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주요 개념 : Duchenne Muscular Dystrophy, Cytogenetic analysis, molecular approaches

Duchenne Muscular Dystrophy에 관한 세포유전학 및 분자유전학적 연구

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Cytogenetic and Molecular Genetic Studies on Duchenne Muscular Dystrophy

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국문요약

Purpose ; 본 연구는 X-염색체와 관련된 장애 중에서 가장 흔하고 심한 Duchenne Muscular Dystrophy(DMD)의 세포유전학 및 분자유전학적 특성을 설명하기 위해서 DMD에 영향을 받고 있는 두 가계의 13명을 대상으로 가계도 분석과 염색체 분석 및 DNA 분석을 하였다.

Method ; DNA분석은 DNA probe을 이용한 Southern blotting method로써 RFLPs와 DMD유전자 부위의 exon소실 유무를 조사하여 아래와 같은 결과를 얻었다.

Conclusion ;

A. 염색체 분석 : 말초혈액과 양수를 표본으로 High-Resolution GTG염색에서 A가계와 B가계의 염색체 분석에서 12명의 염색체는 정상 X-염색체였으나 B가계의 Ⅰ-2(DMD여성)에서 46, x,-x,+t(2:x)(q 21.1 : p21.2)로 나타난다.

B. DNA분석3 :

1) RFLPs의 분석

J66,XJ-1.1,754-11로써 B가계의 RELPs(Restriction Fragment Length Polymorphisms)에서 J66/Pst Ⅰ은 1.7hb(E), 1.6kb(e)을 보여 주었고 XJ-1.1/Taq Ⅰ은 3.6kb(F), 3.0kb(f), 754-11/EoR Ⅰ은 4.2kb(G), 2.0kb(g)의 대립인자를 나타내었다. 이상의 결과를 바탕으로 영향을 받고 있는 남자 (Ⅱ-2_의 haplotype는 보인자인 어머니의 한쪽 인자를 받았으며 어머니와 딸은 보인자이고 임산부의 태아는 남아였고 태아의 인자들은 그의 할아버지로부터 물려받아 DMD에 영향을 받지 않은 것으로 진단되었다.

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2) DMD 유전자의 exon 소실에 대한 분석

cDNA probe 8과 cDNA probe 2b-3으로써 소실에 대한 진단은 영향을 받은 남자(Ⅱ-2)는 cDNA probe 8에서 12, 7.3, 6.6, 4.2kb에 소실이 있고 cDNA 2b-3은 1.7kb에 소실에 나타났다.

Key words : Duchenne Muscular Dystropy, Chromosome Analysis, RFLPs(Restriction Fragment Length Polymorphisms)

I. INTRODUCTION

Duchenne muscular dystrophy(DMD) is one of the most common and serious X-linked recessive disorders with a frequency of 1 in 3,000-5,000 newborn males (Gardner-medvin, Duchenne, 1980).and was first described in the mid-1800s(Meryon, 1852; Duchenne, 1868). The muscular dystrophies are a heterogenous group of both human and animal hereditary diseases whose primary manifestation is progressive muscle weakness due to intrinsic biochemical defects of muscle tissue (Mastaglia and Walton, 1982). The DMD has recently been shown to be caused by the deficiency of a large abundance protein called dystrophin (Hoffman et al., 1987; 1988) and a mutation rate of approximately 1 in 10,000 (Moser, 1984). The DMD protein, dystrophin, is shown to be approximately 400kd and to represent 0.002% of total striated muscle protein. The major distribution of dystrophin is on the cytoplasmic face of the plasma membrane of muscle fibers. and possibly on the cytoplasmic face of the plasma membrane of muscle fibers, and possibly on the contiguous t-tubules membranes. The dystrophin is a component of the membrane cytoskeleton in myogenic cells.

1. Clinical manifestation

Myofibro-necrosis in patients affected with Duchenne muscular dystrophy was probably the result of plasma membrane instability. This genetic disorder in affected boys exhibited no obvious clinical manifestation until the age of 3 to 5 years, when proximal muscle weakness was first observed. The clinical manifestation illustrated the Gowers sign, the characteristic "Climbing up himself" maneuver by which the child rises from the prone position(Duvowitz V., 1978). The ensuring progressive loss of muscle strength usually leaves affected individuals wheelchair-bound by the age of Ⅱ, and results in early death due to respiratory failure.

2. Genetic lesions

The genetic lesions causing DMD have been mapped to the short arm of the chromosome, band Xp21, by detection of structural chromosome abnormalities(Greenstein et. al, 1980; Jacobs et al., 1981), Those DNA loci that were mapped physically within or near-by Xp21 exhibited linkage to the DMD mutation, further substantiation the

Xp21 location of the DMD mutation(de Martinvill et al., 1985). Recently, a male patient has been described who exhibited the phenotype of DMD associated with the additional X-linked phenotypes of chronic granulomatous disease(CGD), retinitis pigmentosa(RP), McCleod red cell phenotype, and a small interstitial deletion within XP(Franckeet al., 1985). The observed instability of this locus and the basic biochemical defect responsible for the Duchenne phenotype are not yet understood.

3. Genetic studies

For many years, carrier detection for DMD been based on pedigree analysis and on serum creatin kinase(CK) levels, even though CK determination displays both variability(Greenstein et al., 1980), and overlaps between normals and carriers in obligate carriers. Since Davies et al.(1983) described the first RELPs located in XP and flanking DMD, other RFLPs in Xp have been reported to be potentially useful for diagnosis of DMD(Aldridge et al., 1984; Hofker et al., 1985).

DNA of RFLPs have recently been used for diagnosis of various diseases including DMD. Kunkel et al.(1985) have isolated DNA fragments, pERT87(DXS 164) from the XP 21 region deleted in a DMD male patient. they successfully used subcloned fragments (pERT87-1, pERT87-8, and pERT87-15) from the p ERT87 locus as probes to detect RFLPs diagnostic of DMD. It is likely that pERT87 is located within the DMD region itself, based upon a series of partial deletions at this locus.

In about 8% of all DMD patients, this sequence (pERT87) or subclones from it, are deleted (Monaco et al., 1985; Kunkel et al., 1986). The majority of the mutation sites for DMD, mapped by RELPs analysis, are located distal to pERT87.

However, at least two X; autosome translocation break points are located proximal to the locus DXS 164, and the position of DXS 164 relative to all mutation sites is not clear. Koenig et al. (1987) have recently cloned and characterized the full length 14kb Duchenne muscular dystrophy gene cDNA spanning about 2,000kb of genomic DNA and encoding a large muscle specific transcript.

5. Purposes of the research

The purpose of this study is to find out the genetic characteristics of DMD on the basis of DMD on the basis of cytogenetic analysis and molecular approaches.

II. MATERIALS AND METHODS

First of all, the careful documentation and analysis of family structure is essential in deciding how molecular approaches are likely to be helpful for prediction in a particular family. With a well documented pedigree available, the important decisions as to which family members should have blood and amniotic fluid samples taken for chromosome analysis and DNA analysis.

Chromosome studies are essential if DMD

is diagnosed in a girl, whether or not part of a clearly X-linked kindred.

In DNA analysis, this study was investigated for RFLPs(Table 1) for heterozygotes of families by the Southern blotting method (1975).

The Southern blotting technique is the standard way to analyze the structure of DNA cleaved by restriction enzymes. The DNA fragments are separated by agarose gel electrophoresis according to their size, small fragments moving much more rapidly than larger ones. After DNA denaturation to separate the DNA strands, the gel is overlaid with a nitrocellulose filter, and the DNA fragments are transferred to filter by blotting. To identify the fragment of interest among the numerous piece of DNA on the filter, a specific radioactively labeled probe is used. The probe is allowed to anneal to the complementary DNA sequence on the filter, which is then autoradiographed to show the position of the fragment of interest.

This article was family studies with 2 DMD families of Japanese by Southern blotting technique.

Two at risk pregnancies from two different families were studied prenatally. The 13 members of two families with DMD from the Japanese population were investigated for chromosome analysis by High-Resolution Giemsa Trypsin Giemsa banded and DNA polymorphisms and pattern of exon deletions by the Southern blotting method.

1. Pedigree analysis

The family A was composed of 3 brothers and 1 sister(Fig. 4). One brother was diagnosed at age 6 years with DMD and died 17 years old. the other brothers have normal Creatine Kinase level at ages 30 and 25. One sister has a son and is at 20 weeks pregnancy state. In family B the mother of one son with DMD and one daughter with

Table 1. Xp markers

Name of Probe	Locus symbol (HGM8)	Genetic distance to DMD(dM0)	RFLP restriction enzyme(s)	Overall hetero-zygotefrequency	Probe source
RC8	DXS9	17^{11}	Taq I	0.23	Davies
782	DXS85	20^{11}	EcoR	0.50	Pearson
pXUT23	DXS16	18^{11}	Bgi/II	0.42	Pearson
pD2	DXS43	18^{11}	PvuII	0.40	Pearson
99-6	DXS41	15^{11}	Pst I	0.49	Pearson
B24	DXS67	-	Msp I	0.15	Pearson
C7	DXS28	10^{11}	EcoRV	0.25	Pearson
pERT87	DXS164	$2\text{-}5^{10}$	Many	0.9	Kunkel
XJ1.1	NA	-	Taq I	0.45	Worton
754	DXS84	$5\text{-}12^{11}$	Pst I /EcoR I	0.66	Pearson
cX5.7	DXS148	-	Msp I	0.46	Hofker
OTC	OTC	1011	Msp I	0.67	Fenton
LI.28	DXS7	2011	Taq I	0.45	Pearson

-No data available

DMD carrier was an obligate carrier. The affected individual was diagnosed at age 6 years, and found to have the classical form of DMD confirmed by muscle biopsy and with serum Creatine Kinase levels of 7000(normal 13-78u). The father and one son were normal. The daughter was pregnant at 12.5 weeks gestation.

2. Cytogenetic analysis

Chromosome analysis of Family A and B blood and amniotic fluid specimen were provided by Dr. Norio Niikawa's laboratory. This study was carried out in this laboratory following the methods of Hogge et al.(1985) for analysis of cultured cells.

Prometaphase chromosomes in their relatives of family A and B were studied on 3 days cultures of peripheral blood lymphocytes by adding ethidium bromide (10ug/ml) for 1hr prior to harvest. The chromosomes were High Resolution GTG banded. The pregant women requested prenatal

diagnosis, amniotic fluid cells cultured at 20 and 12.5 weeks of gestation were analyzed cytogenetically.

3. Molecular analysis

DNA was extracted and purified from leukocytes in heparinized blood and amniotic fluid by the method described by Blin and Stafford(1976). The genomic DNA obtained (5ug) was then digested by appropriate restriction enzymes, and the DNA fragments were separated by electrophoresis on a 0.7% agarose gel in TE buffer(0.04M Tris acetate containing 1 mM EDTA, PH8.0) (Fig. 1) and

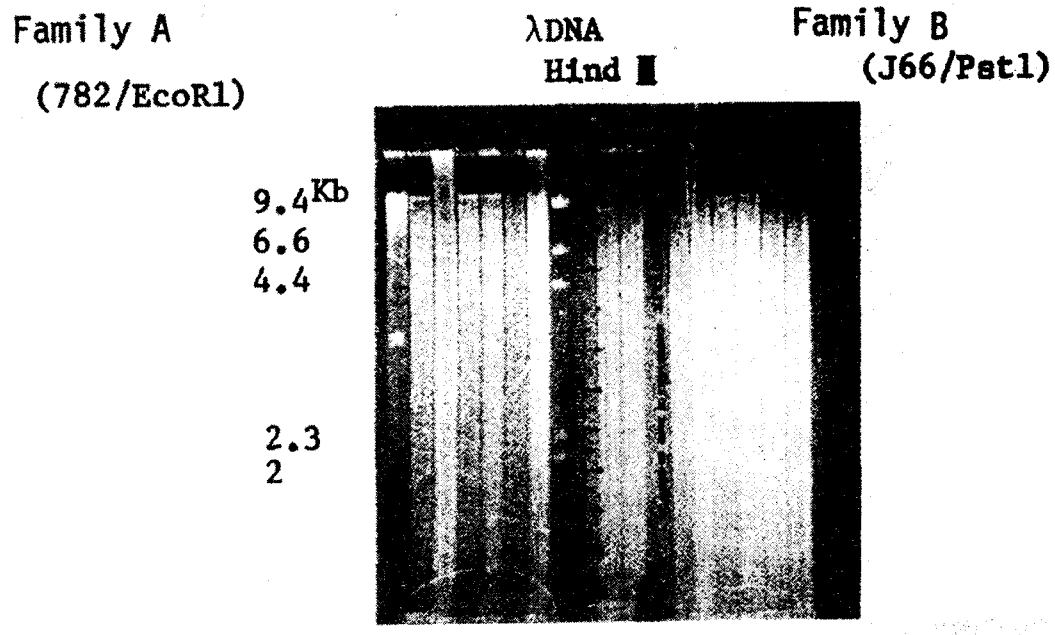


Fig. 1. The DNA fragments were separated by electrophoresis on a 0.7% agarose gel in TE buffer.

transferred onto a nitrocellulose membrane filter (Membrane filter BA85, Schleicher and Schuell) by Southern blotting method. Hybridization was performed with the nick translated DNA probe(final specific activity lx108 cpm/ug) using Amersham Nick Translation kit(Amercham) under the same condition as described by Bakker et al.(1985). After hybridization, the filter was washed twice at 65℃ in 2 X SSC(20X SSC: 3M NaCl, 0.3M Sodium Citrate) containing 0.1% SDS for 15min. The spots were visuallized by autoradiography at -70℃ for 1-3days using Sakura X-ray films and intensifying screens. The Hind III digests of lambda phage DNA were as molecular size markers. The following probes were used in this study. DNA proves of Xp marker(Table 1) were kindly supplied by Dr. P. L. Pearson(782, 99-6, J66, 754-11). Dr.L Kunkel (p ERT87-15) and Dr. R. G. Worton(XJ 1.1). The cDNA probes 2b-3, 8were obtained from the American Type Culture Collection (Rockwille, M.D.).

III. RESULTS

1. Chromosome analysis

Chromosome analysis of peripheral blood and amniotic fluid specimen in family A and B was carried out by the method of Hogge et al. (1985) for analysis of culture cell. The 12 members of DMD families studied showed normal X chromosome, where female DMD (family B, I-2) showed 46,X, -X, +t (2;X) (q21.2;p21.2) (Fig. 2).

2. Molecular analysis

The peripheral blood specimens were obtained from various members of the 2 families including the affected individuals. Confirmation of the fetus genotypes was obtained with the DNA purified from cultured amniocytes.

A. Analysis of Family A

The result of Southern hybridization studies are shown in Figs. 3 and 4. The RELPs for DNA probes on the short arm of X chromosome among Japanese was compiled from HGM9(Table 2).

1) The results of RELPs analysis with DNA probes(782, 99-6, pERT87-15) : To perform detection of DMD, this study was investigated RFLPs with 3 polymorphic probes on the short arm of X-chromosome for the basis of linkage analysis of X-linked disorders in japan. In the results of RFLPs analysis, the polymorphic bands for 782 probes after digestion with a restriction enzyme EcoRI in family A were calculated as 6kb(allele, A) and 8kb(allele, a). This probe (782)was mapped on Xp 222-223.

The 99-6/Pst 1 polymorphism shown in Figs. 3 and 4 was characterized by allelic fragment of approximately 23kb(B) and 15kb(b). The mother(I -2) is heterozygous. The daughter(II-3) is heterozygous. The one son(II-1) inherited the b allele. The pERT87-15/Xmn I polymorphism was characterized by allelic fragment of approximately 2.8kb(C), 1.6kb(c) and 1.2kb(c). The allelic fragment of daughter(II-3) was the same as

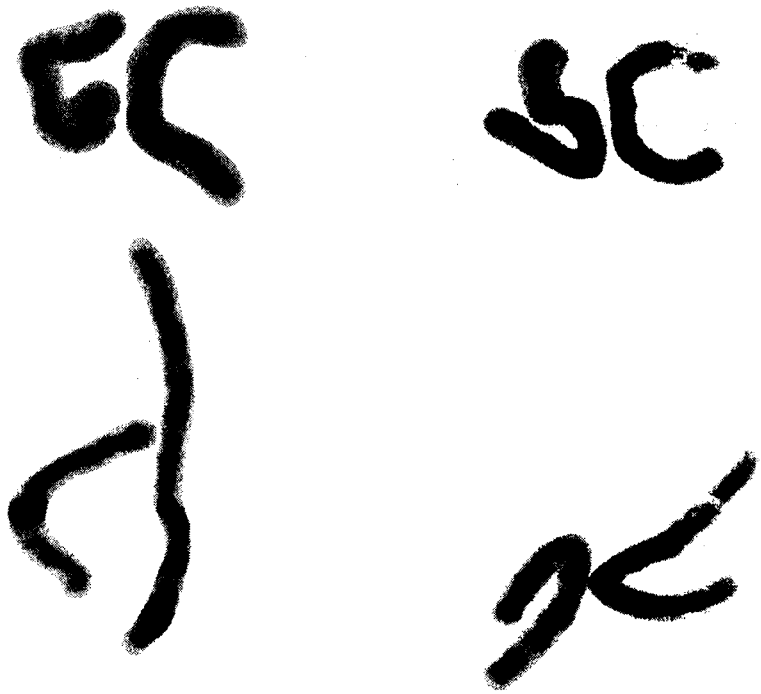


Fig.2 . High-resolution GTG-banded sex chromosomes
Upper row showed normal X chromosome of DMD families.
Low row showed 46, X,-X,+t(2;X) (q21. 1; p21.2) of female DMD (family B, I -2)

that mother. As described above, we have also performed carrier detection of DMD by linkage analysis using RFLPs. Especially, molecular analysis of the second pregnancy's amniotic fluid specimen(Ⅲ-2) was showed that the male embryo inherited the grandfatherly derived chromosomal region, and consequently would not be affected(≥ 99% certainty).

2) Analysis for molecular deletions

The recognition of a molecular deletion in an affected individual means that specific prenatal diagnosis is now feasible for relatives regardless of pedigree structure. Thus, while recognizing that this situation will apply only to a minority at present, the detection or exclusion of such deletions is now first priority, since this will completely determine the subsequent steps. Thus, in this study, all individuals of family A carried out deletion were identified by cDNA probe 8(Fig. 5).

This result clearly demonstrated carrier status for the daughter(Ⅱ-3), because the fragment in detection of female carriers with cDNA probe 8 was of two copy intensity in Ⅰ-2, Ⅱ-3 and female control F. The male fetus had inherited the same grandfatherly, The predicted normal fetus (Ⅲ-2) did not carry the deletion.

Table 2. RELPs for DNA probes on the short arm of S chromosome

Probe	Restriction enzyme	Japanese		precious reports *	
		Size(kb)	frequency(n)	Size(kb)	Frequency(n)
782	EcoR I	16.0	0.35	14.0	0.60
		8.0	0.65 (168)	7.0	0.40 (200)
99-6	Pst I	22.0	0.83	22.0	0.71
		13.0	0.17 (162)	130	0.29 (26)
J66	Pst I	1.7	0.57	1.5	0.60
		1.6	0.43 (158)	1.4	0.30
				1.35	0.10 (29)
P20	EcoR	7.5	0.27	7.5	0.40
		7.0	0.73 (26)	7.0	0.60 (30)
	Msp I	6.8	0.63	6.8	0.60
		3.5	0.37(52)	3.5	0.40 (30)
		2.1	0.39		
		1.8	0.61(28)		
pERT 87-30	Bgl II	30.0	0.85	30.0	0.63
		10.0	0.15 (84)	8.0	0.37
pERT 87-15	Xmn I	2.8	0.55	2.8	0.32
		1.6,1.2	0.45 (170)	1.6,1.2	0.68 (75)
	Taq I	3.3	0.07	3.3	0.33
		3.1	0.93 (74)	3.1	0.67 (75)
pERT 87-8	Taq I	3.6	0.70	3.8	0.26
		2.6,1.0	0.30 (114)	2.7,1.1	0.74 (105)
pERT 87-1	Xmn I	8.7	0.45	8.7	0.69
		7.5	0.55 (187)	7.5	0.31(105)
	BstN I	3.2	0.42	3.1	0.65
		2.6,0.6	0.58 (142)	2.45,0.65	0.35 (105)
XJ1.1	Taq I	3.6	0.44	3.8	0.28
		3.0	0.56 (80)	3.1	0.72 (130)
754-11	EcoR I	4.2	0.77	4.2	0.84
		2.4	0.23 (178)	2.4	0.16 (50)
LI.28	Taq I	12.0	0.59	12.0	0.68
		9.0	0.41 (98)	9.0	0.32 (50)

* Data compiled from HGM9 (Cytogenetics and Cell GEnetics 1987 46:497-499).

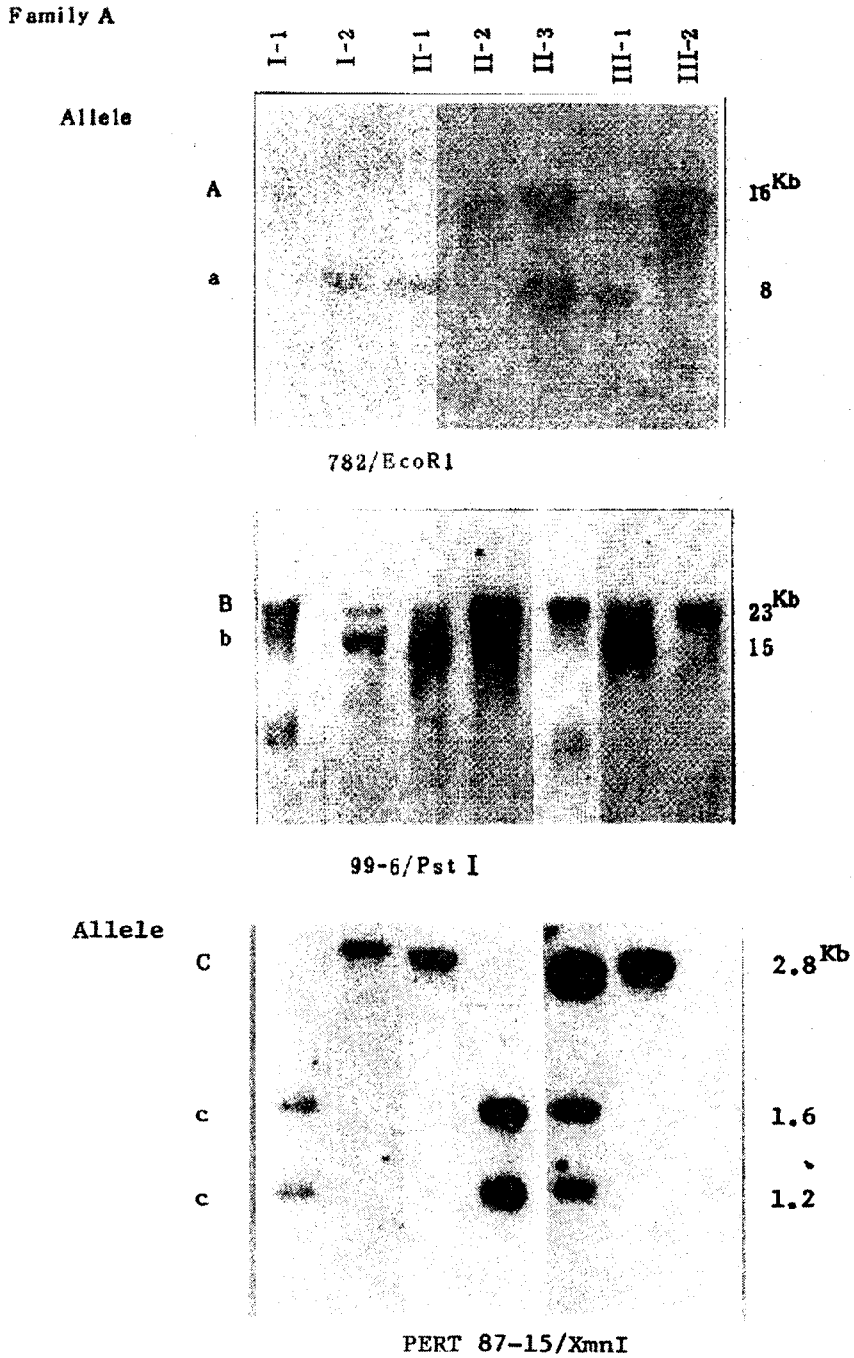


Fig. 3. Restriction fragment pattern of DNAs from 7 members of family A. Alleles detected by the 3 polymorphic probes when DNA was cut with the appropriate restriction enzyme.

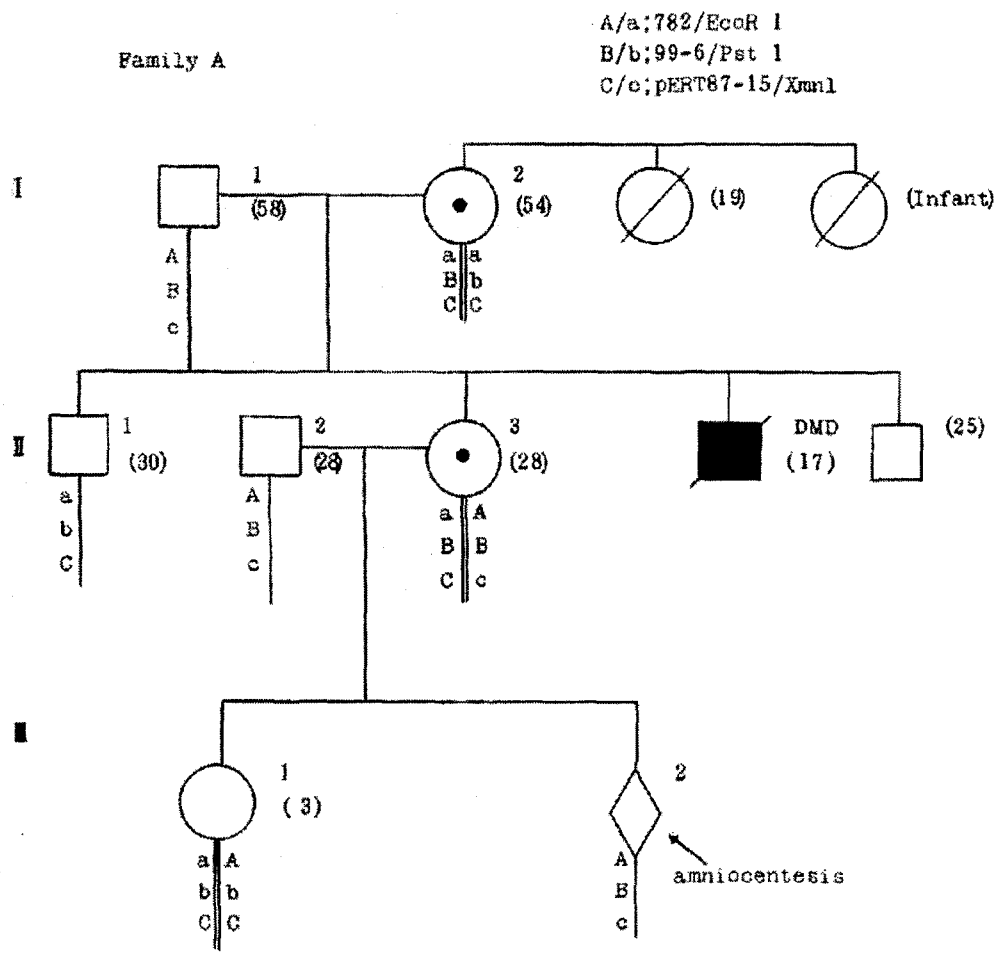


Fig. 4. DNA polymorphism study of family A.
Each vertical row represented an Xp chromosome region and each letter designated a different polymorphic DNA segment.

B. Analysis of Family B

1) The results of RELPs analysis with DNA probes(Figs.6 and 7) (J66, XJ-1.1, and 754-11). Fig.12 was showed informative for RELPs of family B detected by these probes. In this study, when using J66 probe, Pst I digests of family B showed 1.7kb(allele, E) and 1.6kb fragments(allele, e). The polymorphic bands for XJ-1.1/taq 1 in family B were calculated as 3.6kb (allele, F) and 3.0kb (allele, f)

EcoRL fragment of 754-11 sequence had a 4.2kb(allele, G) and 2.0kb (allele, g). As described above, haplotype analysis showed that affected male(II-2) had received from his mother(I -2), transmitted without apparent crossover. Therefore, his mothershould be a carrier, and now his sister (II-1) also

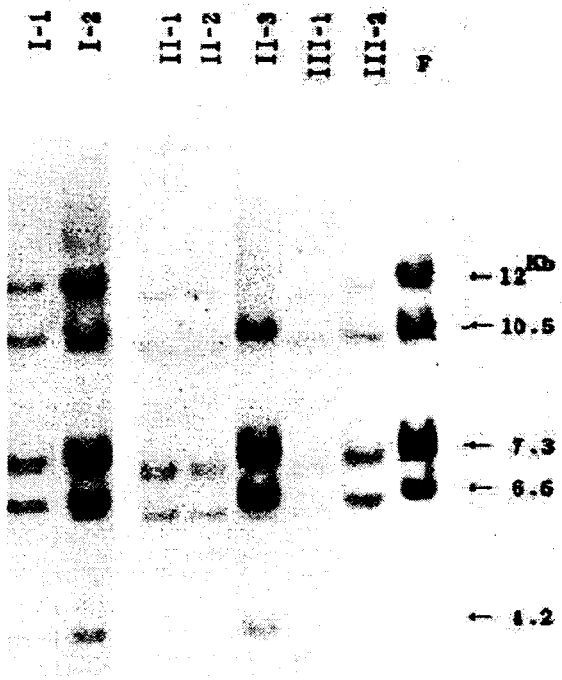


Fig. 5. Hind III restriction fragment patterns documented in DNAs from family A and control individuals with cDNA segment 8.

This fragments (black arrow)were of two copy intensity in I-2, II-3 and female control F.

should be a carrier. The fetus(III-1) showed a male fetus by chromosome analysis and the polymorphism pattern of fetus(III-1) inherited the grandfatherly and consequently would not be affected.

2) Analysis for molecular deletions

The result for detection of deletion with the cDNA probe 8 and cDNA probe 2b-3 showed in figure 13 As indicated in figure 13 by solid arrows, the adjacent cDNA probes 8 and 2b-3 discovered several restriction fragments that were missing in the DNA of the affected male(II-2) when compared to the patterns of his family. This deletion extending into two adjacent fragments of the cDNA was interpreted to represent the molecular basis of the DMD phenotype in the affected male.

IV. DISCUSSION

New molecular developments in our understanding of DMD are affected in the practical approach to genetic counseling, carrier detection, and prenatal prediction for these disorders. Such an approach now allows accurate carrier for most women at detection for a proportion of carrier women.

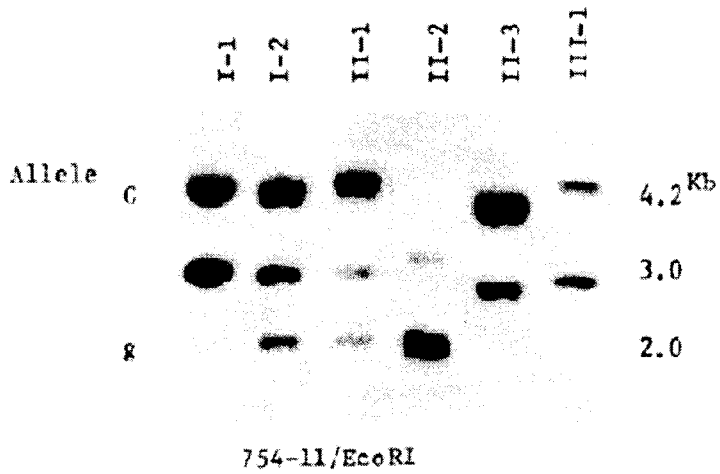
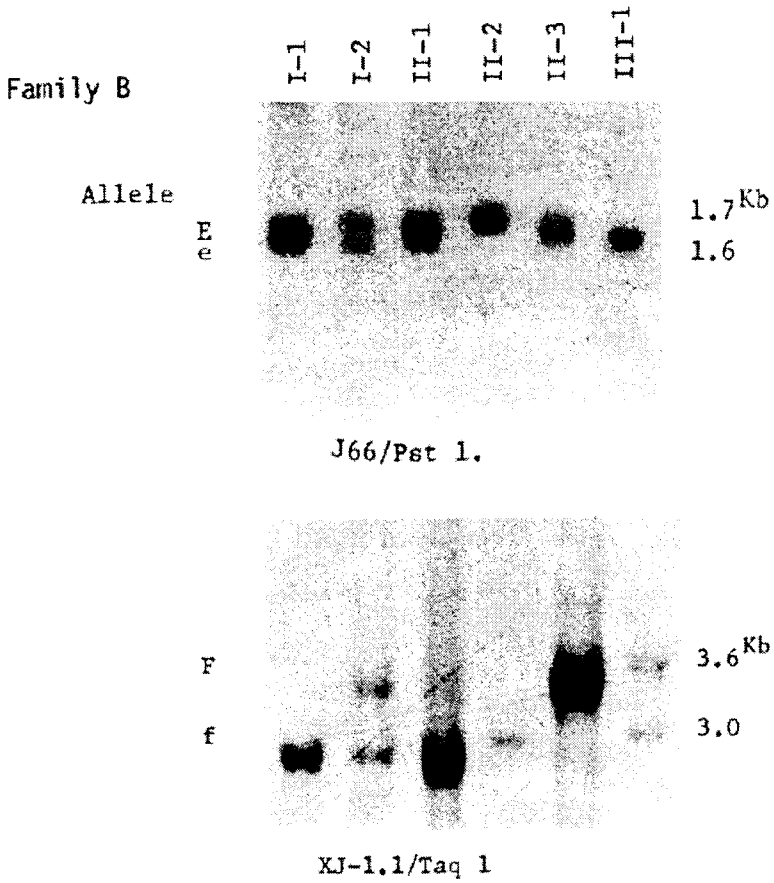


Fig. 6. Restriction fragment pattern of DNAs from 6 members of Family B Alleles detected by the 3 polymorphic probes when DNA was cut with appropriate restriction enzyme.



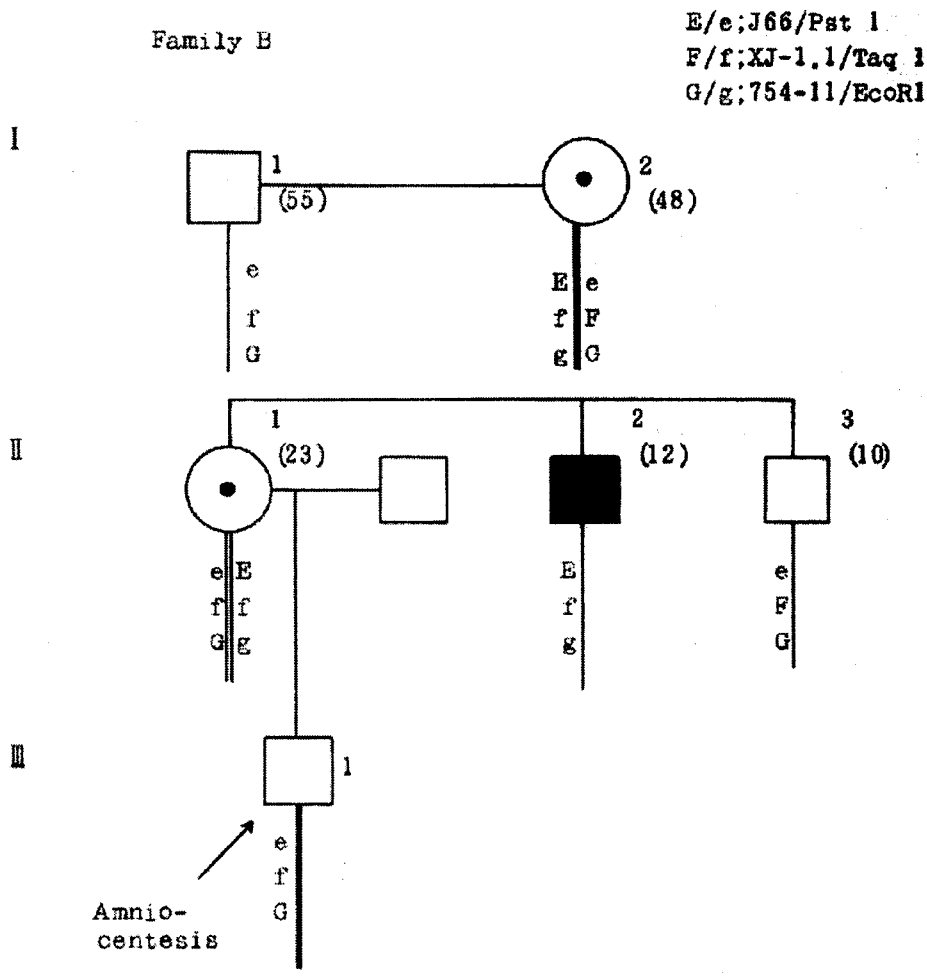


Fig. 7. DNA polymorphism study of family B
Each vertical row represented an Xp chromosome region and each letter designated a different polymorphic DNA segment.

The established approach to genetic counseling and carrier prediction for the X-linked muscular dystrophies preformed by the combined use of pedigree information from the extended family and biochemical information on the carrier status of female relatives. The development n molecular genetics over the past 5 years initially the accurate gene localization of DMD, and more recently the detection of specific abnormalities at the DNA level, have been proved of major practical as well as theoretical importance and are rapidly finding an integral place in clinical practice.

This article detected DMD and carrier detection to reassess the approach to genetic counseling of DMD patient and their relatives. However, there are fundamental

limitations and drawbacks in the molecular methods. This study was confirmed by high-resolution chromosome analysis and Sothern hybridizaion analysis. The availability of RELPs markers on the short arm of the X chromosome near DMD locus greatly improved the reliability of carrier detection and prenatal diagnosis of X-linked muscular dystrophy (Bakker et al. 1985). Representative data of chromosome analysis in the family A and B were shown normal X chromosome in the 12 family individuals and female DMD (family B, I-2) showed 46, X, -X, +t (2;X) (q21.2; p21.2)

RELPs analysis was possible by the three probes. In the family A, a daughter (II-3) who has received haplotypes from her mother and the fragment in detection of female carriers with cDNA probe 8 was of two copy intensity in I-2, II-3 and female control F. This result clearly demonstrated carriers status. Prenatal diagnosis of male fetus inherited the grandfatherly derived polymorphisms and would not be affected. We decide to prolong pregnancy. In family B reliable answered regarding carrier status and DMD were obtained for individuals. DNA polymorphism study of family B was performed with J66 probe, XJ-1.1 and 754-11. Haplotype analysis showed affected male (II-2) had received from his mother (I-2), transmitted without apparent

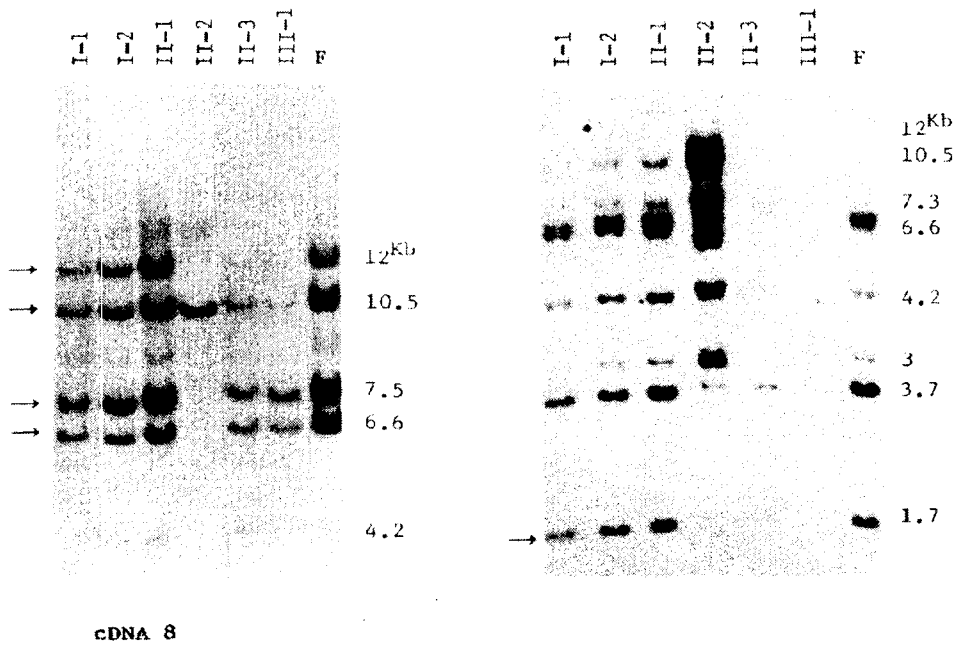


Fig. 13. The detection of deletion in affected male (II-2, family B) with cDNA segment 8 and cDNA segment 2b-3

A : The deletion identified in II-2 by the missing 12kb, 7.3, 6.6, 4.2 Hind III fragment.

B : The deletion identified in II-2 by the missing 1.7kb Hind III fragment.

crossover. Therefore, his mother should be a carrier, and now pregnant woman(I -1) also should be a carrier. The fetus(III-1) showed a male fetus by cytogenetic analysis. The result for detection of deletion in affected male and carrier DMD with cDNA probe 8, 2b-3 represented two copy intensity(black arrow) in I -2, II-1. We discovered several restriction fragments that were missing in the DNA of the affected male(II-2) with cDNA probe 8, 2b-3. The deletion in DMD gene did not occur randomly along cDNA but most of them are detected with probe 8 and I -3 (Koening et al., 1987).

The probes currently being used in DNA diagnostic studies include closely linked flanking markers(e.g. CX5.7, 754, C7, B24) (Hofker et al., 1985; 1986; Camerino et al., 1984; Ray et al., 1985). Probes now known to be intragenic were either derived from the cloning of the chromosomal breakpoint region from a t(X;21) translocation in a girl affected with DMD (Ray et al., 1985) or isolated by subtractive hybridization(e.g. pERT84, pERT87) (kunkel et al., 1985)using DNA from a male with DMD and other X-linked disorders. who had a cytogenetically visible deletion at Xp21, or by cloming of deletion endpoints(e.g. J-Bir) (Monaco et al., 1987) which allowed "Jumping"over unknown distances from the pERT87 locus(Monacoet al., 1985) with combinations of these flanking and intragenic probes, genotype predictions can be made in informative families with probabilities as high as 95%. As the result of this study, carrier detection and prenatal diagnostic studies can be extremely accurate when a deletion or any rearrangement is detected. Counseling issue will vary as each family is independently evaluated. Counseling prior to molecular studies should involved a discussion of the molecular evaluation as an evolving process. This process may require re-evaluation with new techniques as they become available so that a detectable defect for the particular family would not be overlooked.

V. SUMMARY

Many genes and phenotypes have been mapped to the human X chromosome because of their characteristic sex-linked mode of inheritance in families. It has been possible to construct a map spanning the entire human X chromosome. Markers are sufficiently well distributed along the chromosome to enable the mapping of almost any common sex-linked disorder.

In particular, some of these have been used for the development of markers for the carrier detection and antenatal diagnosis of Duchenne muscular dystrophy (DMD).

Molecular biological techniques have led to the identification of the primary biochemical defect in an important hereditary human disease, DMD. The identification of this defect was solely based on the chromosomal location of the DMD locus. Deletion of part or all of the DMD locus is one of the major causes of DMD on the human population.

The purpose of the present study is to find out the genetic characteristics of DMD by some biological methods such as family

pedigree analysis, chromosome analysis and DNA analysis. and two pregnancies from two different families were studied prenatally.

The results obtained were as follows; chromosome analysis of family A and B showed normal X chromosome, and female DMD (family B, I-2) showed 46,X, -X, +t (2;X) (q 21.1; p 21.2). The result of restriction fragment length polymorphisms analysis with DNA probes, especially molecular analysis of the second pregnancy's anmiotic fluid specimen (family A, III-2) showed the male embryo inherited the grandfatherly derived chromosomal region, and consequently would not be affected(99% certainly). And this result clearly demonstrated carrier status for the daughter (family A, II-3)

because the fragment in the detection of female carriers with cDNA probe 8 was of two copy intensity in I-2, II-3 and female control F. In the family B, the fetus (III-1) showed a male by cytogenetic analysis and the polymorphism pattern of fetus inherited the grandfatherly derived chromosomal region and consequently would not be affected. And the adjacent cDNA probes 8 and 2b-3 discovered several restriction fragments that were missing in the DNA of the affected male (II-2).

REFERENCES

Aldridge, J., L. M. Kunkel, and G. Bruns(1984). A strategy to reveal high frequency RELPs along the human chromosome. Am. H. Hum. Genet. 36 : 546-564.

Bakker, E, M. H. Hofker, N. Goor, K. Wrogemann, L. M. Kunkel, W. A. Fenton, D.Majorr-Krakauer, M. G, Jahoda, G. J. B. Van Ommen, J. L . Mandel, K. E. Daves, H. F. Willard, L. Sandkuyl, A. J. V. K. E. Davies, H. F. Willard, L. Sandkuyl, A. J. V. Essen, E. S. Sachs, and D. L. Pearson(1985). Prenatal diagnosis and carrier detection of Duchennemuscular dystrophy with closely linked RELPs. Lancet. 1 : 655-658.

Blin, N., and D. E. Stafford(1976). A general method for isolation of high molecular weighter DNA from eukaryotes. Nucleic Acid Res. 3 : 2303-2308.

Bostein, D., R.L. White, M. H. Scolnick, and R. W. Davis(1980). Constructionn of genetic linkage map in man using restriction fragment lengh polymorphism Am. Hum. Genet. 32 : 314

Bullock, D. G., F. M. McSweeny, T. P. Whitehead, and J. H. Edwards(1979). Serum creatine kinase activity and carrier status for Duchenne muscular dystrophy. Lancet. 3 : 1151.

Camerino, G., M. Koening, J. P. Moisan, I . Oberl'e, F. Morle, M. Jaye, H. Salle, D, Weil, B. Hellkuhl, K. H. Grzeschik, and J.L. Mandel(1984). Regional mapping of coaguation factor IX gene and of several unique DNA sequences on the human X chromosome. Cytogenet Cell Genet. 37 : 431-432.

Davies, K. E., P. L. Pearson, and P. S. Harper(1983). Linkage analysis of two cloned DNA sequences flanking the Duchenne muscular dystrophy locus on the short arm of the human X-chromosome.

Nucleic Acid Res. II : 2302-2312.

Drayna, D., and R. White(1985). The genetic linkage map of the human X-chromosome. Science. 230 : 753.

Dubowits, V.(1978). Muscle disorders in childhood. Philadelphia, W. B. Saunders Company. 26.

Duchenne, G. B(1868). REcherches sur la paralysie muscular pseudo-hypertrophic. Arch. Gen. Med. II : 421-552.

Franke, U., H. D. Ochs, B. de Martinvle, J. Giacalone, V. Lindren, C. Disteche, R. A. Pargon, M. H. Hofker, G. B. Van Ommen, P. L. Pearson, and R. J. Wedgwood(1985). Minor Xp21 chromosome diletion of Duchenne muscular dystrophy, chronic granulomatous disease,retinitis pigmentosa and McLeod syndrome. Am. J. Hum. Genet. 37 : 250-267.

Gardner, Medwin, D(1980). Clinical features and classification of the Muscular dystrophies. Br. Med. Bull. 36 : 109-115.

Greenstein, R. M., M. P. Reardon, T. S. Chan, A. B. Middleton, R. A. Mulivor, A. E. Greene, and L. L. Coriell(1980). An(X;11) translocation in a girl with Duchenne muscular dystrophy. Cytogenet Cell Genet. 7 : 268.

Hoffman, E. P., R. H. Brown, and L.M. Kunkel(1987). Dystrophin : The protein Product of the Duchenne muscular dystrophy gene. Cell. 51 : 919-928.

Hoffman, E. P., K. H. Fischbeck, and R. H. Brown(1988). Dystrophin analysis in muscular dystrophy patients. New Eng. J. Med. 318 : 1363-1368.

Hoffman, E, P., C. M. Knudson, K. P. Campbell, and L. M. Kunkel(1987). Subcellular fractionation of dystrophin to the triad of skeletal muscle. Nature. 330 : 754-758.

Hofker, M. H. A. A. B. Bergen, M. I. Skraasted, E. Bakker, U. Francke, B. Wieringa, J. Bartley, C. J. B. Van Omern, and P. L. Pearson(1986). Isolation of a random cosmid clone, cxs, which defines a new polymorphic locus DXS 148 near he locus for Duchenne muscular dystrophy. Hum. Genet. 74 : 275-279.

Hofker, M. H., M. C. Wapenaar, and N. Goor(1985). Isolation of probes detecting RELPs from X-chromosome specific libraies : Potential use for diagnosis of Duchenne musculardystrophy. Hum. Genet. 70 : 148-156

Hofker, M. H., M. C. Wapenaar, and N. Goor, E. Bakker, G.J.B. Van Ommen, and P. L. Pearson(1985). Prenatal diagnosis and carrier detection of Duchenne muscular dystrophy with closely linked RELPs. Lancal. 1 : 655-658

Hogge, W. A., S. A. Schonberg, and M. S. Golbus 1985. Prenatal diagnosis by chorionic villus sampling : Lessons of The first 600 cases. Prenat Diagn. 5 : 393-400.

Jacobs, P. A., P. A. Hunt, and R. D. Mayer(1981). Duchenne muscular dystrophy(DMD) in a female with an X/autosome translocation : Futher evidence that the DMD locus is at Xp21. Am. J Hum. Genet. 33 : 513-518.

Koening, M., E. P. Hoffman, C. J. Bertelson, A. P. Monaco, C. Feener, and L. M. Kunkel 1987. complete cloning of the Duchenne muscular dystrophy(DMD) cDNA and preliminary genonic organization of the DMD gene in normal and affected individuals. Cell. 50 : 509-517.

Koening, M., A. P. Monaco, and L. M. Kunkel(1988). The complete sequence of Dystrophin predicts a rod-shaped. Cytoskeletal Protein. Cell. 53 : 219-228.

Kunkel, L. M., and Coauthors(1986). Analysis of deletions in DNA from patients with Becker and Duchenne muscular dystrophy. Nature. 332 : 73-77.

Kunkel, L. M., A. P. Monaco, W. Middlesworth, H. D. Ochs and S. A. Latt(1985). Specificcloning of DNA fragments abscent from the DNA of a male patient with an X chromsome deletion. Proc. Natl. Acad. Sci. USA 82 : 4778-4782.

de Martin Vile, B., K. J. Blakemore, M. J. Maoney, and U. Francke(1984). DNA analysis offirst-trimester chroionic villous biopsies. Test for maternal contamination. Am. J. Hum. Genet. 36 : 1357-1368.

Mastaglia, F. C., and S. Walton(1982). Skeletal muscle pathology. New York. Churchill Livingstone. 3 : 33-37.

Mckusick, V. A., and F. H. Ruddle(1977). The status of the gene map of the human chromosomes. Science. 196 : 390.

Meryon, E.(1852). On granular and fatty degeneration of the voluntary muscles. medico chirurgical Trans. London. 35 : 63.

Monaco, A. P., C. J. Bertelson, C. Colletti-Feener, and L.M. Kunkel(1987). Localization and cloning of Xp21 deletion breakpoints involved in muscular dystrophy. Hum. Genet. 75 : 221-227.

Monaco, A.P., C.J. Bertelson, W. Middlesworth, C.A.Colletti,J. Aldridge, K.H. Fischbeck, R. Bartlett, M.A. pericak-Vance. A. D. Roses, and L.M. Kunkel,(1985). Detection of deletions spanning the using a tightly linked DNA segment. Nature. 316 : 842-845.

Monaco, A. P., R. L. Neve, C. Colletti-Feener, C. J. Bertelson, D. Kurnit, and L. M. Kunkel(1986). Isolation of candidate dDNAs for portions of the Duchenne muscular of dystrophy gene. Nature. 323 : 646-650.

Moser, H.(1984). Review of studies on the proportion and origin of new mutants in Duchenne muscular dystrophy. In ten kate L, ed. Research in the origin and treatment of musculardystrophy. Hum. Genet. 66 : 17-40.

Ray, P. M., B. Belfall, C. Duff, C. Logan, V. Kean, M. W. Thompson, J. E Sylvester, J. L.,Gorski, R. D. Schmickel, and R. G. Worton(1985). Cloning of the breakpoint of an X; 21translocation associated with Duchenne muscular dystrophy. Nature. 318 : 672-675.