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Original Article

Neurocognitive Profiles in Duchenne Muscular Dystrophy and Gene Mutation Site

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ABSTRACT

The presence of nonprogressive cognitive impairment is recognized as a common feature in a substantial proportion of patients with Duchenne muscular dystrophy. To investigate the possible role of mutations along the dystrophin gene affecting different brain dystrophin isoforms and specific cognitive profiles, 42 school-age children affected with Duchenne muscular dystrophy, subdivided according to sites of mutations along the dystrophin gene, underwent a battery of tests tapping a wide range of intellectual, linguistic, and neuropsychologic functions. Full-scale intelligence quotient was approximately 1 S.D. below the population average in the whole group of dystrophic children. Patients with Duchenne muscular dystrophy and mutations located in the distal portion of the dystrophin gene (involving the 140-kDa brain protein isoform, called Dp140) were generally more severely affected and expressed different patterns of strengths and impairments, compared with patients with Duchenne muscular dystrophy and mutations located in the proximal portion of the dystrophin gene (not involving Dp140). Patients with Duchenne muscular dystrophy and distal mutations demonstrated specific impairments in visuospatial functions and visual memory (which seemed intact in proximally mutated patients) and greater impairment in syntactic processing.

Introduction

Duchenne muscular dystrophy is a fatal, recessive, X-linked muscular disease affecting about 1 in 3500 liveborn human males [1,2]. In Duchenne muscular dystrophy, the body is unable to produce the dystrophin protein as a result of a large variety of mutations/deletions of the dystrophin gene. The protein is essential for muscle contraction, and its absence leads to progressive muscle weakness, chronic degeneration, and replacement of the muscle with fat and endomysial fibrosis.

The presence of nonprogressive cognitive impairment is widely recognized as a common feature in a substantial proportion of patients. Interestingly, delay in global developmental and language disorders can constitute the signs of onset in this disease [3].

A meta-analysis performed by Emery and Muntoni [4] documented intelligence quotients in 721 patients with Duchenne muscular dystrophy, and indicated that the overall mean intelligence quotient was 82 (approximately 1 S.D. below the population mean). Nineteen percent demonstrated an intelligence quotient below 70 (i.e., the generally accepted cutoff point for a diagnosis of mental retardation), and 3% demonstrated an intelligence quotient of less than 50 (indicating moderate to severe mental retardation).

A discrepancy between verbal intelligence quotient and performance intelligence quotient, with greater impairment of verbal components, is widely described [5]. Verbal disability consisting of poor expressive verbal abilities, deficits in short-term memory, and specific disabilities in learning to read, write, and calculate, with relatively intact visuospatial cognitive abilities, are more frequently reported cognitive deficits in English-speaking and French-speaking children with Duchenne muscular dystrophy [6-9]. Some authors point to deficits in verbal working memory [9] and in phonologic processing [10,11] as the main sources of difficulty in these patients' verbal processing.

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Because this muscular disease is caused by an absence of dystrophin, a 427-kDa protein associated with sarcolemma in skeletal and smooth muscle and two alternative 427-kDa isoforms are also expressed in the cerebral neocortex. In the cerebellum, dystrophin appears to play a role in normal neuronal function or development. Two carboxy-terminal dystrophin proteins (Dp), Dp71 and Dp140, are both expressed in the brain, in addition to full-length central nervous system dystrophins, and are initiated between exons 62 and 63, and upstream from exon 44, respectively [12-14].

Rearrangements in the second part of the dystrophin gene tend to be more commonly associated with cognitive impairment, and several reports described mutations in the Dp71 coding region as a factor that contributes to the severity of mental retardation, and may account for shift in intelligence quotient of 2 S.D.s downward [13-15].

Moreover, a lack of the Dp140 isoform is thought to play a significant role in cognitive performances in both Duchenne muscular dystrophy [16,17] and Becker muscular dystrophy [18]. However, apart from general intellectual abilities, a lack of consensus exists on the correlation between dysfunction in the dystrophin gene and a specific neuropsychologic profile.

The present study investigated possible similarities and differences in the cognitive profiles of Italian-speaking, school-age children with Duchenne muscular dystrophy, with different mutation sites along the dystrophin gene, i.e., distal vs proximal (downstream and upstream from exon 44, involving or sparing the expression of Dp140, respectively). We hypothesized that different mutations along the dystrophin gene not only influence intellectual levels, but also determine specific neuropsychologic profiles.

Materials and Methods

Participants

Forty-two children affected with Duchenne muscular dystrophy and 10 boys affected with spinal muscular atrophy and osteogenesis imperfecta (severe muscular impairments not related to a deficiency of dystrophin) were enrolled in the study. All parents gave informed consent. This study was approved by our local Human Ethics Committee, according to the declaration of Helsinki.

All patients had clinical, histologic, and immunohistologic features compatible with a diagnosis of Duchenne muscular dystrophy. The identification of the responsible abnormalities in the dystrophin gene confirmed the diagnosis [2,19]. At the time of their evaluation, 19 children with Duchenne muscular dystrophy were ambulant, and 23 were wheelchair-bound.

The control group comprised children with a diagnosis of spinal muscular atrophy (defined via clinical and neurophysiologic signs, molecular alterations in the Survival Motor Neuron 1 gene) [20,21] or of osteogenesis imperfecta (defined via clinical signs, radiologic findings, and genetic or biochemical analysis) [22,23]. All patients were boys, including six with osteogenesis imperfecta (four were wheelchair-bound, and two were ambulant) and four with spinal muscular atrophy (two were wheelchair-bound, and two were ambulant), and all demonstrated motor impairments similar to those in the group of children with Duchenne muscular dystrophy. The mean age in the group with Duchenne muscular dystrophy was 9.1 years (S.D., 1.6 years). The mean age in the control group was 9.6 years (S.D., 1.6 years) ($t(50) = -0.845$, no significance).

For both groups, the inclusion criteria comprised an age ranging between 6-12 years, normal hearing, and the absence of severe visual impairment (the reliability of results of cognitive and linguistic tests may be impaired by visual deficits). The age range of subjects was chosen to allow for the administration of cognitive and linguistic tests, standardized for an Italian population.

None of the children were habitually bilingual. All were attending mainstream schools. Twenty percent of the children in both groups with Duchenne muscular dystrophy (with a deletion in the distal and the proximal portions of the Duchenne muscular dystrophy gene; see below) received psychoeducational support.

Home and educational environments were comparable for all children. In every group, most of the parents (ranging from 65-80%) had graduated from high school, and around 10% had graduated from college. The sociodemographic characteristics of the families were similar across groups.

Genetic analysis

We extracted DNA from peripheral blood samples according to standard procedures, using a commercial kit (Flexi Gene DNA Handbook, Qiagen [Hilden, Germany]). Deletions were defined via multiplex polymerase chain reactions. In some patients, multiplex ligation-dependent probe amplification [24] was also performed, to screen for deletions and duplications. A direct sequence analysis of all dystrophin gene-coding exons and surrounding splicing sites was performed to detect point mutations and other microrearrangements.

On the basis of the localization of molecular abnormalities along the dystrophin gene, mutations located in (or extending to) the genomic region corresponding to exons 45-55 of the dystrophin gene are considered to affect Dp140 (as well as Dp427 and Dp260; these proteins are not relevant to our study), but not Dp71. Mutations in the dystrophin gene, located upstream from exon 44, are predicted to preserve Dp140 and to affect only the expression of Dp427 and Dp260 [15].

Patients with Duchenne muscular dystrophy were further subdivided into two groups: 17 children ("Duchenne muscular dystrophy proximal") carried mutations in the 5′ end of the Duchenne muscular dystrophy gene (upstream from exon 44), with 1/17 duplications, 4/17 point mutations, and 12 deletions; 25 children ("Duchenne muscular dystrophy distal") carried mutations in the 3′ end of the Duchenne muscular dystrophy gene (downstream from exon 44), with the following distribution: 1 /25 duplications, 2/25 point mutations, and 22 deletions.

The group of distally deleted children consisted of 24 boys bearing mutations predicted to affect all dystrophin products, including Dp140 but not Dp71, and one boy with a mutation affecting the expression of Dp140 and Dp71.

Fourteen children in the Duchenne muscular dystrophy distal group were wheelchair-bound, and 11 were ambulant. In the Duchenne muscular dystrophy proximal group, nine were wheelchair-bound, and eight were ambulant. Only one patient in the Duchenne muscular dystrophy distal group presented with mild cardiac involvement, and one child in the Duchenne muscular dystrophy proximal group presented a very severe clinical phenotype with mild respiratory insufficiency at the time of his examination.

The mean age in the two groups was comparable (Duchenne muscular dystrophy distal, mean age, 8.8 years; S.D., 1.4 years; Duchenne muscular dystrophy proximal, mean age, 9.5 years; S.D., 1.8 years; $t_{40} = -1.25$, no significance). A similar percentage of children treated with steroids was present in the two groups, i.e., 16 of 25 children of the distal group (35%), and 12 of 17 children in the proximal group (32%).

Cognitive assessment

General intelligence was assessed using the Wechsler Intelligence Scale for Children-Revised. This version of the Wechsler scales was used because it was the only version currently adapted and normed for the Italian population as of the time we initiated the study [25]. The results are expressed as intelligence quotient scores (mean, 100; S.D., 15) for the Verbal and the Performance scales, and as scaled scores (mean, 10; S.D., 3) for single subtests. Further neuropsychologic and neurolinguistic tests were administered to test specific functions.

Language abilities

A battery of standardized tests for the assessment of language development in Italian children was used, i.e., the Batteria 4-12 (a battery for the linguistic assessment of children aged 4-12 years) [26]. A number of single tests are included in the battery.

In terms of comprehension, the verbal auditory discrimination of subjects was assessed by the Same-Different Judgment Test, a phonemic identity judgment task. Semantic comprehension was assessed by means of a picture identification test, adapted from the British Picture Vocabulary Scale [27]. Morpho-syntactic comprehension was assessed with the Test of Grammatical Comprehension for Children [28], a sentence-picture matching test. Syntactic comprehension was assessed with the Italian version of the Token Test [27].

Production was assessed with a naming task requiring subjects to name 36 object pictures [27] and the Test of Semantic Fluency, in which subjects are prompted to name, in the course of 90 seconds, as many words as possible belonging to the "animals" and "home objects" semantic categories. An additional test of derivational morphology, taken from the Test of Morpho-Syntactic Development [29], requires subjects to produce derived forms of given terms.

Repetition abilities were assessed by means of a Sentence Repetition task [30,31]. An additional test of Word and Nonword Repetition was taken from the Test of Morpho-Syntactic Development [29].

Reading abilities

Text reading was assessed with the Prove di Rapidità e Correttezza Nella Lettura del Gruppo MT (a test for speed and accuracy in reading, as developed by the Memory Training Group) [32]. Scores in speed and accuracy are recorded.

Single word/nonword reading subtests were taken from the Batteria per la Valutazione della Dislessia e Disortografia Evolutiva (the Battery for the Assessment of Developmental Reading and Spelling Disorders) [33]. Scores of speed and accuracy in reading lists of words and nonwords were recorded.

Neuropsychologic assessment: Attention

Visual attention was assessed via the Visual Attention Subtest of the Developmental Neuropsychological Assessment (NEPSY) [34], a visual search task in which total scores are computed from speed and accuracy scores.

Auditory attention was assessed with the NEPSY [34] Subtests of Auditory Attention and Response Set, requiring subjects to displace sets of colored tokens from one box to another one while listening to a series of spoken words.

Neuropsychologic assessment: Memory

Visual/verbal cross-modal memory was assessed by means of the Memory for Names Subtest of the Test di Memoria e Apprendimento battery (an Italian adaptation of the Test of Memory and Learning) [35], requiring subjects to learn and recall the names of eight children whose faces are depicted in line drawings.

Verbal learning, supraspan memory, and resistance to interference were assessed via the List Learning Subtest of the Test of Memory and Learning [35]. This test assesses the immediate recall of word lists over repeated trials with or without interference, as well as a delayed recall after 30 minutes.

Visual memory was assessed with the Abstract Visual Memory Subtest from the Test of Memory and Learning [35], requiring the immediate recognition of abstract, nonverbalizable pictures. All scores are expressed as *z*-scores.

Data analysis

The two groups of children with Duchenne muscular dystrophy with distal and proximal mutations were compared in terms of various cognitive and neuropsychologic measures. Subsequent analyses were performed to better define: (1) the degree of impairment of the two subgroups compared with control patients, and (2) the relationship of the deficits highlighted in either subgroup with other theoretically relevant cognitive and neuropsychologic functions. SPSS software (SPSS, Inc, Chicago, IL) was used for all analyses.

Results

Our study showed that in Italian-speaking children with Duchenne muscular dystrophy, the intelligence quotient is approximately 1 S.D. below the population average, with an overall mean full-scale intelligence quotient of 86.43 ± 13.7, and a discrepancy between verbal intelligence quotient (86.26 ± 14.9) and performance intelligence quotient (89.98 ± 14.8). The control group of patients with spinal muscular atrophy or osteogenesis imperfecta (severely motor impaired) did not manifest any cognitive deficits, with a full-scale intelligence quotient of 107.7 ± 10.45, a verbal intelligence quotient of 108 ± 9.34, and a performance intelligence quotient of 105.6 ± 16.

Separate analyses, taking into account genetic alterations in the dystrophin gene (Duchenne muscular dystrophy distal and Duchenne muscular dystrophy proximal), indicated that the verbal intelligence quotients of both groups were significantly lower than those of the control children, whereas only distally mutated children with Duchenne muscular dystrophy demonstrated significantly lower performance intelligence quotients (Table 1).

In the Duchenne muscular dystrophy distal group, 24 of 25 patients carried mutations predicted to affect all dystrophin products, including Dp140 but not Dp71. Only one patient carried a mutation also affecting Dp71. That patient did not exhibit deficits in any neurocognitive function. Moreover, his full-scale intelligence quotient was above 100.

We further analyzed data from four additional patients who carried mutations affecting the Dp71 region, but who did not meet to the inclusion criteria of this study. Two were older than 12 years of age, and two were younger than 6 years of age at the time of their evaluation. Their full-scale intelligence quotients ranged from 62-88. We emphasize that the lowest full-scale intelligence quotient score (51) in the whole sample was observed in a child carrying a mutation affecting the expression of Dp140, but not the expression of Dp71.

A more detailed analysis of the Wechsler subscales revealed some interesting and completely new differences in cognitive profiles in the two subgroups of patients with Duchenne muscular dystrophy (Fig 1). Patients in the Duchenne muscular dystrophy distal group scored significantly lower than patients of the Duchenne muscular dystrophy proximal group in Digit Span ($t(26.83) = -3.627$, $P = 0.001$), Picture Arrangement ($t(32) = -2.419$, $P = 0.021$), and Object Assembly ($t(32) = -2.075$, $P = 0.046$). Children in the Duchenne muscular dystrophy proximal group tended (albeit not significantly) to score lower than those in the Duchenne muscular dystrophy distal group on Comprehension ($t(38) = 1.777$, $P = 0.08$). All these differences tended to retain (for Digit Span, Picture Arrangement, and Object Assembly, $P = 0.009$, $P = 0.025$, and $P = 0.064$, respectively) or even improve (Comprehension subtest, $P = 0.006$) their significance when full-scale intelligence quotient was used as a covariate in the analysis (to assess the specific components of reported impairments). No Bonferroni correction was applied, because the variables are obviously intercorrelated.

Compared (using *t* tests) with control subjects, patients in the Duchenne muscular dystrophy distal group scored significantly lower ($P < 0.03$) on all verbal subtests except on Comprehension, while patients in the Duchenne muscular dystrophy proximal group scored lower ($P < 0.03$) on all verbal subtests except Digit Span. In terms of Performance subtests, the children with distally mutated Duchenne muscular dystrophy performed significantly worse than control subjects in Picture Completion, Picture Arrangement, Block Design, and Coding ($P < 0.05$), whereas the children with proximally mutated Duchenne muscular dystrophy did not differ from control subjects on any subtest ($P > 0.05$).

Concerning scores obtained on language tests, comparisons between subgroups were repeated, controlling for the effect of full-scale intelligence quotient (set as a covariate in an analysis of variance).

As depicted in Fig 2, both subgroups demonstrated deficits in all linguistic functions, with distally mutated patients obtaining generally lower scores than proximally mutated patients. Only scores in

Table 1. Scores of Wechsler Intelligence Scales in two groups of children with Duchenne muscular dystrophy (Duchenne muscular dystrophy distal and Duchenne muscular dystrophy proximal) and the control group

	Duchenne Muscular Dystrophy, Distal	Duchenne Muscular Dystrophy, Proximal	Control Subjects	Pairwise Comparison (Tukey Post Hoc Tests)
VIQ	86.8 ± 16.8	85.5 ± 12.2	108 ± 9.3	0.001* 0.001†
PIQ	86.5 ± 13.8	95.1 ± 14	105.6 ± 16	0.002*
FSIQ	84.8 ± 14.5	88.9 ± 12.6	107.7 ± 10.4	<0.001* 0.002†

Abbreviations:
FSIQ = Full-scale intelligence quotient
PIQ = Performance intelligence quotient
VIQ = Verbal intelligence quotient
Mean scores obtained on the Wechsler Intelligence Scale-Revised are indicated as means ± S.D. Different symbols indicate pairs that demonstrated significant differences ($P < 0.05$).
* Duchenne muscular dystrophy distal group vs control group.
† Duchenne muscular dystrophy proximal group vs control group.

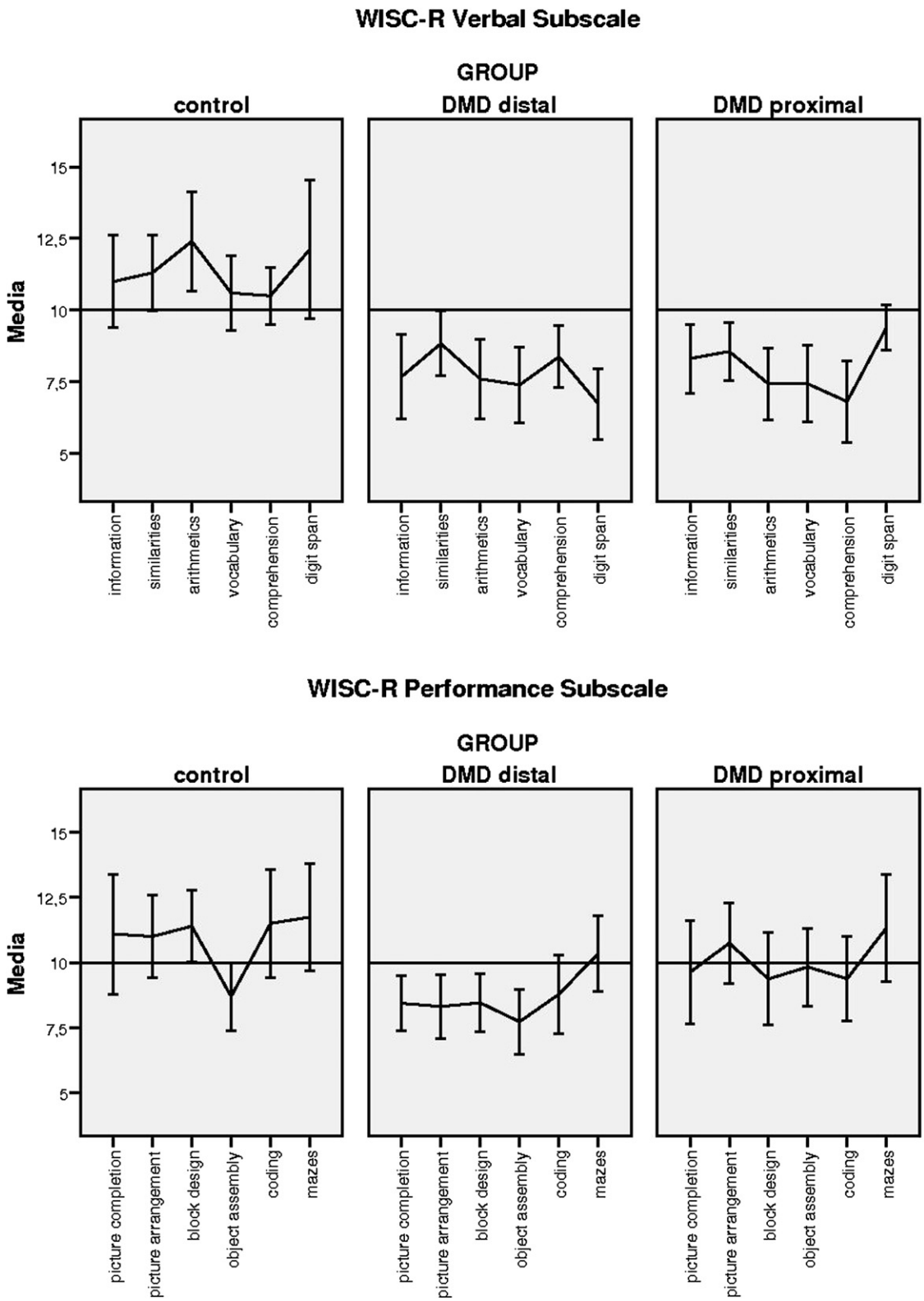

Figure 1. Top: WISC-R verbal subscale. Mean scaled scores (y-axis) were obtained in verbal scale subtests (x-axis) in each group (DMD distal, DMD proximal, and control group). Bottom: WISC-R performance subscale. Mean scaled scores (y-axis) were obtained in performance scale subtests (x-axis) in each group (DMD distal, DMD proximal, and control groups). DMD, Duchenne muscular dystrophy; WISC-R, Wechsler Intelligence Scale-Revised.

Grammatical Comprehension proved significantly different in the two subgroups (F (1.40) = 5.667, P = 0.02), and this difference was confirmed when intelligence quotient was entered as a covariate into the analysis (F (1.39) = 5.07, P = 0.03). In comparison with control subjects, including intelligence quotient as covariate, we observed that all differences became nonsignificant, with the exception of Fluency for patients with distally mutated Duchenne muscular dystrophy (though yielding z-scores greater than 0).

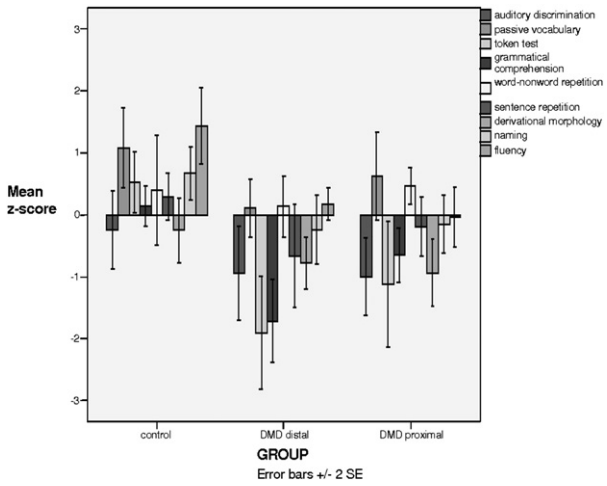

Figure 2. Performance of DMD subgroups (DMD distal and DMD proximal) and control group in linguistic tests. DMD, Duchenne muscular dystrophy; SE, standard error.

Regarding the results of our neuropsychologic assessment (with Bonferroni correction, $\alpha = 0.01$) in the two subgroups of patients with Duchenne muscular dystrophy (Fig 3), patients with both distally and proximally mutated Duchenne muscular dystrophy performed at a lower level than control subjects in Visual Attention, whereas only patients with distally mutated Duchenne muscular dystrophy exhibited deficits in Visual Abstract Memory. The difference in Visual Memory between the two subgroups was not significant ($t(40) = -1.936$, $P = 0.06$), and trended further from significance when intelligence quotient was entered as a covariate ($P = 0.10$). Moreover, the differences between patients with distally mutated Duchenne muscular dystrophy and control subjects concerning Auditory Attention (though with z-scores greater than 0; $t(32) = 2.603$, $P = 0.012$), Visual Attention ($t(33) = 3.476$, $P = 0.001$), and Visual Memory ($t(33) = 3.552$, $P = 0.001$) became nonsignificant ($P > 0.25$) when intelligence quotient was included as a covariate. Children with proximally mutated Duchenne muscular dystrophy performed (almost significantly) worse than control subjects on Visual Attention only ($t(25) = 2.452$, $P = 0.022$), but this difference, in contrast with the findings for the Duchenne muscular dystrophy distal group, appeared to be independent of influences from intelligence quotients. Interestingly, no deficits in Auditory Attention, List Learning, and Memory for Names were evident in any of the subgroups.

Finally, the two groups of control patients and patients with Duchenne muscular dystrophy were compared on tests of reading accuracy and speed. The results are reported in Fig 4. No Bonferroni correction was applied, because all measures were highly intercorrelated. The children with Duchenne muscular dystrophy appeared to be particularly slow in reading text and words, and partly slow in reading nonwords. However, the reading performances were rather similar in the two groups with Duchenne muscular dystrophy, and no significant differences were evident between distally and proximally mutated children. Taken separately, the Duchenne muscular dystrophy distal group performed at a significantly lower level than control subjects in text reading speed ($t(30) = 2.135$, $P = 0.041$), and the same held true for the Duchenne muscular dystrophy proximal group ($t(25) = 2.229$, $P = 0.035$). In both groups, variations in intelligence quotient, entered in the analysis as a covariate, explained all differences.

Correlations

The patterns of correlations between reading skills and other linguistic or neuropsychologic functions were also investigated, to evaluate whether different sources of impairment were identifiable in the two subgroups of children with Duchenne muscular dystrophy. The scores of different language tests pertaining to a common language domain were averaged into global scores. We ensured that the variables considered to be part of the same domain, beyond theoretical justifications, were indeed characterized by high

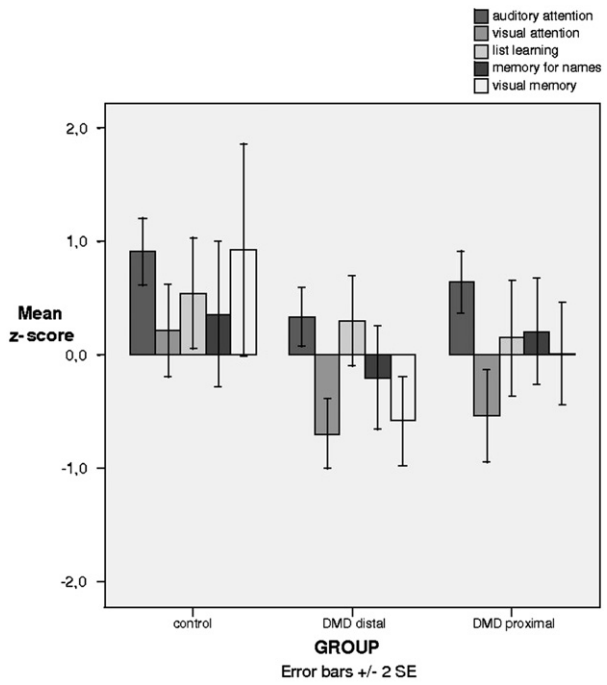

Figure 3. Neuropsychologic profiles of control, DMD distal, and DMD proximal patients. DMD, Duchenne muscular dystrophy; SE, standard error.

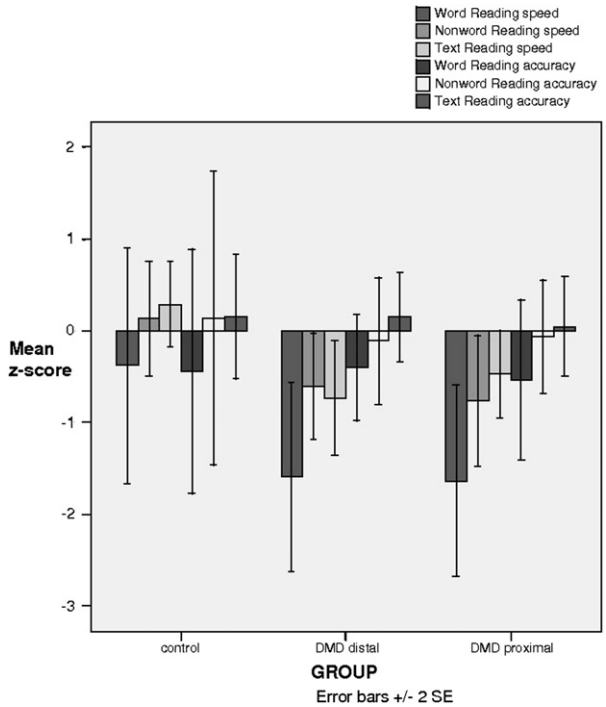

Figure 4. Reading performances in control, DMD distal, and DMD proximal patients. DMD, Duchenne muscular dystrophy; SE, standard error.

intercorrelations. In specific terms, Auditory Discrimination and Word and Nonword Repetition scores were averaged to express a Phonological Skills score. The Token Test, Grammatical Comprehension, and Sentence Repetition scores were averaged to express a Syntactic Skills score. Passive Vocabulary, Naming, Derivational Morphology, and Verbal Fluency were averaged to express a Lexical Skills score. Similarly, reading scores from Word, Nonword, and Text Reading were averaged into a Reading Speed and a Reading Accuracy global score.

First, Pearson's correlations were computed for reading scores with subtests of the Wechsler Intelligence Scale for Children-Revised, revealing interesting associations for the Duchenne muscular dystrophy distal group between reading accuracy and information ($r = 0.476$, $P = 0.022$), as well as between reading speed and Picture Arrangement ($r = 0.487$, $P = 0.025$). In the Duchenne muscular dystrophy proximal group, only one significant correlation emerged between reading accuracy and arithmetic ($r = 0.557$, $P = 0.025$). Further associations emerged, in the proximal group only, between reading speed and lexical skills ($r = 0.558$, $P = 0.02$), phonologic skills ($r = 0.492$, $P = 0.045$), and visual memory ($r = 0.616$, $P = 0.009$), and also between reading accuracy and syntactic skills ($r = 0.657$, $P = 0.004$). No significant correlations emerged for the distal group ($r < 0.31$, in all cases). The predicted correlations between Reading Speed and Digit Span scores, which were highly significant for the control group ($r = 0.755$, $P = 0.03$), appeared to be negligible for both groups with Duchenne muscular dystrophy ($r < 0.3$ in all cases, for both speed and accuracy in reading).

Discussion

A number of findings suggest that rearrangements located in the second part of the dystrophin gene are more often associated with cognitive impairment than mutations in the proximal part. Indeed, distal macrodeletions in the dystrophin gene (altering Dp140 expression) are usually associated with cognitive impairment [16-18], and mutations involving the Dp71 region are often associated with severe cognitive impairment [13,15,36,31].

To investigate the possible relationship between mutations in the dystrophin gene affecting the Dp140 brain dystrophin isoform and specific cognitive profiles, 42 school-age children with a clinical and molecular diagnosis of Duchenne muscular dystrophy were first subdivided according to site of mutations, and then accurately characterized at the cognitive level through a battery of tests tapping a wide range of intellectual, linguistic, and neuropsychologic functions.

Intelligence quotients in the whole group were in general accordance with data reported in the literature, with an overall mean full-scale intelligence quotient approximately 1 S.D. below the population average, although the discrepancy between verbal intelligence quotient and performance intelligence quotient was more limited than that described in some previous studies [5,9,14]. Our control group of patients with spinal muscular atrophy and osteogenesis imperfecta was severely motor impaired but did not exhibit any cognitive deficits, thus confirming that motor impairment does not influence intellectual abilities, as already demonstrated by Billard et al. [10].

Separate analyses taking into account the genetic alterations in the dystrophin gene (Duchenne muscular dystrophy distal and Duchenne muscular dystrophy proximal) indicated that the verbal intelligence quotients in both groups were significantly lower than those of control children, whereas only children in the distally mutated Duchenne muscular dystrophy group showed significantly lower performance intelligence quotients.

Patients with distal mutations were generally more severely affected and manifested different patterns of strengths and impairments, in comparison to patients with proximal mutations.

In particular, distal mutations seem to produce greater deficits in verbal short-term memory, as expressed by low Digit Span scores (and possibly working memory, which may also be responsible for the low performances in the Picture Arrangement subtest), in visual memory, and in visuospatial organization, as expressed by lower scores on the Performance subtests of the Wechsler Intelligence Scale for Children-Revised, especially in Object Assembly, and also in logical sequencing (Picture Arrangement). On the other hand, patients with mutations in the proximal portion of the dystrophin gene demonstrated relative strengths in verbal short-term memory (as measured by the Digit Span subtest) and in the Performance subtests of the Wechsler scales, especially Object Assembly, Mazes, and Picture Arrangement (requiring visuospatial organization and planning), whereas they exhibited some difficulties in social judgment and the critical appreciation of general statements (as measured by the Comprehension subtest). Furthermore, dystrophic children with distal mutations manifested clear difficulties in syntactic processing, as expressed by both Token Test and Grammatical Comprehension scores. Finally, lower scores in Visual Memory were also an exclusive characteristic of patients with distal mutations, whereas deficits in Visual Attention were common to both subgroups.

Analyses controlling for the influence of general intellectual deficits on specific linguistic, neuropsychologic, and academic functions revealed that most of the deficits were substantially explained by variations in intelligence quotients. The only exceptions were difficulties in Syntactic Comprehension, which were characteristic of patients with Duchenne muscular dystrophy distal mutations, and deficits in Visual Attention among patients with proximal mutations.

Surprisingly, reading performances did not reflect the same pattern of differences. Children with distal and proximal mutations demonstrated very similar patterns and degrees of impairment in reading. Interesting differences, however, appeared in the patterns of correlations of reading skills with other cognitive and neuropsychologic functions. Children with distal mutations in the Duchenne muscular dystrophy gene exhibited positive associations between reading accuracy and long-term memory functions (in the Information subtest of the Wechsler Intelligence Scale for Children-Revised), as well as between reading speed and logical sequencing abilities (Picture Arrangement). Children with proximal mutations in the Duchenne muscular dystrophy gene, on the other hand, demonstrated associations between reading speed and lexical and phonologic competence, and with visual memory, whereas reading accuracy correlated with syntactic skills and some computational skills (working memory and auditory attention were excluded, because no associations were evident with their specific measures) measured by the Arithmetic subtest of the Wechsler Intelligence Scale for Children-Revised.

In dystrophic patients with distal mutations, deficits in academic ability seem to involve primarily verbal long-term memory, and these deficits seem to be relatively independent of their (severe) limitations in linguistic and visuospatial abilities.

The great amount of heterogeneity usually described for cognitive and intellectual functions in the population with Duchenne muscular dystrophy may thus be largely dependent on the two genetic and functional types being intermingled within groups.

In summary, apart from a general greater impairment in all cognitive functions for dystrophic patients with distal mutations, specific differences concern visuospatial functions and visual memory, which seem to be intact in proximally mutated patients,

and syntactic processing, which is impaired in both groups, but more severely in the distally mutated group.

Thus, the present data, obtained directly through a thorough and wide-ranging cognitive assessment (different from previous analyses based on academic achievement), support the hypothesis of a relationship between cognitive impairment and a lack of Dp140. In particular, the lack of Dp140 seems to produce specific deficits in visuospatial abilities, verbal and visual memory, and syntactic skills, whereas general verbal deficits are also evident in the presence of Dp140. The precise, differential effects of different mutation sites on the expression of dystrophin-related products in the brain remain to be clarified.

The expression of the dystrophin gene in the central nervous system is complex, and is characterized by alternate Duchenne muscular dystrophy mitochondrial RNA transcripts produced from different promoters that, together with tissue-specific alternate mitochondrial RNA splicing, produce a complex of dystrophin gene-related protein expression [37]. The developmental stage, distribution, and function of Duchenne muscular dystrophy gene products in the central nervous system, although not well characterized, are thought to be different for each isoform. We postulate that differences in neuropsychologic profiles among our patients are attributable to the number and type of brain-expressed isoforms affected. The brain-specific Dp140 is expressed mainly in fetal tissue and in low quantity in adult brain [38], and is suggested to play a role in the regulation of neuroglial-specific gene expression of the 5′ flanking region of genomic DNA adjacent to the Dp140 first exon, containing a variety of transcription factor-binding motifs [39]. On the other hand, the expression of Dp71 gradually increases from the embryonic to the adult stage. Dp71 becomes the major product of dystrophin in the brain, particularly in the hippocampus and some layers of the cerebral cortex. The function of Dp71 remains unknown [40,41], and it is mainly recovered in synaptic membranes, microsomes, and to a lesser extent, synaptic vesicles and mitochondria [42]. Studies of Dp71-deficient mice suggest a role of this brain isoform in the formation or stabilization of the dystrophin-associated complex [43] and in signaling complexes at glutaminergic synapses and in synaptic maturation and function [44].

The present data may shed some light on the great heterogeneity observed in cognitive functions of the population with Duchenne muscular dystrophy. As mentioned by Taylor et al. [36], however, even if the site of a mutation in the Duchenne muscular dystrophy gene constitutes an important determinant for the risk of cognitive impairment, the variability in cognitive deficits among children with Duchenne muscular dystrophy does not allow for a classification of the risk of cognitive disabilities based on structural features (deletions before or after a specific exon). In the present sample, both the lowest and highest full-scale intelligence quotients were observed in children with a mutation causing a lack of Dp140, but sparing the expression of Dp71. On the other hand, the second highest verbal intelligence quotient (i.e., 118) was observed in a child with mutations affecting both the Dp140 and Dp71 isoforms. Our results are not in complete agreement with those of Muntoni et al., who reported that all patients with a lack of Dp71 demonstrated severe cognitive deficits [45]. Furthermore, the clear impairments of both verbal and visual memory functions in dystrophic children with distal mutations (lacking Dp140 but not Dp71) suggest that Dp140, and not only Dp71, is related to hippocampal functions.

Our results suggest that the relationship between specific dystrophin isoforms and cognitive impairments is complex, and that the resultant deficits are not simply the sum of negative effects from either isoform. We also think it reasonable to presume that other elements beyond Dp140 and Dp71 must be considered, to understand the neuropsychologic effects of a lack of dystrophin in the brain.

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