RESEARCH ARTICLE

POLYMERASE CHAIN REACTION OF DMPK GENE FOR DIAGNOSIS OF MYOTONIC DYSTROPHY TYPE 1 PATIENT

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ABSTRACT

Myotonic Dystrophy type 1 (DM1) is an autosomal dominant disorder. It is estimated with the annual incidence of 1:8000. It is characterized by progressive muscle weakness, wasting of muscles, myotonia, and muscle stiffness. Myotonic dystrophy is due to the expansion of CTG triplet repeats in the 3' untranslated region (UTR) of dystrophia myotonica protein kinase gene (DMPK) located on the chromosome of 19q13.3. Molecular analysis of the repeats in DM1 & normal controls were performed. This repeat is subject to expansion and has 5-35 repeats in normal individuals, 50-80 repeats in mildly affected patients, >200 repeats in severely affected patients. In the severest, neonatal form of diseases, the number of repeats can reach 2000 repeats and more. PCR technique was performed. The genomic primer sequences used in PCR assay for the (CTG) n repeat were Primers - DM1-5'-GAA GGG TCC TTC TAG CCG GGA A-3' and 5'-CAG AGC AGG GCG TCA TGC ACA A-3' - Reverse primer.

INTRODUCTION

Myotonic Dystrophy type 1 (DM1) is an autosomal dominant disorder with the estimated annual incidence of 1:8000. It is the most common form of late onset inherited muscular dystrophy. It shows a characteristic pattern of wasting of muscles, myotonia and muscle stiffness. The penetrance is high nearly 100% by age of 50 years, in all manifestations of the disease. However, mild cases with only cataracts may be missed (moxley and meola 2008). DM1 is multisystem disorders, sharing comparable core diagnostic criteria and multi-organ involvement (Harper, 2001; Harper, 2004). There is a wide range of symptoms in DM1, ranging from mild features, such as baldness and cataracts, to very severe features including muscle weakness that may involve the heart. Many of the severely affected patients die of cardiac failure or respiratory distress. The cardiac involvement in DM1 may range between asymptomatic ECG abnormalities and sudden death. (Groh et al. 2008). Mild DM1 is typical of mildly symptomatic patients in whom premature cataracts and baldness may be the only clinical features. A late-onset myopathy may develop and myotonia may only be detectable on electromyography. In Classical or adult-onset DM1 the age of onset is typically in the 2nd decade of life. The most frequent symptoms are distal weakness, involving the long finger flexors of the arms and the dorsiflexors of the legs, leading to symptoms relating to the strength of hand grasping and higher frequencies of stumbling. In addition to cataracts and baldness as in minimal DM1, clinical myotonia, gastro-intestinal symptoms and fatigue may occur.

In Muscle the predominant symptom is distal muscle weakness, leading to foot drop or gait disturbance and difficulty with performing tasks requiring fine dexterity of the hands. The typical facies is mainly caused by weakness of the facial and levator palpebrae muscles. Myotonia may interfere with daily activities such as using tools, household equipment, or doorknobs. Handgrip myotonia and strength may improve with repeated contractions (Logigian 2005). A juvenile DM1 form resembles the classical form of myotonic dystrophy. However, it is more clearly associated with cognitive and behavioral abnormalities, (e.g) difficulties in learning and socialization at school. Muscle involvement may be minimal in the juvenile presentation. In the Congenital DM1 the Polyhydramnios and poor fetal movements precede the birth of an infant with congenital DM1. The affected parent is virtually always the mother and congenital DM1 occurs in a quarter of offspring of affected DM1 mothers (Harper, 7 2001). The main features are severe generalized weakness, hypotonia, and respiratory compromise. Typically, affected infants have an inverted V-shaped (also termed 'tented or 'fish'-shaped) upper lip, which is characteristic of significant facial dysplasia (weakness). Mortality from respiratory failure is high. Affected children are usually able to walk, however a progressive myopathy occurs eventually, as in the classic form (Harper 2001). These individuals may develop any of the typical features of DM1 including weakness, myotonia, cataracts, and cardiac problems.

Intellectual disability is present in 50-60% of individuals with congenital DM1. The cause of the intellectual disability is unclear, but cerebral atrophy and ventricular dilation are often evident at birth. Intellectual disability may result from a combination of early respiratory failure and a direct effect of the DMPK mutation on the brain (Spranger 1997). Facial and jaw muscles are weak and produce a tented upper lip with difficulties in feeding and sucking. Mental retardation and developmental delay are
common. Muscle biopsies of patients with congenital DM1 may reveal only variability of the size of the fibers. The disease characterized by myotonia, weakness and atrophy affecting the face and sternomastoid as well as the distal, rather than proximal, muscles of the limbs, frontal baldness, lens opacity, gonadal atrophy in the male patients, cardiomyopathy and mild endocrine changes (Harper, 1995). This clinically ranges very mild disease with cataracts, polyhydramnios and akesinesia during pregnancy, and tented upper lip, respiratory inadequacy and pulmonary hypoplasia, motility and mental retardation in rare cases of survival. The molecular biology of the disease was elucidated in 1992 (Brook et al., 1992; Mahadevan et al., 1992)

MATERIALS AND METHODS

Patient selection

Cases: Total of 11 cases of previously molecularly confirmed Myotonic Dystrophy patients were take in Clinical Genetics Department [CMC, Vellore]. Blood sample were collected from those DM1 patients includes males of ages from 30-40 years from all races and strata.

Controls: 4 healthy controls were selected from the general population, matched in all aspects except the absence of the disease.

Collection of Blood sample: 2 ml of peripheral blood was collected after informed consent and stored at −20 °C till DNA was extracted.

DNA ISOLATION

DNA was extracted using commercially available DNA extraction kit (QIAGEN) and stored at 20°C till it was used for the analysis. DNA was isolated from Patient & normal person. Analysis of the DMPK gene by PCR. The gene were analysed using the HOT start PCR. PCR reagents are added in the PCR tubes & kept it in the PCR thermo cycler. The amplification products of DMPK loci were analyzed in 3% agarose gel electrophoresis and viewed under gel documentation ultra violet box which is used to visualize ethidium bromide stained DNA in gels.

RESULTS

All the 11 patients were taken. All were males and the range of age onset was 30-42 years.

Patients

All the 11 patients were screened for DMPK1 gene mutation by PCR technique. Figure 3 shows results for patients, corresponding to a number of CTG repeats more than 50 repeats.

The patient sample shows a single band i.e. a normal allele. The affected allele is not amplified.

Normal subjects

In 4 controls, a size of bands less than 310bp corresponding to a number of CTG less than 37 repeats, showing two bands for the normal individuals.

The range of PCR products of the normal DMPK alleles varied from 205 bp-310 bp for a number of repeats from 5-37. The Patients P1, P2, P3, P4, P5, P6, P7, P8, P9, P10 and P11 were showing only one band shows the normal allele. The healthy control H1, H2, H3 and H4 are presenting two bands which is in the normal allele ranges.

Pedigree analysis

Autosomal dominant inheritance could be documented in all, except in those patients who had no family history of the disease. Transmission of the disease was paternal in some cases and maternal in very few patients. It was confirmed that all affected subjects with DM1 show expansion of the CTG repeat. Out of 11 patients 1 were having family history consistent with Autosomal Dominant inheritance, other families failed to give a positive family history.

DISCUSSION

Myotonic dystrophy is the most common neuromuscular diseases with a worldwide prevalence of 2.1-14.3/1,00,000 inhabitants; incidence is 1 in 8000 births (Ausubel FM 1992). The multisystem disease caused by an expanded and unstable trinucleotide CTG repeat localized to the 3’ untranslated region of the dystrophia myotonica protein kinase (DMPK) gene on chromosome19q13.3.(Meola 2000). PCR method is a rapid and
cheap method for initial screening. The standard PCR amplify the normal allele smaller than 310 BP. As the results two bands of distinct size less 310pb or an intense band which corresponds to two overlapping bands of the same size.

In patients, it obtained only one band corresponding to the normal allele. PCR fails to differentiate between true homozygous and affected. PCR could not amplify the large size alleles. The TP-PCR can detect the presence of long allele size without determining the total size of the expansion or the exact number of CTG Expansions. (Madhumita RC et al 2006). Normal alleles have the range between 5 and 29 CTG repeats, while the normal range has been reported as 5-37 repeats (Brook JD 1992., Mahadevan M 1992). Numerous DM patients have been reported in which transmission of the affected (DM) chromosome 19 is accompanied by a decrease in length of the triplet repeat. (Shelbourne p, 1993). Studies of a large number of DM patients have shown a definite correlation between the extent of amplification and the age onset and severity of DM. (Hunter A 1992). PCR based screening for DM1 is reliable and should be used as an initial screening test for all patients with DM1.

CONCLUSION

Myotonic Dystrophy type 1 is an autosomal dominant with the annual incidence of 1 in 8000. It is most common form of late onset inherited muscular dystrophy. It is due to the expansion of CTG repeats in the 3' untranslated portion of the DMPK gene. PCR can identify the alleles up to 500 repeats with the hot start method with accuracy. The method is easy high throughput and less time consuming. The PCR method could not differentiate between the true homozygous and affected individuals. It fails to amplify the alleles larger than 500 repeats. It is a good screening method to screen normal from affected. For confirmation of diagnosis other methods like TP-PCR or Southern Blotting are required. The samples that fail to amplify the very large allele, so they are undetectable by short PCR. The large alleles can be detected by TP-PCR, for the repeats above 500-2000. For large expansion this TP-PCR will act as a screening tool.

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