

With this study, starting from the clinical symptoms, such as aggression and insensitivity to pain, the genes related to aggression and pain insensitivity were investigated. It is predicted that these genes may be highly associated with autism. The aim of our study was to explain the differences in candidate gene expression in whole blood samples of ASD patients. It is not easy to obtain postmortem brain tissue; this study was performed on the whole blood samples. We also aimed to reveal the relationship between genes and the clinical aspects of autism and assess genes as potential disease markers. In our previous study, we investigated *OPRL1*, *TACR1*, and *HTR1E* gene expression in a small ASD group and we have indicated that these genes have a decreased expression in patients. To our knowledge, the expression of these genes in children with ASD has not been previously investigated in the literature.

Methods

Study Design and Patient Collection

The study was approved by the ethical committee of the Faculty of Medicine, Erciyes University (2014/26). Written informed consent forms were received from the parents. The study conformed to the code of ethics stated in the Declaration of Helsinki. The clinical evaluation of the ASD patients in the present study was based on the clinical history from clinical examination, and neuropsychiatric assessment. Among the individuals diagnosed with ASD, 40 children with monitored ASD or with the first complaints of speech, social, and communication delays were selected after an evaluation according to the DSM-V criteria in the outpatient clinic of Child Psychiatry and Child Neurology, Erciyes University Medical Faculty. Patients who enrolled in the study were not taking any medication when blood samples were collected and according to their examination, that there was no clinical infection or endocrine findings. Exclusion criteria included a history of other developmental disorders, psychiatric diseases or genetic disorders, such as Fragile X Syndrome, Rett Syndrome, cerebral palsy, chronic seizures, and other congenital diseases. Taking into account the patient's age and sex, 50 healthy individuals between the ages of two and six without chronic medical, psychiatric and genetic conditions and not taking psychiatric medication were used as controls. All of the control subjects were without any psychiatric condition as confirmed by a child psychiatrist according to their examinations and parental interviews. None of the controls had delays in speech or psychomotor development as per parental interview. Additionally, the patients and control group were selected from the same location and ethnic origin in this work. Information about the demographic and clinical characteristics of patients was collected using a sociodemographic form and scale which were prepared by the researchers.

Study Measurements

In addition, the degree of the disease severity was assessed by using the Childhood Autism Rating Scale (CARS). The CARS score is widely used for investigating the severity of ASD in clinical and general populations (Geier et al. 2013). In CARS, scores of 38–60 points constitute an autistic group showing severe symptoms, scores of 30–38 points constitute an autistic group showing mild to moderate symptoms, while scores of 15–29 constitute a non-autistic group. In addition to CARS, the Ankara Developmental Screening Inventory (ADSI) was also used in the patients. The ADSI is a developmental screening instrument that assesses development in

children between 0 and 6 years of age in 154 items and five categories (communication-cognitive, fine motor, gross motor, social self-care skills and total skills), based on information obtained from parent interviews. The intellectual profile of the patients was determined with the Wechsler Intelligence Scale for the Children-Revised (WISC-R) scale.

Gene Expression Studies with Quantitative Real-Time PCR

All of the genetic studies were performed in the Erciyes University Genome and Stem Cell Center (GENKOK). Two milliliter blood samples were taken before the patients' treatment. Peripheral blood samples were collected in the morning between 09:00 and 11:00. After collection, the total blood was immediately used for RNA isolation. Total RNA was extracted from the whole blood samples with TRIzol Reagent (Roche, Germany). The quantity (absorbance at 260 nm) and quality (ratio of absorbances at 260 nm and 280 nm) of RNA were evaluated with a BioSpec-Nano Spectrophotometer. All total RNA concentrations were > 40 ng/μl. Following isolation, RNA samples were stored at –80 °C prior to expression studies. First-strand complementary DNA was synthesized from the total RNA with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Germany). Gene expression was assessed by quantitative Real-Time PCR (qRT-PCR) with RotorGene (Qiagen, Germany). Cycling conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. All of the the samples were studied in duplicate. *ACTB* (beta-actine) and *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) were used as the housekeeping genes. The changes in gene expression between the case and control groups were determined by the $2^{-\Delta\Delta Ct}$ method of relative quantification. Target gene copy numbers were normalized using both housekeeping genes (Bustin et al. 2009).

Statistical Analysis

Histogram and q-q plots were examined; Shapiro-Wilk's tests were used to assess data normality. The Levene test was used to check the variance homogeneity. To compare the differences among groups, either a one-way analysis of variance (ANOVA) or Kruskal-Wallis H test was used for continuous variables; chi-square analysis was used for categorical variables. We applied the Bonferroni correction for the correlation of CARS scores and gene expression patterns. All interpretations of the correlation coefficients were classified according to Evans JD (Evans 1996). Analyses were conducted using R 3.2.0 (www.r-project.org) software. A *p* value less than 5% was considered as statistically significant.

Table 1 Clinical and demographic findings of the study group

Variables	Control (N = 50)	Autism spectrum disorders (N = 40)	<i>p</i> value
Age (years)	3.64 ± 1.19	3.64 ± 1.17	0.988
Gender (male/female)	37(74.0%)/13(26.0%)	33(82.5%)/7(17.5%)	0.335
ID	–	29 (72.5%)	
ADHD	–	15 (37.5%)	
Insensitivity to pain	–	5 (12.5%)	

ADHD attention deficit hyperactivity disorder, ID intellectual disability

Results

We recruited 40 patients with the diagnosis of ASD and 50 healthy controls. In the study group, there were 33 (82.5%) males and 7 (17.5%) females. There were 37 (74%) male controls and 13 (26%) female controls. The mean age was 3.64 ± 1.17 in the study group and 3.64 ± 1.19 in the control group. There was no significant difference between the groups for gender or age ($p = 0.335$, $p = 0.988$). When the patients were evaluated according to their mental condition, 29 (72.5%) of the ASD patients were diagnosed with ID. There were ten cases with ADHD (25%) and ten cases with no additional features. However, 15 ASD cases (37.5%) had aggression, and 5 (12.5%) had insensitivity to pain. The clinical and demographic features of the groups are presented in Table 1.

When the expressions of the *OPRM1*, *PRKG1*, *SCN9A*, *DRD4*, *HTR1E*, *OPRL1* and *TACR1* genes studied in the ASD patient group were compared to the control group, the results were found to be statistically significant ($p = 0.014$, $p = 0.007$, $p < 0.001$, $p = 0.002$, $p = 0.010$, $p < 0.001$ and $p = 0.029$, respectively); the expressions of the *OPRM1*, *PRKG1* and *HTR1E* genes were low in the ASD cases, while the *SCN9A*, *DRD4*, *OPRL1* and *TACR1* gene expressions were high when compared to the control group (Fig. 1). No significant differences were recorded in the *CRHR1* and *SLC6A4*

gene expressions between the ASD patients and controls ($p = 0.372$, $p = 0.955$).

Next, we compared the ASD group by dividing the patients into five subgroups based on clinical findings including the presence of ID, ADHD, aggression, insensitivity to pain and the ADSI scores. Comparisons of the *DRD4* and *OPRL1* gene expressions between the ID positive and negative groups, the *OPRM1* and *DRD4* gene expressions between the ADHD positive and negative groups, and the *HTR1E* gene expression between the aggression positive and negative groups were statistically significant (Table 2).

A positive and strong correlation was observed between the *PRKG1* and *OPRM1* genes, the *PRKG1* and *SLC6A4* genes, as well as the *OPRL1* and *DRD4* genes. Other positive or negative correlations are summarized in Table 3. The *p* value of the correlation coefficient between the CARS score and the *HTR1E* expression was detected as 0.054. This probability value is pretty close to the significance level, $\alpha = 0.05$, and thus can be considered statistically significant.

We also compared the groups according to gender. When we analyzed the gender, we could not detect significant differences in gene expression in the female patients compared with the female controls. In contrast to this result, there were significant differences in the male patients and controls. *OPRM1* and *PRKG1* gene expression levels (0.014 and 0.024) were significantly lower and *DRD4* and *OPRL1* gene

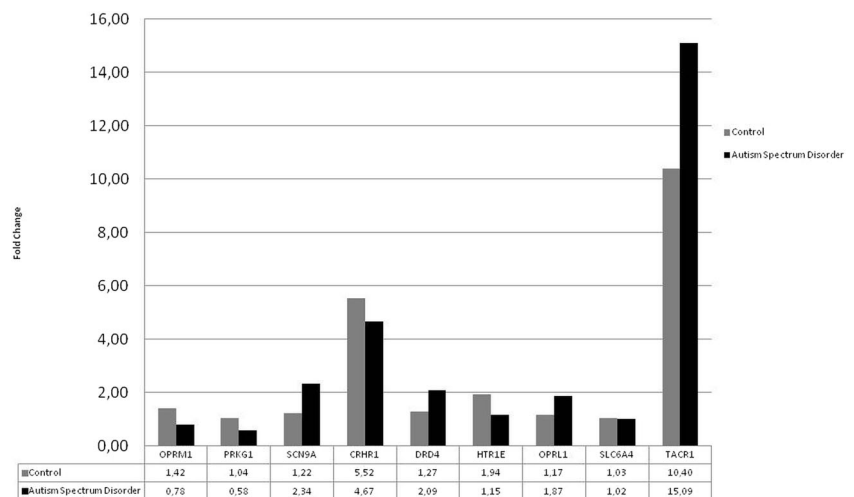
Fig. 1 Comparison of the nine gene expressions between the ASD patients and controls

Table 2 Comparison of the gene expressions and clinical findings of the ASD group

Variables	CARS score	OPRMI	PRKGI	SCN9A	CRHRI	DRD4	HTRIE	OPRLI	SLC6A4	TACRI
ID										
Negative	32.00 (30.00-46.00)	0.28 (0.08-0.96)	0.32 (0.08-1.13)	3.07 (2.00-3.66)	5.09 (1.60-7.98)	2.84 (2.44-4.27)	0.60 (0.24-2.24)	2.35 (2.04-2.83)	0.63 (0.37-1.40)	14.14 (11.89-20.13)
Positive	38.50 (32.50-43.00)	0.85 (0.34-2.63)	0.62 (0.38-1.18)	2.20 (1.27-2.95)	4.28(1.83-12.70)	1.60 (1.18-2.38)	1.23 (0.54 2.49)	1.62 (1.12-2.04)	1.13 (0.56-1.54)	15.49 (11.23-21.93)
<i>P value</i>		0.054	0.093	0.178	0.683	0.006	0.168	0.038	0.198	0.750
ADSI										
Normal	32.00 (30.00-32.00)	0.15(0.08-0.94)	0.21 (0.08-0.32)	3.44 (3.07-3.66)	2.08 (0.79-5.09)	3.82 (2.81-4.73)	0.24 (0.21-0.45)	2.83 (2.04-3.53)	0.60 (0.37-0.63)	18.89 (12.15-19.24)
Suspicious	32.25 (31.00-39.25)	1.34 (0.46-2.12)	0.82 (0.48-1.79)	1.48 (1.10-2.049)	6.07 (2.08-8700.79)	1.41 (1.20-2.22)	2.06 (1.03-2.56)	1.57 (1.07-2.41)	1.18 (0.76-1.41)	14.28 (10.09-16.68)
Abnormal	39.00 (34.50-45.00)	0.77 (0.28-2.63)	0.62(0.37-1.22)	2.30 (1.27-3.07)	5.06 (1.83 12.70)	2.08 (1.15-3.28)	1.20 (0.54-2.68)	1.74 (1.20-2.14)	1.15 (0.53-1.55)	15.49 (11.23-21.93)
<i>P</i>		0.348	0.083	0.092	0.260	0.070	0.055	0.359	0.109	0.717
ADHD										
Negative	36.00 (31.00-44.00)	0.71 (0.15-1.83)	0.47 (0.22-1.09)	2.18 (1.49-3.43)	4.67 (1.83-7.98)	2.21 (1.42-3.56)	0.75 (0.45-2.24)	1.97 (1.20-2.79)	0.99 (0.55-1.42)	14.08 (11.23-19.11)
Positive	37.75 (32.00-42.00)	3.23 (0.34-6.22)	0.90 (0.54-1.82)	2.81 (1.27-3.07)	7.81 (1.77-20.97)	1.18 (0.99-2.81)	2.05 (0.74-3.76)	1.64 (1.09-2.04)	1.20 (0.53-2.20)	25.07 (12.44-58.71)
<i>P</i>		0.034	0.118	0.553	0.553	0.031	0.070	0.169	0.512	0.061
Aggression										
Negative	33.00 (30.50-40.00)	0.85 (0.17-1.93)	0.54 (0.34-1.13)	2.20 (1.27-3.21)	2.91 (1.69-7.41)	2.23 (1.15-3.28)	0.74 (0.40-1.75)	1.92 (1.20-2.35)	0.97 (0.56-1.52)	13.88 (11.89-20.13)
Positive	41.50 (36.00-46.00)	0.66 (0.23-2.76)	0.90 (0.14-1.82)	2.51 (1.49-2.98)	6.75 (2.81-17.26)	1.71 (1.27-3.56)	2.37 (0.56-3.22)	1.62 (1.16-2.43)	1.15 (0.53-1.42)	18.50 (10.72-21.16)
<i>P</i>		0.944	0.696	0.625	0.142	0.967	0.037	0.989	0.878	0.586
Insensitivity to pain										
Negative	36.00 (30.50-44.00)	0.77(0.17 -1.96)	0.54 (0.34-1.13)	2.20 (1.27-3.21)	4.28 (1.77-9.88)	2.08 (1.22-3.28)	1.10 (0.45-2.24)	1.74 (1.12-2.35)	1.02 (0.55-1.46)	14.14 (11.23-20.69)
Positive	41.50 (38.00-43.00)	1.41 (0.35-5.76)	1.22 (0.10-2.90)	2.67 (2.51-2.98)	5.75 (3.51-42.98)	3.21 (1.63-3.56)	3.22 (0.56-3.90)	2.14 (1.89-3.17)	1.19 (0.53-2.26)	19.11 (18.50-58.71)
<i>P</i>		0.526	0.474	0.526	0.402	0.526	0.136	0.212	0.668	0.316

Values are expressed as *n*(%) or median(1st–3rd quartiles). Different superscripts in a column indicate statistically significant difference
ADHD attention deficit hyperactivity disorder, ADSI Ankara developmental screening inventory

Table 3 Correlations between CARS scores and gene expressions

Genes	CARS	CRHR1	DRD4	HTR1E	OPRL1	OPRM1	PRKG1	SCN9A	SLC6A4	TACR1
CARS	1.000									
CRHR1	0.034	1.000								
DRD4	0.030	-0.262	1.000							
HTR1E	0.428**	0.410**	-0.137	1.000						
OPRL1	0.122	-0.104	0.666**	-0.036	1.000					
OPRM1	-0.067	0.545**	-0.426**	0.467**	-0.222	1.000				
PRKG1	-0.090	0.488**	-0.505**	0.478**	-0.309	0.771**	1.000			
SCN9A	-0.092	0.132	0.166	-0.128	0.083	0.191	0.104	1.000		
SLC6A4	-0.046	0.354*	-0.138	0.238	-0.015	0.454**	0.686**	0.173	1.000	
TACR1	0.016	0.338*	0.116	0.250	0.323*	0.425**	0.260	0.452**	0.214	1.000

* $p < 0.05$, ** $p < 0.001$

expression levels (0.001 and 0.004) were found to be increased in the males controls.

Discussion

In this study we assessed the clinical symptoms of ASD such as aggression and insensitivity to pain in relation to candidate gene expression in children with ASD. Except for *CRHR1* and *SLC6A4*, we obtained significant differences between the patients and the control groups. In our preliminary study, we studied the gene expression of *OPRL1*, *TACR1*, and *HTR1E* in 22 patients with ASD. Positive and strong correlations were obtained between the three gene expressions in the ASD group. The expression levels of the three genes were found to be statistically significant between the groups (Sener et al. 2017). In this study, we supported our previous results regarding related gene expressions.

Recently, the knock-out mu opioid receptor gene (*Oprm1*^{-/-}) was proposed as a monogenic mouse model of autism, based on severe deficits in social behavior and communication skills (Becker et al. 2014). The expression of these genes was statistically significant ($p < 0.001$); thus, our data support the relationship between insensitivity to pain and autism. In *MECP2*-TG mice, reducing *Crh*, or its receptor, *Crhr1*, suppresses anxiety-like behavior; in contrast, reducing *Oprm1* improves abnormal social behavior. Reducing the levels of either of these two genes specifically corrected the respective phenotypes (Samaco et al. 2012). In our study, it was observed that the expression of *CRHR1* and *OPRM1* were decreased in the controls. This finding is consistent with the results of the above-mentioned study. However, the lower expression of the *CRHR1* gene did not give a significant result between the groups. Therefore, we can think that the low expression of these genes makes a positive contribution to the autism phenotype. The decreased expression of the *CRHR1* gene can be interpreted as a reduced CRH level or a lack of an adequate

HPA axis response. When genders were considered, lower *CRHR1* expression was detected in males than in females. Based on this finding, the *CRHR2* gene may play a more active role in male patients. We suggest that a clearer finding should be obtained by further investigating CRH levels and *CRHR2* gene expression.

In a study that conducted interviews with families, aggression in patients with ASD was found to be 65% independent of mental retardation (Totsika et al. 2011). Again, the method of interviewing the families of ASD patients was selected in another study. The parents of 67 children were interviewed, and it was reported that two-thirds of these children had severe temper tantrums and one-third had past aggression (Dominick et al. 2007). A broader study shows that in more than 50% of the ASD patients physical aggression was seen as a snapshot of clinical findings, while 70% stated that aggression against caregivers was present in their lives (Kanne and Mazurek 2011; Mazurek et al. 2013). Many studies in ASD have emphasized that there is no change in the level of aggression with age (Dominick et al. 2007; Lecavalier 2006). However, 15 ASD cases (37.5%) had aggression in our study group. The involvement of substance P (SP) and its potent tachykinin receptor (*TACR1* or *NK1*) in the induction of both defensive rage and predatory attack appears to be a consistent finding. An overall understanding of the nature of the SP involvement in the induction of aggression has not been fully achieved yet (Katsouni et al. 2009). *TACR1* expression, which was the first aggression-related gene, differed significantly among the groups in our study.

The first evidence for DRD4 receptor mRNA expression in ASD patients is an increase in peripheral blood lymphocytes compared to controls (Emanuele et al. 2010). This study was in accordance with our findings, and the expression of the *DRD4* gene was also increased in our patient group. In a recent study, *DRD4* mRNA expression in whole blood was significantly lower in ADHD and ASD children compared to healthy controls (Taurines et al. 2011).

Low serotonergic activity correlates with increased impulsive-aggressive behavior, while the opposite association may apply to cognitive impulsiveness (Oades et al. 2008). The *SLC6A4* gene modulates serotonergic neurotransmission by the active reuptake of serotonin from the synaptic cleft into the presynaptic nerves (Whyte et al. 2014). In our study, while the data were not significantly different, the low gene expression of *SLC6A4* in the patient group implied that there is an abnormality of serotonin reuptake. *HTR1E* encodes one of the highly conserved serotonin receptor gene families (Shoval et al. 2014). The serotonin receptors are involved in several behavioral functions (e.g. nociception, aggression, and stress). This gene expression was significantly lower in the ASD patients compared to the controls. We can suggest that serotonin receptors have potential roles in the pathophysiology of these cases, as is consistent in the literature. In our study, the *HTR1E* gene expression pattern was significantly different in our groups. As a result of our findings mentioned above, *TACR1*, *HTR1E* and *DRD4* expression patterns may be useful tools in the diagnostic procedures of ADHD and ASD in future studies.

Additionally in this study, a positive and strong correlation was observed between the *PRKG1* and *OPRM1*, *PRKG1* and *SLC6A4*, *OPRL1* and *DRD4* genes. These positively correlated genes may be thought to have contributed to the autism phenotype by interacting on the same or similar pathways.

Finally, our study revealed significant results with the clinical findings of ASD. This indicates a need for replication of this study both with mRNA and protein levels and for contributing influences to be explored and better understood. To the best of our knowledge, the expressions of the candidate genes that we tested have not yet been investigated in ASD patients displaying aggression and insensitivity to pain. Alterations in the mRNA expression patterns of the *HTR1E*, *OPRL1*, *OPRM1*, *TACR1*, *PRKG1*, *SCN9A* and *DRD4* genes provide further evidence for a relevant effect of the respective candidate genes on the pathophysiology of ASD. Low expression of the *OPRM1* and *CRHR1* genes can make a positive contribution to the autism phenotype. We suggest that CRH levels and the *CRHR2* gene may be further investigated in the same way. The presence of expression differences between genders also provides clues about the disease phenotype. Given their potential as markers, mRNA expression patterns may be useful tools in the diagnostic procedures of ASD. Future studies may use larger sample groups to determine the sensitivity and specificity of these putative markers in the mRNA and protein expression profiles of these genes, including other neuropsychiatric disorders.

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Compliance with Ethical Standards

Conflict of Interest The authors have no conflicts of interest or other disclosures to report.

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