

Original Article

## A PRELIMINARY INVESTIGATION OF HTR1A GENE EXPRESSION LEVELS IN AUTISM SPECTRUM DISORDERS

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### ABSTRACT

**Objective:** This study was conducted to explore the expression levels of HTR1A gene in a sample of Egyptian autistic children.

**Methods:** Thirty autistic patients (18 boys, 12 girls) and 20 controls were enrolled in the study. From each child, we isolated RNA samples from whole blood. Quantitative Real-Time PCR (qRT-PCR) was used to measure the gene expressions of HTR1A and normalized to the housekeeping gene, beta-actin.

**Results:** The HTR1A gene expression of healthy controls and ASD subjects were varied significantly ( $p=0.0062$ ). As compared to control of healthy subjects, the HTR1A expressions were greatly reduced in samples of ASD.

**Conclusion:** HTR1A gene expression level is a candidate gene for further studies to explore its potential roles in ASD related pathways.

**Keywords:** Autism spectrum disorders, Gene expressions, HTR1A gene expression

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### INTRODUCTION

It is well established that a complex interaction between genetic, epigenetic and environmental factors derives Autism's etiology. Chromosomal rearrangements at almost all chromosomes have been noticed among individuals with autism spectrum disorder (ASD) [1]. Unfortunately, little is known about the genetic variants and their contribution to the development of the ASD disorder. However, studying autistic related symptomatology may add to our knowledge about related genes and may help to further understanding of these disorders. For example, many serotonin polymorphisms were correlated with the level of IQ [2].

Various biological processes, besides, variations in gene expression levels between different biological tissues are implicated with ASD individuals. Moreover, some studies utilize gene expression analysis pattern to categorize autism into different subcategories. A list of genes found to be significantly dysregulated in ASD is needed to help further researchers to define any abnormalities useful for diagnosis or treatment [3].

The gene coding the 5-HT<sub>1A</sub> receptor is 1.27kb and intronless. It is located on the long arm of chromosome 5. The family of G protein-coupled receptors (GPCRs) includes serotonin 1A receptors which are involved in behavior, development and cognition [4]. Consequently, the serotonin 1A receptor serves as an important target for neuropsychiatric disorders; for example; anxiety, depression and neuronal developmental defects [5].

High levels of serotonin in platelet were the first observation to relate 5-HT to ASD [6, 7]. In 25-50% of ASD patients, hyperferritinemia was observed [6]. In the prefrontal cortex, high concentrations of serotonin 1A receptors are distributed. Due to its ability to reverse drug-induced cognitive deficits, HT<sub>1A</sub> receptor antagonists may be a good tool to cure age-related cognitive impairment [8]. The serotonin 5HT<sub>1A</sub> receptor has gained extensive investigations on their role in development, plasticity, and memory processes in the human brain [9].

HTR1A receptors are found as an auto receptor in the soma and dendrites of serotonergic neurons. And hence, reduction of the HTR1A autoreceptor activity results in an increase in serotonin transmission, suppress the function of target neurons mediated by HTR1A and results in a reduction in the potency of neural

transmission [10]. Many transcriptional regulators of the 5-HT<sub>1A</sub> receptor have been identified [11].

The serotonin 1A receptor, as well as, serotonin neurotransmitter system was extensively involved in the pathophysiology of mood and anxiety disorders. However, the researchers found that there is a variation in the genetic correlations [12]. Gross *et al.* [13] found that a varied pattern of up-regulated fear-related behaviors was showed by knock-out 5-HT<sub>1A</sub> receptor in mice.

Gene expression studies are helpful in the comparison between groups of subjects with ASD and healthy control samples in order to elucidate genes that are interrupted in the ASD group. And hence, this study was carried out to investigate HTR1A gene expression levels in ASD children. This also will be useful in increasing our knowledge about understanding autism development. Moreover, this will help in tailoring treatment strategies targeting the serotonergic system in the near future.

### MATERIALS AND METHODS

#### Patient selection

This study was carried out on 30 children with autism (group I), their ages ranges between 6-13 y receiving care at Neuro-Rehabilitation Clinic, Center of Excellence of Medical Research, Department of Research on Children with Special Needs, Medical Division- National Research Centre. Group II included 20 normal children matched for age, sex and socioeconomic status with group I. The study was approved by the ethical committee of Medical research division, National Research Centre, approval number 18-173. An informed consent was taken from each child's guardian.

#### The inclusion criteria

Diagnosed cases using 3 psychometric assessments; Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV), Childhood Autism Rating Scale (CARS) and Autism Diagnostic Interview-Revised (ADI-R). Their age range between 6-13 y,

#### Exclusion criteria

Cases received vitamins 3 mo before the study; Cases with auditory, vestibular, visual and respiratory defects; Cases with identified

metabolic, genetic, or progressive neurological disorders, based on screening by clinical staff; and Secondary autistics with Genetic/Medical conditions.

#### Quantitative real-time gene expression analysis

This was done using SensiFast® syberGreen one-step RT-PCR (Bioline). Briefly, total RNA was extracted from peripheral blood cells after RBC's lysis using Qiazole lysis reagent (Qiagen, USA) according to the manufacturer instructions. Primer sequence for  $\beta$ -actin gene is: Forward, CCTTCCTGGGCATGGAGTCCT; Reverse, GGAGCAATGATCTTGATCTTC; for HTR1A: Forward, GGCAACAACACCACATCACC, Reverse, and GACGGTCACGTCGGAGATAC. The copy numbers were normalized to 100,000 copies of the housekeeping beta-actin gene. The RT and subsequent PCR cycling conditions were as follows: 50 °C for 10 min, 95 °C for 5 min, 60 °C for 30 s and then 95 °C for 15 s; the number of cycles were 40 cycles. BioRad Miniopticon™ real-time PCR cyler was used for quantitative estimation.

#### Statistical analysis

The data were analyzed using Microsoft Excel. All the data are expressed as mean±standard error mean. Analysis of the data was done using student t-test to detect the significant difference between the studied groups. A level of  $P < 0.05$  was defined as statistically significant. Data are represented as mean±sem.

#### RESULTS

In the study subjects, there were 18 males and 12 females ADS patients, besides, 10 females and 10 males' healthy controls. The average age ranged between  $9.0 \pm 0.33$  in the patient group and  $9.1 \pm 1.4$  in the control group. No significant differences were recorded between the groups for Gender ( $P = 0.3$ , 0.92 for ADS patients and control, respectively) or age ( $P = 0.98$ ). HTR1A gene expression levels were normalized to 100000 copies of beta-actin. We found a high significant difference ( $p = 0.0062$ ) in HTR1A gene expression between children suffering from ASD and healthy children group (table 1).

**Table 1: The expression levels of HTR1A gene and demographic features of ADS patients and control group**

Variables	ASD (n=30)	CONTROL (n=20)	P value
Age	$9.0 \pm 0.33$	$9.1 \pm 1.4$	0.976426
Gender			
Male	$8.8 \pm 4.4$	$9.1 \pm 0.6$	0.3
Female	$9.4 \pm 0.5$	$9.0 \pm 0.7$	0.92
HTR1A expression Levels (normalized Copy numbers)	$1950 \pm 395$	$6468 \pm 1037$	0.0062

Data are represented as mean±SEM

#### DISCUSSION

Zafeiriou *et al.* [6] suggested that in ASD, serotonin (5-HT) system have been disrupted. A variation in sequence in the serotonin genes is well-replicated leading to this disruption. Consequently, their involvement in the development of ASD has gained extensive studies [14]. The 5-HT<sub>1A</sub> receptors is considered as a molecular target for the action of serotonin [15]. In the current investigations, the gene expression levels of HTR1A gene were markedly reduced in ASD patients as compared to healthy controls. This finding is consistent with that of Sener *et al.*, [11] who reported that the expression of HTR1A was undetectable in autistic Turkish children.

In the central nervous system, the rate-determining enzyme tryptophan hydroxylase 2 (TPH2) are secreted by the brain 5-HT system and evolves from neurons of the raphe nuclei [16]. In non-neuronal cells, the expression of 5-HT<sub>1A</sub> could be knocked down by a group of repressor elements in the upstream from the minimal promoter. Besides, in neuronal cell lines that express 5-HT<sub>1A</sub> receptors, the transcription of 5-HT<sub>1A</sub> could be down-regulated [17]. However, not all subcategories of neurons contain the 5-HT<sub>1A</sub> receptors, and consequently, more down regulators are needed to reduce its expression to exact neurons [18].

In the prefrontal cortex (PC), the main cause of the suppression of neuronal output by 5-HT<sub>1A</sub> receptors is the hyperpolarizing potassium current acceleration. Meanwhile, the output is mediated by 5-HT<sub>2A</sub> receptors through phospholipase induction [19]. Moreover, serotonin 1A receptor agonists were reported to mediate the release of dopamine in the PC. Besides, it can moderate the effect of dopamine D2 receptor blockers on the secretion of dopamine [20]. Diaz-Mataix *et al.* [21, 22] reported that dopaminergic neurons in the ventral tegmental area could be potentiated by activation of PFC 5-HT<sub>1A</sub> receptors, and also the mesocortical release is accelerated by it.

The 5-HT<sub>1A</sub> autoreceptor signaling in raphe neurons was explored in previous work [23]. 5-HT<sub>1A</sub> was found to inhibit adenylyl cyclase activity, and hence negatively regulates serotonergic neuronal activity. The 5-HT<sub>1A</sub> autoreceptor chronic desensitization is needed to gain increased serotonergic neurotransmission [24] and to activate serotonin synthesis [25]. Building on this fact in humans, the 5-HT<sub>1A</sub> autoreceptors level is inversely associated with raphe 5-HT formation in human [26].

When 5-HT<sub>1A</sub> auto receptor expression is increased, the activity of 5-HT neurons is suppressed. Meanwhile, an abolished behavioral response to 5-HT may be caused by suppressed post-synaptic 5-HT<sub>1A</sub> receptors. Mental illness susceptibility is regulated by the receptor of 5-HT<sub>1A</sub>. However, further explorations for the exact pathway causing variable changes in the receptor of 5-HT<sub>1A</sub> are needed [10].

Increasing in 5-HT neuron firing rate and accelerated 5-HT output were recorded in Mice with a 30% suppression in 5-HT<sub>1A</sub> auto receptors [27]. Endogenous 5-HT<sub>1A</sub> receptors were expressed in some neuronal cell lines. Particularly, the rat raphe RN46A cells which express the receptors of 5-HT<sub>1A</sub>. These cells are serotonergic and could be utilized as a model for 5-HT<sub>1A</sub> auto receptor handling [28].

#### CONCLUSION

This study concluded that the HTR1A gene expressions were greatly reduced in samples of ASD. This finding could suggest the importance of enrolling this gene in further studies in order to determine its exact role in ASD etiology.

#### AUTHORS CONTRIBUTIONS

Dr. Shaymaa MM Yahya: Conducting laboratory experiments, statistical analysis, Manuscript writing, and submission, Dr. Ola Gebril: Basic concept for research, physical examination and diagnosis of subjects, Prof Dr. Ehab Abdelraouf: physical examination and diagnosis of subjects, Dr. Mohamed E. Elhadidy: Conducting laboratory experiments, statistical analysis, Manuscript writing.

#### CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interests.

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