Homozygosity for CCTG mutation in myotonic dystrophy type 2


Summary
Myotonic dystrophy type 2 (DM2) is caused by a dominantly transmitted CCTG repeat expansion in intron 1 of the zinc finger protein 9 (ZNF9) gene on chromosome 3q. DM2 patients with two mutant alleles have not been reported so far. In one large consanguineous family from Afghanistan, we found three homozygotes for the DM2 mutation. The oldest patient was clinically more severely affected, compared with the two younger homozygotes, but for the clinical course of symptoms all three homozygotes were within the range expected for heterozygotes. Further investigations, such as mutation repeat length, muscle histology, anti-muscleblind-like 1 stainings or brain imaging studies, at least at short-term observation, showed no differences between homozygotes and heterozygotes. Twenty of 24 children, aged 2–21 years, were available for clinical examination. None of these children have signs or symptoms of disease until the age of 18 years. Homozygosity for the DM2 expansion does not seem to alter the disease phenotype as compared with the heterozygous state.

Keywords: CCTG expansion; mutational homozygosity; muscle biopsy; myotonic dystrophy type 2; proximal myotonic myopathy (PROMM)

Introduction
Myotonic dystrophy is an autosomal dominant, multisystemic disease with a core pattern of clinical presentation including myotonia, muscular dystrophy, cardiac conduction defects, posterior iridescent cataracts and endocrine symptoms. Steinert’s disease, myotonic dystrophy type 1 (DM1), was first described 100 years ago (for a review see Harper, 2001). In 1992, DM1 was shown to be caused by an expanded CTG repeat in the 3’-untranslated region of the dystrophia myotonica-protein kinase (DMPK) gene located on chromosome 19 (Brook et al., 1992; Buxton et al., 1992; Fu et al., 1992; Harley et al., 1992; Mahadevan et al., 1992). Recently, another multisystemic disorder was reported with similar clinical features lacking the DM1 repeat expansion (Ricker et al., 1994; Thornton et al., 1994). In Europe, the disease in these families has been called proximal myotonic myopathy (PROMM; OMIM*160900) (Ricker et al., 1994) or proximal myotonic dystrophy (PDM) (Udd et al., 1997), and in the USA these families initially were described as having ‘myotonic dystrophy with no CTG repeat expansion’ (Thornton et al., 1994; Day et al., 1999). The myotonic dystrophy type 2 (DM2) locus was mapped to 3q21 (Ranum et al., 1998) and subsequently the mutation was identified as a CCTG expansion in intron 1 of the zinc finger protein 9 (ZNF9) gene (Liquori et al., 2001). All the families previously reported to suffer from PROMM, PDM or DM2 have the DM2 CCTG expansion (Bachinski et al., 2003; Day et al., 2003; Liquori et al., 2003). Clinical and molecular parallels between DM1 and DM2 indicate that CUG and CCUG microsatellite expansions in pre-mRNA can be pathogenic and cause the multisystemic features of both diseases (Day et al., 1999, 2003; Ricker, 1999; Liquori et al., 2001; Ranum and Day, 2002).

The DM2 repeat tract (marker CL3N58) is a complex repeat motif with an overall configuration of (TG)n(TCTG)n(CCTG)n. The sequenced expanded alleles contain an uninterrupted
elongation of the CCTG portion of the repeat tract (Liquori et al., 2001). Expanded DM2 alleles show extreme somatic instability with significant increases in length over time (e.g. 2000 bp/3 years) and often appear as a difficult to discern smear of multiple bands on Southern blots (Liquori et al., 2001; Bachinski et al., 2003; Day et al., 2003). Parts of the repeat tract and flanking intron sequences are conserved in chimp, gorilla, mouse and rat, suggesting that the repeat tract originally found in humans may have a conserved biological function (Liquori et al., 2003).

Recent haplotype and linkage disequilibrium studies suggest that the DM2 expansion originated from a single or a few founder mutations (Liquori et al., 2001, 2003; Bachinski et al., 2003). In one study, the DM2 expansion mutation was estimated to have arisen ~200–540 generations ago (Bachinski et al., 2003). In a recent study, a family of apparent Afghan/Tajik ancestry, described in detail here, was shown to share the common European haplotype, suggesting that the DM2 expansion occurred prior to the Aryan migration of Indo-Europeans that settled Aryana (ancient Afghanistan) in 2000–1000 BC (Liquori et al., 2003).

We now present clinical and genetic data on this large consanguineous DM2 family from Afghanistan in which three affected siblings are homozygous for the DM2 expansion. We also report the phenotype of 20 children of the Afghan family.

Methods

Study sample

We studied three family members that have CCTG expansion mutations in the ZNF9 gene on both alleles. These three patients belong to a 47-member sibship originating from Kabul, Afghanistan. During the past 15 years, most family members have settled in Germany. In addition, we studied another six adult members of this sibship with CCTG mutations in the ZNF9 gene on one allele and in another two family members a CCTG mutation was excluded. The two eldest brothers were not investigated; one died in the Afghan war and the other refused any investigation. The onset of the disease was defined as the time when a symptom first became notable to the patient, e.g. myotonia, myalgia, proximal weakness, cataracts, diabetes mellitus or hyperhidrosis. Twenty children younger than 21 years of age participated. All participants (or their legal guardians) gave informed written consent to participate in the study. The study was carried out in agreement with guidelines of the ethics committee of the Ludwig Maximilians University Munich and the Declaration of Helsinki. Generation III will be presented anonymously for sex, age and family structure in this report.

Clinical assessment

Full neurological exams including electromyography (EMG), blood analysis of creatine kinase, glucose, liver enzymes, immunoglobulins, sex and thyroid hormones were performed on 10 members of the Afghan family from generation II. Seven affected family members had slit-lamp examination for cataracts and five had echocardiograms. Additionally, 20 children younger than 21 years of age were neurologically examined. Only in an affected 20-year-old female was EMG and creatine kinase analysis performed.

Neuromaging

The three homozygous DM2 patients underwent a 1.5 Tesla brain MRI (Siemens Symphony) using the standard head coil. T2-weighted multi-echo [TR (repetition time) 5800 ms, TE (echo time) 15/75/135 ms, 2 mm slice thickness] and T1-weighted spin-echo (TR 11.08 ms, TE 4.03 ms, 0.9 mm slice thickness) pulse sequences as well as fluid-attenuated inversion recovery (FLAIR) sequences [TR 9000 ms, TE 110 ms, TI (inversion time) 2500 ms, 6 mm slice thickness] were obtained in the axial and sagittal planes. MRI brain scans, available in the three homozygotes, were compared with reports of heterozygotes given in the literature (Hund et al., 1997; Meola et al., 1999; Kassubek et al., 2003).

Myopathology

Muscle biopsies were taken from the vastus lateralis and biceps brachii of two homozygous females (subject II.14 and subject II.16). Using identical methods (immunohistochemistry and histomorphometry), we compared this histology with reported muscle biopsy findings in heterozygous DM2 patients (Vihola et al., 2003; Schoser et al., 2004).

Muscle cell culture

A part of the vastus lateralis muscle biopsy (subject II.14) was used to establish primary muscle cell cultures according to standardized protocols (Lochmüller et al., 1999). We investigated the expression of the RNA-binding protein muscleblind-like 1 (MBNL1) by an indirect immunofluorescence technique. Briefly, after 5 days of differentiation, myotubes were fixed with 2% paraformaldehyde and 70% ethanol, blocked with 4% BSA (bovine serum albumin) in PBS (phosphate-buffered saline), and stained with an anti-MBNL1 antibody (1:100, Dr M. S. Swanson, Florida) at room temperature. After rinsing for 15 min in PBS, the sections were incubated with horse serum (dilution 1:300, Dr M. S. Swanson, Florida) at room temperature. After rinsing for 15 min in PBS, the sections were incubated with fluorescein rhodamine-conjugated goat anti-mouse serum (Dianova, Hamburg, Germany, dilution 1:100). For the final wash, 4',6-diyanminido-2-phenylindole dihydrochloride (DAPI; dilution 1:1000, Dr M. S. Swanson, Florida) was added to counterstain the myonuclei. After mounting, a Leica epifluorescent microscope with a Zeiss Axiosvert imaging system was used for visualization. For comparison, normal controls, as well as heterozygous DM2 and DM1 myotube cultures were investigated for muscleblind staining.

Genetic assessment

DNA was extracted from white blood cells obtained from 31 members of the Afghan family (members of generations II and III) after written informed consent. All members of generation I had already died. After exclusion of a CTG repeat expansion at the DMPK locus on chromosome 19, we analysed the CCTG tract in intron 1 of the ZNF9 gene (Liquori et al., 2001, 2003). We did not use DNA from tissues other than white blood cells.

Haplotype analysis

DNA marker CL3N58 containing the CCTG repeat stretch was typed as described (Liquori et al., 2001). For amplification of the flanking polymorphic marker CL3N59 (119 kb in the 3' direction of the ZNF9 gene, Liquori et al., 2003), we developed a new primer pair (forward, 5'-AGCTCCGGAGTCCAAGT; reverse, 5'-CCAACCCCTCCTCTTTCAA): ~200 ng of DNA, 0.4 μM of each primer, 0.22 μM of each
dNTP, 1.5 mM MgCl₂, 2 µl of DMSO (dimethylsulfoxide) and 1 U of Taq polymerase (Invitrogen, Karlsruhe, Germany) were added to 25 µl of the supplier’s 1× PCR buffer, annealing temperature 57°C. The high-risk allele of CL3N59 in the German population was subcloned using a TA cloning kit (Invitrogen, Karlsruhe, Germany) and sequenced with a vector primer (four different clones).

**Southern blot**

Equal amounts of DNA were cleaved by restriction enzyme TaqI. Separation of cleaved DNA was carried out on 0.7 % agarose gels in 1× TAE buffer for 18–20 h at 1.2 V/cm. DNA was transferred to Hybond N⁺ membranes (Amersham Biosciences, Freiburg, Germany) and hybridized with the radioactively labelled probe ZNF9-1 kb-HiIII and a surplus of small cut human placental DNA for background suppression (Jakubiczka et al., 2004). Bands were visualized by autoradiography.

**Long-range PCR repeat expansion assay (LR-RA)**

The conditions for the long-range polymerase chain reaction (PCR) were: primer pair forward, 5'-CCA TGC AAA TGT GTC CAT TAA GTT G; reverse, 5'-CGG GCA TGC GCC TGT AAT. The PCR with 200 ng of DNA, 0.4 mM of each primer, 0.22 mM of each dNTP, 1.5 mM MgCl₂, 2.5 µl of DMSO and 2 U of polymerase (Expand long template, Boehringer, Mannheim, Germany or a mixture of Taq/Pfu polymerase 14 : 1) in 25 µl of 1/2 buffer (50 mM KCl, 10 mM Tris–HCl pH 8.4) was run applying the cycler programme: 1 × 96°C/10 s, 1 × 95°C/3.5 min, 31 × (95°C/30 s, 58°C/30 s, 72°C/3.0 min + 15 s increment), 1 × 68°C/18 min. The PCR product was separated on a 0.7% agarose gel with 1/2 TAE buffer for 2.5 h at 3 V/cm. DNA was transferred to Hybond N⁺ membranes (Amersham Biosciences) and hybridized with the radioactively end-labelled oligo 5'-GGC AGG CAG GCA GGC AGG CAG GCA in Church buffer at 65°C. After washing, smears were visualized by autoradiography (2–6 h at room temperature). Hybridization, washing and autoradiography were likewise calibrated to eliminate any smear in healthy control samples (heterozygous for CL3N58).

**Results**

The pedigree of the family from Afghanistan is presented in Fig. 1. The parents of generation II were reported as first-degree cousins. According to family history, the father (subject I.1) had a mild proximal weakness and could not climb stairs. He died at the age of 69 years. The mother (subject I.2) was reported to have a severe proximal weakness with myalgia. She died of a heart attack at the age of 65 years.

**Homozygous phenotype**

**Patient 1**

The oldest homozygous patient (subject II.5) is a 52-year-old woman showing clinically a rather severe DM2 phenotype, but for the clinical course of symptoms within the range seen in some DM2 heterozygotes so far. The first symptoms of proximal leg weakness occurred at age 40 years. Diabetes mellitus and cardiac ventricular arrhythmia with dilatative cardiomyopathy [New York Heart Association (NYHA) III] was detected at age 46 years, and a pacemaker was implanted at age 52 years. Myalgia and bilateral cataracts occurred at age 48 years. Between the age of 49 and 52 years, the patient complained about unintended weight loss of >15 kg. She has moderate frontal

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**Fig. 1** Pedigree and haplotype analysis of the Afghan family. The abbreviated pedigree of the Afghan family generation III is presented anonymously for sex, age and family structure. The two chromosomal segments of each haplotype are shown. A number is assigned to each allele according to the size (largest allele has number 1). 'E' indicates an expanded allele: CL3N58 typing gave no signal; thus a child inherited virtually no allele from the affected parent. Allele 1 from the CL3N59 marker is the rare allele in linkage disequilibrium with the disease comprising 28 CA repeats.
balding, temporal muscle atrophy, ptosis, myalgia, myotonia, proximal weakness of the neck flexors [sternocleidomastoideus, Medical Research Council (MRC) 4/5], elbow extensors (extensor carpi radialis, MRC 4/5), hip flexors (iliopsoas, MRC 3/5) and bilateral posterior iridescent cataracts. She could not get up from the ground in a squatting position. A mild muscle atrophy of the quadriceps femoris is notable. In addition, she describes daytime drowsiness and generalized hyperhydrosis. She has no signs of mental retardation or cognitive changes. Nerve conduction velocities were normal. EMG showed myotonic runs in limb muscles. Creatine kinase levels ranged between 354 and 683 U/l (normal <175 U/l), liver enzymes were elevated [γ-glutamyltransferase (GGT) 310 U/l, aspartate aminotransferase (AST) 50 U/l, alanine aminotransferase (ALT) 29 U/l, lactate dehydrogenase (LDH) 265 U/l], gonadotrophins were elevated [luteinizing hormone (LH) 36.2 U/l, follicle-stimulating hormone (FSH) 34.6 U/l], oestradiol (10 pg/ml) and progesterone (0.4 pg/ml) were decreased, with elevated fasting glucose (140–207 mg/dl), haemoglobin (Hb) A1c (9.4%), and fructosamine (322 μmol/l), and high triglyceride (395 mg/ml) and cholesterol levels (287 mg/dl).

She had no abortions and did not complain about worsening of symptoms, e.g. stiffness, myotonia or weakness, during the course of pregnancies or delivery of her two children (obligatory carrier of the mutation). Her first child, aged 20 years, developed mild proximal weakness (iliopsoas, MRC 4), mild myalgia and hyperhydrosis at age 18 years. EMG and creatine kinase levels were normal. Her younger child, aged 14, was clinically and electrophysiologically investigated and showed completely normal findings. Creatine kinase levels, split-lamp, electrocardiography and echocardiography were also normal.

**Patient 2**

The younger homozygous sister (subject II.14) is a 47-year-old woman showing a milder DM2 phenotype compared with her older sister. Onset of proximal leg weakness began at age 25 years, onset of myalgia and myotonia at age 30 years, and bilateral cataracts were noticed at age 36 years. She has grip myotonia, myalgia, weakness of the neck flexors (sternocleidomastoideus, MRC 4/5), elbow extensors (extensor carpi radialis, MRC 4/5), hip flexors (iliopsoas, MRC 3/5) and bilateral posterior iridescent cataracts. She could not get up from the ground in a squatting position. A mild muscle atrophy of the quadriceps femoris is notable. In addition, she describes daytime drowsiness and generalized hyperhydrosis. She has no signs of mental retardation or cognitive impairment. Her electrocardiographic and echocardiographic examinations were normal. Nerve conduction velocities were normal. EMG showed myotonic runs in limb muscles. Creatine kinase levels ranged between 300 and 450 U/l (normal <174 U/l). There was slight liver enzyme elevation (ALT 39 U/l, AST 59 U/l), high triglyceride 230 mg/ml and cholesterol levels (312 mg/dl), but normal fasting glucose (100 mg/dl), HbA1c (5.5%) and sex hormone levels.

She had no abortions. She reported aggravation of proximal leg weakness and myotonia during the course of her two pregnancies. Due to difficulties during delivery, she underwent a Caesarean section twice.

Her older child, aged 11 years, had normal cognitive and motor milestones. The child only complained about transient exercise-induced proximal myalgia, starting at the age of 10 years. Clinically, the child showed a normal exam. EMG was not performed. Creatine kinase, glucose and GGT levels were normal. The younger child, aged 5 years, revealed normal age-appropriate findings. EMG and other laboratory tests were not performed.

**Heterozygous adults**

All six heterozygous adults present a mild clinical phenotype of DM2 with proximal limb weakness and mildly elevated creatine kinase in the blood, diabetes and hypercholesterolaemia. Clinical myotonia was seen in two, cataracts in three, leg pain in one, and hyperhydrosis in two. None of the heterozygous adult patients had signs and symptoms of cognitive dysfunctions. All family members reached higher academic degrees. None of the heterozygous women reported abortions or difficulties during delivery.

**Children**

Twenty of 24 children, aged 2–21 years, were available for clinical examination. None of these children have signs or symptoms of mental retardation or cognitive impairment. All had normal motor milestone development. None of the children had clinical evidence of disease up to the age of 18 years.

At the age of 40 years, she underwent an extirpation of the uterus that was enlarged by multiple myoma. The unmarried patient has no children.

**Patient 3**

The youngest homozygous sister (subject II.16) is a 37-year-old woman with a mild DM2 phenotype. Onset of proximal leg weakness was at age 25 years, and onset of myotonia and myalgia was at age 30 years. In addition, she describes mild daytime drowsiness for the past 4 years. She has grip myotonia, weakness of the neck flexors (sternocleidomastoideus, MRC 4/5) and hip flexors (iliopsoas, MRC 3/5). She could not get up from the ground in a squatting position. A mild muscle atrophy of the quadriceps femoris is notable. She has neither signs of mental retardation nor cognitive impairment. No cataracts were present. Her electrocardiographic and echocardiographic examinations were normal. Nerve conduction velocities were normal. EMG showed myotonic runs in limb muscles. Creatine kinase levels ranged between 300 and 450 U/l (normal <174 U/l). There was slight liver enzyme elevation (ALT 39 U/l, AST 59 U/l), high triglyceride 230 mg/ml and cholesterol levels (312 mg/dl), but normal fasting glucose (100 mg/dl), HbA1c (5.5%) and sex hormone levels.

She had no abortions. She reported aggravation of proximal leg weakness and myotonia during the course of her two pregnancies. Due to difficulties during delivery, she underwent a Caesarean section twice.

Her older child, aged 11 years, had normal cognitive and motor milestones. The child only complained about transient exercise-induced proximal myalgia, starting at the age of 10 years. Clinically, the child showed a normal exam. EMG was not performed. Creatine kinase, glucose and GGT levels were normal. The younger child, aged 5 years, revealed normal age-appropriate findings. EMG and other laboratory tests were not performed.
Southern blot analysis and repeat assay

Southern blot analysis (Fig. 2) and an LR-RA (Fig. 3) for the family members was carried out for comparison (Udd et al., 2003). The size of the expansion could be estimated in most cases on the Southern, especially if there are discrete expansion bands. The faster LR-RA does not allow reading expansion sizes, but is very helpful when the Southern shows smears only. The specificity of the new LR-RA was controlled by assay calibration as described in Methods. Before establishing the

Fig. 2 Southern blot of genomic DNA cleaved with TaqI. In lanes 1–20, 10 μg of DNA from the family members indicated was run; C = 10 μg of DNA of a healthy control person; c = 5 μg of DNA of the control; M = size standard λ/HindIII. Patients without expansions show about the same intensity of the constant band (1.8 kb) as C; patients having an expansion show the same intensity as c. Only those expansions which can be correctly sized are given: 1 = III.1, 2 = II.5, >23 kb; 3 = III.3, 4 = III.4, 20 kb; 5 = II.7, 6 = II.12, >23 kb; 7 = III.8, 18 kb; 8 = III.9, 9 = II.12, 10 = III.14, 9 kb, 11 = III.15, 20 kb; 12 = III.16, 13 = II.16, >23 kb; 14 = III.17, 7 kb; 15 = II.18, smear; 16 = III.21, 17 = II.20, 18 = II.21, 19 = II.6 (19 = unaffected family member without DM2 mutation), 20 = II.14. Homozygotes are shown in lines 2, 13 and 20 (asterisk).

Fig. 3 Long-range PCR repeat expansion assay (LR-RA) for those family members indicated: 1 = III.1, 2 = II.5, 3 = III.3, 4 = III.4, 5 = II.7, 6 = II.6 (6 = unaffected family member without a DM2 mutation), 7 = II.8, 8 = II.10 (8 = unaffected family member without a DM2 mutation), 9 = II.12, 10 = II.14, 11 = II.16, 12 = II.18, 13 = II.20, 14 = II.21, 15–19 = controls, M = marker λ/HindIII. The band with the lowest molecular weight (~300 bp) is the normal (non-expanded) allele. It is absent in the homozygotes (lines 2, 10 and 11 (asterisk). Additional discrete bands may be due to the special PCR conditions and are partly dependent on the Taq polymerase used in the assay.
LR-RA as a diagnostic tool, the sensitivity was proven by analysing 65 patients from the large German DM2/PROMM pedigrees definitely linked to the ZNF9 locus (Day et al., 2003). There was no contradiction between haplotype and LR-RA results. Furthermore, we never observed any discrepancy between Southern blot results and the assay. In cases with doubtful Southern blot results, the repeat assay was predictive.

**Neuroimaging in homozygotes**

MRI studies of the brain of subject II.5, performed at age 52 years, showed a left parietal subcortical white matter lesion, assumed to be a residual clinically silent lacunar infarct without significant brain atrophy, while subjects II.14 and II.16 revealed normal findings (Fig. 4).

**Muscle biopsy in homozygotes**

A muscle biopsy was taken from the right quadriceps femoris from subject II.14 at the age of 37 years. Histology showed a non-dystrophic myopathic pattern consistent with an increase in internalized myonuclei and pronounced fibre size variability. In addition, pyknotic nuclear clumps and scattered atrophic myofibres were seen. In ATPase stains at different pH values, an atrophy of both fibre types was notable. Highly atrophic myofibres belonged predominantly to type 2 myofibres. Using a monoclonal antibody against myosin heavy chain type 2 confirmed the predominant non-selective type 2 fibre atrophy. Fibre type grouping, necrosis or increase in connective tissue was not detectable. Ringfibres, sarcoplasmic masses, targets and mitochondrial abnormalities were absent (Fig. 5). A second muscle biopsy taken from the biceps brachii muscle of subject II.16 revealed no further aspects.

**Expression of MBNL1 in human myotubes of homozygous DM2, heterozygous DM2 and heterozygous DM1 patients**

To investigate further differences in hetero- and homozygous DM2 muscles, we compared primary myotubes of

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**Fig. 4** Brain MRI of two homozygous DM2 patients. T2-weighted (A and B) and FLAIR (C and D) axial and sagittal cranial MRI in homozygous DM2 patients. MRI scans of patient II.5, performed at age 52 years (A and C) show a left parietal subcortical white matter lesion (clinically silent lacunar infarct residuum, marked by an asterisk) without significant brain atrophy. MRI scans of patient II.14 (B and D) reveal normal findings.
heterozygous DM2 and DM1 patients and one homozygous DM2 patient (subject II.14). Immunofluorescence stains with antibodies against human MBNL1 did not reveal obvious differences in size or count of the marked ribonuclear protein inclusions between hetero- and homozygous DM2 and heterozygous DM1 myotubes (Fig. 6).

Discussion
This study reports for the first time DM2 patients with homozygous DM2 mutations. In a large, consanguineous family originating from Afghanistan, three homozygous and six heterozygous adults for the CCTG repeat expansion were identified and a large group of symptom-free children are reported. The precise mechanism by which (CCTG)n expansion in intron 1 of the ZNF9 gene causes DM2 is not known, although substantial evidence has accumulated indicating that the ribonuclear inclusions in the cell nuclei in DM1 and DM2 cause transdominant splicing alterations in other genes (Philips et al., 1998; Savkur et al., 2001; Charlet et al., 2002; Mankodi et al., 2002). We did not see a large variance in age of onset, pattern of symptoms, disease progression, brain imaging studies, muscle pathology or size of the CCTG expansions. Moreover, no additional clinical symptoms were detected. In addition, in the myotubes which we examined, no obvious large differences in sizes and numbers of ribonuclear inclusions stained for muscleblind between homozygous DM2, heterozygous DM2 and homozygous DM1 patients was found. These results are consistent with a gain-of-function effect of the mutated DM2 allele, and do not argue for haploinsufficiency. Eleven subjects homozygous for DM1 expansions have been reported so far (Cobo et al., 1993; Roeder et al., 1994; Martorell et al., 1996; Murata et al., 1997; Akbas et al., 2001; Abbruzzese et al., 2002). All homozygous DM1 patients were offspring of consanguineous marriages. Clinically, these patients did not differ from heterozygotes in the short term, and no new clinical features have been reported in these patients. In addition, homozygosity in DM1 does not appear to affect
In summary, although there is a high degree of interfamilial variation of the disease manifestation in all repeat expansion disorders, DM1 appeared to be a truly dominant disorder (Zlotogora, 1997). For both DM1 and DM2, it will be important to identify additional homozygous patients and to perform long-term studies to follow the clinical consequences of a homozygous state, and compare the impact of a possibly increased, toxic pre-mRNA production containing CUG and CCUG expansions. Comparably, Huntington’s disease was thought to be true dominant, but recent long-term studies on a cohort of eight homozygous and 75 heterozygous Huntington’s disease patients showed that all homozygous patients have a more severe clinical course and decline in motor, cognitive and behavioural symptoms combined with progressive neurodegeneration (Squitieri et al., 2003).

One of the 11 reported homozygous DM1 patients was reported to have children (Akbas et al., 2001). In contrast to DM2, pregnancy in DM1 may be associated with multiple obstetric complications such as miscarriages, premature onset of labour, polyhydramnios, stillbirths, difficulties during the evacuation, atonic post-partum haemorrhage and anaesthetic accidents. Two of our three women homozygous for DM2 expansions had delivered two healthy children each. There are no reports of multiple obstetric complications such as multiple miscarriages in heterozygous DM2 female patients in this study or in previous reports (Harper, 2001; Day et al., 2003).
Concerning the offspring in heterozygous DM1 patients, there is a correlation between expansion size and clinical severity of the disease, and the expansion tends to increase fatally when transmitted from one generation to the next, e.g. resulting in congenital DM1 or stillbirth (Harper, 2001). In contrast, in DM2, there is no obvious evidence for intergenerational expansions, and no severe juvenile or congenital cases are found. Recently, in two Huntington’s disease patients, expansion mutations were shown to occur before meiosis is completed and were already present in pre-meiotic cells (Yoon et al., 2003). How pre-meiotic expansions arise is not known, but slippages during cell division-dependent DNA replication or repair of DNA lesions induced by tri-/tetranucleotide repeat secondary structures might contribute to human pre-meiotic expansion mutations. This pre-meiotic expansion may also occur between the time of primordial germ cell determination and puberty, but no experimental data are available (Yoon et al., 2003).

In summary, the oldest of the three homozygous DM2 patients we report here appears more severely affected than the average heterozygous DM2 phenotype. No additional disease symptoms or earlier symptom onset were found. Further molecular investigations including these homozygous DM2 patients may help to unravel the enigmatic RNA disease mechanisms in DM2.

Acknowledgements

We wish to thank the patients, their families, and Drs Temor Alam and Thomas Kaufmann for very kind cooperation, Dr Sibylle Jakubiczka for providing the DNA probe, Drs Gerhard Meng and Timo Grimm for discussing the haplotype analysis, Mrs Eva Wiens and Ms Eva Schmidtmeier for expert technical assistance, Dr Maurice S. Swanson, Gainesville, Florida for the kind gift of the MBNL1 antibody, and Dieter Pongratz and Laura P. W. Ranum for helpful advice. This study was supported in part by the German network on muscular dystrophies (MD-NET, 01GM0302) funded by the German Ministry of Education and Research (BMBF, Bonn, Germany). Human myoblast cultures were obtained from the Muscle Tissue Culture Collection at the Friedrich-Baur-Institute (Department of Neurology, Ludwig-Maximilians-University, Munich, Germany). The Muscle Tissue Culture Collection is a partner of Eurobiobank (coordinator: C. Jaeger, Paris, France) funded by the EC within the 5th framework (QLRT-2001-02769).

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