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Analysis of Serotonin Transporter Gene (5HTT) Variants Association in Children with Autistic Disorder

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ABSTRACT Autism is a complex neurodevelopmental disorder and the prevalence was estimated to be 4 in every 10,000 children. Autism cannot be traced to a Mendelian mutation and thought to be a complex multifactorial disorder. 5-HTT gene is responsible for the reuptake of serotonin into the presynaptic cell after it has been released into the synaptic cleft to signal the adjacent neuron. 5-HTTLPR is a degenerate repeat polymorphic region in 5-HTT gene, located on chromosome 17 and has been implicated in some human mental disorders. In the present study the researchers screened for the association of serotonin transporter gene variants in children's with autism. The DNA was isolated using salting out method and PCR was performed for the amplification of the gene of interest and the products were run on 2% agarose gel and the band pattern were analyzed. The study analysis, revealed no evidence for an association of 5-HTT gene variants and autism.

INTRODUCTION

Autism is a complex neurodevelopmental disorder impacting development in the areas of social interaction, communication skills and behaviour and they do not follow the typical patterns of development and autistics have been described as being in their "own world" (Abrahams and Geschwind 2008). Prevalence was estimated to be four in every 10,000 children, and the ratio of affected males to females is 3: 1(APA 1994). It is characterized by marked social deficits, deviant language, difficulties in verbal and non- verbal communication, social awkwardness, social deficits and unusual responses to the environment, such as a restricted range of stereotyped repetitive behaviors, circumscribed interests and sensory issues usually occurring within the first 3 years of life and associated with subtle abnormalities in specific structures or functions in the brain (Christine et al. 2008). It is strongly genetic, heritable but the specific causes of autism are unknown; although many genetic and environmental causes of autism have been proposed, its theory of causation is still incomplete. Teratogens are also related to the risk of autism in rare cases (Piven et al. 1997).

Serotonin is an important neurotransmitter, one of the short-range signaling molecules with which one neuron communicates with another and it is involved in inducing sleep, sensory perception, temperature regulation and control of mood. The serotonin transport protein (5-HTT-*5-hydroxytrptamine transporter*) also called as *SLC6A4* is a monoamine transporter protein with 12 transmembrane domains and is involved in the transport of serotonin in the serotonergic neurons of central nervous system. It has 14 exons and it is located on chromosome 17 (17q11.2). Some individuals with autism were found to have abnormal levels of serotonin in their blood stream platelets **(**Cuccaro et al. 1993). 2 major polymorphisms in 5HTT are a 44bp insertion/deletion in the 5HTTLPR promoter region and VNTR in the intron 2 of 5HTT. 5-HTTLPR is a degenerate repeat polymorphic region in 5-HTT, and it has been implicated in some human mental disorders. The alleles of the 5-HTT gene are called "short" and "long," the terms "short" and "long" actually refer to two different lengths of the sequences in the gene's regulatory region. The polymorphism causes decreased gene expression and fewer serotonin transporters in the membrane of the cell. Thus, rate of reuptake of serotonin is

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reduced and the difference lies in the structure of the promoter, the regulatory region that turns the gene on and off. 5HTTLPR alleles are most commonly composed of either fourteen (short or "S" allele) or sixteen (long or "L" allele) repeated elements, each of 20 to 23 bp which alters the promoter activity (Lesch et al. 1997). The short (S) 5HTTLPR variant of the 5HTT gene has been reported to be associated with lower basal and transcriptional efficiency of the 5HTT resulting in lower serotonin uptake activity which produces significantly less 5-HTT mRNA and protein, than the long (L) variant, in the synaptic cleft (Cook et al. 1997). In the human population, the frequency of the long allele of 5-HTT is about 57% that of the short allele is 43 % (OMIM 2003).

With the exception of chromosomes 14 and 20, rest of the chromosomes have been associated with autistic behavior. Abnormalities of chromosome 15 and structural and numerical abnormalities of the sex chromosomes have been the most frequently documented (**Janine et al.** 2000). Some of the common syndromes associated with autism are Asperger's syndrome, Rett syndrome, Prader-Willi Syndrome, Angelman Syndrome and Fragile X syndrome (Zafeiriou et al. 2007) and the candidate genes for autism are FOXP2, located on chromosome 7q31, found to be an important regulator of embryogenesis and neural functioning. RELN, on chromosome7q22.2, which is expressed during embryonic development and play a crucial role in cortex lamination. GABA (A), on chromosome 15q11-q13 is responsible for synaptic inhibition in the adult brain and UBE3A, for normal sensory and cognitive abilities (Klauck et al. 1997).

The potential role of the serotonergic system in the etiology of autism is still under investigation but several serotonin transporter (5-HTT) re-uptake inhibitors have been demonstrated to be partially successful in treatment of autistic symptoms. In a study conducted by Betancur et al. (Betancur et al. 2002) people with autism found to have irregularities and abnormal levels of serotonin and other neurotransmitters in the brain, serotonin is of interest to autism research-ers because some individuals with autism have consistently been found to have abnormal levels of serotonin in their blood stream platelets and this suggest that autism could result from the disruption of normal brain development early in fetal development caused by defects in genes that control and regulate brain growth. There-fore, 5-HTT seems to be a good candidate for association studies in the serotonin transporter gene. The current study is replicating these analyses, on the importance of the serotonin transporter gene as a genetic risk factor in autism. Previous studies have provided conflicting evidence regarding the association of the serotonin transporter (5-HTT) gene with autism and hence the genetic contribution to autism was studied by performing a molecular analysis on the blood samples of children with autism to identify the role of serotonin transporter gene (5HTT) in autistic disorder

Aim

The main aim of the study was

(i) To identify the association of serotonin transporter gene variations(5HTT) in children with autism using PCR technique.
(ii) To estimate the frequency of 5HTT gene variation in children with autistic disorder.

METHODOLOGY

Subjects

The study enrolled 23 - Autistic subjects , 7 - Age matched controls, attending the Department of speech, language and hearing Sciences at Sri Ramachandra University, Porur, Chennai, India.

Subjects of both the sexes were taken in to consideration. Pedigree was charted out by obtaining family history.

Sample Collection

Ethical clearance was obtained from the committee for student's proposal at Sri Ramachandra University, in order to proceed with the sample collection. A formalized informed consent form will be used to obtain approval from the subject's guardian to proceed with the sampling. Demographic and family information were obtained from the subject's respective guardians.

Experimental Design: DNA Isolation and PCR

DNA Isolation

Genomic DNA was isolated from the blood samples using high salting out method (Miller

et al. 1988). 5 ml of peripheral blood from the subject was collected in a sterile K_2 – EDTA vacutainer to that double the volume of red cell lysis buffer was added followed by 6 drops of Triton –X100 and incubated at 37°C for about 5 to 7 minutes. The tube was then centrifuged at 2000g for 15 minutes at 4°C. The supernatant was discarded and pellet was retained and to that 1ml of nucleated cell lysis buffer and 20µl of 10% SDS was added and incubated at 55°c for 20mins – 2 hours. After incubation 400µl of 5M NaCl was added and centrifuged at 10000 g for 15 minutes, the supernatant was transferred to a sterile 15ml centrifuge, to which double the volume of ice cold ethanol was added. The tube was then gently inverted several times until DNA in the form white strands is precipitated out. The DNA strand was then picked up and washed with 70% ethanol, the pellet was air dried and resuspended in TE buffer. Quality and quantity of DNA was checked using agarose gel electrophoresis and nanodrop.

Polymerase Chain Reaction (PCR)

The isolated DNA was then subjected to PCR (Vijayalakshmi et al. 2005). A master mix was prepared containing nuclease free water, Taq buffer (1X), dNTPs (0.2mM), Forward and reverse primers (0.1-1µM), Taq DNA polymerase (1.5U/ µl), and template DNA (10ng/ µl) and the tubes were placed in the thermocycler. A gradient PCR was first performed before running the standard PCR in order to determine the optimum temperature for primer annealing. After the optimum annealing temperature was identified, a standard PCR was performed and on completion of the standard PCR, the products were run on 2% agarose gel and the bands patterns were analyzed using a gel documentation system.

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 salting out method. The isolated DNA was run on a 0.8% agarose to check the quality of the DNA and nanodrop was used to check quantity and also quality of DNA in the sample. On an average the quality of the DNA was found to be 1.77 in 13 samples and in rest of the 10 patient samples we were not able to isolate the DNA as strand and the DNA were seen as fragments, and also no bands were observed when it was run on 0.8% agarose gel. Initially a gradient PCR was carried out to check the optimum annealing temperature. Annealing temperature was found to be 64.5°C. Same annealing temperature was followed for standard PCR reactions in all 23 patient's samples. The PCR was also done in 10 Patient in which the DNA was seen as fragments by increasing the concentration of the DNA. Out of 23 samples screened, only 13 patient samples showed a band at 528 bps corresponding to the long allele of 5HTTLPR and in 10 patient samples no band were observed, which may be due to the absence of required DNA quantity for amplification (Fig. 1).

DISCUSSION

The serotonin transporter (5-HTT) gene 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 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 5HTT has been reported to be a quantitative trait locus for anxiety disorder and short variant of 5-HTTLPR is preferentially transmitted from parents to autistic patients and short variant of 5HTT is also associated with lower serotonin uptake activity which produces significantly less 5-HTT mRNA and protein, and the gene is less active in individuals with shorter promoter.

In the present study the researchers examined the association between autistic disorder and serotonin transporter gene variations using PCR technique. The study results have shown the band length corresponding to the longer allele of 5HTT gene. Based on the literature survey it has been found that the short variant of the 5*HTT* gene is found to be preferentially transmitted and hence the study analysis revealed no evidence of association of serotonin transporter gene variant and autistic disorder. The negative results obtained in the present study could

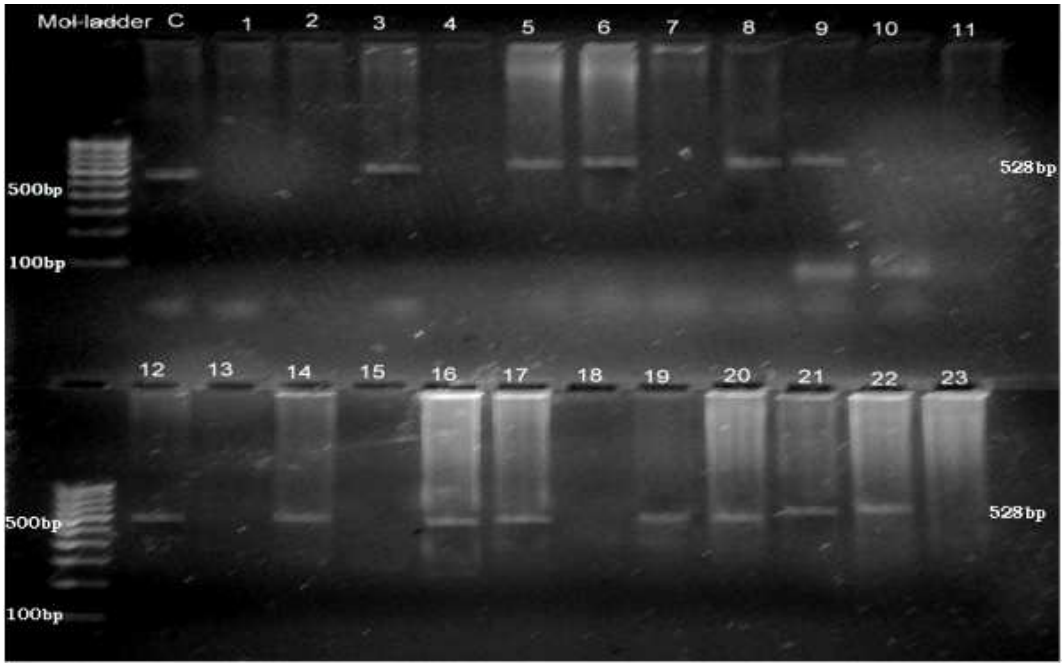

Fig. 1. Band patterns of agarose gel electrophoresis after PCR
Lane 1 &14 – DNA ladder
Lane 2 – Normal control (C)
Lane 3-13 – Patient's sample
Lane 15-26 – Patient's sample
The No: 1- 23 represents patient sample

mainly be attributed to the fact that autism is a complex anomaly with a multifactorial inheritance. Manifestation of the disease in individuals could be due to some environmental factors during their embryogenesis or during their development. Patient samples also reflect on difference in selection of patients and etiological heterogeneity.

CONCLUSION

To conclude, the 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. 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 cytogenetics, and psychiatry will help us to understand the genetic background of autism in the future.

REFERENCES

Abrahams BS, Geschwind DH 2008. Advances in autism genetics on the threshold of a neurobiology. *Nature Reviews Genetics,* 9: 341–355.

American Psychiatric Association 1994. *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV).* 4th Edition. Washington DC: American Psychiatric Press.

Betancur C, Corbex M, Spielewoy C, Philippe A, Laplanche JL, Launay JM et al. 2002. Serotonin transporter gene polymorphisms and hyperserotonemia in autistic disorder. *Molecular Psychiatry,*7: 67–71.

Christine HH, Santangelo Susan L 2008. Autism and serotonin transporter gene polymorphisms. A systematic review and meta-analysis. *American Journal of Medical Genetics Part B. Neuropsychiatric Genetics,* 147B: 903–913.

Cook EH, Courchesne R, Lord C, Yan S, Lincoln A, Haas R, Courchesne E, Leventhal BL 1997.Evidence of linkage between the serotonin transporter and autistic disorder. *Molecular Psychiatry,* 2: 247–250.

Cuccaro ML ,Wright HH, Abramson RK, Marstellar FA, Valentine J 1993. Whole- blood serotonin and cognitive functioning in autistic individuals and their first-degree relatives. *The Journal of Neuropsychiatry and Clinical Neurosciences,* 5: 94- 101.

Janine A, Lamb Moore J, Bailey A, Monaco A 2000.Autism-recent molecular genetic advances. *Human Molecular Genetics,* 9: 861-868.

Klauck SM, Poustka F, Benner A, Lesch KP, Poustka A 1997. Serotonin transporter (5-HTT) gene variants associated with autism? *Human Molecular Genetics*, 6: 2233-2238.

Lesch KP, Meyer J, Glatz K 1997. The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) in evolutionary perspective: Alternative biallelic variation in rhesus monkeys. *Journal of Neural Transmission*, 104: 1259–1266.

Miller SL, Dykes DD, Polesky HF 1988.A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, 16: 1215.

Online Mendelian Inheritance in Man OMIM (TM) 2003. Johns Hopkins University.MIM Number: 182138. From <http: //www.ncbi.nlm.nih.gov/omim/> (Retrieved August 15, 2003).

Piven J, Palmer P, Jacobi D, Childress D, Arndt S 1997.Broader autism phenotype evidence from a family history study of multiple-incidence autism families. *American Journal of Psychiatry*, 154 (2): 185–90

Vijayalakshmi K, Vettriselvi V, Krishnan M, Sunil S, Vishwanathan KN et al. 2005. Polymorphism at GSTM1 and GSTP1 gene loci and risk of prostate cancer in a south Indian population. *Asian Pacific J Cancer Prev*, 6: 309-314.

Zafeiriou DI, Ververi A, Vargiami E 2007. Childhood autism and associated comorbidities. *Brain and Development*, 29: 257-272.