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Assessment of Genetic Mutations in Genes *DSM-IV, DRD4, SERT, HTR1B, SNAP25, GRIN2A, ADRA2A, TPH2* and *BDNF* Induced Attention Deficit Disorder and Hyperactivity in Children

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Abstract

In this study we have analyzed 1200 people, 580 ADHD and 620 control groups. The genes *DSM-IV, DRD4, SERT, HTR1B, SNAP25, GRIN2A, ADRA2A, TPH2* and *BDNF* analyzed in terms of genetic mutations made. In this study, people who have genetic mutations were targeted, with nervous disorders, ADHD. In fact, of all people with ADHD, 360 children had a genetic mutation in the genes *DSM-IV, DRD4, SERT*, and *HTR1B*. And 100 children had a genetic mutations in SNAP25, GRIN2A genes were a genetic mutations in the genes *ADRA2A, TPH2* and *BDNF* and 70 children, respectively. Any genetic mutations in the target genes control group, did not show.

Hypothesis and objectives: In this study, we further understand the genes involved in ADHD children with the genetic mutations discussed. The aim of this study was to evaluate genetic and epigenetic closer to induce hyperactivity disorder in children.

Keywords: Genetic study; ADHD; Mutations; The genes *DSM-IV; DRD4; SERT; HTR1B; SNAP25; GRIN2A; ADRA2A; TPH2; BDNF*

Introduction

Today, hyperactivity or attention deficit disorder in children, neurological disorders are incurable and usually fatal disease. Hyperactivity always caused by genetic mutations that have the potential to be transmitted from mother to child. If the mother during pregnancy, the drug as well as strong antibiotics and sedatives as well as taking abortion drugs, the probability that a child is hyperactive, very much.

Attention deficit hyperactivity disorder (ADHD)

Is a mental disorder of the neurodevelopmental type [1,2]. It is characterized by problems paying attention, excessive activity, or difficulty controlling behaviour which is not appropriate for a person's age [3]. These symptoms begin by age six to twelve, are present for more than six months, and cause problems in at least two settings (such as school, home, or recreational activities) [4,5]. In children, problems paying attention may result in poor school performance [3]. Although it causes impairment, particularly in modern society, many children with ADHD have a good attention span for tasks they find interesting [6].

Despite being the most commonly studied and diagnosed mental disorder in children and adolescents, the cause is unknown in the majority of cases [7]. The World Health Organization (WHO) estimated that it affected about 39 million people as of 2013 [8]. It affects about 5% to 7% of children when diagnosed via the DSM-IV criteria [9,10] and 1% to 2% when diagnosed via the ICD-10 criteria [11]. Rates are similar between countries and depend mostly on how it is diagnosed [12]. ADHD is diagnosed approximately three times more often in boys than in girls [13,14]. About 30% to 50% of people diagnosed in childhood continue to have symptoms into adulthood and between 2% to 5% of adults have the condition [15,16]. The condition can be difficult to tell apart from other disorders, as well as to distinguish from high levels of activity that are still within the normal-range [5].

Materials and Methods

In this study, 580 patients with ADHD and 620 healthy controls were studied. Peripheral blood samples from patients and parents with written permission control were prepared. After separation of serum, using Real Time-PCR technique of tRNA molecules was collected. To isolate Neuroglial cells erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solution in ratio of 1 to 5 with the peripheral blood of patients and controls were mixed. After 60 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 14 min at 400 Gera. The cell sediment with PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrate ratio of 1 to 2 on ficole (Ficol) was poured in the 480G was centrifuged for 34 minutes. Mono nuclear Neuroglial cells also are included, has a lower density than ficole and soon which they are based. The remaining erythrocytes have a molecular weight greater than ficole and deposited in test tubes.

The supernatant, which contained the mononuclear cells was removed, and the 400 Gera was centrifuged for 12 minutes. Finally, the sediment cell, the antibody and Neuroglial cells was added after 34 minutes incubation at 5°C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the

column LSMACSS pam Stem cell culture medium containing the transcription genes *DSM-IV, DRD4, SERT, HTR1B, SNAP25, GRIN2A, ADRA2A, TPH2* and *BDNF* were kept (Figures 1-8).

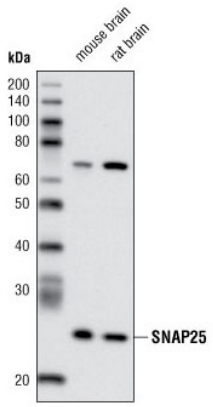

Figure 1: *SNAP25* gene pattern band formation in patients with ADHD.

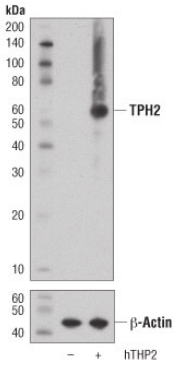

Figure 2: *TPH2* gene pattern band formation in patients with ADHD.

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose, approximately 4.5 × 103 Neuroglial cells were transfer red to 1.5 ml Eppendorf tube and then were centrifuged at 2000 rpm for 7 minutes a time. Remove the supernatant culture medium and there maining sediment, 100 µl of PBS buffer was added. After adding 5-10 µl CD4 + PE monoclonal anti body to the cell suspension for 60 min at 4°C, incubated and read immediately by flow cytometry. For example, rather than control anti body Neuroglial cells PE, IgG1 negative control solution was used.

Total mRNA extraction procedure includes

1) 1 ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200 µl chloroform solution to target mix, then transfer the micro tubes were added, and the shaker well was mixed for 15 seconds. The present mix for 4 minutes at room temperature and then incubated for 20 min at 4°C and was centrifuged at 13200 rpm era. Remove the upper phase product were transferred to a new microtube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hours at -20°C was incubated.

2) Then for 45 min at 4°C, it was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1 ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4°C and by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placed at room temperature until completely dry deposition. The precipitate was dissolved in 20 µl sterile water and at a later stage, the concentration of extracted mRNA was determined.

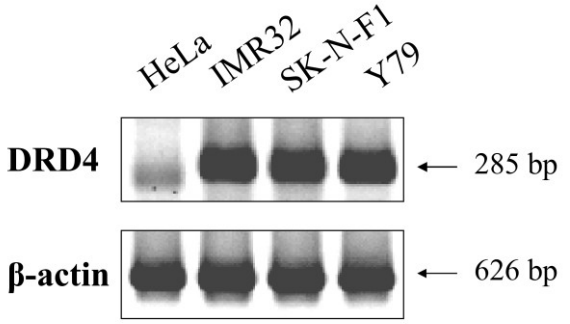

Figure 3: *DRD4* gene pattern band formation in patients with ADHD.

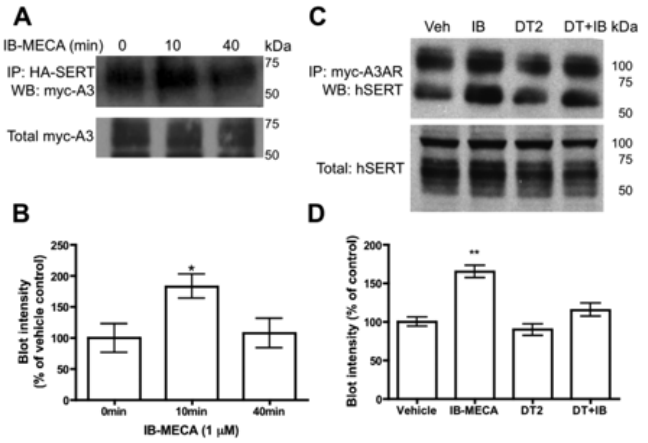

Figure 4: *SERT* gene pattern band formation and diagram in patients with ADHD.

To assessment the quality of mi-RNAs, the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentas K1622) and 1 µl oligoprimers 18(dT) was performed. Following the PCR reaction 2 µM dNTP, 1µg cDNA, Fermentas PCR buffer 1X, 0/75 µM $MgCl_2$, 1.25 U/µl Tag DNA at 95°C for 4 min, 95°C for 30s, annealing temperature 58°C for 30s, and 72°C for 30 seconds, 35 cycles were performed. Then 1.5% agarose gel, the PCR product was dumped in wells after electrophoresis with ethidium bromide staining and color was evaluated.

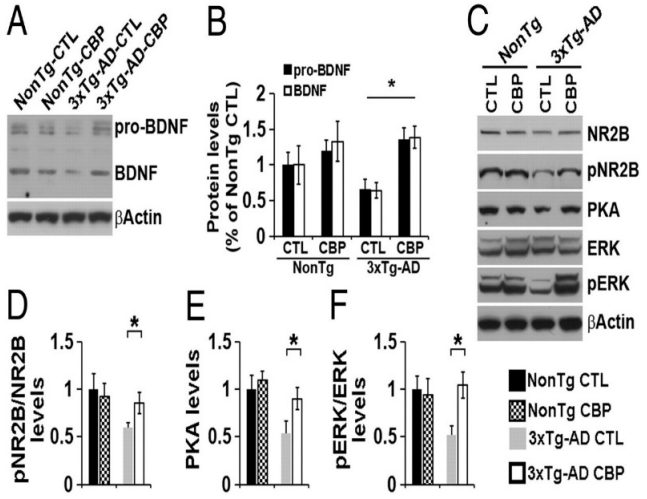

Figure 5: *BDNF* gene pattern band formation and diagram in patients with ADHD.

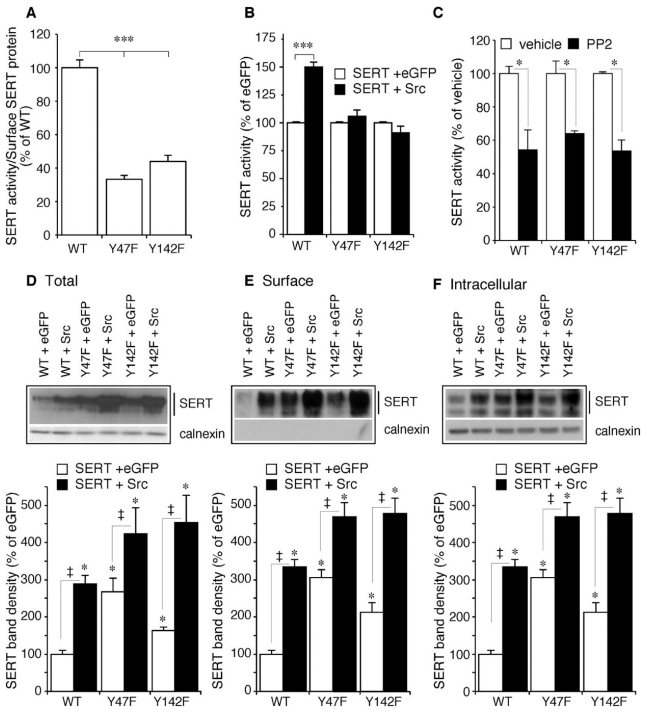

Figure 6: *SERT* gene pattern band formation and the density diagram in patients with ADHD.

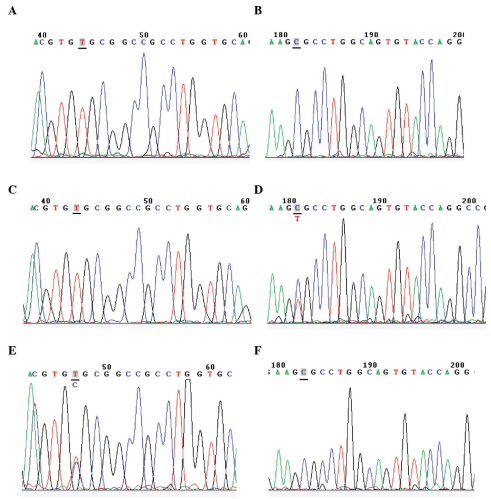

Figure 7: Schematic view of genes mutations in patients with ADHD.

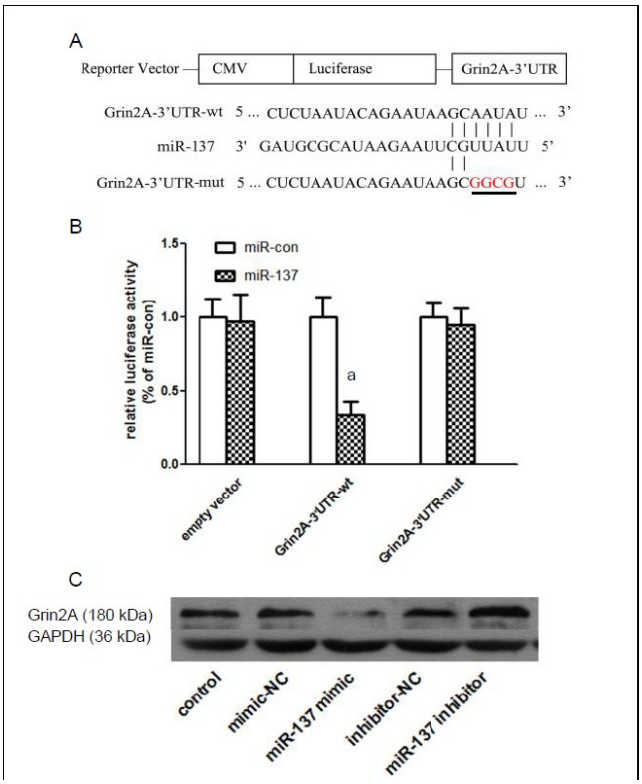

Figure 8: *GRIN2A* gene pattern band formation and the density diagram in patients with ADHD.

Discussion and Conclusion

According to the results of sequencing the genome of patients with ADHD, and the genetic mutations *DSM-IV, DRD4, SERT, HTR1B, SNAP25, GRIN2A, ADRA2A, TPH2* and *BDNF* genes found that

about 91% of patients with ADHD, they have these genetic mutations. Patients with ADHD, unusual and frightening images in the process of ADHD, experience. Lot epigenetic factors involved in ADHD. But the most prominent factor to induce ADHD, mutations is *DSM-IV, DRD4, SERT, HTR1B, SNAP25, GRIN2A, ADRA2A, TPH2* and *BDNF* genes. These genes can induce the birth and can also be induced in the adulthood. This study is the largest genetic study of ADHD in the country and one of the largest global research in the genetics of psychiatric illness is more active. This study shows that many genes and lifestyle, and diet of the mother during pregnancy induced hyperactivity disorder in children is involved. We hope researchers in the field as well as additional genes are examined for the disease (Figures 1-8).

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