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Molecular Genetic Analysis of TH and COMT Gene Polymorphism in Mentally Retarded Patients

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Abstract

Mental retardation is a variable and heterogeneous manifestation of central nervous system dysfunction characterized by significant sub average intellectual functioning.

In India the incidence of mental retardation is reported to be 2-3% of these 30% cases of severe mental retardation are genetically determined due to many reasons viz- chromosomal aberrations, X linked and subtelometric abnormalities and mutations in genes associated with nervous system function viz- TH ,COMT, MTHFR, PPP1R1B, MECP2.

Tyrosine hydroxylase (TH) gene is located on chromosome number 11 and is coding for rate limiting enzyme in the synthesis of dopamine. Changes in TH gene expression or function influence the process or behavior modulated by dopamine, any mutation in TH gene modulate dopamine and its function.

Catechol-o-methyl transferase (COMT) gene is located on chromosome number 22 and plays an important role in the metabolism of neurotransmitters. Low levels of COMT expression leads to mental retardation.

The present study was carried out to study polymorphism in TH and COMT and its possible association with mental retardation. The detection technique includes isolation of DNA from peripheral blood of the mentally retarded patients of Surat and Anand regions of Gujarat state. DNA was isolated by standard phenol: chloroform method. PCR-RFLP was used for detection of polymorphism. Analysis of TH and COMT gene polymorphism in mentally retarded patients revealed that most observed genotype in normal as well as in mentally retarded patients is TT and HH for TH and COMT loci respectively.

Highlights

In India 30% cases of severe mental retardation are genetically determined hence it is important to study genes associated with mental retardation.

Keywords: TH gene mutation, COMT gene mutation, Mental retardation.

Mental retardation is a variable and heterogeneous manifestation of Central nervous system dysfunction. It is characterized by significantly sub average Intellectual functioning, existing concurrently with related limitations in two or more of the following adaptive skill areas: community use, self-direction, Health and safety, functional academics, leisure, and work. Various gene mutations has been identified which leads to the manifestation of Mental Retardation. Tyrosine hydroxylase (TH) encodes rate limiting enzyme in the synthesis of dopamine. Changes in TH gene expression or function may influence the processes or behavior modulated by dopamine. Tyrosine hydroxylase (TH) consist of 14 exons and map to chromosome 11. TH gene is the rate limiting enzyme in the synthesis of dopamine to non epinephrine. Catechol-O-methyl transferase (COMT) is a major

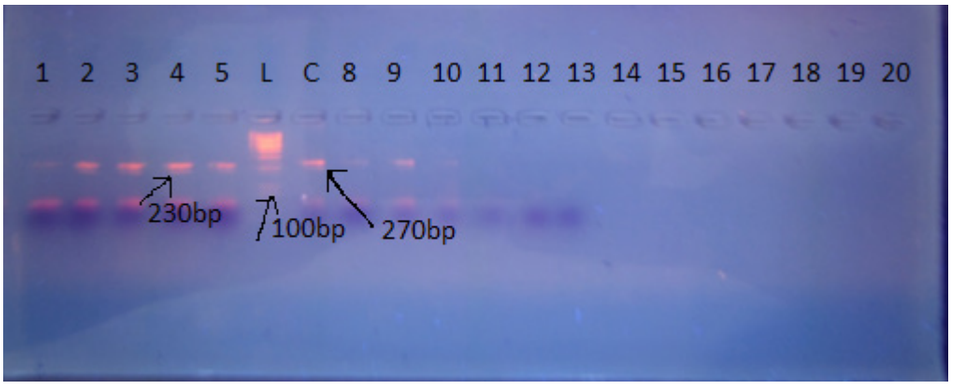

Plate 1 Restriction Digestion of TH exon 14 locus with *PstI* electrophoresed on 3% agarose in 0.5X TBE at 100V. Lane 3, 4, 5 - 230 bp and 40bp, L -100bp ladder and C- PCR product

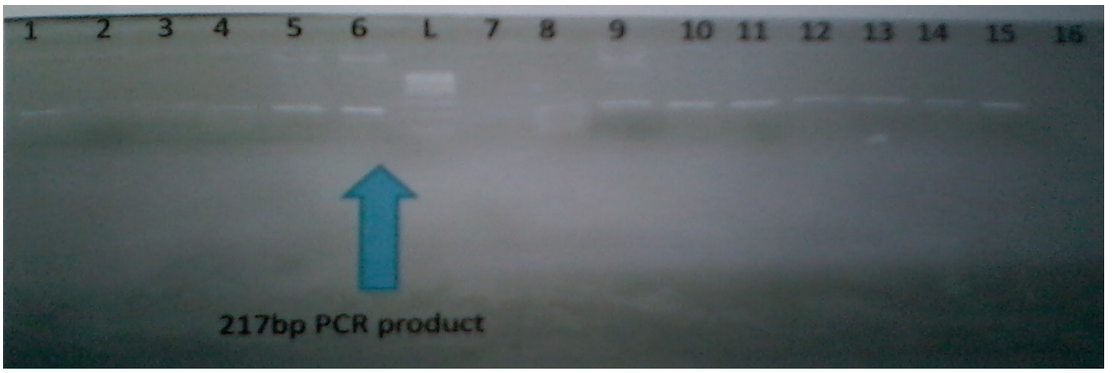

Plate 2. Restriction digestion of COMT exon 4 locus fragment electrophoresed on 3% agarose in 1X TBE at 100V L- ladder Lane 1 to 15 digested COMT product restriction enzyme *NlaIII*

enzymatic inactivator of the neurotransmitter dopamine. COMT catabolizes more than 60% of the dopamine in the prefrontal lobe thus regulating the duration of dopamine effect. The metabolism of neurotransmitters is influenced by different *COMT* activities and is involved in the changes of the prefrontal lobe cognitive function, which is represented by the individual's clinical characteristics or the cognitive and behavioral differences (Zang *et.al., 2006)*.

As very scanty reports are available regarding study of TH and COMT gene polymorphism in mentally retarded patients in Indian population hence, the present study was undertaken with objectives to study TH and COMT gene polymorphism in mentally retarded patients

Materials and Methods

The blood samples for the study were obtained from the unrelated mentally retarded patients of Mitra Rehab Centre, Mogri, district Anand, Singapur Vadi Trust, Manav kalian trust, Manar Trust, Surat, Gujarat. Normal individuals from students volunteers, P.G Department of Genetics, ARIBAS College new V.V Nagar; Shradha pathology Lab Borsad with informed consent. Methods of collection and use of human samples were approved by the institutional ethics committee. A minimum of 25 blood samples of normal as well as 25 blood samples of mentally retarded patients were obtained and brought to laboratory on ice for further use. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform method

(Sambrook, and Russell,2001). The TH and COMT gene was amplified by Polymerase chain reaction (PCR) using primers reported by Awad and Tarras, (2011) and Zang *et.al.,* (2006) respectively. Amplified PCR product of TH of 270 bp exon14 fragment was screened for *PstI* RFLP. The fragment of 217bp of COMT exon4 was digested with restriction enzyme *NIaIII*.

Results and Discussion

Locus specific primers were used for PCR amplification of TH gene exon 14. The fragment of 270 bp of TH exon 14 was amplified by PCR, using primers reported by Awad and Tarras, (2011) and digested with restriction enzyme *PstI (*Plate 1). Awad and Tarras, (2011) reported that polymorphic *PstI* digest of TH yields two fragment of 230bp and 40bp(TT) was wild type and mutated allele remains uncut (CC). Only two restriction patterns were observed for TH gene in mentally retarded patients and normal individuals with genotypes TC and TT showing absence of mutant phenotype. In the present study genotypes observed in mentally retarded patients are 5% TC, 85% TT and in normal patients 75% TT and 25% TC. Here the most observed genotype in normal as well as in mentally retarded patients is TT i.e. no significant difference between genotypes observed in normal and mentally retarded patients. The result of the present study are in accordance with Awad and Tarras, (2011) reported that most common genotype only in mentally retarded patients is TT.

The fragment of 217bp of COMT exon4 was amplified by PCR, using primers reported by Zang *et.al.,* (2006) and digested with restriction enzyme *NIaIII*. All the samples of mentally retarded patients as well as normal individuals showed identical restriction pattern consisting of absence site in 217bp fragment i.e. wild type genotype (Plate 2). The result indicated no *NIaIII* RFLP at COMT exon 4. The study revealed only one allele H fixed in population studied with an allele frequency 1.0. The result of present study i.e. higher frequency of allele H in mentally retarded patients is in accordance with Zhang *et.al.,* (2006).

In the present study as there was no polymorphism for TH and COMT gene in mentally retarded patients as well as in normal individuals, association analysis of RFLP with mental retardation was not warranted.

Conclusion

In the present study for TH gene the most observed genotype in normal as well as in mentally retarded patients is TT. For COMT gene the most observed genotype in normal as well as in mentally retarded patient is HH.

As there was no polymorphism for TH and COMT gene in mentally retarded patients as well as in normal individuals, association analysis of RFLP with mental retardation was not warranted

As the study concluded on small sample size and hence, for mutation detection and association studies of with mental retardation , study can be verified by taking large sample number of normal as well as mentally retarded patient.

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