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Association of CTG Trinucleotide Repeat (TNR) Allele Polymorphism of Gene CTG B33 with Schizophrenia Expression in North- Indian Population

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Abstract: Known as CTG B33 /RPL14 (L14; RL14; hRL14; CTG-B33; CAG-ISL-7, this gene contains a polymorphic Trinucleotide (CTG) repeat tract. As reported from research studies, many genes with such trinucleotide repeats have been associated with Schizophrenia. We studied this gene as its association was reported positive in some studies carried out on Caucasian population. Named as **Sam1** in our study, we could not find association between the CTGB33 gene polymorphism and Schizophrenia in North-Indian population.

Key words: Polymorphic, CTGB33, Schizophrenia, Sam1.

Introduction

Known as CTG B33 /RPL14 (L14; RL14; hRL14; CTG-B33; CAG-ISL-7) Sam1- this gene encodes a ribosomal protein that is a component of the 60S subunit. Located in the cytoplasm, this protein belongs to the L14E family of ribosomal proteins and contains a basic region-leucine zipper (bZIP)-like domain. Localized on chromosome 3(3p22-p21.2) and 12(12q14.2), this gene contains a trinucleotide (CTG) repeat tract whose length is highly polymorphic. Genes with triplet repeats are putative candidates for causing Schizophrenia, thus this is one of the studied genes for Schizophrenia reported by studies in Caucasian population.

Material and Methods

High intrabreeding status due to demographic, cultural and religious preferential options provided a chance of pure gene pool and possibility of preservation of population-based significant genes in North-Indian population. There has been shown strikingly distinct increase in the neuropsychiatric diseases during past decade or so, analysis of patients registered at psychiatric hospitals' show that with decrease in the age onset, there is predisposition of the neuropsychiatric diseases such as Schizophrenia thus making this study of great importance.

Sampling : To ensure the pure-line sample collection, blood samples of only those normal individuals and Schizophrenic subjects were collected who were of pure North-Indian origin, atleast upto four generations. Both normal and patient age group averaged between 21-50 years.

Ethnic Status : For broad-spectrum observation, subjects - Normal individuals and Schizophrenic patients representing the greater part of North India were included randomly. Without any ethnic bias, blood samples of subjects from UP, Kashmir, Punjab, were collected. Samples included one male patient from Ladakh and one female patient from a sub-ethnic Gujjar population of Kashmir. Samples were collected strictly in accordance with the institutes' ethical committee, with the

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informed consent of the donors/ volunteers or their guardians in all cases for taking blood sample.

Collection of Blood : Our study was based on the DNA analysis isolated from blood collected from the forty (40) Schizophrenic patients of pure North-Indian origin –twenty (20) of the subjects were males and twenty (20) females. For comparative study, blood samples from normal twenty (20) male and twenty (20) female subjects also of pure North-Indian origin were collected randomly. The normal individuals included in the study were healthy laboratory volunteers with no history of any psychiatric illness upto four generations. The samples were collected strictly in accordance to the clinical diagnostic for disease criteria (DSM-IV) of the disease established by the consultants and medicos in service. Patient blood samples were made available from Department of Mental Health Care and Psychiatry Hospital, Srinagar, Kashmir, Psychiatric Department of Jawaharlal Nehru Medical College (JNLMC), Aligarh Muslim University, Aligarh.

For DNA isolation, using 0.5M EDTA, approximately 5 ml of peripheral blood sample was collected from each of the subject involved in our study. Blood samples from forty (40) patients – twenty (20) males and twenty (20) females with Schizophrenia anomalies were obtained for studying the polymorphic status of trinucleotide repeat expansions in the Schizophrenic patients specifically. Of these, twenty (20) subjects were suffering from Chronic Schizophrenia and twenty (20) subjects were in the range of Mild-Average type of Schizophrenia, SCZ (Mild-Avg.). Also forty (40) blood samples from healthy individuals with no mental illness records in either themselves or a first-degree relative were collected. Of those samples, twenty (20) were normal males and twenty (20) normal females.

Isolation of Genomic DNA : Total genomic DNA was isolated from blood following standard protocol (Miller SA, *et al;* 1988) as per the high-salt, phenol-chloroform method.

Oligonucleotides for Southern Hybridization and PCR : Oligonucleotides were purchased from Biobasic Inc (Canada) and Microsynth (Balgach) and purified on polyacrylamide gel electrophoresis.

Polymerase Chain Reaction: Reactions were carried out in a total volume of 25 µl having 25 ng (1 µl) DNA, 2.5µl of 10X PCR buffer (100 mM Tris HCl pH 8.8; 500 mM Kcl; 0.8% Nonidet P40), 1.5 µl of 25 mM Magnesium chloride, 4.0 µl dNTP mix (1.25 mM), ~ 10 picomoles sense primer, ~ 10 picomoles antisense primer and 0.25 units Taq DNA polymerase.

Volume was made upto 25 µl with triple distilled water. Gently mix and microfuge the contents for volume accumulation at the base of the tube. The mixture was incubated for 95-96^{0}C for 2-15 minutes to denature. The subsequent 32-35 cycles included denaturation at 94-96^{0}C for 30 seconds-2 minutes, annealing at appropriate Tm for 30 seconds-1 minute and extension at 72^{0}C (30 seconds-1 minute per kb to be amplified). Final extension was carried out at 72^{0}C for 7-10 minutes as per the primer involved. The reaction mixture was stored at 4^{0}C until agarose gel electrophoresis.

Agarose Gel Electrophoresis of Resultant Amplicons : PCR generated amplicons were resolved on 1- 2% agarose (depending on the amplicon size) in 1 X TAE buffer containing Ethidium bromide at 20 volts/cm following standard protocols (Sambrook *et al.,* 1989).

The amplicon size was determined using a molecular size marker run alongwith the samples. The gel was photographed and the precise molecular weight of the amplicons analysed using gel-documentation system UVIDoc Mw Version 99.03 for Windows.

Sequencing Reaction : Sequencing reactions were performed with dsDNA isolated from extraction and subsequent purification of PCR product using Sequenase Version 2.0 PCR Product Sequencing kit (USB) following Sanger's dideoxychain termination method (Sanger *et al.,* 1977).

Polyacrylamide Gel Electrophoresis : For resolving sequencing reaction, 6% polyacrylamide gel was prepared by dissolving 5.7g Acrylamid, 0.3g Bis acrylamide, 48g Urea, and 10 ml 10X TBE (890 mM Tris-Cl; 890 mM Boric acid; 2 mM NaEDTA, pH 8.2) in 40 ml Distilled water.

The acrylamide solution was prepared fresh and volume adjusted with water to 100 ml. Sequencing gel apparatus, (Bio-Rad, USA) of 38 cm X 50 cm with 0.4 mm spacers and 20 well 0.4 mm comb was used. The gel was prepared using 40 ml of acrylamide solution with 225 l of 10% APS and 25 l of TEMED and allowed to polymerize for 2-3 hours.

The gel was pre-electrophoresed for 1 hour at 1900 constant volts to achieve the 50^0C temperature. Prior to loading the reaction mixture on gel, samples were heated at 90^0C for 10 min. and 5 l of each sample was loaded for electrophoretic separation at the above mentioned temperature. After electrophoresis, the gel was transferred onto 3MM Whatman sheet, covered with saran wrap and dried at 80^0C under vacuum (Savant, Gel drying system). Gel was exposed to X-ray films (Konica, India or Hyperfilm -max, Amersham) at RT for 16-48 hours. Developing and fixing of autoradiograms was done following the protocols described earlier.

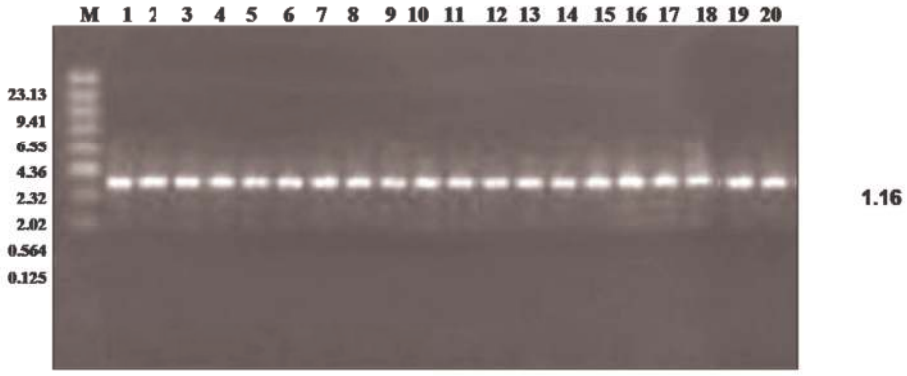
PCR products were used for automated sequencing on the commercially available automated sequencer (ABI, 377) using automated sequencing facility at Eukaryotic Gene Expression Laboratory, NII.

Sequence Analysis : The PCR contig of Sam1 was analysed for the presence of short tandem repeats using programs as DNAsis (LKB/Pharmacia, Sweden) and GeneRunner programme (Hastings Software, USA). A database search was performed on the NIH mail server using BLASTX, BLASTN and FASTA programs for the sequence homology with the others in the Genbank using sites on default blast server as http://www.ncbi.nigh.gov.cgi-bin/Blast/nph-newblast

Statistical Analysis : The frequency distributions of the genotypes and alleles studied between patients and normal controls were statistically compared using Chi square test and Fisher's exact test. Fisher's exact test is specifically used for the small samples especially for molecular base study which equally and precisely represents the result in overall population study. Analysis was done using SPSS version 10.01 for Windows. Our study on North-Indian population agreed to the polymorphic status of the loci as had been reported by many earlier studies on different populations (Ashkenazi Jews, Caucasian, Japanese, and Chinese).

Results

The Sam1 (CTG-B33) CTG repeat locus was highly polymorphic with Twelve (**12**) (CTG)(n) repeat alleles in the range of 9-



Undigested DNA fragment amplified by PCR with primer Sam1

Lane M : Marker ; λ(Lambda)
Lane 1-5: Normal Male Sample ;5-10:Normal Female Sample
Lane 11-15: Patient Male Sample ;11-12-Chronic SCZ;13-15:Mild-Avg.SCZ
Lane 16-20:Patient Female Sample;16-18:Chronic SCZ;19-20:Mild-Avg.SCZ.

20 CTG TNR in Normal and Schizophrenic subjects of our study sample. The allele with 15 CTG repeats was most prominent in both samples (25%), allele with highest repeat 20 was observed in only patient samples of our study (5%) (Fig. 1). Statistical analysis as per Chi Square test at .01 level (P=0.588) rendered the allele frequency difference between the Normal and Schizophrenic patients insignificant (Table 1). Also, using Fisher's exact test (Table 1) at all the repeat numbers, a non-significant difference was persistent throughout.

Conclusion

It is concluded that susceptibility to Schizophrenia was not influenced by polymorphic variation at the Sam1 (CTG-B33) repeat locus as per our study sample. We found absolute lack of association of Trinucleotide Repeat -TNR Allele Polymorphism with expression of Schizophrenia in North-Indian population

1. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag
3' 5'

2. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag
3' 5'

3. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag
3' 5'

4. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag
3' 5'

5. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag
3' 5'

6. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag
3' 5'

7. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag
3' 5'

8. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag
3' 5'

9. 5' 3'
tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**agaag

3’ 5’

5’ 3’

10. tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctg ctgctgctgctgctgctgctgctgctgctgctgctg***ctaaagttcca gcaaaaaagatcaccgccgcg**tcatttttccgagcgcctggggg**ag aag 3’ 5’

5’ 3’

11. tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgctgc tgctgctgctgctgctgctgctgctgctgctgctg***ctaaagttc cagcaaaaaagatcaccgccgcg**tcatttttccgagcgcctgggg**a gaag

5’ 3’

12. tctcc**caaaaaagcacctggtactaa**gggta***ctgctgctgctgc tgctgctgctgctgctgctgctg ctgctgctgctgctgctgctg***ctaaagttccagcaaaaaagatcac cgccgcg**tcatttttccgagcgcctgggg**agaa 3’ 5’

Sam1 (CTG-B33): Repeats present at Sam1 gene locus shown with flanking primer sequences

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Locus	Repeat Observed	Allele	No. of Repeats	No. of Chromosomes	Frequency % age	No. of Chromosomes	Frequency % age	Fishers exact P- value	Chi square P- value
Sam_1	CTG	1	9	4	5	4	5	0.640	0.588
		2	10	4	5	4	5	0.640	
		3	11	4	5	4	5	0.640	
		4	12	8	10	8	10	0.603	
		5	13	16	20	12	15	0.267	
		6	14	4	5	0	0	0.060	
		7	15	20	25	20	25	0.572	
		8	16	4	5	4	5	0.640	
		9	17	4	5	4	5	0.640	
		10	18	8	10	12	15	0.237	
		11	19	4	5	4	5	0.640	
		12	20	0	0	4	5	0.060	

Table 1.1: Allele distribution of candidate loci in Normal Indivduals and Schizophrenic patients and P values using Fishers exact and Chi square test.

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