Genotype-Phenotype Correlation among patients with Dystrophinopathies

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Abstract:

Objective: To correlate the site and size of dystrophin gene deletion with the clinical picture in patients with dystrophinopathies.

Design: The dystrophin gene is one of the largest known genes. More than half of the dystrophinopathy cases are associated with intragenic deletions. The importance of the study arises from the fact that dystrophin cDNA probes provide a direct method of genetic diagnosis. This is the first study in an Arab population and only the second to use a three multiplex PCR method.

Setting: Kuwait Medical Genetic Centre and Faculty of Medicine – Ain Shams University, Egypt

Subjects and Methods: Fifty-two patients with dystrophinopathies (50 with Duchenne muscular dystrophy (DMD) and 2 with Becker muscular dystrophy (BMD) from both Kuwait and Egypt were ascertained. Dystrophin gene deletions were detected using three multiplex reactions.

Results: DNA analysis showed that 71.4% of the patients had deletion of the dystrophin gene while 28.6% showed no deletion. 24.5% had two deleted exons while 14.3% had only one deleted exon. The most common deleted exons among the Kuwaiti patients were 81, 45 & 48 while exons 19, 45, 48 & 51 were found more commonly deleted among the Egyptian patients. The onset of walking was not changed by the number of exons deleted except when five exons were deleted. However, delayed onset of walking was observed when exon 48, 51 and 45 were deleted (r=0.6078, and p=0.110). On the other hand, the average onset of weakness was neither correlated to the number of the exons deleted or to the deletion sites. Similar results were obtained regarding the average onset of wheel chair dependency. There was a slightly lower IQ with deletion of exons 48, 45 and 12 but in general there is no correlation between the IQ and the site or the frequency of the deletion. Study of the intragenic deletions in 25 exons of the dystrophin gene using three different multiplex PCR sets revealed that 78%, 76% and 12% of DMD patients had deletion of each of the three sets separately. With all three sets together, the detection of deletion rate was increased to 86%. Fifty percent of the deleted exons were located in the distal hot spot, 8% in the proximal hot spot while 42% were scattered over both sides of the hot spot.

Conclusion: No significant correlation was found between the size/site of the dystrophin deletions and the clinical severity. A multi centre larger study is recommended for a better understanding of the genotype-phenotype correlation.

Introduction:

Duchenne and Becker muscular dystrophies (DMD & BMD) are among the most common X-linked disorders. They are caused by mutation of the dystrophin gene. The estimated incidence varies considerably and ranges from 130-390/million male livebirths for DMD while the incidence of Becker muscular dystrophy is 10 times lower than DMD. The dystrophin gene is one of the largest known genes. It comprises 75 exons, spans a region of 2.3 Mb and is located on the short arm of the X-chromosome at Xp21. More than half of the DMD cases are associated with intragenic deletions. The high incidence of deletion mutations in-patients that can be identified by means of dystrophin cDNA probes has provided a direct method for genetic diagnosis. The aim of the present study is to correlate the site and size of the gene deletion with the clinical picture.

Subjects and Methods:

Fifty two patients with dystrophinopathies (fifty patients with Duchenne muscular dystrophy (DMD) and two with Becker muscular dystrophy (BMD) were ascertained from both Kuwait and Egypt. Patient ascertainment was based on previously described criteria. An informed consent was obtained from all patients and /or parents before inclusion in this study. Venous blood was collected, anticoagulated with EDTA and DNA was extracted by conventional methods. Dystrophin gene deletions were detected using three multiplex PCR reactions. The products of multiplex I and II were analyzed by 1.4-% agarose gels and those with
multiplex III were analyzed by 2% gels using TBE buffer. More details of the molecular aspect were discussed in a separate paper.(5) The data were keyed in an IBMPC and analyzed using the statistical package for social science (SPSS) for Windows version 7. The value \( p = 0.05 \) was used as a cut off level for significance. The normal Z test, Chi Square test and Fisher exact test were used for statistical analysis. The Pearson correlation coefficient was used to study the extent of association between two continuous variables. The student T Test was used to compare the means of two groups while one way analysis of variance was used to compare the means of more than two variables. The following results were obtained.

**Results:**

In the present study, DNA analysis showed that 71.4% of the patients had deletion of the dystrophin gene while 28.6% showed no deletion. 24.5% of the patients had two deleted exons (table I). The most common deleted exons among the Kuwaiti patients were exons 61,45 & 48 while exons 19, 45, 48 & 51 were found more commonly deleted among the Egyptian patients (table I and fig. 1).

The onset of walking was not changed by the number of exons deleted except when five exons were deleted (table II). In both patient groups, there was delayed onset of walking when exons 48,51 & 45 were deleted, \((r=0.6078 & P=0.110)\) (table III). The average onset of weakness was neither correlated to the number of the exons deleted nor to the deletion sites (table IV). The same results were obtained regarding the average onset of wheelchair dependency (tables V and VI).

In general there is no correlation between the IQ and the site or the frequency of the deleted exons (tables VII & VIII). However there was a slightly lower IQ level with deletion of exons 48,45,12.

![Figure 1. (see text )](image)

**Discussion:**

Dystrophinopathies the second most common X-linked genetic disorder in humans, are caused by mutations of the dystrophin gene located on the X chromosome at Xp21.\(^{(10)}\) They represent a group of primary muscular disorders, which are genetically determined and are characterized by progressive muscle wasting and weakness. Duchenne muscular dystrophy (DMD) & Becker muscular dystrophy (BMD) are allelic and considered the two major dystrophinopathies. Other X-linked forms of muscular dystrophy, such as Emery Dreifus, are rare. The estimated incidence of DMD and BMD varies considerably and ranges from 130-390 million male livebirths for DMD while the incidence of BMD is 10 times less frequent than DMD.\(^{(6-4,12)}\) The prevalence rate was found to be \(34x10^{-8}\) and the mutation rate was found lower than \(50x10^{-8}\).\(^{(13)}\) The dystrophin gene is one of the largest known genes, comprises 75 exons and spans a region of 2.3-Mb.\(^{(9)}\) About 55-65% of DMD cases are associated with intragenic deletions and 5-10% with duplications.
### Table I: DNA analysis of both Kuwaiti and Egyptian groups

<table>
<thead>
<tr>
<th>DNA result</th>
<th>Kuwait</th>
<th>Egypt</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Deletion</td>
<td>17</td>
<td>68</td>
<td>18</td>
</tr>
<tr>
<td>No deletion</td>
<td>8</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td><strong>N° of exons deleted</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 exon</td>
<td>2</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>2 exons</td>
<td>8</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>3 exons</td>
<td>2</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>4 exons</td>
<td>3</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>5 exons</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>6 exons</td>
<td>1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>7 exons</td>
<td>7</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>No exon deleted</td>
<td>8</td>
<td>32</td>
<td>6</td>
</tr>
</tbody>
</table>

**Deletion frequency of each amplified region**

<table>
<thead>
<tr>
<th>Exon number</th>
<th>Frequency</th>
<th>Average onset of walking (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>7</td>
<td>15.7</td>
</tr>
<tr>
<td>Exon 2</td>
<td>12</td>
<td>13.8</td>
</tr>
<tr>
<td>Exon 3</td>
<td>5</td>
<td>13.6</td>
</tr>
<tr>
<td>Exon 4</td>
<td>7</td>
<td>12.4</td>
</tr>
<tr>
<td>Exon 5</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Exon 6</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Exon 7</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>

### Table II: Distribution of patients according to average onset of walking and the number of deleted exons

<table>
<thead>
<tr>
<th>Exon deleted</th>
<th>Frequency</th>
<th>Average onset of walking (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>7</td>
<td>15.7</td>
</tr>
<tr>
<td>Exon 2</td>
<td>12</td>
<td>13.8</td>
</tr>
<tr>
<td>Exon 3</td>
<td>5</td>
<td>13.6</td>
</tr>
<tr>
<td>Exon 4</td>
<td>7</td>
<td>12.4</td>
</tr>
<tr>
<td>Exon 5</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Exon 6</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Exon 7</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>

### Table III: The mean onset of walking in months related to different deleted exons.

<table>
<thead>
<tr>
<th>N°. of Exons deleted</th>
<th>Kuwait</th>
<th>Egypt</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N°</td>
<td>Mean</td>
<td>N°</td>
</tr>
<tr>
<td>48</td>
<td>12</td>
<td>14.7</td>
<td>11</td>
</tr>
<tr>
<td>51</td>
<td>5</td>
<td>15.3</td>
<td>11</td>
</tr>
<tr>
<td>17</td>
<td>6</td>
<td>13.8</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>14.5</td>
<td>8</td>
</tr>
<tr>
<td>45</td>
<td>9</td>
<td>15.2</td>
<td>9</td>
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<td>12</td>
<td>4</td>
<td>14.5</td>
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<td>9</td>
<td>14.5</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>15.0</td>
<td>-</td>
</tr>
</tbody>
</table>

\( r=0.6078 \ p=0.110 \)

### Table IV: The distribution of patients according to onset of weakness and the number of deleted exons.

<table>
<thead>
<tr>
<th>Exon deleted</th>
<th>N°. of patients</th>
<th>Onset of weakness (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 exon</td>
<td>7</td>
<td>2.7</td>
</tr>
<tr>
<td>2 exons</td>
<td>12</td>
<td>3.1</td>
</tr>
<tr>
<td>3 exons</td>
<td>5</td>
<td>4.2</td>
</tr>
<tr>
<td>4 exons</td>
<td>7</td>
<td>3.9</td>
</tr>
<tr>
<td>5 exons</td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td>6 exons</td>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td>7 exons</td>
<td>1</td>
<td>2.0</td>
</tr>
</tbody>
</table>
### Table V: The mean onset of wheelchair dependency (in years) correlated with different deleted exons.

<table>
<thead>
<tr>
<th>Exon deleted</th>
<th>Kuwaiti N°</th>
<th>Mean</th>
<th>Egyptian N°</th>
<th>Mean</th>
<th>Total N°</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>48</td>
<td>4</td>
<td>9</td>
<td>7</td>
<td>10.4</td>
<td>11</td>
<td>9.7</td>
</tr>
<tr>
<td>51</td>
<td>1</td>
<td>9</td>
<td>7</td>
<td>10.7</td>
<td>8</td>
<td>9.9</td>
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<td>17</td>
<td>3</td>
<td>9</td>
<td>2</td>
<td>11.5</td>
<td>5</td>
<td>10.3</td>
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<tr>
<td>19</td>
<td>2</td>
<td>9.5</td>
<td>7</td>
<td>10.9</td>
<td>9</td>
<td>10.2</td>
</tr>
<tr>
<td>45</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>11.0</td>
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<td>9.5</td>
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<td>12</td>
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<td>7.3</td>
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<td>11.6</td>
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<td>9.5</td>
</tr>
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<td>4</td>
<td>8.5</td>
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<td>9.5</td>
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<td>9</td>
</tr>
<tr>
<td>4</td>
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<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

\[ r = 0.607 \quad p = 0.111 \]

### Table VI: The average onset of wheelchair dependency (in years) related to the number of deleted exons.

<table>
<thead>
<tr>
<th>Exon deleted</th>
<th>N° of patients</th>
<th>Average onset of wheelchair</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 exon</td>
<td>3</td>
<td>9.7</td>
</tr>
<tr>
<td>2 exons</td>
<td>6</td>
<td>8.7</td>
</tr>
<tr>
<td>3 exons</td>
<td>2</td>
<td>9.5</td>
</tr>
<tr>
<td>4 exons</td>
<td>3</td>
<td>9.7</td>
</tr>
<tr>
<td>5 exons</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>6 exons</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>7 exons</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

*No significant difference.*

### Table VII: Average IQ of patients according to the number of deleted exons*

<table>
<thead>
<tr>
<th>Exon deleted</th>
<th>N° of patients</th>
<th>Average IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 exon</td>
<td>7</td>
<td>94.7</td>
</tr>
<tr>
<td>2 exons</td>
<td>12</td>
<td>91.3</td>
</tr>
<tr>
<td>3 exons</td>
<td>5</td>
<td>67.8</td>
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<tr>
<td>4 exons</td>
<td>7</td>
<td>97</td>
</tr>
<tr>
<td>5 exons</td>
<td>2</td>
<td>87.5</td>
</tr>
<tr>
<td>6 exons</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>7 exons</td>
<td>1</td>
<td>87</td>
</tr>
</tbody>
</table>

*No significant difference.*

### Table VIII: Mean IQ correlated to different exons deleted.

<table>
<thead>
<tr>
<th>Exon deleted</th>
<th>Kuwaiti N°</th>
<th>Mean</th>
<th>Egyptian N°</th>
<th>Mean</th>
<th>Total N°</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
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<td>80.2</td>
<td>11</td>
<td>85.7</td>
<td>23</td>
<td>82.9</td>
</tr>
<tr>
<td>51</td>
<td>5</td>
<td>85.6</td>
<td>11</td>
<td>91.5</td>
<td>16</td>
<td>88.6</td>
</tr>
<tr>
<td>17</td>
<td>6</td>
<td>80.2</td>
<td>2</td>
<td>92.5</td>
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<td>86.4</td>
</tr>
<tr>
<td>19</td>
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<td>85.6</td>
<td>8</td>
<td>93.7</td>
<td>11</td>
<td>89.7</td>
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<td>84.3</td>
<td>5</td>
<td>93.2</td>
<td>9</td>
<td>88.8</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>82.5</td>
<td>1</td>
<td>93.2</td>
<td>10</td>
<td>87.9</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>83.5</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>83.5</td>
</tr>
</tbody>
</table>

\[ r = 0.248 \]

Alex J Pediatr, 13 (2), July 1999
The availability of genomic DNA probes flanking the DMD gene, intragenic genomic probes and cDNA (Dystrophin) probes have revolutionized our ability to make a precise diagnosis of DMD and BMD. Variation in the clinical picture of DMD and BMD is known. But there is no simple relationship between the extent of a mutation and the resultant clinical features. The effect on the phenotype depends not only on the extent of the deletion/duplication but also on whether or not it disrupts the reading frame. Mutations that shift the mRNA translational reading frame of three nucleotides for each amino acid soon result in a termination codon with premature termination of translation. Frame shift mutations typically lead to the synthesis of a truncated dystrophin molecule where the carboxy terminus is missing. This results in impaired membrane attachment of dystrophin leading to severe dystrophin deficiency and Duchenne phenotype. The proper function and stability of dystrophin depends on the C terminal domains. With in-frame deletions, the position and the functional significance of the deleted region also affect phenotype severity. Those deletions involving the distal rod domain (exons 45 to 47 or 45 to 48) are associated with relatively high levels of dystrophin (40% to 70% of the normal) and a mild Becker phenotype. On the other hand those involving the N terminal are associated with a relatively low level of dystrophin (10% of the normal) and tend to result in a more severe phenotype.

In the present study no correlation was found between the size or site of the deletion and the clinical severity which was scored by several signs and symptoms: (onset of walking, onset of weakness, onset of wheelchair dependency and average IQ. The onset of walking (12 to 15 months) was found more or less the same for the different number of exons deleted. However the onset of walking was delayed to 21 months when 5 exons were deleted. This finding cannot be generalized due to small sample size (only 2 patients). The same is true for the onset of weakness, that ranges between 2-4 years. An exception was found when 5 exons were deleted where the onset of weakness was shifted to 5.5 years (2 patients). Similarly, early onset of wheelchair dependency was observed where there were 5 deleted exons. However we could not find any specific exon in common. Several authors have found positive correlation between the extent, site of deletion and the phenotypic severity. However, negative correlation was found by others. Deletion of exons 33 to 34 & 33 to 35 were associated with BMD and not with DMD.

Deletions of exons 3 to 7 have been found in patients with intermediate phenotypes and rarely in patients with BMD. A very close correlation between a mild clinical phenotype and deletion of exons 45 to 47 and 45 to 48 and 45 to 49 have been found. Meanwhile the same deletions were found by others in DMD patients. On the other hand, Hart et al., 1989 found no simple correlation of position or extent of deletions with DMD or BMD although the authors found that deletion of a specific region towards the 5’ end of the gene may be more often associated with a milder phenotype. On the other hand Baumbach et al., 1989 observed that similar deletions within the proximal and distal regions of the gene are associated with different disease phenotypes. The very large deletions within the gene do not necessary result in more severe forms of muscular dystrophy. The most interesting finding is that deletions of the central region of the gene which remove almost 50 percent of the dystrophin gene can result in a very mild phenotype.

The present study showed an average IQ of more than 80 except for those patients with 3 deleted exons, (exon 45,48,51) where the IQ was found less than 67.8. This means that not only the size of the deletion is responsible for low IQ but most probably the site of the deletion. An involvement of exons 45 & 48 was associated with lower IQ than the involvement of exon 51, which was associated with average IQ. In general, about 30-50% of DMD patients have a moderate to severe non progressive mental retardation. It is not yet clear why mental retardation occurs in some DMD cases and not in others and if there is any correlation between site and size of DNA deletion and IQ impairment of cases of DMD. An association between a mutation involving exons 50 to 52 and low IQ has been reported. In the present study, exon 51 was deleted in 4 patients with low IQ (67.8). However deletion of exons 45 & 48 were also recorded. Among the Egyptian, patients, the average IQ was above 90 but involvement of exons 45 & 48 were associated with IQ ranges from 85-86. However no significant correlation between the site/size of the gene deletion and IQ was found.

In conclusion, dystrophinopathies are the second most common X-linked genetic disorders in human. The present study showed no significant correlation between the size / site of the dystrophin deletion and the clinical severity [onset of walking, weakness and wheelchair dependency]. The non significant correlation observed in the presence of several deleted exons (3 or more) might be due to small sample size. Involvement of exons 45 & 48 were
associated with low IQ. Among Arab DMD patients, the pattern of the deleted exons [50% distal to hot spot, 8% proximal to the hot spot and 42% scattered over both the proximal and distal hot spot] is different from other previously reported patterns. A larger Multicentre study including different Arab countries will hopefully enable us to gain a better understanding of the genotype-phenotype correlation of DMD and BMD in Arab patients.

References:
18. Bastaki L: Comparative clinical genetic study for Xp21 muscular dystrophies in Kuwait and Egypt: Thesis submitted for partial fulfilment of the MD Degree in Medical Genetics, Faculty of Medicine, Ain Shams University, Egypt 1997.

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