CASE REPORT

Dystrophin Deletion Mutation Pattern and Cardiac Involvement in 46 Cases of Dystrophinopathies

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ABSTRACT

Dystrophinopathies are X-linked recessive disorders which includes Duchenne muscular dystrophy and Becker muscular dystrophy. Forty-six cases were included in our study. Our main aim was to identify the exon deletions in these cases, determine the extent of cardiac involvement by two dimensional echocardiography and whether a correlation exists between the two. It was found that the cases having left ventricular ejection fraction ≤40% majorly showed deletions in the proximal exons as compared to the distal exons.

Keywords: Dystrophinopathy, Duchenne muscular dystrophy, Becker muscular dystrophy, cardiac

Dystrophinopathies are X-linked recessive disorders caused by mutations in the duchenne muscular dystrophy (DMD) gene, encoding the dystrophin protein. The dystrophin gene consists of 79 exons over 2.2 Mb of genomic DNA, which encodes a huge 427 kDa membrane-associated protein found in some neurons and all muscle cells.[1] Becker muscular dystrophies (BMD)/DMD are collectively termed as dystrophinopathy and are estimated to occur in 1:12,000 and 1:3,500 male births, respectively. The majority of mutations in dystrophin are exonic or multiexonic deletions (around 60% in DMD and 80% in BMD); less frequent are duplications (around 10%), and the remaining mutations are single nucleotide changes generating nonsense (DMD) or missense mutations (BMD). There are two mutational hotspots: 30% of mutations occur at the proximal hotspot (exons 3-7) and 60% distally (exons 44-55). Mutations in DMD usually disrupt the open reading frame, whereas mutations in BMD retain the open reading frame, generating an internally truncated dystrophin molecule with sufficient biological activity. Hence, patients with BMD exhibit milder progression with ambulation maintained into the teenage and adult years. On the other hand, patients with DMD tend to lose independent ambulation by 10-15 years of age depending on their gene deletion and succumb to death by the end of the second decade usually due to cardiorespiratory compromise.[2]

Cardiac involvement in DMD and BMD includes cardiomyopathy and arrhythmias. The incidence of cardiomyopathy increases with age in DMD patients. Cardiomyopathy can be evident at 10 years of age and is nearly universal in DMD patients over the age of 20. Approximately 70% of boys with BMD have cardiac involvement by age 20.

To study the deletion patterns and the cardiac conditions in dystrophinopathy, we hereby present 46 cases of the same.

MATERIAL AND METHOD

A total of 46 male cases of dystrophinopathy were included in the study, which comprised of 43 DMD cases and 3 BMD cases. The age of the DMD cases ranged from 4.5 to 19 years with an average of 11.5 years, while the age range of the BMD cases was from 24 to 33 years with an average of 26.5 years. Except for 1 BMD case all the other cases were sporadic with no family history. Their gene deletions and duplications were recorded and their cardiac function was found using two dimensional echocardiography (Table 1).

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Table 1. Showing the Gene Deletions and LVEF in 46 Cases of Dystrophinopathy

Age (years)	Gene deletion	LVEF (%)
13	45-49	60
13	21	75
11	8-19	45
9	51 and 52	55
9	8-13, 17,19	55
17	No deletions	50
10	No deletions	60
8.5	No deletions	60
6	48-50	60
12	Promotor gene	74
10	No deletions	60
11	8-11	40
10	8-44	55
13	46-51.	60
4.5	49-52	72
8.5	49-51	55
10	49-50	60
11	48-50	55
33	12	40
17	Nonsense mutation	55
11	No deletions	60
8	46-47	65
11	No deletions	55
13	47 and 50	60
13	53-55	55
9	49-50	65
13	53	33
11	45-50	60
13	49-50	60
18	5 -13	40
7	45-52	60
11	8-13, 17, 19, 20, 21, 43, 45, 46, 47	55
14	48-50	60
18	3-7	30
10	50	73
10	50	67
10	44	65
10	6, 8, 12, 13, 17, 19	70
11	49 and 50	46
30	45-48	50
23	57-61	55
24	No exon del	45
19	3, 4, 8, 12, 17	60
7	No deletion	55
8	46-51	60
17	51	30

RESULTS

Thirty-eight out of 46 (82.6%) cases showed deletions in one or more exons while, in the other eight cases no deletion was detected. Duplication of the exons 57-61 was detected in only one out 46 cases (2.1%). The majority (25 out of 46 i.e. 54.3%) had deletions in the distal rod domain between exons 45-55. Eight out 46 (17.3%) showed deletions in the proximal rod domain between exons 3-21. Two out of 46 (4.3%) showed large deletions spanning both the regions from exon 3-55. One case (2.1%) showed nonsense mutation and promoter gene deletion was also detected in only (2.1%) case out of the total 46 cases.

On studying the ejection fractions of the 46 cases, it was found that four cases had left ventricular ejection fraction LVEF ≤40% while 42 cases had LVEF ≥41%. Our analysis showed that the cases having LVEF ≤40% majorly showed deletions in the proximal exons as compared to the distal exons.

DISCUSSION

Dystrophinopathies are due to mutations in the dystrophin gene on chromosome Xp21.1 and comprise the allelic entities DMD, BMD and X-linked dilative cardiomyopathy (XLDCM). These are X-linked disorders affecting the synthesis of dystrophin, a large, sarcolemmal protein that is absent in DMD[3] and reduced in amount or abnormal in size in BMD patients.[4] Dystrophin provides the connection between a large, multimeric complex of glycoproteins in the muscle cell membrane and intracellular actin filaments, thereby transmitting forces generated by sarcomere contraction to the extracellular matrix.[5,6] Dystrophin has an important role in stabilizing the cell membrane of both skeletal and cardiac myocytes[7,8] and its absence produces sarcolemmal fragility, muscle cell degeneration and leads to conformational changes in stretch-activated calcium channels, resulting in pathologic leakage of calcium in the muscle cytosol.[9] Intracellular calcium accumulation then leads to protease activation, increased reactive oxygen species production, and cell death.[10,11] Finally, impaired vasoregulation occurs via marked reduction in membrane-associated neuronal nitric oxide synthase in both cardiac and skeletal muscle.[12] Without dystrophin, neuronal nitric oxide (NO) synthase mislocalizes to the cytosol, this greater distance between neuronal nitric oxide synthase and the sarcolemma may impair NO diffusion through the myocyte membrane to the microvasculature. As a consequence, insufficient NO

release follows muscle contraction, resulting in muscle ischemia.[13] Unopposed vasoconstriction may, therefore, explain the necrosis observed in skeletal and cardiac muscle of dystrophinopathy patients. Microvasculature abnormalities have also been shown to result primarily from the absence of dystrophin or sarcoglycan components of the dystrophin-glycoprotein complex in cardiomyocytes.[14,15]

Subclinical or clinical cardiac injury is present in about 90% of the DMD/BMD patients but is the cause of death in only 20% of the DMD and 50% of the BMD patients.[16] Correlations between dystrophin mutations and the onset of cardiomyopathy have been noted. Rita Wen Kaspar et al have analyzed 78 BMD and XLDCM patients with common deletion mutations predicted to alter the dystrophin protein and correlated their mutations to cardiomyopathy age of onset. They observed that deletions affecting the amino-terminal domain are associated with early-onset dilated cardiomyopathy (DCM; mid-20s), whereas deletions removing part of the rod domain and hinge 3 have a later-onset (DCM; mid-40s).[17] Some mutations result in only cardiomyopathy without skeletal myopathy.

X-chromosome inactivation, the random process by which 1 of the 2X chromosomes in female cells becomes transcriptionally inactive, may result in cardiomyocytes with an active X chromosome with the abnormal dystrophin gene. The X chromosome containing the normal dystrophin gene may become inactivated in cardiac muscle to a greater degree than in skeletal muscle, causing female carriers to develop dystrophinopathic cardiomyopathy. The exact prevalence and severity in the carrier population are uncertain.[18-21]

Almost all DMD patients who survive to the third decade of life display cardiomyopathy.[22] Recognition may be delayed by relative physical inactivity obscuring symptomatology and also because, in most cases, the patient presents at an early stage of the disease. BMD patients, whose skeletal myopathy occurs later and progresses more slowly, experience worse cardiomyopathy than do DMD patients: Upto 70% have left ventricular (LV) dysfunction on echocardiography. Perhaps because of less skeletal muscle weakness, these patients can perform more strenuous exercise with dystrophin-deficient myocardial muscle fibers and have earlier manifestations of myocardial disease.[23] Most cardiac magnetic resonance imaging (CMR) data in MDs currently exist for patients with DMD and BMD. The pathology of cardiomyopathy in patients with dystrophinopathy classically produces subepicardial fibrosis of the inferolateral wall,[24] remarkably similar to the pattern observed in some patients with viral myocarditis. Cardiac screening has been recommended for female DMD/BMD mutation carriers, particularly beginning after the teenage years, as these individuals are known to be at risk for developing cardiomyopathy.[25] Interestingly, CMR has revealed a pattern of myocardial fibrosis in mutation carriers similar to that seen in DMD patients.[26] Because myocardial damage in carriers has been observed even in the absence of clinically apparent muscular weakness, cardiac screening should be considered in female relatives of DMD/BMD patients. In patients predisposed to cardiomyopathies because of dystrophinopathy, occult regional cardiac dysfunction can be diagnosed with CMR tagging. This method of strain imaging analysis may offer a sensitive approach for delineating the presence and progression of cardiovascular disease and for assessing therapies designed to modulate the onset and course of heart failure.[27,28]

Our aim was to identify the exons, which are most commonly affected in cases of DMD/BMD, to determine the extent of cardiac involvement in these cases and to identify if a correlation exists between the exons affected and the cardiac involvement in these patients.

In conclusion, LVEF ≤ 40% in dystrophinopathy patients can be correlated to the deletions in the proximal exons. Cardiac involvement, though present in most cases might be difficult to detect solely based on LVEF, and hence continued monitoring of these patients is essential. Newer imaging techniques like CMR may help to detect the cardiac abnormalities earlier and in the follow-up of these patients. Although, the accurate diagnosis of DMD/BMD by DNA methods represents a considerable challenge because of the unusual characteristics of this gene and its mutations; genetic counseling, carrier detection and DNA analysis will dramatically improve the early detection and prevention of these disorders.

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