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Analysis of Dp71 contribution in the severity of mental retardation through comparison of Duchenne and Becker patients differing by mutation consequences on Dp71 expression

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The presence of variable degrees of cognitive impairment, extending from severe mental retardation to specific deficits, in patients with dystrophinopathies is a well-recognized problem. However, molecular basis underlying mental retardation and its severity remain poorly understood and still a matter of debate. Here, we report one of the largest study based on the comparison of clinical, cognitive, molecular and expression data in a large cohort of 81 patients affected with Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) bearing mutations predicted to affect either all dystrophin products, including Dp71 or all dystrophin products, except Dp71. In addition to the consistent data defining molecular basis underlying mental retardation in DMD, we show that BMD patients with MR have mutations that significantly affect Dp71 expression or with mutations located in exons 75 and 76. We also show that mutations upstream to exon 62, with DMD phenotype, predicted to lead to a loss-of-function of all dystrophin products, except Dp71 isoform, are associated, predominantly, with normal or borderline cognitive performances. Altogether, these reliable phenotype–genotype correlations in combination with Dp71 mRNA and protein expression studies, strongly indicate that loss-of-function of all dystrophin products is systematically associated with severe form of MR, and Dp71 deficit is a factor that contributes in the severity of MR and may account for a shift of 2 SD downward of the intelligence quotient.

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INTRODUCTION

The presence of non-progressive cognitive impairment is now widely accepted and well recognized as a common feature in a substantial proportion of patients with Duchenne muscular dystrophy (DMD), a disorder that is caused by mutations in the dystrophin gene. Duchenne himself had already noted a 'character obtus' of many of the DMD children. More specific studies, such as the meta-analysis carried out by Emery documenting intelligence quotient (IQ) in a total of 721 DMD patients had also provided valuable information (1). This meta-analysis showed that the overall mean IQ was 82 (approximately one standard deviation below the population mean), 19% had an IQ lower than 70 (generally accepted cut-off point for a diagnosis of MR) and 3% had an IQ of less than 50 (severe MR). In this meta-analysis, a specific deficit in verbal rather than performance IQ, with a mean difference of seven or eight point was also highlighted. Weakness of the IQ with a tendency that verbal IQ (VIQ) is more affected than performance IQ (PIQ) was subsequently reported by Billard and coworkers (2–4). In patients with Becker muscular dystrophy (BMD), which is milder than DMD in terms of muscular disability but is caused by defects in the same gene, cognitive deficit seems to be less frequent and ~10% have an IQ lower than 70 (5,6).

Several lines of evidence suggest that the weakness in IQ is likely to be related to mutations in the dystrophin gene (1,7,8). With the identification of the dystrophin gene, our understanding of the molecular pathology of the muscular deficit spectrum has been greatly improved, but the aetiology of the intellectual deficit associated with DMD/BMD remains elusive. As 60–70% of patients with DMD and BMD have an intragenic deletion (9,10), there have been several genotype–phenotype studies, but no consistent correlation between type, site or extent of deletion and IQ has emerged (3,11,12). One might argue that these early studies did not take into account the complex genomic organization of the dystrophin gene and the diversity of isoforms produced either by alternative or internal promoters (7). In addition to the full-length dystrophin isoforms (427 kDa) that derive from three independent alternative promoters, named as muscle, brain and Purkinje promoters that regulate spatio-temporal dystrophin expression in muscle tissues, central nervous system (CNS) structures and cell types (13–15), the dystrophin gene has at least four internal promoters that give rise to shorter dystrophin products (Dp260, Dp140, Dp116 and Dp71) lacking the actin binding terminus but retaining the cystein-rich and C terminus domains. The most abundant dystrophin product in the brain is Dp71 (71 kDa product) that has its promoter region and specific first exon between exons 62 and 63 (7 kb upstream to exon 63) (16–18). Even though these findings are taken into consideration, it is still difficult to predict the consequences of defects either in the full-length dystrophin and/or in shorter isoforms on the development of cognitive functions. However, a number of findings have suggested that rearrangements located in the second part of the gene seem to be preferentially associated with cognitive impairment than mutations in the proximal part (7,11,12,19,20). In particular, a large study of 66 DMD patients comparing deletions involving the Dp140 isoform with deletions presumably not altering Dp140 expression revealed a significant association between distal macrodeletions and cognitive impairment (21). Potential implication of Dp140 in cognitive impairment was also highlighted in a study reported by Bardoni *et al*. (22), who showed a statistically significant correlation between the absence of Dp140 promoter and the presence of MR in BMD patients. However, in all these phenotype–genotype studies, no systematic correlation was found and only trends for association were pointed out. More recently, consistent correlation with MR has emerged for mutations located in Dp71 coding region. Indeed, several reports (8,19,23–25) described limited number of mutations that extend 3′ of exon 63 and almost all of them highlighted the presence of severe mental retardation. However, to our knowledge, no large studies comparing consequences on cognitive functions of mutations differing exclusively by their consequences of Dp71 expression have been reported. Here we present clinical, cognitive, molecular and expression data on a cohort of 81 DMD/BMD with mutations predicted to affect either all dystrophin products including Dp71 or all dystrophin products except Dp71. Interestingly, phenotype/genotype correlations provide convincing data, indicating that severe MR results from loss-of-function of all dystrophin products, and Dp71 deficit appears as a worsening factor of cognitive impairment, which contributes in the severity of MR and accounts for the equivalent of 2SD downward shift of the IQ.

RESULTS

In order to assess the potential contribution of Dp71, in the pathogeny of the severity of cognitive impairment frequently observed in dystrophinopathies, we undertook a retrospective comparative study in two large groups of patients differing by mutations consequences on Dp71 expression and function. The two groups of patients were defined on the basis of molecular abnormalities in the DMD gene. In the first group of patient, (Group I) mutations in the DMD gene are predicted to affect all dystrophin products, including Dp71, and in the second group (Group II), mutations in the *DMD* gene are predicted to affect all dystrophin products except Dp71 (Table 1).

Molecular and neuropsychological data in BMD and DMD patients with mutations predicted to affect all dystrophin products (BMD and BMD patients of Group I)

The first group corresponds to a cohort of 54 patients, 42 DMD and 12 BMD, with molecular abnormalities in the *DMD* gene extending to, or located in the genomic region corresponding to Dp71 isoform (Table 1). For some of these patients (indicated in Table 1 by asterisks), we have previously reported clinical phenotypes and molecular data (19,24,26). In this study, we expanded the cohort of patients and discussed potential correlations between DMD mutations and cognitive deficit.

For BMD patients described in this study, and in accordance with the clinical phenotype, analysis of available muscle biopsies (deltoid or quadriceps) of the 12 Becker-type patients

Table 1. Clinical, molecular and neuropsycological features of DMD and BMD patients included in this study

Patient ID/ age at last examination	Phenotypic group	Age at loss of ambulation	Dystrophin protein/dystrophin mRNA data	Mutation in the *DMD* gene and relative exonic position	Age at evaluation of intellectual performances	IQ/clinical evaluation of intellectual performance	Mutation origin and comments
BMD patients of Group I with normal cognitive development							
4620/26*	BMD	19	Slightly reduced amount with normal MW. One mRNA isoform: c.10102G>C	c.10102G>C Asp3368His exon 70	Clinical follow-up and schooling performances	Performances at high school and university within average	Sporadic case, carrier mother
6534/31*	Cramps and myalgia	Still ambulant	Reduced amount with an apparent normal MW. Two mRNA isoforms: mRNA with c.10231dupA and in frame mRNA lacking exon 71	c.10231dupA exon 71	Clinical follow-up and schooling performances	Performances at high school and university within average	Familial, affected uncle
1932/19*	BMD	Still ambulant	Reduced amount with an apparent normal MW. Two mRNA isoforms: c.10225delCCCGT, in frame mRNA lacking exon 71	10225_10229 delCCCGT exon 71	Clinical follow-up and schooling performances	Performances at high school and university within average	Familial, affected grand father and uncle
4295/26*	BMD	Still ambulant	Reduced amount with abnormal MW. Two mRNA isoforms: c.10279C>T, in frame mRNA lacking exon 72	c.10279C>T exon 72	Clinical follow-up and schooling performances	Performances at high school and university within average	Sporadic case, carrier mother
R1181/32	BMD	18	Reduced amount with abnormal MW. Two mRNA isoforms: c.10620T>A, in frame mRNA lacking exon 74	c.10620T>A exon 74	Clinical follow-up and schooling performances	Performances at high school and university within average	Sporadic case, carrier mother
6847/39*	BMD	21	Reduced amount with abnormal MW. Two mRNA isoforms: c.10454dupC in exon 74, in frame mRNA lacking exon 74	c.10454insC exon 74	Clinical follow-up and schooling performances	Performances at high school and university within average	Sporadic case, carrier mother
1038/30*	BMD	26	Reduced amount with abnormal MW. Two mRNA isoforms: c;10498delAG, in frame mRNA lacking exon 74	c.10498_10499delAG; exon 74	Clinical follow-up and schooling performances	Performances at high school and university within average	*De novo*
BMD patients of Group I with cognitive impairment							
5539/28	BMD	19	Reduced amount with abnomal MW. Two mRNA isoforms: in frame full-length mRNA, out of frame mRNA with an insertion of a cryptic exon of 67 nt (intron 62)	c.9225-647A>G exon 63	21	WISCR, IQ45	Sporadic case, carrier mother
5945/16	BMD	Still ambulant	Decreased amount with normal MW. Two mRNA isoforms: in frame full-length mRNA, out of frame mRNA with an insertion of a cryptic exon of 207 nt (intron 62)	c.9225-160A>G exon 63	Clinical follow-up and schooling performances	Neuro-developmental and schooling delay compatible with mild MR	Sporadic case, carrier mother

Continued

Table 1. *Continued*

Patient ID/ age at last examination	Phenotypic group	Age at loss of ambulation	Dystrophin protein/dystrophin mRNA data	Mutation in the *DMD* gene and relative exonic position	Age at evaluation of intellectual performances	IQ/clinical evaluation of intellectual performance	Mutation origin and comments
2205/17	BMD	Still ambulant	DYS1 antibody: decreased amount with abnormal MW, DYS2 antibody: dystrophin not detected mRNA: out of frame mRNA isoform lacking exons 75 and 76	c.10554-10921del out of frame deletion of exons 75 and 76	4 Years and 6 months	Mc Carthy, IQ 67, mild mental retardation	Sporadic case, carrier mother
7434/32	BMD	Still ambulant	DYS1antibody: decreased amount with abnormal MW, Dys2 antibody: protein not detected; mRNA: detection of only one out of frame mRNA with an insertion of an intronic 17 nt upstream to exon 75 (stop codon in exon 75 at c.10580)	c.10554-18C>G IVS74-18C>G	31	WISC-R, IQ 65	Familial, one uncle with BMD and severe MR
6651/9*	BMD	High CK, very mild muscle weakness	DYS1 antibody: decreased amount with abnormal MW, DYS2 antibody: dystrophin not detected; One mRNA isoform with C>A mutation: c.10910C>A; S3637X	c.10910C>A exon 76	7	WISC-III, IQ 70, mild mental retardation	Familial, a second affected brothers: DQ = 56 at the age of 2 years 8 months, and 47 at the age 3 years and 5 months with behavioural problems
DMD patients of Group I							
L9605/14	DMD	10	Absence of dystrophin	Out of frame deletion of exons 45–79	9	WISC-III, IQ 45	ND
6509/10	DMD	10	Not analysed	Out of frame deletion of exons 55–63	7	WPPSI tests, IQ 51	Familial, 13 years affected brother with significant neurodevelopmental
D118/14	DMD	8	Absence of dystrophin	In frame deletion of exon 64 (c.9287-9361)	7	WPPSI-R, IQ < 45	Familial
00349/11	DMD	9	Not analysed	c.9333delA exon 64	6	WPPSI-R, IQ 47	Sporadic case, status of the mother: not investigated
3475/19*	DMD	14	Absence of dystrophin	c.9319insAA exon 64	12	WISC-III, IQ 46	Familial
2297/17	DMD	12	Absence of dystrophin	c.9337C>T exon 64	15	Vinland, IQ 47	Sporadic case, carrier mother
D181/11	DMD	Severe muscle weakness	Not analysed	9445C>T exon 65	8	IQ < 50	Carrier mother
D93/20	DMD	8	Absence of dystrophin	c.9568C>T exon 66	8.5	WISC-R, IQ 52	*De novo*
0944/29*	DMD	Severe form (did not walk)	Absent	c. 9649+1G>A IVS66+1G>A	17	WISC-R, IQ 47	Familial, one affected brother with severe language and learning impairment (died at the age of 16)
7898/13*	DMD	7	Absence of dystrophin	c.9974+1G>A IVS68+1G>T	9	WISC-R, IQ 54	Familial
T1056/23**	DMD	12	Absence of dystrophin	c.9568C>T exon 66	11	WISC-III, IQ 46	Familial: one affected brother. Severe neurodevelopmental delay and behavioural difficulties
T1309/10	Severe muscle weakness (DMD)	Very limited ambulation	Absent	c.9958 DelC exon 68	4 ans	Brunet-Lézine, DQ35 behavioural difficulties	Familial, one affected brother. WPPSI-R tests at the age of 7: IQ 40, (with behavioural difficulties)
2893/17	DMD	10	Absence of dystrophin one mRNA isoform: c.10033C>T	c.10033C>T exon 69	7	WISC-III, IQ 50	Familial

D264/12	DMD	12	Decreased amount with normal MW	c.10227G>A 3340Cys>Arg exon 69	10	WISC-III, IQ 52 behavioural difficulties	ND
D80/18	DMD	9	Absence of dystrophin	c.10294+5G>C exon 69	6.4	WPPSI, IQ 55	*De novo*
T-53/25**	DMD	<14	Not analysed	c.10033C>T exon 69	5, 10 and 15	Borel Maisony at 5: IQ 50 Columbia at 10: IQ 48 WISCIII at 15, IQ 46	ND
T70/20**	DMD	<14	Not analysed	c.10171 C>T exon 70	16	WISC-R, IQ 45	ND
LD115/20	DMD	11 ans	Absence of dystrophin	c.10171C>T exon 70	15	WAIS, IQ 46	Sporadic case, carrier mother
D174/12	DMD	9	Absence of dystrophin	c.10171C>T exon 70	11	WISC-III, IQ 51	Familial
4062/17*	DMD	12	Absence of dystrophin; out of frame mRNA lacking exon 70	c.10223+1G>A IVS70+1G>A	10	WISC-III, IQ 70	Familial, 7-year-old affected nephew with muscle weakness and severe cognitive deficit (DQ < 50)
S2895/17	DMD	8	Not analysed	c.10223+1G>A IVS70+1G>A	15	WISC-III, IQ 50	ND
1511/17	DMD	12	Absence of dystrophin; two mRNA isoforms: traces of full-length mRNA, out of frame mRNA lacking exon 70	c.10223+1G>A IVS70+1G>A	7	WISC-III, IQ 45	Familial
T2210/15	DMD	9	Absent	Out frame deletion of exon 70	12	WPPSI, IQ 55	Sporadic case, carrier mother
4248/10	Severe muscle weakness (DMD)	Still ambulant at the age of 6	Absent	c.10223+1G>A IVS70+1G>A	6	Terman, Columbia and WPPSI-R, IQ 57	Sporadic case, carrier mother
5740/16*	DMD	12	Not analysed	c.10135 A>T exon 70	11 years	KABC, IQ 47	Sporadic case, status of the mother: not investigated
DMD and BMD patients of Group II							
3541/49	BMD	Still ambulant	Not analysed	c.6913-8937del in frame deletion of exon 48–59	46	81	ND
196/22	DMD	8	Not analysed	c.6913_9163del out of frame deletion of exons 48–61	7	WPPSI, IQ 70	ND
0710/20	DMD	12	Not analysed	c.6291_9224del out of frame deletion of exons 45–62	7	WPPSI, IQ 77	Sporadic case, carrier mother
214/15	DMD	8	Absent	c.7310_9085del out of frame deletion of exons 51–60	8	WISC-III, IQ 80	*De novo*
4114/15	DMD	14	Absent	7543_9163del out of frame deletion of exons 52- 61	13	WISC-III, IQ 52	Familial
4466/15	DMD	11	Traces with abnormal MW	7543-9224del out of frame deletion of exons 55–59	12	WISC-III, IQ 92	ND
418/22	DMD	9	Absent	c.8028_8937del out o.d. frame deletion of exons 55–59	13	WISC-III, IQ 73	Familial
0311/20	DMD	12	Not analysed	8218_9224dup out of frame deletion of exons 56–62	15	WAIS, IQ 78	Familial
1736/19	DMD	10	Absent	c.8218-8390del out of frame deletion of exon 59	8 and 14	Columbia tests at the age of 8, IQ 78; WISC tests at the age of 14, IQ 72	ND
5937/13	DMD	12	Absent	c.8628G>A exon 57	7	WPPSI tests, IQ 62	Sporadic case, carrier mother

Continued

Table 1. *Continued*

Patient ID/ age at last examination	Phenotypic group	Age at loss of ambulation	Dystrophin protein/dystrophin mRNA data	Mutation in the *DMD* gene and relative exonic position	Age at evaluation of intellectual performances	IQ/clinical evaluation of intellectual performance	Mutation origin and comments
D235/15	DMD	12	Absent	c.8669-8937del out of frame deletion of exon 59	9	WISC-III, IQ 69	ND
1600/19*	DMD	12	Traces with abnormal MW	c.9084+80326G>T IVS60 + 80326G>T	12 and 16	Terman Merill at 12, IQ 55 WAIS, IQ 52	Familial, 24 years affected brother with an IQ of 50
1953-01/18	DMD	9	Not analysed	c.9085-9163del out of frame deletion of exon 61	12	WISC-III, IQ 80	Familial
8179/13	DMD	11	Absent	c.9104insGACCGAG exon 61	11	WISC-III, IQ 89	Sporadic case, status of the mother: not investigated
LD273/10	DMD	Severe muscle weakness	Absent	c.9148C>T exon 61	8	WISC-III, IQ 73	Sporadic case, carrier mother
1478/20	DMD	12	Absent	c.9216C>A exon 62	12	WISC-III, QI>80	Familial
T911/18	DMD	9	Not analysed	c.8938_9224 out of frame duplication of exons 60–62	11 years 1/2	WISC-III, IQ 55	Familial
Cognitive performances and adaptive skills evaluated through clinical follow-up and schooling performances BMD and DMD patients of Group II							
7025/55	BMD	Still ambulant	Reduced amount with abnormal MW	c.6913-8937del in frame deletion of exons 48–59	Clinical follow-up and schooling performances	Absence of significant delay (no MR)	ND
7958/14	DMD	11	Not analysed	Out of frame duplication of exons 50–62	12	Absence of significant delay, able to read and write	ND
D201/70	BMD	Ambulant at adult age	Not analysed	c.6913-8547del in frame deletion of exons 48–57	Clinical follow-up and schooling performances	Absence of significant delay (no MR)	ND
4049/20	DMD	12	Not analysed	c.6291-9084del out of frame deletion of exons 44–60	Clinical follow-up and schooling performances	Absence of significant delay (no MR)	ND
2989/23	DMD	9	Not analysed	c.8028-9163del out of frame deletion of exons 55–61	Clinical follow-up and schooling performances	Language acquisition and learning difficulties compatible with mild MR	ND
0435/22	DMD	12	Not analysed	c.8218_9225dup out of frame duplication of exons 56–62	Clinical follow-up and schooling performances	Language acquisition and learning difficulties compatible with mild MR	Familial
6333/10*	DMD	9	Absent	c.8944C>T exon 60	Clinical follow-up and schooling performances	Absence of significant delay (no MR)	Sporadic case, carrier mother

4298/14	DMD	9	Traces with abnormal MW	c.9164-9224del out of frame deletion of exon 62	Clinical follow-up	Absence of significant delay (no MR)	Familial, 9 years brother, normal neuro-cognitive development and schooling
1463/17*	DMD	11	Absent	c.9164-1G>A exon 62	Clinical follow-up and schooling performances	Language acquisition and learning difficulties compatible mild MR	*De novo*
DJ01/19	DMD	12	Not analysed	del exons 60–63	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	ND
DMD patients of Group I							
4119/ 19	DMD	8	Not analysed	c.7873_11014del out frame deletion of exon 55–77	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR and autistic behaviour	Familial, carrier mother
8065/9	Limited ambulation	Severe muscle weakness	Absent	c.9328-9337 ATGAAACTCCdel exon 64	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	Sporadic case, status of the mother: not investigated
1913/20*	DMD		Traces of dystrophin with of WB/ two mRNA isoforms: ARNms: minor full-length mRNA, and out of frame mRNA with an insertion of a 147 nt pseudo-exon between exons 65 and 66	c.9362-1215A>G IVS65-1215A>G	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	Familial
2753/17	DMD	14	Traces of normal WB Dys1 and Dys2)/two mRNA isoforms: ARNms: minor full-length mRNA, and out of frame mRNA with an insertion of a 147 nt pseudo-exon between exons 65 and 66	c.9362-1215A>G IVS65-1215A>G	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	ND
1585/22*	DMD	10	Absent	c.9568C>T exon 66	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	Familial
1056/22**	DMD	12	Not analysed	c.9568C>T exon 66	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	Familial, one DMD affected brother with severe MR
4205/15*	DMD	10	Absent	c. 9649+1G>A IVS66+1G>A	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	Familial, 12 years affected brother with learning and language difficulties

Continued

Table 1. *Continued*

Patient ID/ age at last examination	Phenotypic group	Age at loss of ambulation	Dystrophin protein/dystrophin mRNA data	Mutation in the *DMD* gene and relative exonic position	Age at evaluation of intellectual performances	IQ/clinical evaluation of intellectual performance	Mutation origin and comments
6248/21*	DMD	12	Not analysed	c.9691C>T exon 67	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	Familial
1015/20*	DMD	10	Absent	c.10086+1G>T IVS69+1G>T	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR and behaviour difficulties	Familial
0955/25	DMD	12	Not analysed	c.10171C>T exon 70	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	ND
LD205/20	DMD	8	Absent	c.10171 C>T, R3391X (exon 70)	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with sever MR and autistic behaviour	Familial
3466/12*	DMD	8	Absent	10108C>T exon 70	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR and autistic behaviour	Familial
T2612/7	Severe muscle weakness (DMD)	Very limited ambulation	Absent with (Dys 1, 2 and 3)	c.10223+1 G>A exon 70	Clinical follow-up	Significant neurodevelopmental and schooling delay compatible with severe MR (behavioural difficulties)	Familial, two affected maternal uncles (died at the age of 12 and 16)
0994/27*	DMD	12	Absent	10171C>T exon 70	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR	Familial, two affected brothers with learning and language impairment (difficulties)
3804/12	DMD	Severe form (did not walk)	Absent	c.10223+3G>C IVS70+3G>C	Clinical follow-up and schooling performances	Adaptive and intellectual performances compatible with severe MR and psychotic behaviour	Familial
4062/7	Severe muscle weakness DMD)	Very limited ambulation	Absence of dystrophin	c.10223+1 G>A exon 70	Clinical follow-up and schooling performances	Significant neurodevelopmental and schooling delay compatible with severe MR	ND

Patients reported in this retrospective study were collected through a network involving eight centres. For some of these patients (indicated by *), molecular data and muscular phenotypes have been reported and discussed by Deburgrave *et al*. (26). Also, for patients indicated by **, molecular and neuropsychological have been reported by Moizard *et al*. (19,24).

showed the presence of significant residual amount of dystrophin with either an apparently normal or reduced molecular weight (Table 1). Besides the patient with a missense mutation (patient 4620, p.Asp3368His), mutations in BMD patients correspond to nonsense (patients 4295, R1181 and 6651, Table 1), frameshift (patients 6534, 1932, 6847, 1038 and 2205, Table 1) or splice mutations (patients 5539, 5945 and 7434, Table 1). Molecular mechanisms underlying dystrophin products expression were uncovered through RT–PCR analyses using muscle total RNA. With the exception of three patients described below, these analyses showed the presence of at least two mRNA species: one non-functional mRNA species resulting from aberrant splicing events, or bearing nonsense (or frameshift) mutations and one functional in-frame mRNA species, resulting from normal (for splice mutations) or alternative splicing and/or skipping of the mutated exon (Table 1). In the remaining three BMD patients with either nonsense (patient 6651, c.10910C>A), splice mutation (patient 7434, c.10554-18C>G), or deletion of exons 75 and 76 (patient 2205, c.10554-10921del), no dystrophin labelling was seen with Dys-2 antibody directed against the most C-terminal amino acids of the protein, while labelling with the antibody specific to the N-terminal domain revealed the presence of a significant amount of dystrophin with a slightly reduced molecular weight (data not shown). Also, for these three patients, dystrophin mRNA analysis by RT–PCR, using total RNA extracted from muscle biopsy, did not reveal any alternative splicing events in the region of the mutated exons (data not shown). Thus the detected dystrophin protein is likely to correspond to 'functional' dystrophin lacking amino acids downstream to the nonsense, frameshift or deleted exons.

As shown in Table 1, cognitive development and adaptive skills evaluated either through clinical follow-up and school performances, or intelligence testing revealed that seven of the 12 BMD patients performed in the normal range, whereas five BMD patients have a significantly reduced IQ ranging from 45 to 70 and are therefore classified as mildly (four patients) to severely (one patient) mentally retarded. Interestingly, mutations detected in these patients correspond to the two cryptic splice mutations in intron 62 (patients 5539 and 5945, Table 1) and to the mutations described above and that led to an undetectable dystrophin with Dys-2 antibody (patients 2205, 7534, 6551, Table 1).

In order to further assess the consequences of mutations detected in BMD patients on Dp71 expression and provide insights into the understanding of the pathogeny of cognitive impairment, we investigated the consequences of some mutations on Dp71 transcripts and protein expression. As Dp71 is known to be expressed in lymphoblasts and fibroblasts, and not in muscle, we used available patients' lymphoblasts and fibroblasts to assess Dp71 expression. Needless to say that these cells are far from being optimal to address the relation between Dp71 expression and cognitive function. However, in the absence of appropriate biological resources, we thought it is reasonable to use these cells to assess mutations consequences on Dp71 expression. Thus, we analysed Dp71 expression by RT–PCR and western blot in available cells corresponding to four BMD patients: two patients with intellectual skills within the normal range and two patients with low IQ. For RT–PCR, we used forward primers located in the specific first exon of Dp71 and reverse primers located either in exon 63 or in exons downstream to this one. For western blot analysis, we used the Dys2 monoclonal antibody (directed against amino acids encoded by exon 78). As one can predict, in the first two BMD (patients 4295 and 6847), we detected significant level of in frame transcripts and protein corresponding to Dp71 (data not shown). However, in the two BMD patients with a low IQ (patient 5539: c.9225-647A>G, IQ 45; patient 7434, c.10554-18C>G, IQ 65) and who are classified as mentally retarded, no potential functional Dp71 transcripts was amplified by RT–PCR and only transcripts with premature stop codons were detected (Fig. 1A and B). In the BMD patient with the c.9225-647A>G splice mutation (patient 5539), RT–PCR using a forward primer in Dp71 specific first exon and reverse primer in exon 63 amplified only the fragment corresponding to the abnormal out of frame transcript with an insertion of the 67 nt cryptic exon (Fig. 1A). In accordance with this result, no Dp71 protein was detected by western blot analysis using the patient's lymphoblasts protein extract (Fig. 1C). This result contrasts with the detection in the patient's muscle biopsy of residual amount of normal dystrophin transcript and protein (data not shown).

In the second BMD patients with mild mental retardation (patient 7434, Table 1) and splice mutation: c.10554–18C>G, RT–PCR experiments using RNA extracted from lymphoblasts detected only an out-of-frame mRNA with an insertion of an intronic sequence corresponding to the 17 nt upstream to the acceptor splice site of exon 75 (Fig. 1B). At the protein level (Fig. 1C), as expected no protein was detected with Dys2 antibody. However, because of the lack of sensitive antibodies directed against the N-terminal part of Dp71, it was not possible to assess whether the mutation results in a truncated Dp71 or an absence of Dp71.

For the three other BMD patients exhibiting a low IQ (patients 2205, 6651 and 5945, Table 1), analysis of Dp71 expression was not carried out because neither fibroblasts nor lymphoblasts were available. However, on the basis of the results detailed above, and positions of the mutations detected in these patients, the following effects could be predicted: (i) Dp71 lacking amino acids corresponding to exons 75–79 (patient 2205 with an out of frame deletion of exons 75 and 76) or 76–79 (patient 6651 with stop codon mutation in exon 76), (ii) out of frame Dp71 transcript resulting from the activation of cryptic-exon located in the vicinity of exon 63 (patient 5945, Table 1).

In addition to BMD patients, Group I includes also 42 DMD patients with mutations predicted to affect all dystrophin products. Mutations detected in these patients include 13 splice mutations, 19 nonsense mutations, four frameshift mutations, three out of frame and one distal deletion (exons 45–79), one in frame deletion encompassing exon 64 and one missense mutation (Table 1). Though at first sight mutations appear to be distributed throughout the distal part of the *DMD* gene, it is worth mentioning the relative high frequency of mutations (17/42) in exon 70 and their flanking splice sites.

Among these 42 DMD patients, cognitive tests were carried out and mean IQ values were available for 25 patients

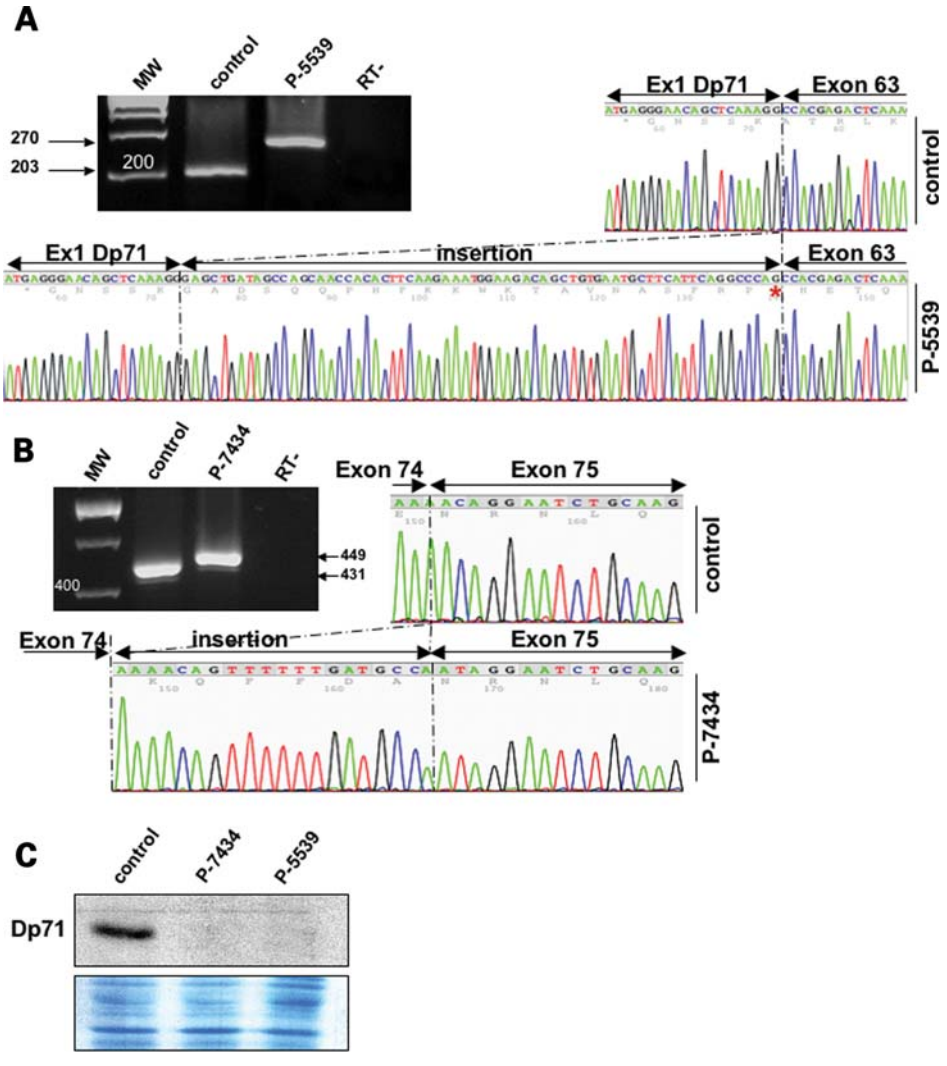

Figure 1. Dp71 mRNA analysis in two BMD patients of Group I with splice mutations in exons 63 and 75 and with cognitive impairment. (**A**) BMD patient 5539 (c.9225-647A>G). Sizing and sequencing analysis of RT–PCR amplified cDNA covering Dp71 specific first exon and exon 63 using lymphoblasts total RNA and showing the presence of a unique out of frame transcript with a cryptic exon corresponding to an intronic 67 nt insertion. (**B**) RT–PCR amplified cDNA fragment covering exons 74–75 showing the presence, in control sample, of the normal transcript (control, 431 nt) and the presence in the patient of a unique transcript with intronic 17 nt insertion (leading to a premature stop codon in exon 75 at c.10580) (P-7434, 449 nt). The partial sequence of Dp71 cDNAs showing the insertion of an intronic 17 nt upstream to exon 75. (**C**) Western blot analysis of Dp71 using Dy2 antibody and protein extracts of lymphoblasts of BMD patients 7434 and 5539 and of a control individual.

(Tables 1 and 2), while for the remaining 17 DMD patients, the level of cognitive development and intellectual performance was estimated through clinical follow-up and school performances. As far as cognitive and intellectual function are concerned, all the 25 tested patients, have a IQ in the range of 35–55, except the patient 4062 bearing the c.10223+1G>A mutation (Table 1). For this patient, the IQ was significantly reduced, but not in the range of those with severe MR and WISC-III tests at the age of 10 years revealed an IQ of 70. Interestingly, this latter patient has 7 years old affected nephew bearing the same mutation associated with severe language impairment, learning delay and an IQ below 50. For the remaining 17 DMD patients, clinical evaluation of cognitive performance, adaptive skills and school performances revealed severe neurodevelopmental and learning delay compatible with severe MR (Table 1).

Analysis of mutations consequences on Dp71 transcripts and protein expression in lymphoblasts or fibroblasts was performed in nine DMD patients of this group (patients 2297, 2893, 4062, 2895, 4248, 5740, 1585, 0994, 1585; Table 1). As predicted, RT–PCR experiments showed the presence of only non-functional transcripts: out of frame transcripts resulting from splicing events or premature stop codon (data not shown). In line with these RT–PCR results, no residual expression of Dp71 protein was detected by western blot in cells of these patients when compared with cells of control individuals, including of DMD patient with stop mutation in the exon 16 (see examples in Fig. 2).

Table 2. IQ distribution in tested DMD patients of Group I (mutations predicted to affect all dystrophin products) and Group II (mutations predicted to affect all dystrophin product, except Dp71)

Range of full-scale IQ	Number of patients Group I (25)	Group II (16)
IQ ≥ 80	0	5
70 < IQ < 80	1 (IQ 70)[a]	6
50 < IQ < 70	8	5
IQ ≤ 50	16	0

[a]Familial case of Group I with an IQ of 70 and who has a 7-year-old affected nephew with severe MR (IQ < 50).

Molecular and neuropsychological data in DMD and BMD patients with mutations predicted to affect all dystrophin products, except Dp71 (DMD and BMD patients of Group II)

This group (Group II) corresponds to a cohort of 27 patients, 24 DMD and 3 BMD, with molecular abnormalities in the *DMD* gene extending to, or located in the genomic region corresponding to exons 56–62. In these patients, and if we consider that their mutations do not effect regulatory elements of Dp71 expression, mutations are predicted to affect all dystrophin products, except Dp71. The three BMD patients have a very mild form of muscular dystrophy and their mutations correspond to in frame deletions encompassing exons 48–59 (patients 3541 and 7025, c.6913-8937del) or exons 48–57 (patient D201, c.6913-8547del). For the patient 3541, the IQ was of 81, and for the two other patients, schooling performances were within similar ranges of children of the same age.

For the 24 DMD patients of this group, identified mutations include 14 out of frame deletions that encompass exons corresponding to Dp116 isoform, three out of frame duplications with a 5′ end located downstream to exon 55, four nonsense mutations, two splice mutations and one frameshift mutation predicted to affect all dystrophin products, except Dp71 expression (Table 1). Among these 24 DMD patients, cognitive evaluations were carried out and IQ values were available for 16 patients (Tables 1 and 2). For the remaining eight DMD patients, the cognitive impairment was estimated through clinical follow-up and school performances (Table 1). Distribution of the IQ in tested DMD patients of Group II shows that most patients of this group have either an IQ within the normal (>80) (5/16, 31%) or borderline range (between 70 and 80) (6/16, 37%). Five of 16 (31%) presented an IQ within the range of mild mental retardation (between 50 and 70). For this group of DMD patients, mutation consequence on Dp71 expression was analysed in lymphocytes or fibroblasts of three DMD patients (3541, 1736, 7958; Table 1), and as illustrated in Figure 2 (patient 7958), a significant amount of Dp71 protein was detected.

DISCUSSION

In order to assess Dp71 contribution in cognitive deficit, we combined in this large study clinical, cognitive, molecular and protein data from two groups of patients with mutations predicted to have different consequences on Dp71 expression.

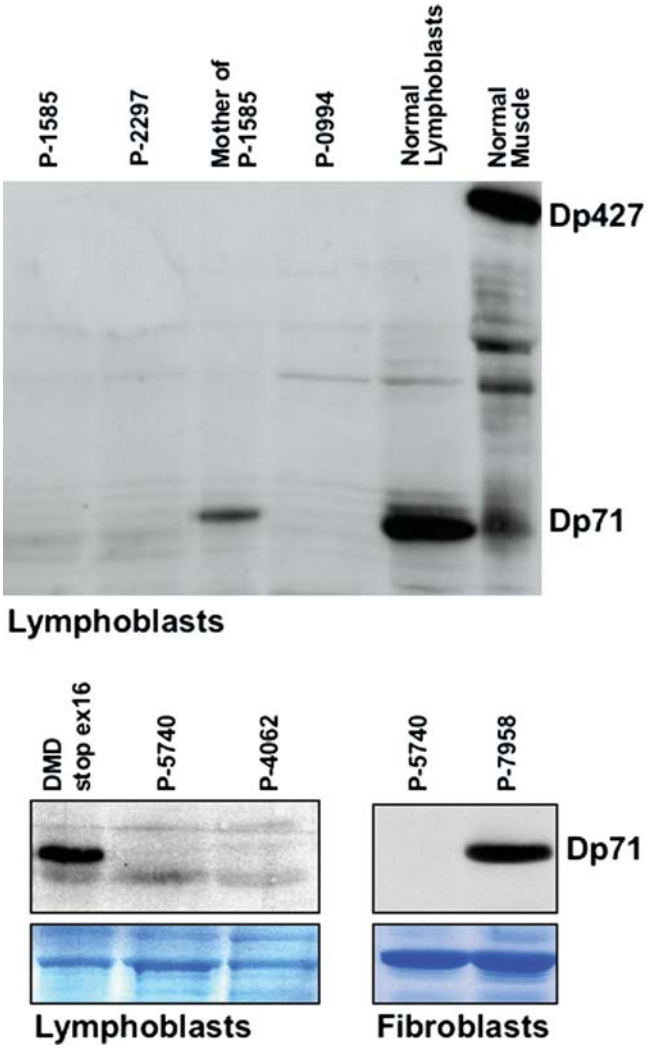

Figure 2. Western blot analysis of Dp71 protein (using Dy2 antibody) in fibroblasts and lymphoblasts of DMD patients of Group I (patients 1585, 2297, 0994, 5740, 4062) and Group II (patient 7958). Normal lymphoblasts and muscle from non-affected individuals, as well as lymphoblasts of DMD patients with nonsense mutation in exon 16 and a carrier mother (of patient 1585) were used as controls.

We found that all 42 DMD patients with mutations extending to or located in the region of Dp71 and that are predicted to affect Dp71 expression are mentally retarded, whereas the majority of patients with mutations disrupting all dystrophin products, except Dp71, has either normal or borderline cognitive performances. If we take into account patients for whom IQ was scored by neuro-psychometric tests, comparison of IQ distribution between DMD patients of Groups I and II indicates clearly that MR (IQ<70) is significantly more frequent in Group I than in Group II: Fisher's test, $P < 0.002$; 24/25 and 5/16 patients for Groups I and II, respectively, and severe MR (IQ < 50) appears to be present only in DMD patients of Group I (Tables 1 and 2).

This finding led us to question if Dp71 deficit is sufficient to affect cognitive development and induce MR or if cognitive dysfunction is linked to the cumulative deficit of all dystrophin products, especially Dp427, Dp140 and Dp71, known to be

Table 3. IQ distribution in DMD patients with mutations predicted to affect or preserve Dp140 expression

Range of IQ	Patients with mutations in exons 45–55 (predicted to affect Dp140 expression)	Patients with mutations upstream to exons 44 (or in exon 44) (predicted to preserve Dp140 expression)
IQ ≥ 80	14	22
70 < IQ < 80	9	15
50 < IQ < 70	14	6
IQ ≤ 50	2	1
Total	39	44

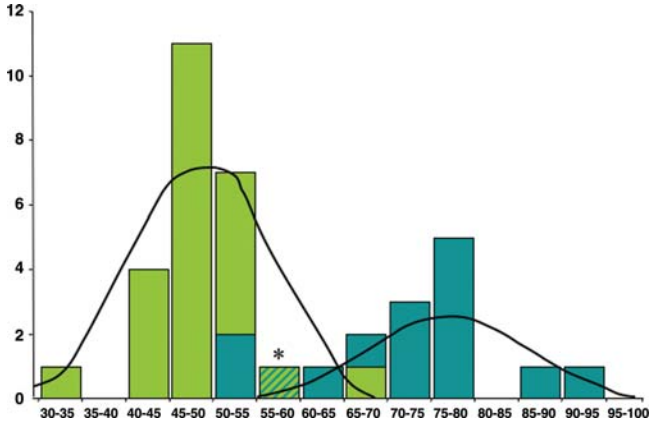

Figure 3. Distribution of IQ in DMD patients. Histograms represent the number of DMD patients of Group I (green) and Group II (blue) at each IQ level (IQ intervals of five). The hatched histogram highlighted by an asterisk indicates that within this range of IQ there is one patient of each group.

expressed in brain. To address this question, we screened the specific first exon of Dp71 in 320 mentally retarded probands (with no muscular deficit) of the European Consortium's cohort of X-linked mental retardation families, and only non-pathogenic changes were detected in this cohort of patients. Though very rare mutations in the first exon and promoter region of Dp71 can not be excluded, this result strongly suggests that severe mental retardation in DMD patients with mutations located in Dp71 region is likely to result from the cumulative effects of inactivation of Dp71 and other *DMD* gene products expressed in brain structures during ontogenesis and adult stages. Among these products, Dp140 had previously been suggested to contribute to mental retardation (21,22). However, as the study described here is focused on Dp71 and all patients included in this study bear mutations predicted to alter Dp140 expression, the contribution Dp140 in cognitive impairment cannot be evaluated. To overcome this limitation, we reviewed clinical and molecular data concerning patients referred to our diagnostic laboratory, and undertook a comparative study in two additional large groups of patients differing by mutations consequences on Dp140 expression (details concerning these patients are available in: http://www.umd.be/DMD/). Mutations in the first group of patients (84 BMD and 39 DMD) are located in (or extend to) the genomic region corresponding to exons 45–55 of the *DMD* gene and predicted to affect Dp427, Dp260 and Dp140, but not Dp116 and Dp71. In the second group (33 BMD and 44 DMD), mutations in the *DMD* gene are located upstream (or extending) to exon 44 and predicted to affect only Dp427 and Dp260 expression. For BMD patients of both groups, our data suggest that BMD mutations, including those located in the region of exons 45–55 (which affect Dp140 expression), do not have significant consequences of cognitive development in BMD patients. For DMD patients, neuropsychometric evaluation with IQ scores was collected for a total of 83 patients: 39 patients with mutations in the region of exons 45–55 of *DMD* gene and 44 patients with mutations located within or upstream to exon 44). Table 3 highlights IQ distribution in tested DMD patients with mutations predicted to affect or to preserve Dp140 expression. As shown in this table, though no systematic correlation between positions of mutations and occurrence of MR, comparison of IQ distribution between DMD patients with mutations predicted to have different consequences on Dp140 expression indicates that mild MR ($50 < IQ < 70$) is significantly more frequent in patients with mutations located in Dp140 regions (14/39 versus 6/44; χ^2 test $P < 0.02$), and severe MR ($IQ < 50$) is very rare in both groups (two DMD patients with mutations in Dp140 region and one patient with mutation upstream to exon 44). Interestingly, distribution of IQ in DMD patients with mutation in the region of exons 45–55 leading to loss-of-function of Dp140 is similar to the one observed in DMD patients with mutations predicted to affect all DMD products except Dp71 (DMD patients of Group II, Table 2). Though mutations leading to loss-of-function of Dp427 and Dp140 do not systematically result in cognitive deficit, these data as well as those reported in the literature (21,22) are consistent with a contribution of Dp140 deficit in the pathogeny of mild cognitive impairment.

The systematic occurrence of mild to severe mental retardation in DMD patients of Group I contrasts with the heterogeneous cognitive profiles observed in the 24 DMD patients with mutations extending to or located in exons 56–62 (or 45–55) which are predicted to affect all dystrophin products except Dp71 (or except Dp71 and Dp116). In order to further assess the contribution of Dp71 in the pathogeny of cognitive impairment, we focused on patients for whom IQ value was available and compared distributions of IQs in DMD patients of Groups I and II. Figure 3 shows histograms indicating the number of patients of Groups I and II at each IQ level. Gaussian distributions generated by StatView software suggest that DMD patients of Group II had an IQ ~75 (2SD below normative data), whereas most DMD patients of Group I had an IQ ~50 (>3SD below normative data and ~2SD below DMD patients of Group II). Though additional studies based on comparison of IQs values obtained by homogeneous cognitive tests in age-matched groups are required, the significant difference in IQ scores distribution between the two different groups of patients suggest that Dp71 contributes in the severity of cognitive impairment and its deficit may account for a shift of 2SD downward of the IQ.

Contribution of Dp71 in the development of cognitive function was further consolidated by the rare, but highly relevant, BMD patients with cognitive impairment. These rare BMD patients include the two patients with splice mutations in

intron 62: c.9225-647A>G and c.9225-160A>G and the three patients with different types of mutations in the genomic region of exons 75 and 76 that result in a truncated dystrophin lacking the C-terminal domain encoded by exon 75 and exons distal to this one and known to be essential for the interaction with DAPC, particularly dystrobrevin protein (27).

Taken together, these data, as well as those reported in the literature, are consistent with a contribution of Dp71 deficit in the severity of cognitive impairment. Understanding of cellular and biological mechanisms underlying this consistent correlation will require further functional investigations to define the specific function of DMD products expressed in the CNS, including dystrophin and Dp71 which represent the major *DMD*-gene product in brain. The full-length dystrophin (Dp427) is derived from three independent promoters that regulate its spatio-temporal expression in muscle, brain structures and other cell types (15,28). Immunocytochemical studies indicating that dystrophin and other factors of DAPC are selectively localized to post-synaptic compartment of neurons in cerebral cortex, hippocampus and cerebellum and colocalize with $GABA_A$ receptor subunit clusters in these regions (29). Also, DAPC was shown to modulate $GABA_A$-receptor clustering in association with Dp427 in central inhibitory synapses (29–31) without being required for membrane anchoring of these receptors. However, as the majority of DMD patients with mutations that leads only to loss-of-function of Dp427, or Dp427 and Dp140 protein do not display MR, it is clear that defects restricted to these proteins are not sufficient to bring about the non-progressive cognitive impairment. In view of these data, we are attempted to propose that loss-of-function of dystrophin (alone or with Dp140) has limited consequences on cognitive development and represents a rather susceptibility factor for cognitive impairment. The occurrence of MR in DMD patients could be hastened by additional deleterious environmental and/or genetic events affecting CNS development and/or function. Mutations located in the distal part of the *DMD* gene are in line with this hypothesis as their occurrence in DMD patients lead to loss-of-function of Dp427, Dp140 and Dp71 proteins.

Dp71 mRNA and protein were shown to be expressed in different brain structures and cell types, including perivascular astrocytes (32), glial Müller cells in the retina (33), neurons of the hippocampus and olfactory bulb (34), in cultures neurons (35) and in post-synaptic densities *in vivo* (36). However, Dp71 function in neuronal cells and synapses, and its accurate role in the development of cognition remains still largely unknown. To address this issue, we have recently carried out complementary approaches, using wild-type and Dp71-null mice, and we found expression of Dp71 at both pre- and post-synaptic compartments of excitatory synapses. We found that Dp71-associated protein complexes interact with specialized modular scaffolds that cluster glutamate receptors and organize signalling in post-synaptic densities. We further showed that Dp71-null mice display abnormal synaptic organization and maturation *in vivo*, abnormally enhanced glutamate transmission and altered synaptic plasticity in CA1 hippocampal area (F. Daoud and J. Chelly, manuscript in preparation).

In conclusion, in addition to the diagnostic consequences, i.e. DMD and BMD patients with severe mental retardation should be first tested for mutations in Dp71 region, these findings suggest that Dp71 is required for development of cognitive functions and provide insights into the understanding of molecular mechanisms underlying cognitive impairment associated with DMD and BMD phenotype.

MATERIALS AND METHODS

Patients

Patients included in this study were identified through a network involving eight paediatric and adult neuromuscular French centres. All patients have clinical, biological and histological features compatible with the diagnosis of Duchenne or Becker muscular dystrophies and were included if they had undergone thorough clinical, cognitive and molecular analysis. In all patients, the diagnosis of DMD or BMD was based on clinical history and examination, elevation of creatine kinases and muscle biopsy findings. The diagnosis was then confirmed by the identification of the responsible abnormality in the *DMD* gene. Molecular inclusion criteria were mainly based on the position of mutations (deletions, duplications and point mutations) in the *DMD* gene that allowed subdividing patients into two different groups. Patients with mutations predicted to disturb the functions of all dystrophin products, including Dp71 function (Group I), and patients with mutations predicted to disrupt the functions of all dystrophin products, except Dp71 function (Group II). Clinical phenotypes and DMD abnormalities corresponding to the two groups of patients are provided in Table 1 and Results section.

Molecular and protein investigations confirming the diagnosis of DMD or BMD phenotypes have been performed in the various French diagnostic laboratories. When muscle surgical biopsy samples were available, dystrophin analysis was performed by immunofluorescence analysis of cryo-sections as described by Bornemann *et al*. (37), and/or multiplex western blot analysis using previously reported procedure (26). In both procedures, monoclonal anti-dystrophin antibodies from Novocastra (Newcastle, UK) were used. For some patients, the diagnostic strategy included dystrophin cDNA analysis and sequencing. Briefly, total muscle RNA extraction using the RNA Plus kit (Q-BiOgene, Carlsbad, CA, USA) was performed according to the manufacturer's protocol. Dystrophin mRNA was amplified by RT–PCR as 14 overlapping fragments, which were subsequently analysed by electrophoresis on a 2% agarose gel, then either scanned by protein truncation test (38), or directly sequenced using a Big Dye Terminator I kit and ABI 377 equipment (Applied Biosystem, Courtaboeuf, France). All mutations detected or suspected through mRNA analyses were confirmed by sequence analysis of targeted genomic regions of the *DMD* gene using patient's genomic DNA. For some patients included in this report (Table 1: patients indicated by asterisks), mutations and clinical data have been described in the previous study (19,24,26).

Dp71 mRNA and protein analysis, and screening of Dp71 first specific exon in MR

Consequences of DMD mutations on Dp71 mRNA and protein expression were assessed by RT–PCR and western blot in patients for whom lymphoblastoid cell lines and primary

cultures of fibroblasts were available. Briefly, total lymphoblasts (or fibroblasts) RNA was obtained by acid guanidium thiocyanate-phenol-chloroform extraction using RNA Plus (Q-BiOgene) in accordance with the manufacturer's protocol. Dp71 mRNA was analysed by RT–PCR using primers located in Dp71 exons, including the specific first exon. The design of these primers was driven by the position of mutations and predicted consequences on transcripts maturation. Amplified RT–PCR fragments were analysed by electrophoresis on 2% agarose gel and direct sequencing. For some patients, real-time quantitative RT–PCR was also used to evaluate levels of residual normal and abnormal transcripts. Real-time quantification of Dp71 mRNAs levels was performed as follows: 1 μg of total RNA was reverse transcribed with Superscript II (Invitrogen) in a final volume of 20 μl. Then real-time quantitative PCR reactions were performed in duplicate on a light-Cycler apparatus using 1 μg cDNA product, appropriate Dp71 primer sets (primer sequences are available upon request) and Light-Cycler-FastStart DNA Master SYBG green I Kit (Roche Diagnosis). We used the *cyclophylin A* gene as the endogenous RNA control, and each sample was normalized on the basis of its *cyclophylin* level. Results were expressed as the amount of target sample relative to the *cyclophylin* gene. For data analysis, the threshold cycle number (Ct) values (cycle number at which the increase in the amount associated with exponential growth of PCR products begins to be detected) were exported and processed according to the comparative ddCt method using the cyclophylin A values for normalization.

Protein analysis was performed on lymphoblasts (or fibroblasts) protein extracts by western blot method using standard protocols and Dys8/6C5 antibody (NCL-DYS2/dystrophin C-ter from Novocastra) directed against the C-terminal part of dystrophin and recognizing Dp71.

For the screening of the specific first exon of Dp71 and its flanking 3′ intronic and 5′ non-coding upstream sequences in XLMR patients collected by the European Consortium, DNA was extracted either from peripheral blood leucocytes or lymphoblastoid cells and sequences corresponding to this first exon were amplified by the following primers: forward primer: 5′ tgccccctgctcgcgccacaagtg 5′ and reverse primer: 5′aaagccccaactgcccagtctctg 3′. PCR products were checked by 2% agarose gel electrophoresis before direct sequencing using the BigDye dideoxyterminator chemistry and ABI700 DNA analyser (Applied Biosystems, Foster City, CA, USA).

Cognitive assessment

In this retrospective study, neuropsychometric evaluation data with IQ scores were collected for a total of 46 patients. Depending on the subject's age, WPPSI (39), WPPSI-R (40), WISC-R (41), WISC III (42) and WAIS-R (43) batteries of test were used. These scales that comprise ten core subtests and five supplemental ones are designed to provide separate IQs for VIQ and PIQ as well as a full-scale IQ (IQ). In four patients, cognitive abilities were assessed by the NEMI-N (44), the Columbia Mental Maturity Scale (45), the McCarthy Scales of Children's Abilities (46), the Terman-Merrill (47) and the K.ABC scales (48) which are highly correlated with the Wechsler's IQ. The diagnosis and severity of mental retardation were classified according to the widely used simplified definitions corresponding to mild mental retardation, defined by an IQ value between 70 and 50, and severe mental retardation defined by an IQ below 50. When patients presented an IQ ranging from 70 to 80, they were classified as 'borderline'.

For 35 additional patients, tests-based evaluation of cognitive function has not been performed and therefore IQ scores were not available. However, for these patients, estimation of intellectual level was based on clinical information (given by neuropaediatricians and psychologists) and on the following adaptive functioning criteria: daily living skills, communication skills, social skills and academic achievement. Patients' evaluation based on these criteria allowed us to roughly estimate cognitive performances and classify these 35 patients into one of three following categories: no MR, mild MR and severe MR.

In this study, we also included seven patients exhibiting MR and severe behavioural problems and psychiatric clinical features for whom it was not possible to score cognitive performances and provide IQ levels. We excluded four mentally retarded DMD and BMD patients with mutations criteria that meet those of Groups I or II patients. However, in two cases, clinical history of the family indicated the presence of a potential X-linked mental retardation condition that independently segregates from the muscular phenotype. For the two other patients, brain imaging investigations revealed the presence of basal ganglia calcifications in one patient, and cerebellar hypoplasia and cortical atrophy in the second one.

Additional information

Molecular and clinical data reported in this paper will also be available in the public LSDB (locus specific database): http ://www.umd.be/DMD/

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Conflict of Interest statement. None declared.

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