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COGNITIVE IMPAIRMENT EVALUATION IN CHILDREN WITH DYSTROPHINOPATHIES

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Abstract. Human dystrophinopathies are X-linked genetic disorders characterized by impairment in the function of dystrophin in a wide range of tissues. The identification of multiple alternative promoters explains the tissue-specific expression of the dystrophin isoforms. Beside the prominent muscular disease, cognitive impairment has been described in dystrophinopathies; however, the genotype – cognitive phenotype relationship is not yet fully clarified. The aim of the present study was to explore the impact of age and genetic lesion on the visuo-motor performance in Romanian pediatric subjects with dystrophinopathies. Duchenne and Becker muscular dystrophies pediatric patients admitted during a 12 months period in "Dr. V. Gomoiu" Children's Hospital, Bucharest were investigated with the use of Raven Progressive Matrices Tests. Most of the studied subjects had exon deletions in the 45-51 range in the dystrophin gene. The obtained results do not support a progressive cognitive impairment in these subjects.
Key words: dystrophinopathies, cognitive function, genetic mutations

Introduction

Human dystrophinopathies are X-linked diseases caused by impairment in the function of the dystrophin protein. The most evident clinical expression of dystrophin impairment is the progressive muscle disorder, manifested as proximal limb muscle weakness, development of calf pseudohypertrophy, Gowers sign (climbing from the floor only possible by walking up one's own body), muscle contractures, defective body positions [1,2] and respiratory difficulty [3].

In what concerns the muscle function impairment, three dystrophinopathy phenotypes have been described: the Duchenne (DMD) and Becker (BMD) muscular dystrophies and an intermediate phenotype [4]. Of them, DMD is the most frequent, with an incidence of 1 in 3500 liveborn males [5], while BMD has an estimated incidence of around 1 in 18000 liveborn males [6] and the intermediate phenotype is even less frequent.

Cognitive deficits have been frequently observed in subjects with dystrophinopathies. They had already been mentioned in the original description of DMD by Duchenne de Boulogne [7].

Later reports confirmed a decrease of the full scale IQ (FSIQ) in DMD subjects with approximately one standard deviation below population mean [8,9]. The cognitive impairment seems to be more severe in verbal than performance tests [9,10].

An increased incidence of attention deficit and hyperactivity disorder (ADHD) and of autism spectrum pathology [11,12] were also reported in DMD children. Deficits in working memory and executive function have also been described [1].

The dystrophin gene is located on the short arm of the X chromosome, in the Xp21.2 region, being composed of 79 exons. The existence of multiple alternative promoters explains the differential tissue expression of dystrophin isoforms [13]. Different gene lesions have specific effects on the expressed isoforms and on the induced pathologic phenotypes.

The most frequent lesions of the dystrophin gene are large deletions encompassing exons (approximately 65% of DMD and BMD patients) [13]. The vast majority of large dystrophin gene deletions cluster around two mutation "hotspots", spanning exons 45-53 (the distal hotspot) and exons 2-20 (the proximal hotspot) [13]. The other types of mutations found in dystrophinopathies are small deletions and point mutations, that generally introduce premature stop codons and are evenly distributed throughout the gene [13].

Besides the 5' promoter that drives the transcription of the largest dystrophin protein isoform Dp427, at least four other internal promoters control the expression of other dystrophin isoforms in the brain (namely Dp260, Dp140 and Dp71) [14-16].

Several reports suggest a role of mutations in Dp140 and Dp71 in the cognitive impairment in subjects with

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distrophinopathies [17-20] and a protective role of an intact brain Dp140 isoform against cognitive impairment in dystrophinopathies [19,20].

The differential role of dystrophin isoforms in the brain is further emphasized by the finding that subjects with mutations in the distal portion of the gene seem to have a greater risk for cognitive impairment in comparison with subjects presenting other genetic mutations [11,17,20,21], while subjects with deletion of the full-length dystrophin promoter at the proximal end of the gene have intelligence within the normal range [11,20,21-23].

The transcription of Dp140 is initiated in intron 44 of the dystrophin gene; the transcript has a long 5' untranslated region (5'UTR) of 1.041 Kbp, the transcription continuing with exon 51 of the gene [20]. Dp71 is initiated in intron 62 of the gene [20].

Mutations in the region spanning exons 45-50 and the portion of exon 51 that lies 5' of c.7381 of the dystrophin gene are considered to affect the sequence of Dp427 and Dp260 and to modify the 5'UTR of the Dp140 isoform, but not to affect the expression of Dp71; on the other side, mutations that are located upstream of intron 44 preserve the expression of Dp140, and Dp71, while affecting the expression of Dp427 and Dp260 [20].

Despite the amount of knowledge accumulated regarding the impact of mutations affecting the expression of Dp140 and Dp71 on the cognitive abilities in subjects with dystrophinopathies, a stringent genotype-phenotype correlation has not been established and predictions about the risk of cognitive disabilities based on structural features are not possible.

The aim of the present study was to investigate the influence of the genetic lesion and of age on the cognitive abilities of Romanian Duchenne and Becker muscular dystrophy pediatric subjects.

Materials and method

Study group

The study was performed on Duchenne and Becker muscular dystrophy pediatric subjects admitted for routine controls at "Dr. V. Gomoiu" Children's Hospital, Bucharest, during a 12 months period (the year 2014).

The diagnosis was established using clinical, biochemical, histological and genetic methods. The genetic lesion was investigated using multiplex ligation-dependent probe amplification (MLPA) and gene sequencing on genomic DNA extracted from peripheral blood lymphocytes.

The study was performed in accordance with the human subjects protection provisions of "Carol Davila" University of Medicine and Pharmacy, Bucharest.

Cognitive function tests

The visuo-motor performances of studied subjects were studied using the Raven Progressive Matrices Tests [24]. The tests consist of a series of increasing difficulty problems in which the subjects indicate missing elements in graphical matrices based on their inference of rules from already present items. The Raven tests are available in colour and black-and-white versions. The intelligence quotient is calculated based on the performance of age-matched pediatric populations.

Data analysis

The location of mutations in the dystrophin gene was inspected in order to determine the regions with higher mutation frequency in the study group.

The correlation of IQ and age was investigated using the non-parametric Spearman's rank correlation test and the parametric Pearson's product moment correlation test. p values lower than 0.05 were considered significant.

Results

The study group included 19 subjects with dystrophinopathies aged between 4.33 and 16.5 years (mean age 9.01, standard deviation 2.95), Romanian language natives, presenting Duchenne and Becker muscular dystrophy phenotypes. Of the 19 subjects, 17 were male and 2 female; 13 subjects (68% of total) presented Duchenne muscular dystrophy and 6 subjects (32% of total) - Becker muscular dystrophy phenotype (Table 1, Figure 1).

The age of subjects in the group with exon deletions in the exon range 45-51 varied between 5.16 and 16.5 years, with a mean age of 10.29, and a standard deviation of 3.24 (Figure 2)

Subjects	Total	Males	Females
Sex	19 (100% of total)	17 (89% of total)	2 (11% of total)
DMD phenotype	13 (68% of total)	12 (92% of DMD subjects)	1 (8% of DMD subjects)
BMD phenotype	6 (32% of total)	5 (83% of BMD subjects)	1 (17% of BMD subjects)
Age	4.33-16.5 years	4.33-16.5 years	10.41 years (twins)

Table I. Structure of the study group

Type of genetic lesions in the study group

Of the studied group, 13 subjects (68%) presented exon deletions, while the rest presented mutations other than exon deletion, including point mutations.

Exon deletions were identified in 10 (77%) of the Duchenne muscular dystrophy subjects and in 3 (50%) of the Becker muscular dystrophy subjects.

Two regions with increased exon deletion frequency were detected: a proximal region (exons 2-7) and a distal one (exons 44-51) (Figure 1).

The majority of exon deletions subjects presented a DMD phenotype. Surprisingly, a subject with a large exon deletion (exons 2-7) presented a BMD phenotype (Figure 1). Of the 6 subjects with mutations other than exon deletions, 3 presented DMD and 3 presented BMD phenotype.

Relation between the visuo-motor performance and the genetic lesion

The IQ values obtained by applying the Raven Progressive Matrices Tests in the study sample were in the 75 to 120 range, with a mean of 91.21 and a standard deviation of 11.59. For the subjects with exon deletions

in the 45-51 region, the IQ varied between 83 and 95, with a mean IQ value of 89.11 and a SD of 4.67 (Figure 3).

The study of the tendency of linear variation of IQ with age for the group of subjects with exon deletions in the 45-51 range suggested a tendency of minor decrease of IQ with age ([x]=age, [y]=IQ, y=-0,64x+96,22) (Figure 4). However, the coefficient of determination for the linear correlation was weak (R2=0.16) and both the non-parametric and the parametric correlation analyses did not reach significance (Spearman's rank correlation p=0.15, Pearson's product moment correlation p=0.34).

Discussion and conclusions

The study sample included subjects admitted for routine controls during a 12 months period in "Dr. V. Gomoiu" Children's Hospital, Bucharest. They were mostly male (89%) as expected from the X-linked character of the pathology. Their age varied between 4.33 and 16.5 years (mean age 9.01, standard deviation 2.95), with a predominance of cases in the 8-11 years old range.

The ratio of DMD to BMD cases in the study sample was 2.16; the difference from the estimated ratio in the general population may be explained by the bias introduced by the different hospitalization necessities of the two phenotypes.

The detected exon deletions in the dystrophin gene were located in two particular regions, namely a proximal region (exons 2 to 7) and a distal region (exons 44 to 51) (Figure 1). The two regions are superposable with the two hotspots already described in the literature [13].

The majority of exon deletions in the 44-51 exon range were accompanied by DMD phenotype (in 9 of 10 subjects). 2 of 3 subjects with exon deletions in the 2-7 exon range presented BMD phenotype (one of them had a longer deletion encompassing the whole 2-7 exon region). Of the group of 6 subjects with mutations other than exon deletions, 3 subjects presented Duchenne and 3 Becker muscular dystrophy phenotype.

The investigation of the visuo-motor performance by Raven Progressive Matrices Tests showed an IQ spanning the interval between 75 to 120 (mean IQ 91.21, SD 11.59) for the entire study sample. The large SD may be accounted for by the varied type of genetic lesions in the study subjects.

For the group of subjects with exon deletions in the exon range 45 to 51 the IQ varied in a narrower range of 83 to 95 (mean IQ 89.11, SD 4.67), suggesting a more uniform group with a stronger genotype-phenotype correlation. The range of IQ values is similar with that reported for subjects with similar mutations in the dystrophin gene located in the region corresponding to the 5'UTR of the Dp140 dystrophin isoform [20].

The slope of the linear model approximating the dependence of the IQ on the age of subjects in the exon 45-51 deletion group was negative (-0.64) suggesting a mild decrease of IQ with age; however, the lack of significance of the results (p higher than 0.05) does not support a correlation of IQ with age. The obtained result is in accordance with published reports suggesting that the cognitive deficits in dystrophinopathies may be non-progressive, in contrast to the progressive aspect of the muscular degeneration [11].

The data presented in the current work do not support a correlation of cognitive impairment with age in pediatric subjects with dystrophinopathies with deletions in the 45 to 51 exon range.

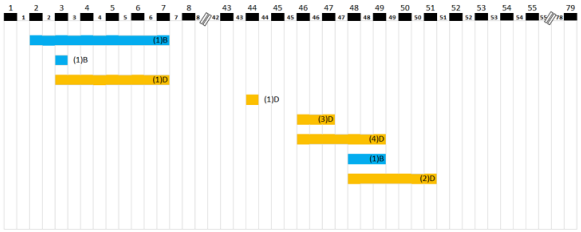

Fig.1. Repartition of the detected exon deletions in the dystrophin gene

The location of the genetic defect in exon deletion subjects was pictured. The horizontal axis maps the location of the deleted exons. Darker color and symbol "D" signifies Duchenne muscular dystrophy, while lighter color and symbol "B"- Becker muscular dystrophy. The numbers in parentheses represent numbers of cases with identical genetic defect and phenotype in the studied group.

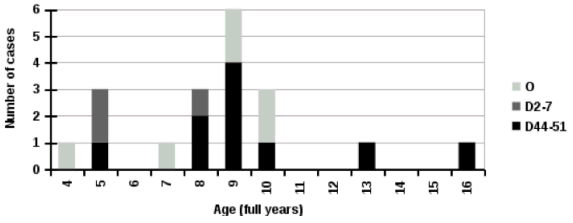

Fig.2. Age distribution of the subjects in the study sample

Number of cases were presented for specific dystrophin gene lesion types: deletions in the exon 44-51 region (D44-51); deletions in the exon 2-7 region (D2-7); other mutations than exon deletions (O).

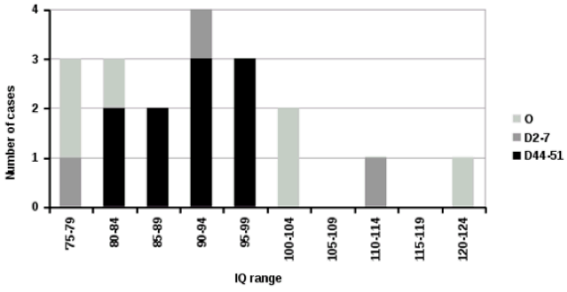

Fig.3. Intelligence quotients (IQ) distribution of subjects in the study sample

Number of cases were presented for specific dystrophin gene lesion types: deletions in the exon 44-51 region (D44-51); deletions in the exon 2-7 region (D2-7); other mutations than exon deletions (O).

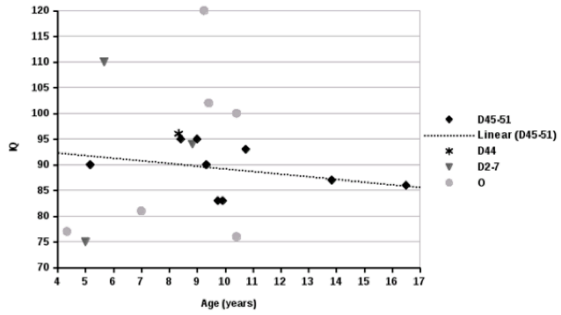

Fig.4. Relation between the visuo-motor performance and the age of subjects

Intelligence quotients (IQ) were measured using Raven tests. Rhombs represent subjects with exon deletions in the region between exons 45 and 51 (D45-51), asterisk- the subject with a deletion of exon 44 (D44), triangles- subjects with exon deletions in the interval between exons 2 and 7 (D2-7), circles- subjects with mutations other than exon deletions (O)

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