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Quantification of brain atrophy in patients with myotonic dystrophy and proximal myotonic myopathy: a controlled 3-dimensional magnetic resonance imaging study

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Abstract

Myotonic dystrophy (DM1) and proximal myotonic myopathy (PROMM or DM2) are two distinct muscular disorders with multisystemic involvement. Both have previously been reported to be associated with cognitive impairment and white matter lesions detected by cerebral magnetic resonance imaging (MRI). In this study, the extent of brain atrophy was investigated *in vivo* in ten DM1 and nine PROMM patients in comparison to age-matched healthy controls for each group. The diagnosis was confirmed by DNA analysis of all patients. As a quantitative marker, the ratio of brain parenchymal to intracranial volume, called brain parenchymal fraction (BPF), was calculated from 3-dimensional MRI data using an automated analysis technique. Compared to age-matched healthy controls (mean BPF 0.852 ± 0.032), the BPF in DM1 patients (0.713 ± 0.031) was highly significantly decreased ($P < 0.001$). In contrast, the PROMM patients (mean BPF 0.792 ± 0.029) showed only slightly decreased BPF values ($P < 0.05$). BPF was not significantly correlated to any of the clinical or genetic parameters in both diseases (disease duration, motor score, educational level, and number of CTG repeats in the expanded allele). In summary, global brain atrophy was demonstrated to occur in both diseases, but was more severely manifested in DM1 patients.

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Myotonic dystrophy (DM1, OMIM 160900) and proximal myotonic myopathy (PROMM, OMIM 600109 or myotonic dystrophy type 2: DM2, OMIM 602668) are progressive multisystemic disorders characterized by myotonia, muscular wasting, and a range of other manifestations of variable severity including cataracts, cardiac conduction defects, gonadal atrophy, and neuropsychological deficits. The genetic defect of DM1 is an unstable expansion of CTG repeats in the 3'-untranslated region of the dystrophica myotonica-protein kinase gene on chromosome 19q13, whereas the mutation causing PROMM was found to be an intronic CCTG repeat expansion located in the zinc finger

protein 9 gene on chromosome 3q21 [6,11,14,17]. DM1 is more common and shows in general a more severe clinical phenotype than PROMM. In particular, cognitive dysfunction ranging from mild neuropsychological deficits to severe mental retardation is frequent in DM1 patients but not a prominent clinical feature of PROMM. In cerebral magnetic resonance imaging (MRI) scans of DM1 patients, abnormalities such as focal white matter lesions, altered T2 relaxometry and magnetization transfer ratios of white matter, diffuse atrophy, and wide Virchow-Robin spaces are found in DM1 as described in several clinical studies (e.g. refs. [4,7,13,15]). The etiology of these MRI findings is still unknown. Leukoencephalopathy with periventricular white matter lesions has been also described in PROMM patients [8,19], but cerebral atrophy has not been investigated so far.

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A systematic analysis of brain atrophy in vivo has not been performed up to now for both disorders.

The present study was initiated to examine the brains of DM1 and PROMM patients with respect to the extent of atrophy assessed from 3-dimensional (3-D) MRI data sets. As a quantitative measure of brain volumes, the brain parenchymal fractions (BPF) were calculated, defined by the ratio of brain parenchymal volume to total intracranial volume [3,18]. Applying a novel, largely automated MRI analysis technique [10], comparisons with age-matched control groups were performed in both diseases.

Ten DM1 patients from eight unrelated families (five males, five females) and nine PROMM patients from four unrelated families (three males, six females) were included in the study. All patients gave their written informed consent to the study protocol which had been approved by the Ethical Committee of the University of Ulm. None of the patients suffered from any former or permanent neurological disease other than DM1 or PROMM. Vascular diseases were largely excluded (exclusion criteria: transient or permanent ischemic events in medical history or more than one cardiovascular risk factor). All patients received a general clinical and neurological examination and electromyography by an experienced neurologist (YGW, AR or HL). The muscle impairment was measured by using a modified form of the muscular disability rate based on a five-point scale (MDRS [12]), grade 1: no clinical muscular impairment, grade 2: minimal signs (myotonia, facial weakness), grade 3: mild muscle weakness (no external help in every day tasks is necessary), grade 4: moderate muscle weakness (external help in every day tasks is necessary), grade 5: severe muscle weakness (confined to wheelchair). The educational level was registered as the number of years of school education.

Diagnosis was confirmed in all cases by the analysis of the CTG repeat on chromosome 19 for the DM1 patients, and of the CCTG repeat on chromosome 3 for the PROMM patients. The mean number of CTG repeats of DM1 patients was 582 ± 402 (range 110–1300); the PROMM patients had expanded CCTG repeats as determined by techniques published by Liquori et al. [11]. The mean ages were 36 ± 14 years (range 18–57) for DM1 and 53 ± 11 years (range 33–71) for the PROMM patients. Disease duration, assessed as the delay from first symptom onset to the time of investigation, was 17 ± 10 years for DM1 and 26 ± 16 years for PROMM patients. MDRS scores were determined as 3.1 ± 0.8 in DM1 and 2.5 ± 0.2 in PROMM patients. In DM1, the mean educational level was 11 ± 2 years, in PROMM patients 12 ± 2 years.

Two groups of 20 healthy subjects each, without any history of neurological, psychiatric or other disease, served as controls. They were investigated following the same MRI protocol as the patients. Two different groups had to be chosen because of the differences in age between the patient groups: one control group (mean age 36 ± 11 years, range 21–59; ten males, ten females) was age-matched to the DM1

patients, the other one to the PROMM patients (mean age 54 ± 12 years, range 33–68; male/female ratio 7/13). All data are given as arithmetic means \pm standard deviation.

For patients and controls, high-resolution volume-rendering data sets of the whole head were collected on a 1.5 T clinical MRI scanner (Magnetom Vision, Siemens, Erlangen, Germany). For data acquisition, a T1-weighted magnetization-prepared rapid-acquisition gradient echo sequence (MP-RAGE, repetition time 9.7 ms, echo time 3.93 ms, flip angle 15° , matrix size $256 \times 256 \text{ mm}^2$, voxel size $1 \times 1 \times 1 \text{ mm}^3$) was used, consisting of 160–180 sagittal partitions depending on head size. Off-line data processing included cropping the MRI data at the spinal cord cut-off level located caudally of the cerebellum. Data were then segmented into the fractions grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF). For creating the probability images of the single fractions, the fully automatic algorithms as implemented in the Statistical Parametric Mapping software (SPM99, Wellcome Department of Cognitive Neurology, London, UK [1]) were used at maximum image inhomogeneity correction. BPF was calculated by dividing the sum of the corrected fractions of GM and WM by the sum of corrected fractions of GM, WM and CSF. Further details of SPM-based data processing were described elsewhere [10]. Differences in BPF values between patients and age-matched controls as well as correlations between BPF values and clinical data (age, disease duration, CTG repeat length) were considered statistically significant at $P < 0.05$; the non-parametric Mann–Whitney U -test was used for comparison of BPF values between patients and controls (Statistical Package for the Social Sciences software (SPSS), Chicago, IL, USA); for correlations, a standard regression analysis using Origin 5.0 (Microcal Software Inc., Northampton, MA, USA) was performed.

BPF values for DM1 and PROMM patients, plotted against age, are shown in Figs. 1A,B compared to the corresponding control populations. In the DM1 patient group, mean BPF was 0.713 ± 0.031 (range 0.645–0.749). Although the PROMM patients were markedly older, their mean BPF was higher (0.792 ± 0.029 , range 0.743–0.839). The BPF values of the DM1 patients showed a tendency to decrease with age, but the correlation was not statistically significant ($P > 0.2$). In contrast, BPF was significantly correlated with age both in PROMM patients ($P < 0.001$) and, as previously described for healthy subjects [3,10], in the control populations ($P < 0.002$). Comparing the BPF values obtained for DM1 patients with those for age-matched controls (mean BPF 0.852 ± 0.032 , range 0.769–0.884), the difference was highly significant (Mann–Whitney U -test, $U = 0$, $P < 0.001$). BPF was markedly decreased compared to controls not only in the group analysis, but in each single DM1 patient. The difference for the PROMM group to their age-matched controls (mean BPF 0.815 ± 0.039 , range 0.743–0.876), was small, but still significant ($P < 0.05$, $U = 59$) (Fig. 1C). BPF was not correlated to any of the clinical or genetic parameters in

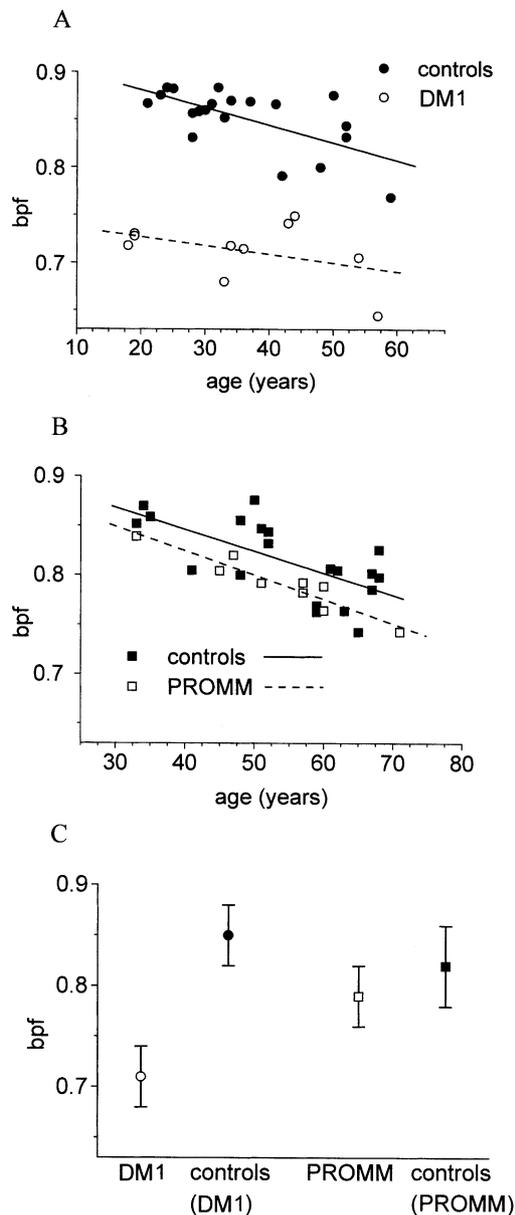


Fig. 1. (A–C) Brain parenchymal fraction (BPF) determined in DM1 and PROMM patients compared to age-matched controls. (A) BPF plotted against age as obtained for DM1 patients (open symbols, $n = 10$) and age-matched healthy controls (filled symbols, $n = 20$), including regression lines for each group. BPF correlated significantly with age for controls ($P < 0.002$), but not for DM1 patients. (B) BPF plotted against age as obtained for PROMM patients (open symbols, $n = 9$) and the corresponding age-matched group of healthy controls (filled symbols, $n = 20$), including regression lines. BPF correlated significantly with age for both PROMM patients ($P < 0.001$) and controls ($P < 0.002$). (C) Mean BPF values and 95% confidence intervals (± 2 SD) for DM1 and PROMM patients in comparison to their age-matched controls; differences are significant for DM1 ($P < 0.001$) and PROMM patients ($P < 0.05$) (Mann–Whitney U -test).

both diseases (disease duration, motor score, educational level and number of CTG repeat).

In summary, we investigated the extent of global brain atrophy in vivo in the distinct multisystem disorders DM1 and PROMM using 3-D MRI and an user-

independent analysis technique. At the methodological level, BPF serves as a straightforward and size-normalized quantification of the brain volume. This ratio had been mainly used to monitor brain atrophy in previous studies, for example in demyelinating diseases such as multiple sclerosis for intraindividual longitudinal monitoring in controlled therapy trials [5]. In addition, BPF has to be considered of general use as a biological marker in neurodegenerative disorders with white or gray matter atrophy, if it is analyzed in comparison to a control population as external reference [10]. It has to be required for that purpose that the control group is age-matched since it had been previously demonstrated that BPF significantly decreases during healthy aging [3]. Gender effects have to be considered as well since females tend to show higher BPF values, but were negligible in the present study since male/female ratios in the patient samples and the corresponding control groups were nearly the same. With respect to the methodology of BPF calculation, all procedures and evaluations should be automatic and observer-independent as far as possible in order to avoid user errors [9]. Consecutively, we used an almost fully-automated postprocessing technique to compute the BPF values from 3-D MRI data sets.

Consistent with the less severe course of the disease compared to DM1, PROMM was associated with a minor extent of brain atrophy. However, our data emphasize the multisystemic character of this disease, including significant cerebral atrophy. As in healthy subjects, BPF values were significantly correlated with age in PROMM patients, but the markedly decreased BPF values in the DM1 patients were not. The most simple explanation for this observation is that younger DM1 patients are generally more severely affected and therefore might have more pronounced brain atrophy compared to their age-matched controls. This is confirmed by a longer CTG repeat length in the younger patients (data not shown).

Global brain atrophy in DM1 had been described in single cases in early computed tomography studies [2] and in neuropathological studies [16]. The controlled analysis of quantitative MRI scans, as performed here, confirms the more prominent involvement of the central nervous system in DM1 compared to PROMM. Our study establishes significant brain atrophy as an in vivo measurable clinical sign and disease marker for both diseases. Although a therapy is currently not available, identification of the genetic defects in both diseases let us hope that this might be the case in the future. The BPF may then serve as an objective longitudinal marker for progression of the disease in the central nervous system.

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