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# Determine Some Mutations in the Foxp1 Gene in Autistic Patients in Baghdad Governorate

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Abstract

Current study targeted 40 autistic children and 10 healthy as a control group ranging from 1 to 12 years old, in Baghdad governorate from children referred to the Central Pediatric Teaching Hospital during the period from January 2019 to July 2019 . The amplification of the targeted region (730 bp) for *foxp1* gene was performed by PCR reaction, which are in 40 autistic patient severe speech difficulty and 10 control. Agarose gel electrophoresis was used to confirm this amplification. The results of DNA sequencing for region (end of intron 16 and the beginning of exon 17 of *foxp1* gene with length of segment 730 bp and a sequence of nitrogen bases by a Pioneer company/ Korean for 20 autistic patients and 5 control. 17 samples with 5 control of DNA sequence for this region appeared to have 100% identity with an expect value of (0.0), meaning that the sequence of DNA in these samples was completely matching the original reference sequence. There were no deletions or insertions in these samples. The sample A and B detected substitution mutation were Transvertion at Location 611606 by Nucleotide A>T in intron region**.**

**Keywords:** autistic**,***FOXP1*gene, Autism Spectrum Disorders

## Introduction

Autism Spectrum Disorders (ASDs) are evaluate as neurological disorders, which means that they affect how the brain functions.Some children are slightly impaired by their symptoms, but others Intellectual disability is seen in over than 50 % the state with ASD .are severely incapacitated

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(Charman*et al. ,2011*, Klin , 2015) and the ASD autism signs also influence speaking abilities and the control of emotions. as well as, manifestations of Autism were mostly related to communing psychiatric illnesses in addition to other behavioral disabilities. (Belardinelli, *et al.,* 2016).

Pervasive Developmental Sickness including the less common Childhood Disintegrative Condition and Rett's Disorder (Baker, *et al.,* 2018).ASD levels in the UK have also been rising: 1 in 200 children was confirmed in 2002 (0.5%)and 1 in 125 children was identified in 2012.Due to the high incidence of ASD in all parts of the world, recently the genetic environment interaction has become the emphasis of intensive ASD (Giarelli*et al*., 2010). Autism incidence rates have been estimated more by Centre for Disease Prevention and Control (Knapp *et al*.,2009). It was 0.2% (20 per10000) children in the United States .It was 0.14% (14.8 per 10000) children in Asia (Blumberg, 2013).

The response with this ever increasing necessity, especially in the US, seems to have been unparalleled (Dawson, 2013). In the United States the Fighting Autistic Act approved US$950 million in autistic funding over five years and offered development programs for States to improve autism detection, early detection and prevention programs for children. (Insel and Daniels, 2011; Singh et al., 2009).

The gene family of FOXP contains 3 genes expressed in the nervous system and brain: *FOXP1, FOPX2, and FOXP4*. This This specific category of transcription factors had recognized roles throughout brain development addition to Language development.Since FOXP1 and FOXP2 interact in regulating developmental mechanisms, it was believed that mutations in FOXP1 may also be correlated with language deficiency. (Verne*set al*,2009).the *FOXP1* include 16 coding exons spanning 600 kb in chromosome 3p14. 1.Expression of *Foxp1* was found in Mantle of cerebral (layers 3–5), thalamus and hippocampus (CA1) (Carr *et al*., 2010; Hamdan*et al*., 2010; Horn *et al*., 2010).while sample C detected Transvertion in Nucleotide C>A at Location 611956 in exon region.

## Materials and Methods

### 1-Patients samples

The blood samples of autistic patients were collected during theperiod from January 2019 to July 2019 in Baghdad governorate fromchildren referred to the Central Pediatric Teaching Hospital a total of 5 ml venous blood was aspirated from each individual and divided to tube containing ethylenediaminetetraacetic acid (EDTA) for DNA extraction and plan tube for obtain of serum by

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centerfugation at 5000 rpm for immunological and biochemical test. The samples were brought to the laboratory in a well-insulated ice box.

**2-Primers of PCR .**

The sets of primer (F1-R1) selected for assessment with clinical samples eventually lead to 730bp amplicons of *HomosapiensFOXP1*gene. The sequences of the primeris as follows: for primer *FOXP1gene* (forward primer), 5'- CAGATATTGCGCAGAACCAA- 3'; R (reverse primer), 5'- GCAAACATTCGTGTGAACCA- 3'. The primer was supplied byBioneer / Korea

**3-Detection of the whole DNA:**

By electrophoresis the Purified DNA was observed in a 1% agarose gel with the addition of ethidium bromide. stain of Methylene blue (loading dye) was applied to the DNA specimen and displays the DNA using U.V. Dark Light.

**4-Agarose Gel Electrophoresis:**

Half gram of agarose was boiled in a 50 ml 1X of TBE buffer, It was left to cool down to 50 degrees 50°C.was added to agarose 5µl of Ethidium bromide and Poured to prepared tray.Comb had been removed after hardening the agarose Leading to the formation of pits

**5- Agarose Gel Electrophoresis Technique: - Sambrook*et al*., (1989)**

It was done by using TBE 1X buffer to the electrophoresis tank, tray with agarose was immersed in electrophoresis tank and buffer was about few milliliters above the surface of agarose. Each well was loaded with 7 µl (5 µl of sample and 2 µl loading dye) and last well with standard molecular weight DNA ladder was loaded. Tank was closed and electrophoresis run at 90 volt of the gel. Agarose was removed from the tank and visualized with the aid of UV transilluminator and photographed.

**6-State of DNA preparation and amplification**

DNA was isolated from 200µl of whole blood or other body fluids by use a available commercially kit for DNA purification and isolation [G-Spin complete DNA extraction (Intron/Korea) as instructed by the supplier. The specimens were examined using a basic PCR targeted polymerase *foxp1* gene. The solution was prepared by mixing 25 µl of Taq for each primer. (1 µl f and1 µl R), 18µl distilled water and 5 µl of DNA template (total 50 µl). The following criteria were applied to *foxp1 gene*: 95°C for 3min, 95°C for 35s,56 °C for 35s, and

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72°C for 45s (40 cycles). The samples were incubated at 72oC for 10 minutes after the final step to fulfillment the reaction. The PCR products were isolated for 15 minutes by electrophoresis on 1% agarose gel stained with ethidium bromide (0.5 g/ml), rinsed and photographed under UV illumination.

## Result and Discussion

### Molecular study of *FOXP1* gene

### DNA extraction and amplification

Agarose gel electrophoresis used following Nanodrop to Ensure the integrity and presence of the DNA extracted (figure 3-1). PCR reaction was performed for the amplification of the targeted region in *foxp1* gene, which are in 40 autistic patient severe speech difficulty and 10 control. Agarose gel electrophoresis was used to confirm this amplification for each region following several optimization experiments when sharp, single bands with the accurate molecular sizes. Such as figure

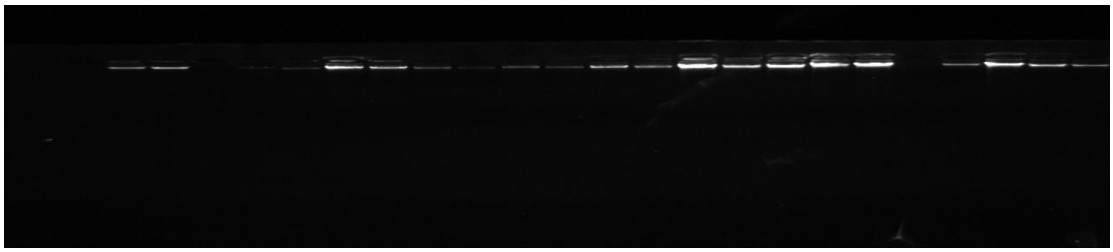

**Figure (2-1): Chromosomal DNA bands extracted from human blood samples on 1% agarose gel for 1 hour at 90 voltages, visualized under U.V light using gel documentation system after staining with ethidium bromide for half an hour for different samples.**

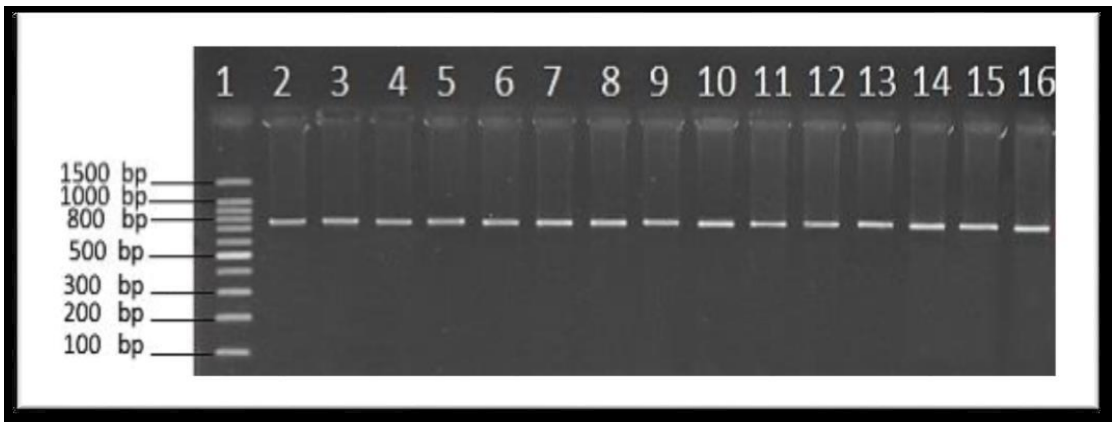


**Figure (2-2): PCR products of *foxp1* of the molecular size 730 bp . Bands were separated by**

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**electrophoresis on 1 % agarose gel for 1 hour at 90 voltages and Viewed first by gel doc method after staining with EtBr. for 30 minutes. Lane 1: DNA ladder (100-1500 bp).**

**Sequencing of FOXP1**

Automated DNA sequencing resulted in a chromatogram (also called electropherogram) computer performance that is the visual image of a sequence of DNA including nucleotide sequences and peaks. Generally, the good series starts about base 20 and is characterized by high independent peaks which have no overlapping. (Dong and Yu, 2011).

The findings of DNA sequencing for all samples were examined and matched by using BLASTn (Basic Local Alignment Search Tool) NCBI nucleotide alignment tool, in which the DNA sequence collected throughout this analysis was compared to the human standard foxp1 gene sequence (NG_028243.1). Alignment using BLAST tool made it easy to detect andlocate mutations along this gene. In this study, DNA sequencing for this region (end of intron 16 and the beginning of exon 17) in 40 autistic patients and 10 control group. BLAST results for 17 patients samples and 10 control samples of DNA sequence for this region appeared to have 100% identity with an expect value of (0.0), meaning that the sequence of DNA in these samples was completely matching the original reference sequence. The sample A and B detected substitution mutation were Transvertion at Location 611606 by Nucleotide A>T in intron region while sample C detected Transvertion in Nucleotide C>A at Location 611956 in exon region.

**Table (2-1) Description and analysis of *FOXP1* gene**

| **No. of sample** | **Type of substitution** | **Location** | **Nucleotide** | **region** | **sequence ID** | **source** |
|---|---|---|---|---|---|---|
| A | Transvertion | 611606 | A>T | Intron | NG_028243.1 | *Homo sapiens FOXP1* gene |
| B | Transvertion | 611606 | A>T | Intron | | |
| C | Transvertion | 611956 | C>A | exon | | |

A part of the fork head box transcription factor family containing a DNAbinding region and a region of protein-protein interaction is encoded by the FOXP1 gene.The primary role of the *FOXP1* gene is to inhibition of transcription (Cosmi*et al*., 2011) and is commonly articulated in

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In the changing and mature brain.It has been proposed that the gene is involved in brain development and function ( Hashimoto *et al*., 2005). Mostly in research, heterozygous removals overlapping the FOXP1 gene were observed in 3 subjects with mental retardation and severe communicative skills defects. (Onore*et al*., 2009). A de novo single base insertion in FOXP1 that introduces a frameshift and a premature stop codon was found in a seriously affected person inside an exome sequencing sample of 40 intermittent Individuals with ASD (simplex ASD). (Cosmi*et al.*, 2011). These results indicate that syndromic or non-syndromic ASD is linked with haploinsufficiency or hypomorphic FOXP1 mutations with diminished expression or defective FOXP1 activity. In this review, we suggested that higher expression of the FOXP1 gene was related to autism in total. (Petalas*et al*., 2012). Jyonouchi*et al.*(2012) A direct interaction between FOXP1 and ASD has been found to broaden our understanding of FOXP1's relationship with autism.Several experiments have discovered FOXP1 deletions, mutations or copy number changes in people with ASD. (Petalas*et al*., 2012). FOXP1 may also suppress CNTNAP2 expression, indicating FOXP family members coordinated transcriptional control of ASD genes (Cosmi*et al.*, 2011). Together all these results indicate that for the communication and signaling pathways involved in ASD, synchronized downstream signaling of FOXP1 and FOXP2 could be important.