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RESEARCH ARTICLE

The expression of the distal dystrophin isoforms Dp140 and Dp71 in the human epileptic hippocampus in relation to cognitive functioning

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Abstract

Dystrophin is an important protein within the central nervous system. The absence of dystrophin, characterizing Duchenne muscular dystrophy (DMD), is associated with brain related comorbidities such as neurodevelopmental (e.g., cognitive and behavioural) deficits and epilepsy. Especially mutations in the downstream part of the *DMD* gene affecting the dystrophin isoforms Dp140 and Dp71 are found to be associated with cognitive deficits. However, the function of Dp140 is currently not well understood and its expression pattern has previously been implicated to be developmentally regulated. Therefore, we evaluated Dp140 and Dp71 expression in human hippocampi in relation to cognitive functioning in patients with drug-resistant temporal lobe epilepsy (TLE) and post-mortem controls. Hippocampal samples obtained as part of epilepsy surgery were quantitatively analyzed by Western blot and correlations with neuropsychological test results (i.e., memory and intelligence) were examined. First, we demonstrated that the expression of Dp140 does not appear to differ across different ages throughout adulthood. Second, we identified an inverse correlation between memory loss (i.e., verbal and visual memory), but not intelligence (i.e., neither verbal nor performance), and hippocampal Dp140 expression. Finally, patients with TLE appeared to have similar Dp140 expression levels compared to post-mortem controls without neurological disease. Dp140 may thus have a function in normal cognitive (i.e., episodic memory) processes.

KEYWORDS

Duchenne muscular dystrophy, hippocampal sclerosis, intelligence, memory, temporal lobe epilepsy

1 | INTRODUCTION

Dystrophin is an important cytoskeletal protein that is transcribed from the *DMD* gene. Its absence results in Duchenne muscular dystrophy (DMD); a severe and progressive muscle wasting disorder. This protein is not only localized in muscles as four of its isoforms (i.e., Dp427, Dp140, Dp71, and Dp40) have been identified in different brain structures (Waite, Brown, & Blake, 2012).

After Dp427 and Dp71 had been identified in the central nervous system (CNS), Lidov and colleagues described a third 140 k-Dalton (kDa) dystrophin transcript in 1995 (Lidov, Selig, & Kunkel, 1995). This

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Abbreviations: AEDs, anti-epileptic drugs; CNS, central nervous system; DMD, Duchenne muscular dystrophy; Dp, dystrophin protein; EEG, electroencephalography; (FDG-)PET, (fludeoxyglucose) positron emission tomography; (f)MRI, (functional) magnetic resonance imaging; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HS, hippocampal sclerosis; IQR, inter-quartile range; kDa, kilo-Dalton; MR, mental retardation; MUMC+, Maastricht University Medical Centre; MWU, Mann-Whitney *U* test; PIQ, performance intelligence quotient; SPECT, single photon emission computed tomography; TLE, temporal lobe epilepsy; VIQ, verbal intelligence quotient; WAIS, Wechsler Adult Intelligence Scale

new alternative C-terminal transcript was first identified in mouse brain and kidney and later also in human brain (Morris, Simmons, & Nguyen, 1995). Immunocytochemically, Dp140 has been found at leptomeningeal surfaces, along penetrating blood vessels as part of the microvasculature (Waite, Tinsley, Locke, & Blake, 2009) but also in the outer nerve layer of the olfactory bulbs (Lidov et al., 1995). It appeared furthermore to be expressed differentially during development with higher expressions in fetal compared to adult brain (Morris et al., 1995). In contrast to Dp427, and to a certain extent in agreement with the expression pattern of Dp71, Dp140 was expressed in different types of glial cells (Lidov, 1996), that is, not only perivascular astrocytes, but also Bergmann glia cells (Blake, Hawkes, Benson, & Beesley, 1999; Lidov et al., 1995; Waite et al., 2009). The absence of dystrophin is supposed to be the underlying causative factor for the increased risk of DMD patients to present with neurodevelopmental deficits such as mental retardation (MR; Ricotti et al., 2016), epilepsy (Pane et al., 2013) or even both (Etemadifar & Molaei, 2004; Hendriksen, Vles, Aalbers, Chin, & Hendriksen, 2018).

In different studies in DMD cohorts, it has been proposed that deletions toward the more distal part of the gene (Bushby et al., 1995)—thereby encompassing the shorter brain isoforms Dp140 and Dp71—are more frequently associated with cognitive deficits (Waite et al., 2012). The link between the latter and Dp140 was subsequently further strengthened by the notion that commonly reported deletions, which may selectively disrupt the expression of Dp140, are not only part of a major deletion hotspot in general—that is, accounting for 70% of the *DMD* gene deletions (Den Dunnen et al., 1989; Koenig et al., 1989; Walmsley et al., 2010)—but are also associated with high incidences of cognitive impairment (Lidov, 1996; Lidov et al., 1995; Lindlof et al., 1989). Although a one-to-one genotype–phenotype relationship has never been demonstrated, the cumulative number of individually affected brain isoforms may result in a propensity to develop (severe) neurocognitive problems (Banihani et al., 2015; Moizard et al., 1998; Taylor et al., 2010; Waite et al., 2012). Multiple studies revealed a significant relationship between the loss of Dp140 and MR in muscular dystrophy patients (Bardoni et al., 2000; Felisari et al., 2000; Moizard et al., 1998). Similarly, DMD patients lacking Dp140 not only scored significantly worse on full-scale intelligence and verbal intelligence quotient (VIQ), but also on verbal learning/memory, and executive functioning compared to DMD patients with preserved Dp140 (Chamova et al., 2013). Moreover, patients without the Dp140 isoform contributed most to the gray matter volume reductions as measured by means of magnetic resonance imaging (MRI; Doorenweerd et al., 2014). Apart from that, Dp71 mutations have also been associated with MR in DMD patients (Daoud et al., 2009; Mehler, 2000).

Patients with chronic epilepsy show an increased prevalence of neurodevelopmental comorbidities (Sillanpää, Besag, Aldenkamp, Caplan, Dunn, & Gobbi, 2016). It is well established that especially patients with temporal lobe epilepsy (TLE) show specific memory deficits with neuropsychological assessment (Bouman, Elhorst, Hendriks, Kessels, & Aldenkamp, 2016; Hendriks et al., 2004). Moreover, there is evidence that memory deficits are more often observed in TLE patients with mesiotemporal structural abnormalities (i.e., hippocampal sclerosis) compared to patients with temporo-lateral neocortical abnormalities. Some studies revealed an association between a left-hemisphere localization and verbal memory performance, whereas other studies related visual memory performance to right-hemisphere localization (Harvey et al., 2008; Moore & Baker, 1996). Two patterns of disruption of memory consolidation are suggested, occurring either at the initial or delayed recall, or continuing progressively through time (Cassel, Morris, Koutroumanidis, & Kopelman, 2016). This accelerated amnesia may lead to a relatively higher decline between the immediate and delayed recall measurements on memory tests. The association between cognitive impairment and epilepsy has to date not been fully elucidated, although three main factors are considered to play a definite role: the structural lesion underlying epilepsy, the transient but continuing seizure effects, and the side-effects of anti-epileptic drugs (AEDs) on the brain (Ijff & Aldenkamp, 2013).

Also, epilepsy is a not uncommon comorbidity in DMD (estimates on the prevalence are ranging from 6.6% to 12.3%). Yet, a straightforward association between affected isoform(s) and the presence of epilepsy could not be identified (Hendriksen et al., 2018; Pane et al., 2013). Conversely, it is tempting to question what happens when the relationship is investigated from the other direction, not least as it has been shown that Dp427 is upregulated in hippocampus of patients with TLE (Hendriksen et al., 2016). DMD patients with epilepsy also presented significantly more often with certain neurodevelopmental disorders compared to DMD patients without epilepsy (Hendriksen et al., 2018). An underlying (triangular) aetiology (i.e., absence of dystrophin may not only result in DMD but also in neurodevelopmental disorders and/or epilepsy in specific cases) may therefore be supposed.

In summary, Dp140 and Dp71 seem implicated in normal neurocognitive functioning. To this end, we studied Dp140 and Dp71 expression levels in resected human hippocampus specimens, obtained after surgery for chronic, drug-resistant TLE. This is a unique setup as it allows molecular analysis of human brain tissue involved in memory formation and evaluation of the respective neuropsychological data. In addition, as epilepsy is more prevalent among patients with DMD, we compared Dp140 levels in hippocampi from TLE patients to those of post-mortem controls without neurological disease as done earlier for Dp427 and Dp71 (Hendriksen et al., 2016).

2 | MATERIAL & METHODS

2.1 | Ethical statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The experiments were undertaken with the understanding and informed consent of each individual participant included in the study.

2.2 | Patients

2.2.1 | Surgery

Human hippocampi were intra-operatively obtained between 2010 and 2015 at Maastricht University Medical Centre (MUMC+). All

patients suffered from medically refractory TLE (Kwan et al., 2010). Extensive pre-surgical evaluation included video-EEG monitoring, neuropsychological testing (Section 2.2.2), and 3T-MRI-imaging in all patients. On indication FDG-PET, SPECT, magnetoencephalography, EEG-fMRI and/or invasive electrode implantation were performed. Surgical hippocampus specimens were evaluated and sclerosis was graded according to the Wyler scale (Wyler et al., 1992) by an experienced neuropathologist, after which the samples were encoded. In total, 15 TLE patients (mean age ± *SEM* = 39 ± 4.1 years; eight males, seven females) were included; nine patients suffered from severe hippocampal sclerosis (HS) and six did not show any signs of HS. Of the former group, six patients were graded as Wyler grade four, whereas three patients were classified as at least grade three, but possibly four. There were six patients without any signs of HS (Wyler grade zero). Age of epilepsy onset ranged from 2.5 until 47.5 years (mean age ± *SEM* = 14.64 ± 3.81 years). In 12 patients the hippocampus on the right side was removed, whereas a left hippocampectomy was performed in three TLE patients.

Hippocampal tissue obtained at autopsy (n = 9; mean age ± *SEM* = 67.3 ± 3.4 years; all male) served as nonepileptic control tissue. Post-mortem delay time was minimized (average time ± *SEM* = 1,112 ± 175 min) and did not correlate with Dp140 expression (r_s = .190, p = .65). Patient characteristics for all 24 patients are provided in the supplemental data section (Hendriksen et al., 2016).

2.2.2 | Neuropsychological assessment

As part of the standard presurgical neuropsychological assessment, intelligence scores were only available for epilepsy patients as measured with the third or fourth version of the Wechsler Adult Intelligence Scale (WAIS-III or WAIS-IV) depending on whether assessment took place before or after 2012, by a clinical neuropsychologist from the Academic Centre of Epileptology Kempenhaeghe & MUMC +. WAIS provides standardised index scores for VIQ and performance intelligence quotient (PIQ) scores.

In addition to verbal and performance intelligence, we measured verbal and visual memory functions with four subtests of the Wechsler Memory Scale-IV (Hendriks, Bouman, Kessels, & Aldenkamp, 2014). Verbal memory was tested with the subtests Logical Memory in which patients had to reproduce two short stories immediately after presentation (Logical Memory I) and after the delayed condition after 20 min (Logical Memory II). Visual memory was tested immediately after the presentation of geometrical figures (Visual Reproduction I) and with a delayed recall condition after, again, 20 min (Visual Reproduction II).

2.3 | Methods

The methods section below has been summarized; for the complete overview of the specific methods and materials (Hendriksen et al., 2016).

2.3.1 | Tissue preparation

Immediately after surgical resection, the hippocampal biopsy was cooled for 1 min at 4 °C in 0.9% saline, and then dissected into two parts perpendicular to the longitudinal axis. One of these parts was immediately frozen on dry ice and stored at −80 °C until Western blot analysis (Aalbers et al., 2014).

2.3.2 | Western blot protocol

Each hippocampus was separately homogenized in lysis buffer (Hendriksen et al., 2016). After having estimated the total protein concentration per sample, proteins were resolved by polyacrylamide gel electrophoresis. Each sample was run in duplicate. Proteins were subsequently transferred to a PVDF membrane. After a 2 hr blocking step in commercially obtained Odyssey blocking buffer (diluted 1:2 with phosphate buffered saline and 5% normal donkey serum), the membrane was incubated overnight at 4 °C with polyclonal rabbit anti-dystrophin (Abcam, Ab15277, Cambridge, UK, AB_301813, diluted 1:100), and subsequently for 1 hr with goat anti-rabbit IRDye 800CW (LI-COR, Homburg, Germany, AB_10796098, diluted 1:10,000) in blocking buffer. Rat muscle (*M. biceps femoris*) was used as a positive control and only showed the Dp427 isoform (not shown). Dystrophin immunoreactive protein bands (Figure 1a) were visualized by an Odyssey infrared imaging system (Li-COR Biosciences, Lincoln, NE; Cooper, Lo, & North, 2003). Optical density values were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), detected by mouse monoclonal anti-GAPDH primary (Fitzgerald Industries International, Acton, MA, diluted 1:2,000,000) and donkey anti-mouse IRDye 680RD (LI-COR, Homburg, Germany, AB_10953628, diluted 1:10,000) secondary antibody (Hendriksen et al., 2016).

2.4 | Analysis

2.4.1 | Western blot quantification

Immunoblots were analyzed with ImageJ software (SCR_003070; Anthony et al., 2014). By means of precisely measuring every individual band on each blot (and its relative distance to the respective marker at 150 kDa) we confirmed we would quantify the bands corresponding to a molecular weight of 140 kDa. Relative pixel intensities were measured and the background signal was subtracted (Taylor, Berkelman, Yadav, & Hammond, 2013). Every dystrophin isoform was corrected for by its respective GAPDH expression and subsequently expressed as a percentage of the average of the post-mortem control patients' expression on that respective gel (Anthony et al., 2014). During quantification, the observer was blinded to the condition to prevent biased assessment.

2.4.2 | Statistical analysis

Quantified blot intensities were averaged of at least two independent experiments (and thus two or more measurements per patients) within groups and were subsequently expressed as the median + interquartile range (IQR) per condition because of non-normal distribution (Figure 1c). Given the abovementioned supposed developmentally regulated expression, Dp140 levels were first correlated with age by means of a nonparametric Spearman test. By making use of the same test, the VIQ, PIQ, and the subtests Logical Memory I and II and Visual Reproduction I and II were correlated with the values found for both Dp140 and Dp71 expression. Furthermore, following Cassel et al. (2016) we analyzed the relative differences between immediate (I) and delayed (II) conditions, expressed as the percentage of lost

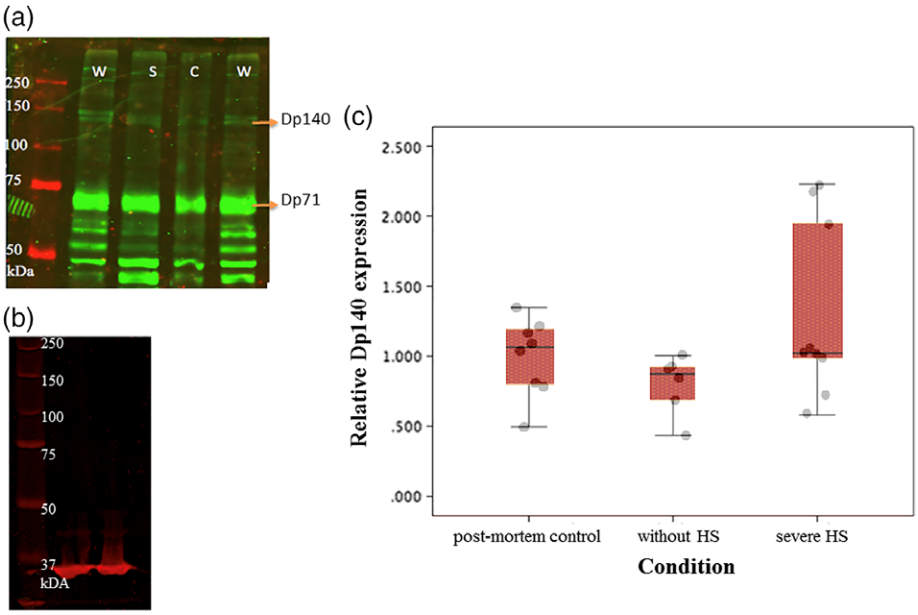

FIGURE 1 (a) Example of a western blot showing dystrophin expression of the distal isoforms (Dp140 and Dp71) in hippocampus of epileptic and nonepileptic patients. In line with the very first paper on Dp140 by Lidov and colleagues (Lidov et al., 1995), Dp140 is substantially less immunoreactive than Dp71. Based on the (red) marker on the left side, the height of the Dp140 bands was graphically verified for each blot. (b) Negative control (performed in human hippocampus tissue) showing no nonspecific protein bands specifically associated with the secondary antibody. (c) Boxplots depicting Dp140 expression in epilepsy patients (both with and without HS) and post-mortem controls. Abbreviations: C = post-mortem control, W = without hippocampal sclerosis, S = with severe hippocampal sclerosis [Color figure can be viewed at wileyonlinelibrary.com]

information (Cassel et al., 2016). Given the fact that visual memory is most associated with the right temporal lobe, we also examined these scores separately for a correlation with distal dystrophin expression in the 12 patients that underwent a right hippocampectomy. As there were only three patients with a left hippocampectomy, verbal memory was not separately correlated with dystrophin levels in this subgroup.

We then compared dystrophin levels in epileptic and nonepileptic tissue, using a Mann-Whitney *U* (MWU) test. To assess the influence of neuronal cell loss and gliosis, we also evaluated the difference in dystrophin expression between epileptic patients with and without HS, according to an alpha level of 0.025 (Bonferroni correction). Additionally, within the epilepsy cohort, correlation analyses (Spearman) were performed between dystrophin (i.e., Dp140 and Dp71) expression—whereby the values for the latter isoform were based on the previous paper as included in (Hendriksen et al., 2016)—and the Wyler degree in order to assess possible effects of astrogliosis in the HS group.

3 | RESULTS

Figure 1a demonstrates a typical Western blot, showing three of the four CNS-associated dystrophin isoforms: Dp427 (not indicated), Dp140, and Dp71. The figure instantly shows that within the human hippocampus, Dp71 is most abundantly expressed. The fourth isoform, Dp40, could not be identified because it does not contain a carboxy-terminal to which the antibody used is directed. Figure 1b shows the negative control (in human hippocampal tissue) and Figure 1c demonstrates the quantified Dp140 expression levels per condition.

3.1 | Distal dystrophin isoform expression and age

To assess if Dp140 expression would be developmentally regulated, we investigated if Dp140 expression levels correlated to the age of the patients and controls (ranging from 16 to 83 years; Figure 2a**)**. Because the expression of Dp140 could not be reliably quantified for one post-mortem control patient, this one was excluded. The analysis learned that there was no correlation (r_s = .102, p = .64, n = 23). Subgroup analysis per condition (i.e., sclerosis, nonsclerosis and post-mortem control) did also not reveal any significant correlations. Although a developmental regulation of Dp71 expression has not been described to date, we examined this suggestion in our dataset. Also the expression of this isoform did not correlate to age (r_s = −.034, p = .88, n = 24).

3.2 | Distal dystrophin isoform expression and cognitive functioning

3.2.1 | Intelligence

Intelligence scores were normally distributed, that is, VIQ scores ranged from 72–116 points (87.8 ± 12.9) and PIQ scores ranged from 62 to 129 points (88.4 ± 16.8) among all TLE patients. Neither intelligence score correlated significantly with hippocampal Dp140 levels (VIQ: r_s = −.227, p = .42; PIQ: r_s = .086, p = .76). In Figure 2b the scatterplot is shown for VIQ. Likewise, VIQ (r_s = −.232, p = .41) and PIQ (r_s = −.218, p = .44) scores did not correlate with the expression of hippocampal Dp71.

3.2.2 | Memory

Verbal memory scores did not correlate with hippocampal Dp140 expression levels (Logical Memory I: r_s = −.404, p = .14; Logical Memory II: r_s = −.122, p = .67). Verbal memory scores did also not correlate with Dp71 expression (Logical Memory I: r_s = .029, p = .92;

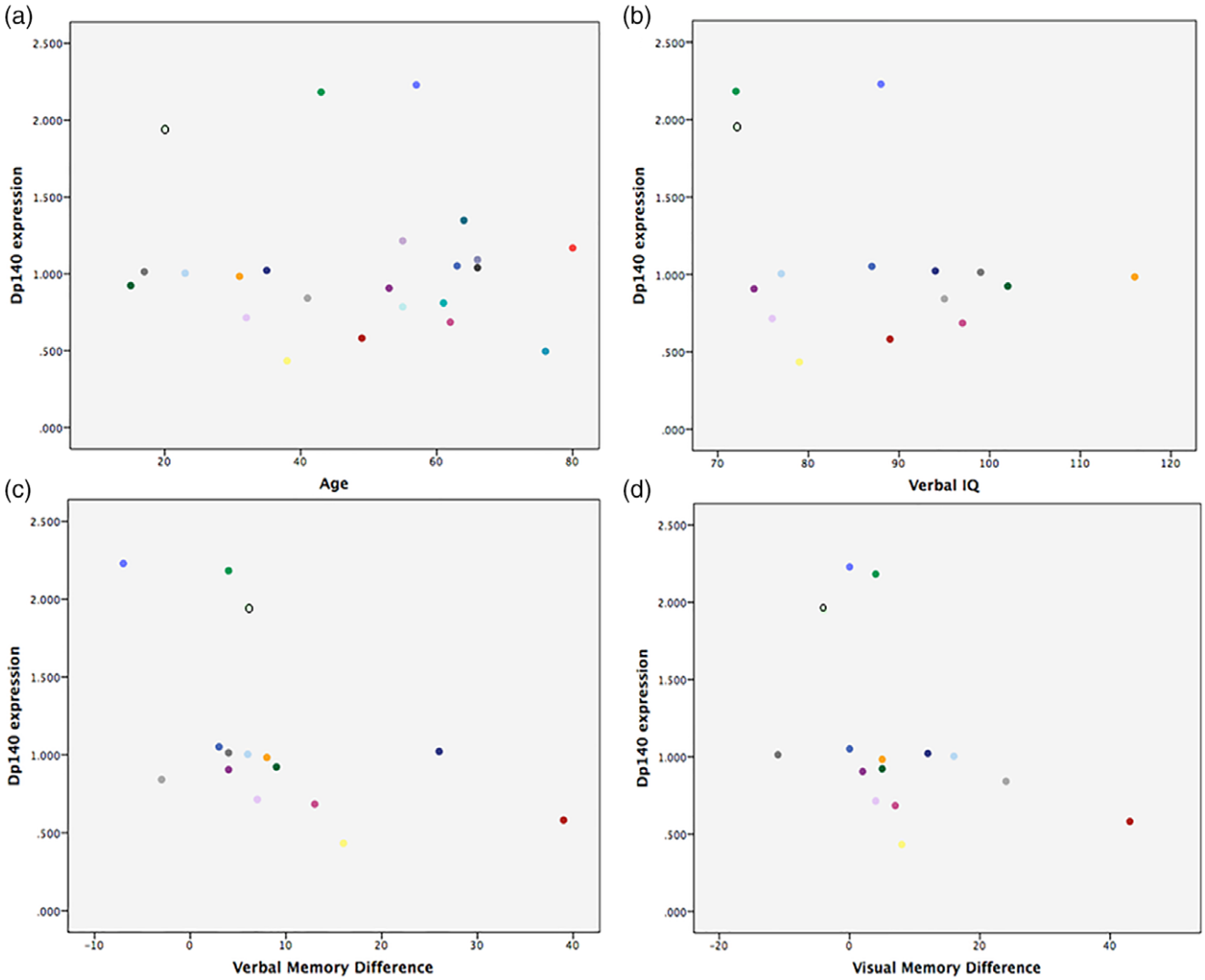

FIGURE 2 Scatter plots for relative hippocampal Dp140 expression, here graphically depicted on the *y*-axis (×100%), with (a) age (b) verbal IQ-(performance IQ is not shown here), (c) verbal memory difference and (d) visual memory difference scores. The different, yet consistent colors of all data point shown here throughout the four graphs represent the same specific individual case in each graph (a–d) [Color figure can be viewed at wileyonlinelibrary.com]

Logical Memory II: r_s = −.002, p = 1.00). Similarly, visual memory did not correlate with Dp140 (r_s = .147, p = .60 for Visual Reproduction I and r_s = .347, p = .21 for Visual Reproduction II) or with Dp71 (r_s = .005, p = .99 for Visual Reproduction I and r_s = −.164, p = .56 for Visual Reproduction II).

When correlating the difference between the immediate and delayed scores (i.e., Logical Memory I vs. Logical Memory II and Visual Reproduction I vs. Visual Reproduction II) to dystrophin expression levels, there was no significant correlation for Dp71 (respective values of r_s = −.075, p = .79 and r_s = .077, p = .79). However for Dp140, a significant correlation was found for the percentage of decay of verbal memory (r_s = −.567, p = .03, Figure 2c). Also, such an association was found for the loss of visual memory (r_s = −.591, p = .02), that is, between the immediate and delayed recall conditions and Dp140 levels (Figure 2d). The observed correlation between Dp140 expression and visual memory difference was maintained when evaluated for the right hippocampal subgroup (n = 12; r_s = −.595, p = .04). Again, for Dp71 no statistical correlations were identified (r_s = .081, p = .80).

3.3 | Dp140 expression and TLE

Dp140 expression did not differ statistically (p = .636) between all epilepsy patients, that is, irrespective of sclerosis (median ± IQR = 0.98 ± 0.34, n = 15), and post mortem controls (median ± IQR = 1.07 ± 0.412, n = 8). However, when studying the effects of sclerosis on Dp140 expression, a trend (p = .05) was identified between patients with severe HS (median ± IQR = 1.02 ± 1.22, n = 9) and without HS (median ± IQR = 0.87 ± 0.32, n = 6; Figure 1c). A correlation was found between Dp140 expression and the degree of HS as measured by means of the Wyler scale (r_s = .6, p = .017). Dp71 levels on the other hand, did not correlate with the extent of HS (r_s = .09, p = .76).

4 | DISCUSSION

In this study we evaluated hippocampal Dp140 and Dp71 tissue expression in relation to cognitive functioning. In addition, as earlier

done for Dp71 (Hendriksen et al., 2017), we compared the expression of Dp140 in the hippocampus of TLE patients to that in post-mortem control hippocampus in order to dissect whether this CNS-specific dystrophin isoform may be related to epilepsy.

4.1 | A developmental regulation of Dp140?

Previous literature suggests—as based on one single study—that Dp140 brain expression is higher in fetal stages compared to adult stages (Morris et al., 1995). We did, however, not find such age-specificity. Although these authors show a clear difference in Dp140 expression in brain by means of the same technique used here, one should still be cautious when generalizing these results as (a) the expression difference has not been quantified and (b) it was based on one 60 year old adult compared to one 3.5 month old fetus (n = 2). This impedes the generalization of such findings. Besides, the brain region was not specified, even though it is known that dystrophin levels can vary per brain region (Lidov, 1996; Lidov, Byers, & Kunkel, 1993). Furthermore, Morris et al. compared an unborn with an adult, whereas we compared persons within an age range of 16–83 years (total mean 49.4 36 $\pm$ 19.8 years). If a developmental effect would exist and the decrease of Dp140 would for instance be highest during the first months or years of development, we would not have been able to detect this. A developmental decrease seems, however, not to be present from the age of 16 years onwards, with a stable value for Dp140 across older ages.

4.2 | Distal dystrophin isoforms in relation to cognitive functioning

The absence of the distal brain isoforms (Dp140 and Dp71) in DMD patients—as based on mutation characteristics—has been repeatedly associated with deficits in cognitive functioning, particularly MR, but recently also with verbal memory (Chamova et al., 2013). Therefore, we investigated verbal and visual memory in relation to the hippocampal expression of Dp140 and Dp71 in epilepsy patients with a functional *DMD* gene. This is especially interesting since evidence exists for the fact that verbal memory is directly related to hippocampal functioning (Sass et al., 1990). As described, the role of Dp140 is largely unknown. It is mainly considered to be located in (perivascular) astrocytes but its function here is undetermined. A (hypothetical) relationship between astrocytes and general cognitive functioning has recently been proposed and consists of the notion that blood–brain barrier breakdown may lead to disturbed cognition in psychiatric disorders (Shalev, Serlin, & Friedman, 2009). Furthermore, astrocytes possess membrane receptors for neurotransmitters, but can at the same time also release so-called gliotransmitters. These astrocyte-specific chemical messengers can communicate with both presynaptic and postsynaptic neurons, resulting in a functional unit called the tripartite synapse that modulates neuronal activity as well as synaptic plasticity (Pereira Jr. & Furlan, 2010). As such, the link between Dp140—which seems to be mainly localized in astrocytes—and cognitive functioning can, at least theoretically, be established.

Considerably more is known about Dp71, which is involved in the maturation and clustering of glutamatergic receptors in hippocampal neurons (Daoud et al., 2009), but also the clustering of water (AQP4) and potassium (Kir4.1) channels in glial cells (Connors, Adams, Froehner, & Kofuji, 2004; Perronnet & Vaillend, 2010). Different studies have implicated a role for the defective functioning of these channels in Dp71 deficient genotypes in the propensity not only to result in epilepsy—albeit theoretically (Hendriksen et al., 2015), but also indirect synaptic alterations and as such cognitive impairment (Daoud et al., 2009; Tadayoni, Rendon, Soria-Jasso, & Cisneros, 2012).

This is, to the best of our knowledge, the first study to correlate the expression of the distal dystrophin isoforms to cognitive parameters. Interestingly, these analyses revealed an association between higher Dp140 levels and less memory loss, which is in line with the fact that DMD patients with intact Dp140 score generally better on verbal memory (Chamova et al., 2013). Dp140 may therefore be supposed to have a function in episodic memory processes and could indeed be related to normal cognitive functioning. For the other cognitive domain tested (i.e., intelligence), no association could be identified. Dp71 expression did not correlate with any of the two cognitive parameters.

One reason that may explain the absence of these correlations was our fairly small sample size. Second, if Dp140 or Dp71 would be related to intelligence as observed in DMD patients without Dp140, it may be the case that only very low—or even absent—levels of this isoform are associated with a decreased intelligence. Nevertheless, in the epilepsy patients studied here, there were still reasonable levels detected (Figure 1c). Third, we studied the expression levels in patients with (another) chronic, therapy-resistant neurological disorder. Although we saw no differences in Dp140 and Dp71 expression between epilepsy patients and controls, we can, however, not completely exclude the effects of the disorder or the AEDs on the hippocampal expression pattern of these isoforms. Furthermore, TLE is also associated with other cognitive problems and lower full-scale intelligence scores (Hermann et al., 2006), which could for instance also influence intelligence distributions as such. Nonetheless by studying cognitive domain scores in solely patients with TLE, we tried to reduce the effects of confounding to some extent. Finally, we studied hippocampal dystrophin levels. Intelligence is a broad cognitive construct that is only very partly related to hippocampal functioning. Other brain regions that contain dystrophin (e.g., frontal cortex, parietal cortex, and cerebellum) are also involved in cognitive processes underlying intelligence, and could alternatively be studied (Jung & Haier, 2007). However, practically, it is ethically impossible to acquire both intelligence scores and dystrophin levels in other brain tissue as the resected hippocampal tissue here.

4.3 | Dp140 expression in relation to epilepsy

In contrast to Dp427, Dp140 expression was not altered when compared to the expression in hippocampi of post-mortem controls, suggesting the absence of causative or compensatory up- or downregulating mechanisms for this isoform in TLE patients. Given its supposed exclusive role and presence in glial cells this was initially not merely expected as stated in the previous publication on Dp427 expression in TLE (Hendriksen et al., 2016). However, around that same time (temporal lobe) epilepsy has become

increasingly implicated as a glial disorder (Robel, 2017; Steinhauser, Grunnet, & Carmignoto, 2016), which framed the hypotheses in this study and precipitated Dp140 assessment in hippocampus of TLE patients.

In line with the previous publication on Dp71 expression (Hendriksen et al., 2016), Dp140 was unchanged compared to the expression in controls. The trend toward higher expression in patients with HS compared to those without could most probably be attributed to astrogliosis, one of the hallmark features of HS. This was subsequently once more suggested by the strong correlation between Dp140 expression and the extent of the sclerosis as evaluated by Wyler's grading scale. As Dp140 is—albeit based on a handful studies—considered to be mainly localized in (perivascular) astrocytes, this would be the most logical explanation. Dp71 on the other hand, which is known to be located in both neurons and glial cells did indeed not correlate with the extent of HS. This might be because astrogliosis and neuronal cell loss balance each other out as also partly reflected by the finding that Dp71 expression did also not differ between TLE patients with and patients without HS (Hendriksen et al., 2016).

4.4 | Limitations and future directions

This study was meant as an incentive for further research into dystrophin isoforms—particularly Dp140—in normal (i.e., not DMD-associated) functioning in order to increase our understanding of the different (distal) CNS-expressed isoforms that are transcribed from the largest human gene. Yet, this explorative study has certain limitations. First of all, Western blot is a technique with intrinsic limitations (i.e., detection limits), especially when examining the expression of a protein that is not highly expressed. Consequently, the results should be ideally further confirmed by future studies by making use of other quantitative techniques such as enzyme-linked immune sorbent assay (ELISA). Apart from that, quantitative immunostainings need to confirm the results presented here from a distributive perspective. Hitherto this has only been done minimally (Lidov et al., 1995; Morris et al., 1995) but has also been reported to be unsatisfactory, for which reason selective in situ hybridization might be a better alternative as this has not been done to date (Lidov, 1996). With regards to the supposed association between dystrophin and epilepsy, gene expression analysis should be performed in order to correlate dystrophin expression to genes involved in epilepsy. Finally, in line with the literature that hitherto appeared, it is worth considering evaluating larger groups of DMD patients with mutations certainly affecting Dp140 and Dp71—yet a mutation affecting the latter isoform is unfortunately rare—in order to increase power and try to establish a straightforward relationship between the absence of distal dystrophin isoforms and cognitive deficits, or not. If such mechanisms cannot be identified, other underlying aetiological (e.g., epigenetic) mechanisms should be brought forward and subsequently evaluated in order to answer the question why a substantial cohort of patients with DMD present with varying degrees of brain involvement, whereas many others do not.

5 | CONCLUSION

The results of this study suggest that higher levels of hippocampal Dp140 are associated with less memory loss (for both verbal and visual memory), which is in line with the finding that DMD patients that lack this isoform, present more often with cognitive deficits such as verbal memory problems. Yet, no correlation was found with the respective intelligence scores, which may be because of the selective resection of hippocampal tissue in our patient group. The expression of the other distal *DMD* gene transcript (i.e., Dp71) did not correlate with intelligence or memory scores. Apart from that, Dp140 and Dp71 expression was found to be consistent among patients with TLE and post-mortem controls without epilepsy. Dp140 may, however, indeed be exclusively located in glial cells as we demonstrated a trend toward increased Dp140 expression in sclerotic hippocampi of TLE patients compared to patients without HS. This was furthermore supported by the statistically significant correlation with the Wyler degree within the collective TLE cohort. For Dp71, neither expression differences between HS and nonHS patients, nor correlations within the group of TLE patients were identified, possibly because it is also known to reside in neurons. Despite the increasing interest for Dp140 in relation to the brain-related comorbidities in DMD, research on this potentially important isoform with regards to cognitive functioning deserves more attention.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

All co-authors agree to having their names listed as authors and that colleagues whose unpublished work is referred to, or who are acknowledged, agree to that.

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