Prenatal diagnosis by direct DNA analysis in facioscapulohumeral muscular dystrophy (FSHD) families

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder which has been clinically shown to cause progressive weakness and result in atrophy of the facial muscles, shoulder girdle and upper arm muscles. The responsible gene for the FSHD has been located on chromosome 4q35-qter. The probes p13E-11 and pFR-1 detect DNA rearrangements associated with FSHD as under 28 kb DNA fragment in genomic southern analysis digested with *EcoR* I and the fragment contains 3.3 kb *Kpn* I tandem repeats. In this study, 4 fetuses with a family history of FSHD were analysed by genomic southern hybridization analysis with probes to determine whether they carried the deleted region. Of the 4 fetuses, three of them had mothers who were FSHD patients and the other one had a father affected with FSHD. After 10-11 weeks of gestation, we performed chorionic villi sampling and extracted DNA from uncultured and cultured tissue cells for the direct DNA analysis. The result of the southern analysis showed two fetuses having received about 15-18 kb of deleted genes from the father and the mother respectively, and found to be FSHD patients. The other two fetuses were shown to have two normal alleles from the parents and found to be normal. Two pregnancies which were determined to be normal were carried to term delivering two healthy babies.

Keywords : Facioscapulohumeral Muscular Dystrophy, 3.3 kb *Kpn* I tandem repeats, Chorionic Villi Sampling

INTRODUCTION

FSHD, which is a regressive neuromuscular disorder, occurs in 10% of progressive muscular dystrophy diseases and one out of every 20,000 newborn infants is affected (Fischbeck *et al.*, 1992). FSHD is an autosomal dominant disease which causes weakness of the muscles in the face, shoulder girdle and upper arms. However, in comparison to Duchenne and Becker muscular dystrophy, regression of FSHD is mild (Williams and Wilkins, 1986; Carpenter and Karpati, 1984). Most are affected between the ages of 10 and 20. Frequency of inheritance is 95% for this disease and it is accompanied by retinal vasculopathy and sensorineural deafness (Brouwer *et al.*, 1991; Padberg *et al.*, 1995). Even within the same family, there is a great difference in the clinical phenotypes and 20-30 % have been found to be new mutations (Zatz *et al.*, 1995).

The FSHD gene is located on the long arm of the chromosome 4q35-ter (Wijmenga *et al.*, 1992). Through positional cloning of FSHD gene region named as D4Z4, the *Eco*RI fragment has been found to contain 3.3 kb tandemly repeated regions. The number of these repeated units can range between approximately 12-96 units within the 4q35 specific fragments (van Deutekom *et al.*, 1993) which is found on the D4Z4 can be detected by *Eco*RI digestion using probe p13E-11. Normal individuals have *Eco*RI fragments longer than 28 kb and as for the FSHD patient, they have re-arranged the fragments between 10-28 kb. Accordingly, with the use of p13E-11 after the *Eco*RI digestion and by

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applying genomic southern hybridization, we were able to detect fragments less than 28 kb for 95% of FSHD patients (Lee *et al.*, 1995).

In this study, we performed a prenatal diagnosis on 4 non-related pregnant women who were either patients or had husbands with a family history of FSHD. The purpose of the prenatal diagnosis was to determine whether the fetuses were affected with FSHD.

MATERIALS AND METHODS

Sample preparation of the subjects

In this study, we performed a prenatal diagnosis on 4 unrelated FSHD patients. For the prenatal diagnosis, gDNA was extracted from fetal cells through chorionic villi sampling during 10-11th weeks of gestation. In one case, due to the insufficiency of chorionic villi cells, the patient's cells were cultured and allowed to multiply (Brambati *et al.*, 1987) until a sufficient amount of DNA could be extracted. To determine whether or not the patient is a true FSHD and also to determine whether FSHD in question was genetically inherited or de novo mutation, blood samples were taken from a total of 27 members of 4 families. For family II, III and IV the pregnant women were FSHD patients. They were also found to have one of their parents who were FSHD patients themselves. Futhermore, more than 50% of their siblings are FSHD patients. For family I, the husband was a FSHD patient. Although, neither parent showed clinical FSHD symptom, most of the siblings were FSHD patients.

DNA extraction and Southern blot hybridization analysis

Prenatal diagnosis and family members testing are referred through the department of obstetrics/gynecology in Samsung Cheil Hospital. In these experiments it will serve as a tool in the exclusion of FSHD. Blood was collected from family members or from both parents and clinically healthy individuals and index patients. DNA was isolated from peripheral blood by use of standard techniques (Sambrook *et al.*, 1989). In the cases of prenatal testing, a chorionic villus sample was taken at 10-11 weeks of gestation. In one case of prenatal diagnosis in which insufficient fetal cells were collected through the CVS, we cultured the fetal cells to obtain sufficient gDNA. And DNA extraction from uncultured and cultured tissue cells were used by direct southern analysis. 10 ug of gDNA was digested with restriction enzyme *Eco*RI according to the manufacturer's instructions (Takara Shuzo) and separated on a 0.4% agarose (Seakem Gold FMC) gel electrophoresis for 92-95 h at 0.5 V/cm in a cold room, and transferred onto Hybond N+ membrane (Amersham). The probe used in this study was p13E-11 (a 0.8 kb fragment insert in plasmid pbluescript isolated by *Sac*I/*Eco*RI double digestion and provided by Dr. K. Arahata). The DNA probe was labelled with α-[^{32}P]dCTP using a random primed labeling system (Takara Shuzo Co., Ltd). Hybridizations were carried out for 24 h at 65°C as described. The membrane filters were washed at 65°C and at a stringency of 1×SSC/0.1% SDS, followed by autoradiography for 1-2 days at -70°C using AGFA film with an intensifying screen.

RESULTS

The results of the southern blot analysis using p13E-11 probe is as follows; 1) the fetuses of family I and IV were detected to have a 15-18 kb fragment with deletions of the 3.3 kb tandemly repeated *Kpn* I units, likely inherited from the mother, 2) the fetuses of family II and III were detected a longer fragment than 28 kb, inherited the normal alleles from one of their parents, confirming that the fetuses were not affected by FSHD (Fig. 1) (Fig. 2).

The genetic analysis of 27 members from their families showed, six members out of eight from family I, six members

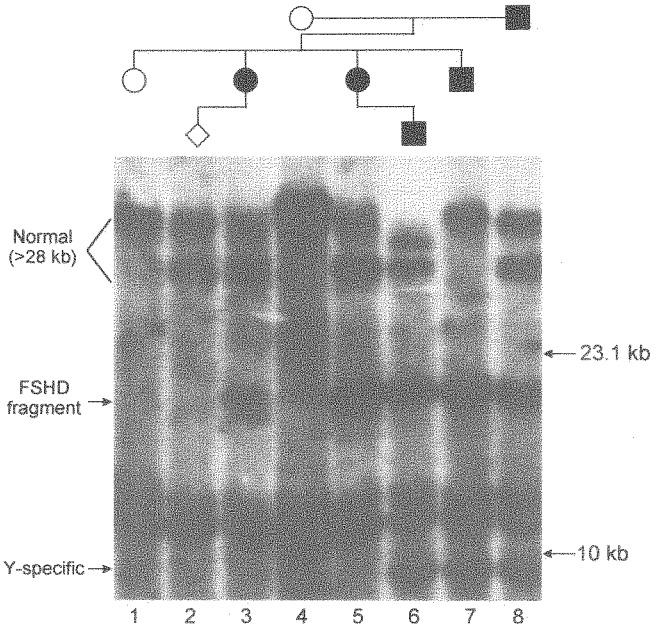

Fig. 1. Detection of the FSHD fragments with probe p13E-11 by genomic southern hybridization in the family II. Lane 1 and 4, healthy members clinically; 2, fetus; 3, pregnant woman; 5,6,7 and 8, FSHD patients. As shown in lane 2, the FSHD fragment was not transmitted from the mother to the fetus.

out of eight from family II, four members out of five from family III, and four members out of six from family IV were determined to be FSHD patients which revealed a high penetration.

DISCUSSION

We performed a prenatal diagnosis on 4 pregnant women as subjects by direct DNA analysis of FSHD patient which is both an incurable inherited disease and a type of muscular dystrophy. Due to the lack of any cure, genetic and pharmaceutical for this incurable genetic diseases, at the present prevention of the birth of such fetuses is the only effective course. Recently, the responsible gene of such an incurable genetic disease has been found and has made it possible for early genetic diagnosis.

Accordingly, it is important to discover the presence of an abnormal gene during early pregnancy for those who have a family case history of the disease. So far, generally, prenatal diagnosis was performed to detect the chromosome abnormalities by chorionic villi sampling (CVS) and amniocentesis during the 9-18th weeks of gestation. However, for the first time in Korea, we were able to perform prenatal direct DNA analysis by the 9-10th weeks of gestation to detect whether the fetuses were affected or not. in pregnant women with a family history of the disease. As for FSHD, it required a large amounts of gDNA making genetic analysis difficult for prenatal genetic diagnosis. This problem could be solved by the development of the LA-PCR (long and accurate PCR) method which does not require large amounts of gDNA and would make diagnosis possible with small amount of fetal samples.

In this experiment, in case of insufficient fetal cells, we conducted an experiment by a cell culture process to obtain the sufficient gDNA. For prenatal diagnosis, because family genetic information is needed, it was necessary to perform DNA analysis on the family members. The frequency of patient in the 4 families was higher than other autosomal dominant diseases families. For family II, III and IV, one of their parents of the pregnant women were found to be FSHD patients showing deleted fragments with clinical symptoms. However, for family I, the parents didn't show clinical abnormalities although the father had deleted fragments. Therefore, it is common for parents of FSHD who have normal phenotype without clinical symptoms but found to have deleted frag-ments. However, offsprings of such parents tend to have severe clinical abnormalities as well as deleted fragments. And so, it has been reported that such families show heterogeneity in same family members.

Generally, the affection and penetration of disease in the FSHD families is higher in comparison to other diseases. In this study, having diagnosed 27 members and finding 20 members to be affected we found 74.1% of FSHD patients.

While it has been reported that frequency of new mutations of FSHD to be 9.6%, one out of every 350,000 newborns (Padberg *et al.*, 1995), some sporadic cases had transmitted the mutation to their progeny (Hsu *et al.*, 1997) we have found all of the cases are familial, inherited from their parents. With direct DNA analysis which we applied to our experiment, size of deleted fragments and normality of the baby can be accurately and easily obtained, making the diagnostic method an effective tool for the prenatal diagnosis.

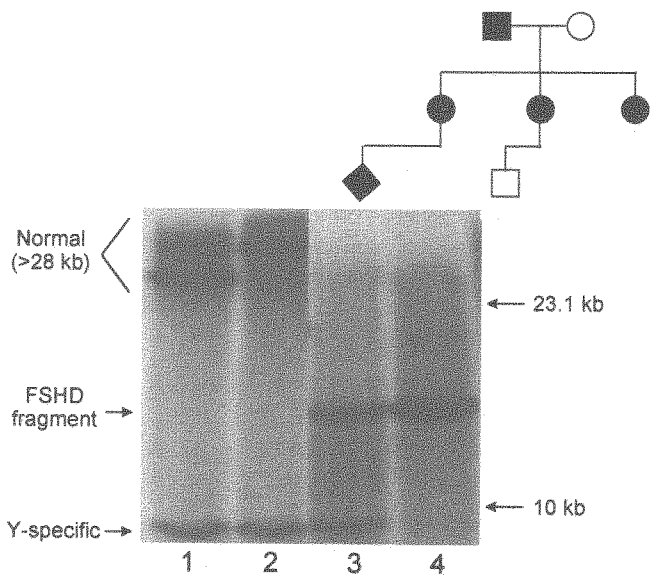

Fig. 2. Prenatal diagnosis of family IV. Southern blot results of *Eco*RI digeted genomic DNA hybridised with the probe p13E-11. Lane 1 and 2, control (unrelated normal individuals); 3, fetus (FSHD patient); 4, pregnant woman (FSHD patient). As shown in lane 3, the FSHD fragment was transmitted from the mother to the fetus.

REFERENCES

Brambati, B., Oldrini, A., Ferrazzi, E. and Lanzani, A. (1987) Chorionic villus sampling: an analysis of the obstetric experience of 1,000 cases. *Prenat Diagn* 7: 157-169

Brouwer, O. F., Ruys, C. J. M., Brand, R., de Laat, J. A. P. M., Grote, J. J. and Padberg, G. W. (1991) Hearing loss in facioscapulohumeral muscular dystrophy. *J Neurol* 41: 1878-81

Carpenter, S. and Karpati, G. (1984) *Pathology of skeletal muscle.* pp. 507-510, Churchill Livingston, New York

Fischbeck, K. H. and Garbern, J. Y. (1992) Facioscapulohumeral

muscular dystrophy defect identified. *Nature Genet* 2: 3-4

Hsu, Y. D., Kao, M. C., Shyu, W. C., Lin, J. C., Huang, N. E., Sun, H. F., Yan, K. D. and Tsao, W. I. (1997) Application of chromosome 4q35-qter marker (pFR-1) for DNA rearrangement of facioscapulohumeral muscular dystrophy patients in Taiwan. *J Neu Sci* 149: 73-79

Lee, J. H., Goto, K., Matsuda, C. and Arahata, K. (1995). Characterization of a tandemly repeated 3.3 kb KpnI unit in the facioscapulohumeral muscular dystrophy (FSHD) gene region on chromosome 4q35. *Muscle Nerve* 2: S6-S13

Padberg, G. W., Brouwer, O. F., de Keizer, R. J., Dijkman, F., Wijmenga, C., Grote, J. J. and Frants, R. R. (1995). On the significance of retinal vascular disease and hearing loss in facioscapulohumeral muscular dystrophy. *Muscle Nerve, Suppl* 2: S73-S80

van Deutekom, J. C. T., Wijmenga, C., van Tienhoven, E. A., Gruter, A. M., Hewitt, J. E., Padberg, G.W., van Ommen, G. J., Hofker, M. H. and Frants, R. R. (1993) FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. *Hum Genet* 2: 2037-2042

Wijmenga, C., Hewitt, J. E., Sandkuijl, L. A., Clark, L. N., Wright, T. J., Dauwerse, H. G., Gruter, A. M., Hofker, M. H., Moerer, P. and Williamson, R. (1992) Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. *Nature Genet* 2: 26-30

Williams and Wilkins, Brooke, M.H. (1986) A clinician's view of Neuromuscular Diseases 2nd Edn. pp. 158-170, Baltimore

Zatz, M., Marie, S. K., Passos-Bueno, M. R., Vainzof, M., Campiotto, S., Cerqueira, A., Wijmenga, C., Padberg, G. and Frants, R. (1995) High proportion of new mutations and possible anticipation in Brazilian facioscapulohumeral muscular dystrophy families. *Am J Hum Genet* 56: 99-105