Analysis of Dystrophin Gene Deletions by Multiplex PCR in Moroccan Patients

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Duchenne and Becker muscular dystrophy (DMD and BMD) are X-linked diseases resulting from a defect in the dystrophin gene located on Xp21. DMD is the most frequent neuromuscular disease in humans (1/3500 male newborn). Deletions in the dystrophin gene represent 65% of mutations in DMD/BMD patients. We have analyzed DNA from 72 Moroccan patients with DMD/BMD using the multiplex polymerase chain reaction (PCR) to screen for exon deletions within the dystrophin gene, and to estimate the frequency of these abnormalities. We found dystrophin gene deletions in 37 cases. Therefore the frequency in Moroccan DMD/BMD patients is about 51.3%. All deletions were clustered in the two known hot-spots regions, and in 81% of cases deletions were detected in the region from exon 43 to exon 52. These findings are comparable to those reported in other studies. It is important to note that in our population, we can first search for deletions of DMD gene in the most frequently deleted exons determined by this study. This may facilitate the molecular diagnosis of DMD and BMD in our country.

INTRODUCTION

Duchenne and Becker muscular dystrophy (DMD and BMD) represent the most common genetic neuromuscular disease of childhood. DMD is relatively frequent, with an incidence of 1 in 3500 male live births. In a typical affected male, clinical symptoms arise around the age of 3 years with progressive muscle weakness. The patient is nonambulatory by the age of 9 or 10 years and usually dies by 20 years of age, following cardiac or respiratory complications. On the other hand, Becker muscular dystrophy (BMD) is a milder form characterized by a slower disease course, with death generally occurring in the third or fourth decade.

DMD and BMD are characterized by an X-linked pattern of inheritance, thus affecting mainly males. However, they have also been occasionally reported in females, in cases of skewed X-inactivation or X chromosome abnormalities [1].

DMD and BMD are allelic variants, produced by a defect in a large gene localized on the short arm of the X chromosome (Xp21). This gene was sequenced by Koenig et al [2], spans approximately 2.4 megabases and consists of at least 79 exons [3, 4]. Its transcript is a 14 kilobase (kb) mRNA encoding the dystrophin protein, a rod-shaped cytoskeletal protein of approximately 427 kda molecular weight, localized to the sarcolemma inside the skeletal muscle fibres [5, 6, 7]. Western blot analysis generally shows that the dystrophin protein is absent or drastically reduced in the muscles of DMD patients, while it is present but reduced in amount and/or in size in BMD patients [8, 9].

In approximately 65% of DMD and BMD patients, deletions in the dystrophin gene have been identified [2, 10, 11]

without correlation between the size of the deletion and the severity of the disorder. In DMD, deletions that result in disruption of the translational reading frame prevent the production of a stable protein, while in BMD, deletions maintain the proper reading frame which results in abnormal but stable and partially functional proteins [9]. Partial duplications are responsible for 6 to 7% of DMD gene mutations [12]. Duchenne cases without deletions, representing one third of all cases [14], are probably the result of point mutations [15].

The screening for DMD/BMD gene deletions can be carried out using Southern blotting with cDNA probes or by PCR. The latter approach allows the detection of 98% of deletions in the DMD gene [13] (64.7% of patients).

In this paper, we report the first DNA analysis of unrelated Moroccan DMD/BMD patients by multiplex PCR. Because of its simplicity, rapidity, reduced cost, and its nonradioactive approach, this technique is very practical for the molecular diagnosis of this disorder in our country.

MATERIALS AND METHODS

Patients

All the patients were referred for DNA analysis from neurological and pediatric departments in Morocco. In some patients, dystrophin analysis by Western blot was also performed.

The diagnosis of DMD or BMD was based on clinical findings, Electromyography (EMG), the transmission of the pattern as an X-linked trait, an elevated serum creatine kinase activity, and dystrophin analysis when parental consent
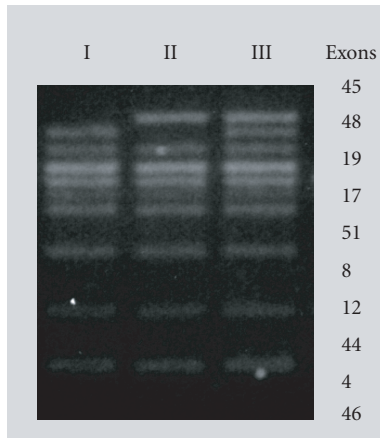
FIGURE 1. Analysis of dystrophin gene by multiplex PCR. I: patient with deletion of exon 45, II: patient with deletion of exon 48, and III: normal control. The numbers at the right indicate the amplified exons.
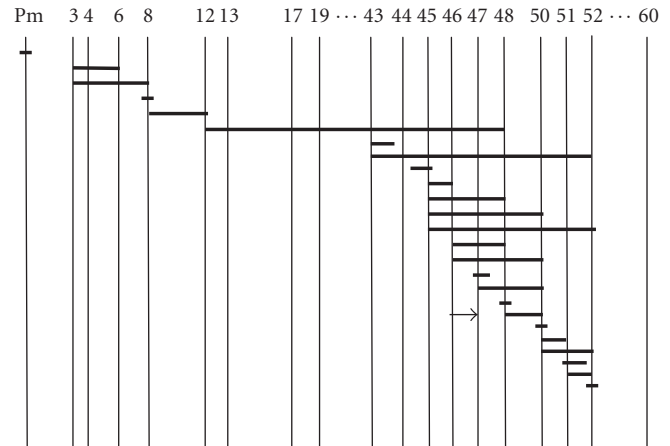

FIGURE 2. Schematic representation of different deletions in the dystrophin gene as detected in Moroccan patients (horizontal lines). The top numbers indicate the amplified exons. The arrow indicates the most frequent deletion.

for a muscle biopsy was obtained. The mean age at referral was 10 years; the age range was between 4 and 32 years.

Multiplex PCR

DNA was isolated from peripheral blood leukocytes using standard phenol/chloroform procedures. Multiplex DNA amplifications of the dystrophin gene were carried out according to Chamberlain et al [16] and Beggs et al [13], using two multiplex PCR assays allowing the amplification of 9 exons each: the Chamberlain reaction using primers for exons 45, 48, 19, 17, 51, 8, 12, 44, 4, and the Beggs reaction performed with primers for the promoter and exons 3, 43, 50, 13, 6, 47, 60, and 52 (Figure 1). In some cases, the Chamberlain reaction modified by Beggs et al [17] allowed amplification of exon 46 plus the original set (because at the onset of this study, the primers of exon 46 were not available). The PCR products were separated on 2% nusieve +1% agarose minigels and the bands visualized by staining with ethidium bromide.

RESULTS AND DISCUSSION

We have analysed, by multiplex PCR, 72 DNA samples from unrelated patients diagnosed with Becker or Duchenne muscular dystrophy cases based on clinical and electrophysiological findings. In 31 cases, the diagnosis was confirmed by dystrophin analysis after Western blotting and immunofluorescence (26 DMD and 5 BMD).

Exon deletions in the dystrophin gene were detected in 37 cases (51.3%). All deletions were found clustered in the two deletion-prone regions identified by Koenig et al [2] (Figure 2). These findings are in agreement with other studies confirming that there are no ethnic differences in the distribution of dystrophin gene deletions [18, 19]. Among the 37 deletions detected, 81% were found in exons 43 to 52, 16% in the promoter region to exon 12 and one patient

mutation covered both hot spots including exon 12 region to exon 48. The most frequent deletion found in this series (detected in 5 patients) is the one spanning the region from exon 45 to exon 48.

The diagnosis of DMD or BMD was confirmed by dystrophin analysis in only 31 patients, 16 of which had dystrophin gene deletions, that is, approximately 50%. In the remaining 42 cases, muscle biopsy could not be obtained either because the parents refused or because the patient was not hospitalized.

Overall, 58 patients had a confirmed dystrophinopathy, following DNA and/or dystrophin analysis, and a family history compatible with X-linked inheritance without deletion (in 6 cases).

Among the 72 patients, 13 cases had a family history and 15 patients were inbred individuals, which represent 20% of cases. Taking into consideration this level of inbreeding, another autosomal recessive muscular dystrophy, particularly a gamma-sarcoglycanopathy, could be suspected in the fourteen remaining cases without deletion and for which no dystrophin analysis was done. With the exception of one case, all those cases were sporadic because the gamma-sarcoglycanopathy is also frequent in Morocco, dystrophin analysis is still important to confirm or exclude the diagnosis of DMD/BMD particularly in sporadic cases without deletion. However, detection of deletions provides accurate information for genetic counselling, and prenatal diagnosis can be proposed to female carriers. It is interesting to note that deletions in Moroccan patients are most frequent in the region from exon 43 to exon 52. For the families of DMD without deletions, linkage analysis using polymorphic markers is still the only possibility proposed to females at risk.

Duchenne muscular dystrophy patients are wheelchair-bound after the first decade. In Morocco, as in many countries, this places a heavy burden on the families of the affected individuals as well as the country's health care system. The

progress of genetic technology and the application of new approaches for DNA analysis, such as multiplex PCR, allow a rapid molecular diagnosis, which, in the absence of therapy, may currently be the only approach for prevention, through genetic counselling and prenatal diagnosis.

ACKNOWLEDGMENTS

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