The CTG repeat polymorphisms of myotonic dystrophy (DM) gene in Korean population

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Myotonic dystrophy (DM) is caused by the expansion of CTG trinucleotide repeat near the 3' end of the gene encoding for a member of protein kinase (DMPK). The normal range of the CTG repeat was determined in 178 normal individuals (141 unrelated individuals and 37 of 9 families) by polymerase chain reaction (PCR), polyacrylamide gel electrophoresis and silver staining method. And the expansion of the CTG repeats in a DM family was analyzed with Southern analysis. In normal population, the range of CTG repeat is between 5 and 34 and 19 different alleles were observed in that range, and $(CTG)_{11-14}$ alleles were predominant. 4 members of an affected family showed the 0.5-2.0 kb size expansion of CTG repeats. In this study we could predict the incidence of DM in Korea as 1 in 20,000 and we could establish the diagnostic procedure for myotonic dystrophy.

Keywords: CTG repeat, Korean population, myotonic dystrophy, polymorphism

INTRODUCTION

Autosomal dominant myotonic dystrophy (DM) is the most common form of muscular dystrophy affecting adults and is known to show variable clinical manifestations. The most common symptoms are neuromuscular, relating to progressive muscle weakness and myotonia (delayed muscular relaxation). And clinically affected individuals present with a highly variable phenotype, ranging from asymptomatic or mild condition such as cataract to severe congenital form that is inherited only by an affected mother. Also phenotype is variable both within and between families (Harper, 1989, 1995).

One of the genetic characteristics of DM is that it shows genetic anticipation, increasing severity and earlier age of onset in successive generations (Harper *et al.*, 1992). The general incidence of DM is variable among ethnic groups, 1 in 8,000 in the European and 1 in 20,000 in the Japanese (Davies *et al.*, 1992). But the incidence in Korean is not known yet.

Recently, the mutation of DM was revealed in molecular level. Like a fragile-X and Huntington disease, the mutation causing DM is the expansion of unstable trinucleotide (CTG) repeat near the 3' end of the gene encoding for a member of protein kinase gene family (DMPK) (Brook *et al.*, 1992; Buxton *et al.*, 1992; Harley *et al.*, 1992). This gene is located at the long arm of chromosome 19, 19q13.2-13.3 (Harley *et al.*, 1991). Normal individuals have the CTG repeats between 5 and 35 repeats but DM patients have an unusual expansion of CTG repeats, n>50. In normal populations, the CTG repeat polymorphisms show some variations among ethnic groups and the differences in the incidence of DM among ethnic groups are caused by this variations.

This study was performed to reveal the CTG repeat polymorphisms, to determine the normal range of CTG repeat in normal Korean population, and to establish the diagnostic procedures for myotonic dystrophy.

MATERIALS AND METHODS

One hundred and fortyone normal individuals and 37 normal individuals from 9 families were analyzed by polymerase chain reaction to determine the exact copy numbers of CTG repeats in DM locus. And 4 members of an affected family were analyzed by Southern hybridization to estimate the size expansion of CTG repeats.

Extraction of genomic DNA

Genomic DNAs were extracted from peripheral blood samples with the methods of Sambrook *et al.* (1989).

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PCR anyalysis and Gel electrophoresis

The genomic segment containing CTG repeat was amplified with flanking primers 101 (5'-CTTCCCAGGCCTGCAG TTTGCC CATC-3') and 102 (5'-GAACGGGGCTCGAAGGG TCCTTGTAGC) reported by Brook *et al.*(1992). Primers were synthesized in Bio Synthesis Inc. (U.S.A.). PCRs were carried out with standard conditions, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 200 μM dNTPs, 1 μM of each primer, 1 μg of DNA, 0.5 unit of *Taq* DNA polymerase (Takara, Japan) in 20 μl reaction volume overlaid with one drop of mineral oil. Cycling conditions were as follow: Initial denaturation at 95°C for 5 min, then 25 cycles of 95°C for 30 sec, 62°C for 30 sec, 72°C for 1 min, and post-extension at 72°C for 5 min. In order to check the efficiency of PCRs, 5 microliters of PCR product was electrophoresed on 2% agarose gel. Then 4 μl of efficiently amplified PCR products were separated in 6% acrylamide gel (20×38 cm, 0.4 mm thick). 2 μl of pBR322/*Msp*I (Gibco-BRL, U.S.A.)was loaded together as a size marker. The bands of CTG repeats on polyacrylamide gel was visualized by silver staining methods (Budowle and Allen, 1991).

Southern blot analysis of DM family members

Twenty micrograms of genomic DNAs from 4 members of an affected family were digested with *EcoR*I. Digested DNAs were electrophoresed on 1% agarose gel in 1× TBE buffer at 30 V for 72 h, depurinated in 0.25N HCl for 15 min, denatured in 0.5 N NaOH, 1.5 M NaCl for 30 min, neutralized in 1.5 M NaCl, 0.5M Tris-HCl (pH 7.2), 0.001 M EDTA for 15 min two times, and transferred to hybond-N^+ nylon membrane (Amersham, U.S.A.) in 20× SSC for over 12 h. The membrane was fixed with 0.4 N NaOH for 25 min and hybridized with ^{32}P labeled pM10M-6 plasmid containing 1.4 kb of DM locus (Brook *et al.*, 1992). Hybridization was performed with Hybrisol (Oncor, U.S.A.) at 42°C overnight in hybridization oven (Hybaid, England). After hybridization, the membrane was washed with 2X SSC, 0.1% SDS at 65°C for 30 min, 1× SSC, 0.1% SDS at 65°C for 15 minutes two times and optionally 0.1× SSC, 0.1% SDS at 65°C for 10 min. The membrane was dried briefly and exposed to X-ray film (Fuji, Japan) overnight.

RESULTS

The CTG repeat polymorphisms in normal population

In the analysis of 159 normal individuals(141 unrelated individuals and parents of 9 families), the CTG repeat in DM locus ranged from 5 to 34 copies, and 19 different alleles were observed in that range. Of the 19 alleles, 7 alleles, $(CTG)_5$, $(CTG)_{11}$, $(CTG)_{12}$, $(CTG)_{13}$, $(CTG)_{14}$, $(CTG)_{15}$ and

Table 1. Allele frequency of the CTG repeat polymorphisms of DM locus in 159 normal unrelated individuals

No. of repeats	No. of chromosome	Allele frequency
5	60	0.189
7	3	0.009
10	1	0.003
11	43	0.135
12	87	0.274
13	64	0.201
14	15	0.047
15	20	0.063
16	10	0.031
17	1	0.003
18	1	0.003
21	3	0.009
22	1	0.003
23	1	0.003
25	4	0.013
28	1	0.003
30	1	0.003
31	1	0.003
34	1	0.003
Total	318	1

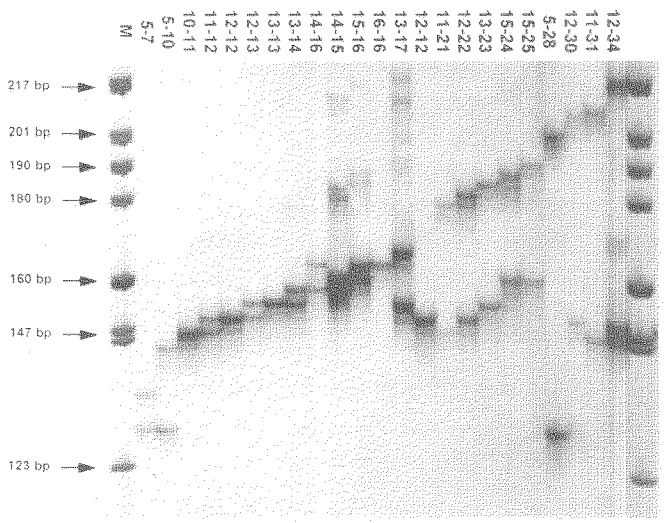

Fig. 1. Polymorphisms of CTG repeats length in normal population. The PCR products were loaded on 8% polyacrylamide gel and bands were detected by silver staining. Two bands of each lane represent a heterozygous individuals. The number of each lane represents the size of CTG repeats of the alleles. Lane M is a size marker (pBR322/*Msp*1 digest)

$(CTG)_{16}$ are predominant. The frequencies of each alleles are presented in Table 1. From the allele frequencies, theoretical heterozygosity ($1-\Sigma P_i^2$, where P_i is the frequency of allele i) was calculated as 0.823 and observed heterozygosity was 0.786.

The results of the polyacrylamide gel electrophoresis of each allele are shown in Fig. 1. In normal families, the CTG repeat alleles showed Mendelian inheritance from generation to generation, and no expansion or compression was seen on normal chromosomes (data not shown).

Southern blot analysis on DM family

The pedigree of DM family and the result of Southern hybridization were shown in Fig. 2. All four affected members of this family had an expanded allele (>10kb) with a normal 9kb allele. We could not estimate the exact size of the expanded alleles, but three of them showed the size expansion of about 500 to 600 CTG repeats and one showed about 150 repeats expansion.

DISCUSSION

In trinucleotide repeat diseases, such as myotonic dystrophy, fragile-X syndrome, spinal and bulbar muscular atrophy (SBMA) and Huntington disease, the determination of the normal and premutation range of triplet repeats is very important in genetic counseling in an affected family. But it is difficult to clear the premutation range in DM because the intervals between normal range (5 to 35 copies) and mutation range (>50 copies) is very short. In general, CTG repeats of more than 30 copies can be considered as a premutation but 31 to 35 repeats alleles were found in normal population (Davies *et al.*, 1992).

In this study, the CTG repeat of DM locus in normal Korean population ranged from 5 to 34 copies, and this range was same as other populations, such as Japanese and European. But the distributions of allele frequencies showed some differences. The distribution of allele frequencies within the normal population is shown in Fig. 3. Like other population, the distribution of alleles showed a trimodal type i.e., there are three main peaks, one is $(CTG)_5$ allele, another is $(CTG)_{11-14}$ alleles, and the other heterogenous alleles, $(CTG)_{>15}$ alleles. Very few alleles were found between 5 and 11 repeats; only three chromosomes of $(CTG)_7$ and one of $(CTG)_{10}$ were observed. The frequencies of $(CTG)_{15}$ and $(CTG)_{16}$ alleles were somewhat higher than in other populations. And in one of 9 normal families, 34 copies of CTG repeats were observed in father and son.

In previous report, the $(CTG)_5$ allele was predominant in Europeans, but in Japanese the $(CTG)_{11-14}$ alleles were predominantly rather than $(CTG)_5$ allele (Davies *et al.*, 1992; Brook *et al.*, 1992). This difference of allele frequencies between two populations are known to cause the differences of the incidence of DM (Watkins *et al.*, 1995). There are some sequence polymorphisms within the DM locus except the CTG repeat polymorphism (Mahadevan *et al.*, 1993). One of them, 1 kb insertion/ deletion polymorphism at intron 8 of DM gene was highly related with DM. One kilobase deletion allele was known to be linked with the $(CTG)_{11-14}$ alleles, and 1kb insertion allele was in complete linkage disequilibrium with an unusual expanded allele of CTG repeat and a $(CTG)_5$ allele and the other normal alleles (Imbert *et*

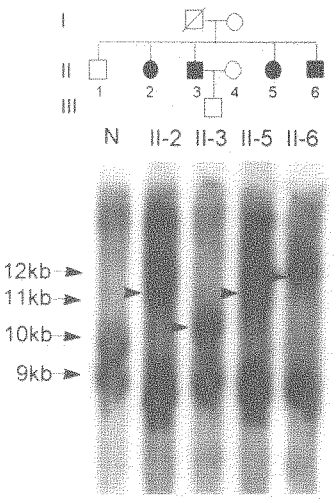

Fig. 2. A pedigree of an affected family (upper) and the result of Southern blot analysis (lower). Four patients had an expanded allele (>10kb) with a normal 9kb allele.

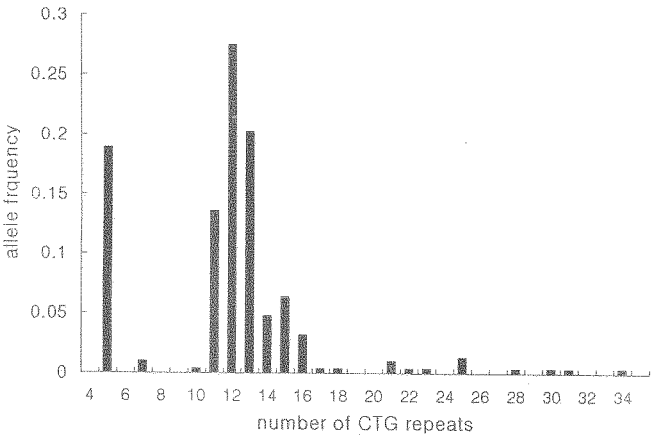

Fig. 3. Distribution of alleles within the normal population. The distribution of CTG repeats allele showed a trimodal type, $(CTG)_5$ alleles, $(CTG)_{11-14}$ alleles and very heterogeneous alleles, $(CTG)_{>15}$.

al., 1993). We could predict that the $(CTG)_{11-14}$ alleles were more stable than any other alleles, and then the higher the frequency of $(CTG)_{11-14}$ alleles is, the lower the incidence of DM is. Until now there are no reports on the incidence of DM in Korean, but we can predict that the incidence is about 1 in 20,000 on the basis that the distributions of CTG repeat alleles in Korean is same as those in Japanese.

In Fig. 3, a pedigree of DM is presented. All 4 sibs of an affected family were showing mild clinical symtoms of DM. In PCR analysis, only normal alleles were amplified. But with Southern blot analysis we could detect the expanded alleles. All 4 affected members showed the expansion of 0.5-2 kb which is classified as E1 (expansion of 0-1.5kb) or E2 (expansion of 1.5-3kb) (Hunter *et al.*, 1992). III-1 is not showing clinical symptoms but presymtomatic diagnosis is necessary.

There was no size change in 9 normal families, but all 4 members of an affected family had the different size of expanded allele. Then we could expect that alleles carrying the normal range of CTG repeat would be very stable, on the other hand a large expanded allele would be prone to dynamic mutation when the allele was transmitted to next generations.

In this study, we can establish the diagnostic procedures for DM patient with Southern analysis, but Southern hybridization is not ideal for prenatal diagnosis because large amount (up to 10 μg) of DNA and relatively long period are needed. Then a development of more rapid and simple diagnostic method is essential for prenatal diagnosis.

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