

## PRECLINICAL DETECTION IN JAPANESE FAMILIES WITH MYOTONIC DYSTROPHY USING POLYMORPHIC DNA MARKERS

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**Summary** Myotonic dystrophy (DM) is a genetic disease inherited by an autosomal dominant trait and characterized by multi-organ disorders. Although its biochemical basis has been unknown, the DM locus is closely linked to D19S19 and APOC2 on the long arm of chromosome 19 both in Japanese and Caucasian populations. Linkage studies of Japanese DM families using these polymorphic DNA markers detected two asymptomatic gene carriers in two unrelated families.

**Key Words** myotonic dystrophy, DNA diagnosis, apolipoprotein CII (APOC2), D19S19, restriction fragment length polymorphisms (RFLPs)

## INTRODUCTION

Myotonic dystrophy (DM), the most common form of adult muscular dystrophy, is inherited in an autosomal dominant fashion. The prevalence of DM has been estimated to be 5 per 10<sup>5</sup> both in Caucasian and Japanese populations (Osame and Furusho, 1983). Neither the gene responsible for DM or its basal disorder has yet been identified. Linkage analysis between the DM locus and APOC2 (apolipoprotein C-II) in Caucasian families has demonstrated that the DM locus is located to the long arm of the chromosome 19 (Shaw *et al.*, 1985). Recently we have reported that in Japanese families the DM locus is also closely linked to D19S19 and APOC2 on chromosome 19 (Takemoto *et al.*, 1989). Therefore there would be no genetic heterogeneity for the DM locus between Caucasian and Japanese populations and the prediction of an affected member of Japanese DM families can be established. Since age of onset and the clinical symptoms and signs are different among not only the DM patients from unrelated DM families but also affected members of the same DM family, it is difficult to detect a pre-symptomatic patient or an asymptomatic carrier in DM families by conventional clinical examination. Clinical application of the molecular genetics enables us to detect an asymptomatic gene carrier in a DM family and to diagnose a fetus at risk for congenital DM whose parent is affected. We have carried out the preclinical detection of a gene carrier in Japanese DM families by linkage analysis using APOC2 and D19S19 as the genetic markers.

## MATERIALS AND METHODS

Peripheral blood samples of DM families were collected by the contribution of the Nishitani's research groups, granted by the Ministry of Health and Welfare of Japan, for the studies of muscular dystrophy. All patients defined as DM satisfied the criteria for DM proposed by the working group on the molecular defect in myotonic dystrophy (Griggs *et al.*, 1989). DNA was extracted from peripheral white blood cells by using the method of Nishisho *et al.* (1986) and digested with restriction enzyme according to conditions of the suppliers (Takara, Kyoto). Samples were run on 0.7% agarose gels and blotted on nylon-membrane filter (Hybond-N, Amersham).

DNA probes were LDR152 (D19S19) and NJ3.6, cDNA of APOC2, kindly gifted by Roses and Humphries, respectively. LDR152 detects a *Pst*I polymorphism with 19 kb and 11 kb alleles (Bartlett *et al.*, 1987). NJ 3.6 detects *Taq*I polymorphic bands with 3.8 kb and 3.5 kb (Myklebost *et al.*, 1984; Shaw *et al.*, 1985). DNA probes were prepared by the oligolabeling method with [<sup>32</sup>P]dCTP. Blots were hybridized with radio-labeled probes in 6 × SSC, 5 × Denharts' solution and 100 μg salmon sperm DNA/ml at 65°C for at least 12 hr. Filters were washed twice at

65°C with 2×SSC-0.5% SDS for 20 min and once at 65°C with 1×SSC-0.5% SDS for 20 min. The final wash of the hybridized blots consisted of 0.1% SSC-0.1% SDS at 65°C for 20 min. Washed blots were exposed to X-ray film (Kodak XS-1) at -70°C for 1-3 days.

## RESULTS

In 16 families including 81 members, 7 and 6 families were informative for LDR152 and NJ3.6, respectively. Two asymptomatic patients from two different families have been detected.

*Family 18.* Both allele A of the *Pst*I polymorphism of LDR152 (Fig. 1) and allele D of the *Taq*I polymorphism of LDR152 (Fig. 1) and allele D of the *Taq*I polymorphism of APOC2 (Fig. 2) cosegregated with the DM gene in this family. II-3, aged 32 years old, is clinically normal. It is more than 95% certain that she has received the mutant DM gene with alleles A and D from her affected father. We expect that she is an asymptomatic carrier, although we can not exclude a rare recombination event between the DM locus and APOC2 as reported by Johnson *et al.* (1988).

*Family 20.* II-3, aged 70 years old, revealed no typical DM features except cataract which had been treated by an ophthalmologist. Differential diagnosis of his cataract was difficult because of combined senile cataract. He should be an obligate gene carrier, because he has three affected children and one affected nephew. Although IV-5, aged 15 years old, is clinically normal, he has inherited the allele A cosegregating with the DM gene and might be an asymptomatic gene carrier (Fig. 1).

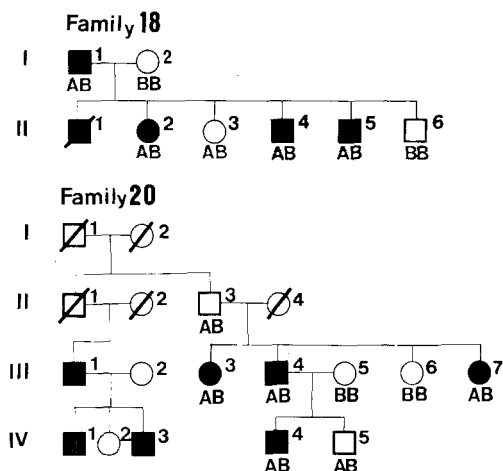


Fig. 1. Hybridization patterns of LDR152 (D19S19) to genomic DNA from members of family 18 and 20 after digestion with *Pst*I. RFLPs are 19 kb (A) and 11 kb (B). II-3 in family 20 is an obligate gene carrier.

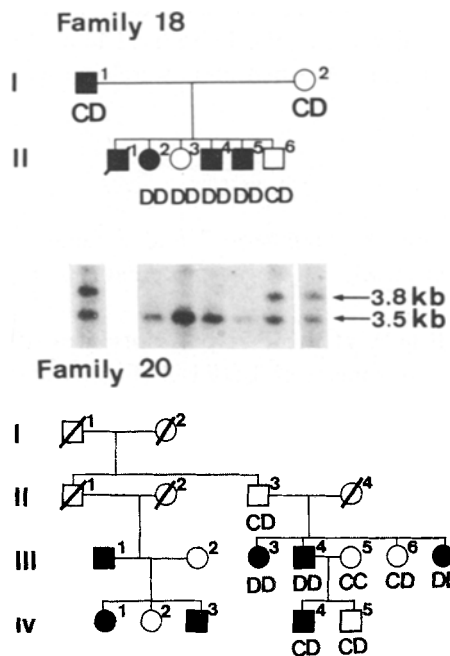


Fig. 2. Hybridization patterns of NJ.3.8 to genomic DNA from members of family 18 and 20 after digestion with *Taq*I. RFLPs are 3.8 kb (C) and 3.5 kb (D).

#### DISCUSSION

Polymorphic DNA markers have become important for the prediction of a gene carrier and prenatal diagnosis in genetic diseases where the affected gene has not been identified and isolated. In the case of DM the gene responsible is unknown and to make a diagnosis we need to use polymorphic DNA markers linked to the DM locus. In Caucasian DM families APOC2 and D19S19 are tightly linked to the DM locus and these DNA markers are frequently used to detect a gene carrier in families with DM by linkage analysis. Since the recombination frequency between APOC2 and the DM locus is 0.04 with a maximum lod score of 23.9, a gene carrier can be detected with a 94.7% accuracy using APOC2 polymorphism (Meredith *et al.*, 1986). Lunt *et al.* (1986) reported prenatal diagnosis of a first trimester fetus at risk for DM by analysis of DNA from chorionic villi.

Recently we have carried out linkage analysis between the Japanese DM locus and the two DNA markers, D19S19 and APOC2, in order to exclude the genetic heterogeneity of the DM gene among races. Because the genetic heterogeneity of genetic diseases such as polycystic kidney disease (Romeo *et al.*, 1988) and Alzheimer's disease (Schellenberg *et al.*, 1988) has been reported. The maximum lod score of 3.38 at 0% recombination between the Japanese DM locus and D19S19

supports that the Japanese DM locus might also be on the long arm of chromosome 19 and that we can predict an affected member in Japanese DM families by linkage analysis (Takemoto *et al.*, 1989).

In family 18 where both APOC2 and D19S19 are informative, we were able to diagnose II-3 as an asymptomatic carrier assuming that the event of crossover between the DM locus and these two markers was negligible. In family 20, the predicted risk of IV-5 carrying the DM gene could be 94.7%. We thought that II-3 would be an obligate gene carrier. He satisfied the criterion 3 for DM, proposed by the working group (Griggs *et al.*, 1989), which mentioned an obligate heterozygote who has a child with definite DM and a parent, sibling, niece or nephew with definite DM. It has been reported a DM family with an elderly person over 70 years old appearing normal with normal EMG who was subsequently determined to be an obligate gene carrier from pedigree analysis (Streib *et al.*, 1987). Osame and Furusho (1983) demonstrated that 4 out of 64 families showed skipped generation and the penetrance of DM in Japanese families was 94% by pedigree analysis.

For the purpose of the more accurate detection of a gene carrier and prenatal diagnosis, we need to isolate the closer polymorphic DNA markers to the DM gene or the DM gene itself.

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