Deletions Analysis of Duchenne Muscular Dystrophy and Becker Muscular Dystrophy Using Polymerase Chain Reaction

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Abstract

The DMD/BMD genes of twenty-four unrelated patients, including 20 with Duchenne muscular dystrophy (DMD) and four with Becker muscular dystrophy (BMD),were analyzed by an extended multiplex polymerase chain reaction (PCR). Deletions were detected in 58% (14/24) of these patients, with a 60% (12/20) detection rate for DMD and a 50% (2/4) detection rate for BMD. PCR detected more than 90% of intragenic deletions, but was uninformative in detecting possible point mutations (42%, 10/24 patients). Since the technique of PCR is rapid and simple, it is ideally suited for preliminary screening of all DMD/BMD patients and for prenatal diagnosis.

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Key words : DMD, BMD, PCR, gene deletions

Introduction

Duchenne muscular dystrophy (DMD), an X-linked recessive disorder affecting about 1 in 4000 live-born males, is characterized by progressive skeletal muscle weakness resulting in death usually in the second decade of life [1]. Becker muscular dystrophy (BMD) is a milder and less prevalent clinical variant of DMD and is known to be caused by a subset of mutations in the DMD gene [2]. Identification of the DMD gene at Xp21 [3,4], and cloning and sequencing of the entire cDNA [5,6], have been important milestones in the understanding of these diseases. Because of the large size of this gene, the majority of mutations are intragenic deletions (60%) [7,8]. The advent of polymerase chain reaction (PCR) technology is revolutionizing approaches to DNA analysis. Chamberlain et al. first described a series of PCR primers that amplify six exons that are detected in many DMD/BMD patients [9]. Since then, serial studies have been performed to detect gene deletions in patients with DMD and BMD [10-13].

The mechanism of PCR is simple and well-known, but the more exons studied the more the gene deletions will be detected. We therefore set out to study 20 exons of the dystrophic gene and discuss the advantages of these methods in diagnostic laboratories.

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Materials and Methods

Twenty-four unrelated patients, including 20

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with DMD and 4 with BMD, attended the pediatric or neurologic clinic at China Medical College Hospital. The diagnosis of DMD or BMD was established on the basis of grossly raised serum creatine kinase activity, pseudohypertrophy of the calf muscles, myopathic electromyographic findings, and dystrophic changes of muscle biopsy. The age at which patients became permanently wheelchair bound was the main clinical parameter used to differentiate DMD and BMD.

Genomic DNA was isolated from peripheral blood lymphocytes. Screening for deletions was carried out as described by Chamberlain et al. [9], and Niemann-Seyde et al. [13]. Our multiplex test contained primers that detect exons 3, 4, 6, 8, 12, 13, 17, 19, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 60, and the muscle promotor. Multiple DNA amplifications at the DMD/BMD locus were performed and subdivided into 6 groups as follows: group A was the muscle specific promotor, exons 49, 12, 52; group B, exons 4, 60; group C, exons 48, 50, 6, 46; group D, exons 45, 17, 43; group E, exons 19, 51, 13, 47; and group F, exons 3, 8, 44. The sequence of oligonucleotide primers used in this study, the size of the amplification products, and the groupings used in PCR are shown in **Table 1.** Genomic DNA was amplified by PCR under the following conditions: an initial denaturation of two minutes at 94 ℃, followed by 28 to 30 cycles of denaturation at 56 ℃ for 30 seconds, annealing at 56 ℃ for 30 seconds, and extension at 65 ℃ for four minutes, followed by a final extension at 65 ℃ for seven minutes. PCR was carried out in reaction volumes of 50 ul, containing 200 mmol/l of each d NTP, 50 p mol of each of the primers, approximately 100 ng of genonic DNA, 1.5 mmol/L $MgCl_2$, PCR buffer (Amersham, Takara, Japan) and two units of Tag DNA polymerase (Amersham, Takara, Japan) in a Perkin-Elmer Cetus 480 thermocycle. The pattern of PCR products was visualized on 3% agarose gel stained with ethidium bromide.

Results

The deletions involving any of the 20 regions assayed were detectable via multiplex amplifications. The amplification products ranged in size from 113 to 547 bp. A total of 20 genomic sequences of the DMD/BMD gene in 6 groups of multiplex reactions was shown in **Fig. 1.** Bands with expected size were clearly visible with ethidium bromide staining. The products of multiplex PCR amplification revealed 4, 2, 4, 3, 4, 3 bands in each group, respectively.

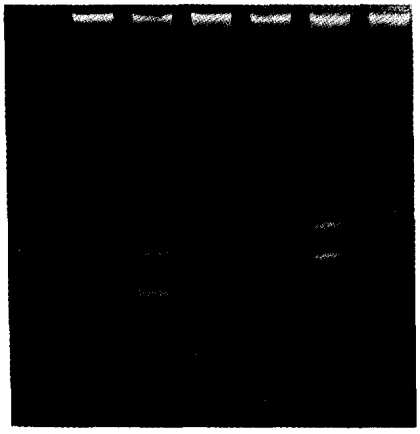

Fig. 1. Multiplex DNA amplification at the DMD/BMD locus. The lane M, DNA ladder (1000, 700, 500, 400, 300, 200, 100, and 50 bp; Amersham); lane A, muscle specific promotor, exons 49, 12, and 52; lane B, exons 4 and 60; lane C, exons 48, 50, 6, and 46; lane D, exons 45, 17, and 43; lane E, exons 19, 51,13, and 47; and lane F; exons, 3, 8, and 44.

The analytical results of the 24 patients are summarized in **Table 2.** The overall detection rate for deletion was 58% (14/24), with a 60% (12/20) detection rate for DMD and a 50% (2/4) detection rate for BMD. From the 14 cases of DMD/BMD diagnosed via PCR 3 were achieved by including exon 43 in the analysis.

Discussion

In a multi-center registration and study of hereditary neuromuscular diseases by Chen et al in Taiwan, 118 cases of DMD and 26 cases of BMD were recorded from 1993 to 1994. Interestingly, the rates of positive family history of DMD/BMD were increased as compared with previous reports, with a 46.6% positive family history rate in DMD patients and a 69.2% rate in BMD patients. Although education and genetic

Table1. Sequence of Oligonucleotides and Amplification Data

Exon	Prime	Sequence	Product size(bp)	Group
pm	PMF:	5'-GAAGATCTAGACAGTGGATACATAACAAATGCATG-3'	535bp	A
	PMR:	5'-TTCTCCGAAGGTAATTGCCTCCCAGATCTGAGTCC-3'		
3	3F:	5'-TCATCCATCATCTTCGGCAGATTAA-3'	410bp	F
	3R:	5'-CAGGCGGTAGAGTATGCCAAATGAAAATCA-3'		
4	4F:	5'-TTGTCGGTCTCCTGCTGGTCAGTG-3'	196bp	B
	4R:	5'-CAAAGCCCTCACTCAAACATGAAGC-3'		
6	6F:	5'-CCACATGTAGGTCAAAAATGTAATGAA-3'	202bp	C
	6R:	5'-GTCTCAGTAATCTTCTTACCTATGACTATGG-3'		
8	8F:	5'-GTCCTTTACACACTTTACCTGTTGAG-3'	360bp	F
	8R:	5'-GGCCTCATTCTCATGTTCTAATTAG-3'		
12	12F:	5'-GATAGTGGGCTTTACTTACATCCTTC-3'	331bp	A
	12R:	5'-GAAAGCACGCAACATAAGATACACCT-3'		
13	13F:	5'-AATAGGAGTACCTGAGATGTACGAGGAAT-3'	238bp	E
	13R:	5'-CTGACCTTAAGTTGTTCTTCCAAAGCAG-3'		
17	17F:	5'-GACTTTCGATGTTGAGATTACTTTCCC-3'	416bp	D
	17R:	5'-AAGCTTGAGATGCTCTCACCTTTTCC-3'		
19	19F:	5'-TTCTACCACATCCCATTTTCTTCCA-3'	459bp	E
	19R:	5'-GATGGCAAAAGTGTTGAGAAAAAGTC-3'		
43	43F:	5'-GAACATGTCAAAGTCACTGGACTTCATGG-3'	357bp	D
	43R:	5'-CTGACCTTAAGTTGTTCTTCCAAAGCAG-3'		
44	44F:	5'-CTTGATCCATATGCTTTTACCTGCA-3'	268bp	F
	44R:	5'-TCCATCACCCTTCAGAACCTGATCT-3'		
45	45F:	5'-AAACATGGAACATCCTTGTGGGGAC-3'	547bp	D
	45R:	5'-CATTCCTATTAGATCTGTCGCCCTAC-3'		
46	46F:	5'-AAGAACAAAAGAATATCTTGTCAG-3'	139bp	C
	46R:	5'-GACTTGCTCAAGCTTTTCTTTTA-3'		
47	47F:	5'-CGTTGTTGCATTTGTCTGTTTCAGTTAC-3'	181bp	E
	47R:	5'-GTCTAACCTTTATCCACTGGAGATTTG-3'		
48	48F:	5'-TTGAATACATTGGTTAAATCCCAACATG-3'	506bp	C
	48R:	5'-CCTGAATAAAGTCTTCCTTACCACAC-3'		
49	49F:	5'-GTGCCCTTATGTACCAGGCAGAAATTG-3'	439bp	A
	49R:	5'-GCAATGACTCGTTAATAGCCTTAAGATC-3'		
50	50F:	5'-CACCAAATGGATTAAGATGTTCATGAAT-3'	271bp	C
	50R:	5'-TCTCTCTCACCCAGTCATCACTTGATAG-3'		
51	51F:	5'-GAAATTGGCTCTTTAGCTTGTGTTTC-3'	388bp	E
	51R:	5'-GGAGAGTAAAGTGATTGGTGGAAAATC-3'		
52	52F:	5'-AATGCAGGATTTGGAACAGAGGCGTCC-3'	113bp	A
	52R:	5'-TTCGATCCGTAATGATTGTTCTAGCCTC-3'		
60	60F:	5'-AGGAGAAATTGCGCCTCTGAAAGAGAACG-3'	139bp	B
	60R:	5'-CTGCAGAAGCTTCCATCTGGTGTTCAGG-3'		

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counseling is essential for families with DMD and BMD, detection of gene deletions and prenatal diagnosis is becoming more important. Recently, Yang et al. used multiplex PCR to detect gene deletion with 11 primers [11]. The target exons chosen were limited to the hot spot regions of the dystrophin gene, and only 37% of DMD/BMD patients proved to have deletions in these regions. These deletions occurred at exons 6 and 8 in 1 patient, at exon 8 in 1 patient, at exon 8,13, and 17 in 1 patients, at exon 17 in 1 patient, at exon 43 in 3 patients, at exon 50 in 1 patient, and at exon 50 and 52 in 3 patients. These patients exhibited a deletion that extended into larger regions not covered by the analysis, limiting the clinical usefulness of their assays.

Recently, Niemann-Seyde et al. extended the locus of the DMD/BMD gene detection to 20 regions [13]. A total of 56 DMD patients and 11 BMD patients were analyzed by multiplex PCR which covered these regions, and deletions were found in 60% of patients. We used these new techniques for the analysis of 20 DMD and four BMD patients. Overall we detected deletions in 14 patients (58%), which is in accord with previous reports on the proportion of detectable deletions [11-13,15]. We noted the different ratios for various subgroups. For DMD patients, we observed a PCR-derived detection rate of 60%. For BMD patients, this proportion was 50%, however, the sample size for this group was too small for statistical analysis.

Approximately 60% of DMD/BMD patients exhibit deletions which can be found by cDNA hybridization [5,7,8]. Another 6% of patients carry duplications which can also be detected by cDNA hybridization of either conventional blots or, more effectively, following pulsed field gel electrophoresis [14]. The remaining one third of patients are thought to carry point mutations and microdeletions either in the coding sequence or in splice site consensus sequences [15]. In our study, results were uninformative for the 42% of all cases that possibly did not display intragenic deletions. Those cases need to be further examined via Southern blot analysis. However, more than 90% of intragenic deletions were detected.

In conclusion, DMD and BMD are allelic neuromuscular disorders caused by mutations to the dystrophin gene at Xp21. Since the technique of PCR is simple and can detect most of the intragenic deletions, it is ideally suited for prelimary screening of all patients and for prenatal diagnosis.

Table 2. Deletions of DMD Gene in Identified Patients

Patients No	pm	3	4	6	8	12	13	17	19	43	44	45	46	47	48	49	50	51	52	60
DMD																				
1			—																	
2				—	—	—														
3					—	—														
4								—	—											
5									—											
6										—										
7										—										
8										—	—	—								
9																—				
10																	—			
11																	—	—	—	
12																			—	
BMD																				
1			—																	
2								—												

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利用聚合酶鏈反應偵測杜現型和貝克型肌失養症之基因缺失

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摘要

本研究利用聚合酶鏈反應來偵測無任何血緣關係之二十位杜現型 (Duchenne muscualr dystrophy) 和貝克型肌失養症 (Becker muscular dystrophy) 病患之基因缺失 (gene delations) 。結果發現 14 位病患 (58%, 14/24) 有基因內缺失 (intragenic deletion) 之現象。其中杜現型肌失養症之偵測率爲 60%(12/20), 貝克型肌失養症之偵測率爲 50% (2/4) 。雖然有 42% (10/24) 之病患可能爲基因突變 (gene mutation) 而無法利用此聚合酶鏈反應偵測出來，但估計大於 90% 之基因內缺失皆可偵測到，因此本方法非常適用於一般檢驗室內對杜現型和貝克型肌失養症之篩檢。

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關鍵詞：杜現型肌失養症、貝克型肌失養症、聚合酶反應、基因缺失

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