Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 10, Issue 1, March 2015 , p.p(71-77)
ISSN 1992 - 0849

Molecular genetic study of the gene in the serotonin transporte 5-HTT for patients with autism in Iraq

Zubaida Adnan Al-jashammy

Department of biology / College of Education for Applied Science / Tikrit University

zubaidabiology@gmail.com

Received date : 3 / 9 / 2014 **Accepted date : 17 / 11 / 2014**

ABSTRACT

Autism is a neuropsychiatric disorders , which have been associated with many causes including genetic causes, which affects 4 out of every 10000 people in the world . In this study, the molecular gene serotonin 5-HTT specially 5HTTLPR area multi-format gene to gene serotonin ,which is located on chromosome 17 which revealed it's association with many of the mental disorders as being responsible for the uptake of serotonin by nerve cell after the synapse and signal transduction of nerve to nerve cells. DNA has been isolated from epithelial cells of the lining of the mouth was studied in fifty child diagnosed clinically with autism in Iraq and conducted PCR technique to amplify the genes and migrated to gel Agaros 2% were analyzed style band was done in an attempt to detect any evidence of a link between autism and gene 5-HTT.

Keyword: *Autism ,5-HTT, 5-HTTLPR, Serotonin*

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دراسة وراثية جزيئية للجين الناقل للسيروتونين 5-HTT لمرضى التوحد في العراق

زبيدة عدنان الجشعمي

قسم علوم الحياة / كلية ا لتربية للعلوم الصرفة / جامعة تكريت
zubaidabiology@gmail.com

تاريخ استلام البحث : 3 / 9 / 2014 تاريخ قبول البحث : 17 / 11 / 2014

الملخص

التوحد هو احد الاضطرابات العصبية النفسية الذي ارتبط بالعديد من الاسباب ومنها الاسباب الوراثية ويصيب هذا المرض 4 من بين كل 10 الاف شخص في العالم . وفي هذه الدراسة تم اجراء دراسة جزيئية لجين السيروتونين 5-HTT على الاخص منطقة متعدد الشكل الجيني 5-HTTLPR لجين السيروتونين والتي تقع على الكروموسوم رقم 17 والذي كشف عن ارتباطه بالعديد من الاضطرابات النفسية كونه المسؤول عن عملية التقاط السيروتونين من قبل الخلية العصبية بعد المشبك العصبي ونقل الاشارة العصبية الى الخلايا العصبية ،حيث تم عزل الحامض النووي منقوص الاوكسجين من الخلايا الطلائية الحرشفية لبطانة الفم لخمسين طفل شخص سريريا أصابتهم بالتوحد في العراق وبأستخدام تقنية PCR لتضخيم الجينات وترحيلها على هلام الاكاروز 2 ٪ حيث تم تحليل نمط الفرقة في محاولة لكشف أي دليل على وجود علاقة بين التوحد وجين 5-HTT .

الكلمات الدالة: التوحد, 5-HTT, 5-HTTLPR,السيروتونين.

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1.INTRODUCTION

Autism is a neurodevelopmental disorder characterized by The clinical trial of social deficits, impaired communication and repetitive restrictive behavior patterns. including genetic causes which affects 4 out of every 10 000 people in the world, with males being affected at least four times more frequently than females [1,2,3,4,5]. Many different biological causes have been implicated in the etiology of autism, but genetic factors appear to be the most important: family studies have revealed are currence risk of 4% among siblings of affected probands and twin studies have shown a significant difference in the concordance rates of autism between monozygotic and dizygotic twins [6] .The serotonin transporter promoter length polymorphism (5-hydroxytryptamine transporter length polymorphism; *5*-HTTLPR) has long been implicated in autism and other psychiatric disorders. The use of selective serotonin reuptake inhibitors (SSRIs) has a positive effect on treating some symptoms of autism. The effects of these drugs vary in individuals because of the presence of the S or L allele of 5-HTTLPR. Studies performed on various autistic populations have found different allele frequencies for the L and S alleles[7,8,9,10,11] .The polymorphism occursin the promoter region of the gene. Researchers commonly report it with two variations in humans: A short ("S") and a long ("L"), but it can be subdivided further [12,13,11] .The purpose of this study was to analyze the genotype of the 5-HTT regulatory region in autism patients to determine the role of this genetic factor in the pathogenesis of autism and possibly to identify patient subgroups. To the best of our knowledge, this is the first report of a genetic clinical study of autisms in Iraq.

2. MATERIALS AND METHODS

In this study, 50 patients with autism have been clinically diagnosed with autism between the ages of 3-15 years and by 44 males and 6 females, collected from three rehabilitation centers in the province of Baghdad. Samples were collected from patients by buccal swabs method and DNA have been isolated by a special kit is equipped with the company Vørnslk. Genetic analysis was carried with sample PCR. Primer PF (5′ATGCCAGCACCTAACCCCTAATGT3′)and PR(5′GGACCGCAAGGTGGGCGGGA3′) were used to amplify a product that consist of 375 base pair (bp) product for the 14-repeat (s) allele and 419 bp product for the 16repeat allele show [14] Applied Bio system Veriti^{TM} .

Thermal Cycler was used for deoxyribonucleic acid (DNA) amplification .The Polymerase chain reaction (PCR) cycling condition consisted of an initial denaturation for 2min at

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95C°,followed by 35 cycles of 95 C°for 1min,62 C°1min ,and 72 C°for 1min,and afinal extension at 72 C°for 4 min .Polymerase chain reaction products were electrophoresed on a 2% agarose gel and visualized under ultraviolet .

3.RESULTS

The association of serotonin transporter gene variations and autism was studied at molecular level using PCR technique and looked for mutations in 5HTT gene .The DNA was isolated by a special kit is equipped with the company Vørnslk. The isolated DNA was run on a 0.8% agarose to check the quality of the DNA and nanodrop 2000 instemal was used to check quantity and also quality of DNA in the sample. On an average the quality of the DNA was fixed on 1.6 -1.8 ng in all samples and also no bands were observed when it was run on 0.8% agarose gel. The PCR was also done in all Patient in which the DNA was seen as fragments by increasing the concentration of the DNA. Out of 50 samples screened, only 38 patient samples showed a band at 419 bps corresponding to the long allele of 5HTTLPR and in 3 patient samples showed a band at 700 bps in addition to main band ,three samples showed two band at 317 , 419 bps and 6 samples no band were observed, which may be due to the absence of required DNA quantity for amplification Figure.(1).

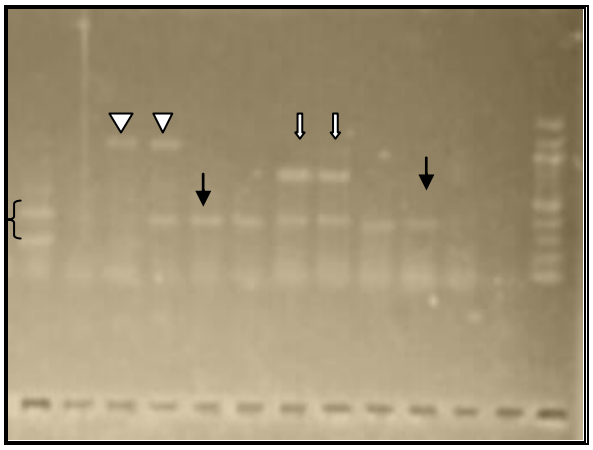

Figure.(1): Band patterns of agarose of gel electrophoresis after PCR
↓ 700pbs , ⇩ 419pbs, ▽ 900pbs, ⊂ Two bands

4.DISCUSSION

The serotonin transporter (5-HTT) is promising candidate for introducing the heritability of interindividual variation in personality and the genetic susceptibility for various psychiatric diseases. Other lines of evidence also suggest that a dysregulation in serotonergic

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neurotransmission might be involved in the pathogenesis of autism and this led us to consider the serotonin transporter gene as primary candidate gene in autistic disorder .Studies have shown that the short variant of 5-HTT has been reported to be aquantitive trait locus for anxiety disorder and short variant of 5-HTTLPR is preferentially transmitted from parents to autistic patients and short variant of 5-HTT is also associated with lower serotonin up take activity which produces significantly less 5-HTT mRNA and protein ,and the gene is less active in individuals with shorter promoter [15]In the current study, we tried to find a relationship between gene of serotonin and Autism using PCR, results showed increased frequency the longer allele compared the short allele although previous studies indicated that the relation shape between high frequency of short allele with neurological disorders including autism cases in a number of studies in different countries but in current study, which included patients with autism in Iraq have shown results contrast with previous studies, this may be due to the role of some environmental factors in addition to genetic factors led to an illness during their embryogenesis or during their development or the cause of neurological disorders in these patients may not return to the serotonin concentration level but to the faulty nerve receptors disorder after neurosynptic ,clearly this result consist with aprevious study in 1961 has shown to increased the whole blood 5-HTT in blood in children with autistic disorder [16].in addition the long allele was showed in halotype in most cases its may be a sign of being linked to the disease. We note the results of previous genetic packages at 700 to 900 pbs and interpreted as a mutation may have occurred on the serotonin gene to regist patients for this mutation observed link autism with hyperactive behaviors for These patients, which may caused by this mutation.

5.CONCLUSION

From the results, it can be concluded that present findings do not support the existence of association between the 5-HTT gene variant and autism in our subjects, since the short variant of 5HTT is transmitted but the mutation in 5-HTT may be due to emergence of symptoms of hyperactivity in patient with gene mutant .The researchers' findings in autism are preliminary and require replication. If replicated with patient samples from same and different ethnic and geographical backgrounds, it is possible that autistic disorder may share common risk at this locus. Integration of results from DNA sequencing, molecular cytogenetic, and psychiatry will help us to understand the genetic background of autism in the future.

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6.ACKNOWLEDGEMENTS

I express my gratitude to all patients for providing buccal swapes samples and their teachers especially miss Sherrin Kalaf for their support in collecting samples and data .I gratefull too much of dr. Akeel Hussain and his group from central lab. In Tikrrit university for excellent technical assistance . I also thank miss Rasha Jamal to help shown by me.

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AUTHOR

Zubaida A. Al-Jashammy: received M. B.Ch.B and Ph.D. in (Human genetic & Bacterial genetic) from Baghdad University /Baghdad – Iraq in 1996 -2007 respectively .During 1996-2002 she worked as amaster in college of eduction (Ibn –Al Hathum) then as adoctor in Tikrite University /biology Dept. from 2003 till now.