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Over Expression Of Foxl 1 In Iraqi Patient With Autism Spectrum Disorder

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Abstract:

Autism spectrum disorders (ASDs) are a collection of diseases marked by difficulties with reciprocal social interaction and communication and limited and repetitive activities. FOXl1is transcription factors have a distinct DNA-binding forkhead domain and are involved in various processes during ontogenesis, including metabolism, gene expression, and cell proliferation.

Materials and Methods

A case-control study was performed of 60 ASD, and 60 age and sex-matched apparently healthy children control group. Their ages ranged between 3 and 16 years 6.66 ± 3. 051(mean ± SD). Study participants at the transcriptional level of FOXL1 (Forkhead Box L1)were quantitated using the qRT-PCR, ΔΔCT method.

Results: The expression of FOXL1 was significantly higher among autistic patients than that of control participants upregulating FOXL1in autistic patients than control one.

Conclusion: FOXL1 is a candidate gene for ASD.

Key word : autism,foxl1, qRT-PCR.

Introduction:

Autism spectrum disorder (ASD) is a severe neurodevelopmental disease in which persons with ASD have difficulty communicating, interacting socially, and engaging in repetitive activities. This illness has yet to be cured. ASD is a neurodevelopmental disorder marked by difficulties with social interaction and communication, as well as limited and stereotypical behavior and interests [1], the prevalence of Autism Spectrum Disorder (ASD) According to the CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network [2], about 1 in 54 children has been recognized. ASD has been reported in people of various races, ethnicities, and socioeconomic backgrounds. ASD is more than four times as frequent in boys as it is in girls.

According to parents' reports, during the research period of 2009-2017,Around one-sixth of all children aged three to seventeen were identified with a developmental impairment. Autism, blindness, attention deficit hyperactivity disorder, and cerebral palsy were only a few of them [3,4]. The ratio of affected males to females is 4:1 [5] due to the disease's rapid onset (within a year). Symptoms generally appear before the age of three years) and there is a dearth of information on the condition. ASD causes severe emotional and behavioral issues as a result of the lack of a suitable therapeutic technique. Financial costs are borne by patients, their families, and society as a whole. The etiology of ASD is thought to include both genetic and environmental risk factors [6,7].

Together, twin and family studies have provided convincing evidence for the participation of several genetic factors in the incidence of autism, with family studies indicating an 8–10 percent recurrence risk of autism in siblings of afflicted probands [8]. Autism diagnosis in the first child was associated with a 10% chance of recurrence in future offspring, a significant improvement over the population level.

The forkhead/winged helix-box (FOX) transcription factor is encoded by this gene. FOX transcription factors are involved in several activities during ontogenesis, including metabolism, gene expression, and cell proliferation. They contain a unique DNA-binding forkhead domain.The gastrointestinal epithelium requires a transcription factor for proper proliferation and differentiation. The hedgehog (Hh) signaling pathway targets the transcription factors GLI2 and GLI3 [9]. foxl1, a transcription factor with a winged-helix, is found in the mesoderm of the gastrointestinal tract, right next to the endoderm-derived epithelium [9]. FOXL1 gene located in chromosome 16 q24.1 locations according to Gene Loci [11]. The proliferation of intestinal epithelial cells was disturbed, leading to a fourfold increase in dividing epithelial cells and a substantial extension of the proliferative area. As a result, the tissue architecture of the stomach and small intestine is skewed, resulting in uneven crypt form, the development of mucin-

filled cysts, and the lengthening of villi [10]. In addition, FOXL1 plays a critical function in the Wnt/APC/-catenin pathway's regulation. Mice homozygous for a Foxl1-null mutation have increased nuclear aggregation of B-catenin in the epithelia of the stomach and intestine [9].

Wnt proteins, which are abundant in subepithelial telocytes, are required for intestinal stem cells to proliferate and induce epithelial renewal. Telocytes are large but uncommon mesenchymal cells that form a subepithelial plexus that extends from the stomach to the colon and are defined by Foxl1 expression and size. Foxl1+ telocytes compartmentalize the synthesis of Wnt ligands and inhibitors while sustaining the whole epithelium, allowing for regional pathway activation [12]. Conditional gene ablation of Porcupine (Porcn), which is required for functional maturation of all Wnt proteins, causes Wnt signaling to intestinal crypts to stop early, loss of stem and transit-amplifying cell proliferation, and inadequate epithelial renewal in Foxl1+ telocytes. Foxl1+ telocytes thus play an important role in giving niche signals to intestinal stem cells [12]. Furthermore, the synthesis of Wnt by Foxl+ telocytes may have an indirect effect on R-spondin gene expression. (which is shown to enhance GIT cell proliferation in mice) [13]. In conclusion, sub-epithelial Foxl1+ telocytes provide essential Wnt signals to intestinal crypt cells, allowing for homeostatic regeneration of the intestinal epithelium.

Material and method

A case-control study was conducted in the " Department of Pathology and Forensic Medicine, Faculty of Medicine – University of Kufa", from December 2020 to March 2020, from both healthy and patient subjects. Their ages ranged between 3 and 16 years 6.66 ± 3. 051 (mean ± SD). The control group consisted of 60 healthy youngsters who were age and sex matched. The cases included in this study were collected from the Teaching Hospitals in the Middle and South Euphrates Region. This group consisted of:-

Selection of Cases

The patients fulfilled the "Diagnostic and Statistical Manual of Mental Disorders" (5th ed.) criteria for autism and were divided into three groups: mild (n=39), moderate (n=13), and severe autistic individuals (n=8). The controls were healthy, typically growing youngsters who were not connected to the autistic patients and did not meet any of the exclusion criteria, which included:

1. Any medical problem that might be a cause of ASD (e.g. Rett syndrome, focal epilepsy).
2. Other than simple non-focal epilepsy, any neurologic condition involves pathology above the brainstem.
3. Evidence of likely newborn brain damage in the present, or unequivocal retrospective evidence.

4. Any CNS-related genetic condition, even if the relationship to autism is unclear.

5. Even after correction, there is clinically substantial visual or auditory impairment.

6. Any variables that might explain the autism image (e.g. severe nutritional or psychological deprivation).

7. Treatment using pharmacological or other substances that are active.

"The local Ethical Committee of the College of Medicine, University of Kufa university Iraq, approved this study".

Blood sampling was carried out from December 2020 to March 2020 from both healthy and patient subjects. 5ml of peripheral venous blood were collected and put into an EDTA tube for RNA extraction for subsequent analysis by qRT PCR for gene analysis.

Primer design

Three primer sets for amplification of FOXL1 were amplified using a Primer-blast online program localized at the server (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) from NCBI (National Center for Biotechnology and Information). Briefly, the following reference sequences: NM_005250.3 and NM_001289746.2 for FOXL1 and GAPDH, respectively, were retrieved from GenBank. Next, the aforementioned reference sequences were used for the design of primer sets for the aforementioned genes. Then, a primer set for each gene was selected based on the criteria of self-complementarity, 3' complementarity, GC content, and Tm. Finally, all designed primers were synthesized in DNA Integrated Biotechnology (IDT Co., Canada).

qRT-PCR protocol

The recipe, primer design, and PCR conditions for qRT-PCR of the target aforementioned genes and the housekeeping gene were displayed in Table 1. For each cDNA sample, four qRT-PCR reactions were performed using the primer designs listed in Table 1. The SYBR Green 2x Master mix (Kappa, Applied Biosystem) was used in all qRT-PCR reactions. The real-time machine was the Rotor-Gene Q-5plex (Qiagen). All fluorescent acquisitions in all qRT-PCR reactions were picked at the extension step using the SYBR Green channel filter.

Gene and primer sequence	PCR recipe	PCR conditions	PCR product (bp)
FOXL1 gene	cDNA: 3.0 µL	1 cycle:	

Fw-FOXL1 5’-GTGCAGTTGAGGTCCTTTCG-3’ Rv- FOXL1 5’-GTCAGGAACAAACCCAGCTG-3’	Fw-primer: 2.0 µL Rv-primer: 2.0 µL 2XPCR master mix: 12.5 µL NFW[a]: 5.5 µL Tot volume: 25 µL	Initial denaturation: 95 °C, 5 min 45 cycles: each cycle Denaturation: 94 °C, 1 min Annealing : 63 °C, 1 min Extension : 72 °C, 25 seconds Hold : 5 min 55-95°C	129
GAPDH gene		1 cycle: Initial denaturation: 95 °C, 5 min 45 cycles: each cycle Denaturation: 94 °C, 1 min Annealing : 60 °C, 1 min Extension : 72 °C, 25 seconds Hold : 5 min 55-95°C	94
Fw-GAPDH 5’-CACTAGGCGCTCACTGTTCT-3’ Rv- GAPDH 5’-GACCAAATCCGTTGACTCCG-3’			

Post qRT-PCR calculations

After PCR termination, melting curves for all conducted qRT-PCR reactions were visualized to assess the specificity of the reactions. For each qRT-PCR for a specific gene, either target or housekeeping gene, a single peak was taken to indicate the specificity of the response. The peak of the melting curve was used to localize the melting temperature (Tm) of the amplified PCR product. The CT (cycle threshold) values for each qRT-PCR reaction was calculated manually using the guidelines settled in the user manual of the real-time PCR machine (Rotor-Gene Q-5plex, Qiagen). All CT values were used to calculate the fold expression of each target gene in each cDNA sample using the ΔΔ CT method. All calculations were estimated using the Excel program of the Microsoft 2013 version.

Result:

The expression of FOXL1 among study participants was quantitated on the transcriptional level through qRT-real- time PCR.

The expression of FOXL1 was significantly higher among autistic patients than that of control participants as shown in Table 2.

Table (2) FOXL1 expression among autistic patients and control participants

Expression fold	Autistic patients	Control	P-value
Min-max	0.02-100.52	0-43.64	0
mean± SD	45.25 ± 27.02	9.52±11.82	
Median	43.9	6.78	

Significance was taken at P<0.05

upregulation of FOXL1in autistic patients than control one.

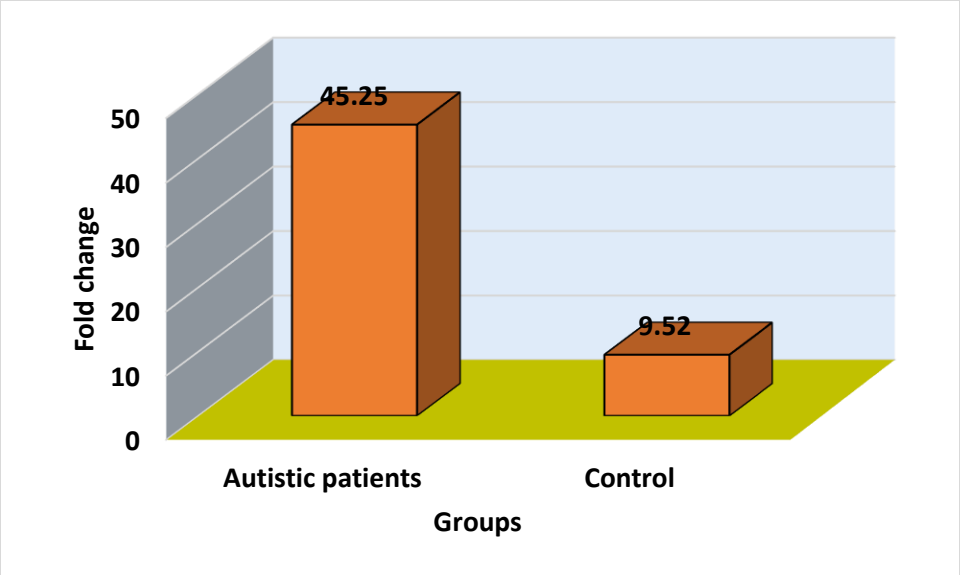

Figure (1) FOXL1 expression among autistic patients and control participants.

Estimation of GIT symptoms with severity of disease showed significant difference with severity .

Table 3 correlation between GIT symptoms in autistic patients and severity.

Number of cases	number	GTI symptom	GIT symptom%	DF	X2	P-value
mild	39	19	48.71	2	9.01	0.011(S)
moderate	13	10	76.92			
sever	8	8	100			
total	60	37	61.66			

S: Significant association (P<0.05).

Discussion:

The expression of FOXL1 was significantly higher among autistic patients than that of control participants, as shown in Table 2. figure 1

Up-regulation of FOXL1in autistic patients than control one.

FOXL1 is a transcription factor that is essential for normal gastrointestinal epithelial proliferation and differentiation. With a significant difference of correlation between GIT symptoms and disease severity, table 3.

Telocytes are big but uncommon “mesenchymal cells that form a subepithelial plexus that runs from the stomach to the colon” and are characterized by Foxl1 and expression. Foxl1+ telocytes compartmentalize the synthesis of Wnt ligands and inhibitors while sustaining the whole epithelium, allowing for regional pathway activation. Shoshkes-Carmel et al. 2018 [12]. In “Foxl1+ telocytes, conditional gene ablation of Porcupine (Porcn)”, which is needed for functional maturation of all Wnt proteins, results in early cessation of Wnt signaling to intestinal crypts, loss of stem and transit-amplifying cell proliferation, and poor epithelial renewal. As a result, Foxl1+ telocytes play a crucial role in providing niche cues to intestinal stem cells.

In conclusion, sub-epithelial Foxl1+ telocytes provide essential Wnt signals to intestinal crypt cells, allowing for homeostatic regeneration of the intestinal epithelium. Change in homeostasis regeneration will affect the intestinal epithelial function, so change in absorption and metabolism. Autistic people frequently have gastrointestinal issues Santocchi, E. et al. 2016 [14], Buie, T. et al. 2018 [15], ovene, M.R et al. 2017 [16]., Berding, K et al. 2016 [17]., Fulceri, F.et al 2016 [18]., Wakefield, A.J. et al. 2002 [19]. The intensity and quantity of GI symptoms in autistic children and their non-autistic siblings were shown to be substantially greater in autistic children and their non-autistic siblings compared to healthy controls in a limited sample size Tomova et al. 2015 [15]. As a result, larger research comparing 230 pre-schoolers found similar results, with ASD patients experiencing much more GI issues than healthy controls. Compared to ASD patients without GI symptoms, those with GI symptoms had higher anxiety difficulties and other somatic complaints and less social contact. According to study Ding, H.T et al. 2017 [20], autistic children's changed behavior, such as aggressiveness, self-injury, or sleep disturbances, might be an expression of stomach pain.

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