

A new form of congenital muscular dystrophy with joint hyperlaxity maps to 3p23-21

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Congenital muscular dystrophies (CMDs) are a heterogeneous group of disorders. A growing number of CMDs have been found to be associated with joint hyperlaxity. We recruited 14 French–Canadian cases belonging to 11 families affected by a novel autosomal recessive congenital muscular dystrophy with hyperlaxity (CMDH). All cases come from the southwestern part of Quebec, suggesting a new French–Canadian founder effect. All patients present muscle weakness, proximal contractures coexisting with distal joint hyperlaxity. Pathological and genetic studies have excluded that mutations in the three genes coding for collagen VI subunits are responsible for this disease. A genome-wide scan established linkage of two CMDH families to a region on chromosome 3p23-21. Further linkage analysis confirmed that all families are linked to the same region (log of the odds score of 5.3). Haplotype analysis defines a 1.6-cM candidate interval and suggests that two common mutations may account for 78% of carrier chromosomes. This study describes and maps a new form of recessive CMD with joint hyperlaxity distinct from Ullrich and Bethlem myopathies with a founder effect in the French–Canadian population.

Keywords: congenital muscular dystrophy; hyperlaxity; linkage; family study

Abbreviations: CMD = congenital muscular dystrophy; CMDH = congenital muscular dystrophy with hyperlaxity; H & E = haematoxylin and eosin; LOD = log of the odds; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; SNP = single nucleotide polymorphism; UCMD = Ullrich congenital muscular dystrophy

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Introduction

Congenital muscular dystrophies (CMD) form a heterogeneous group of disorders characterized by congenital hypotonia, muscular weakness, joint contractures and myopathic or dystrophic changes on muscle pathology (Lampe and Bushby, 2005). To date the combination of contractures and distal joint hyperlaxity has been observed mostly in Ullrich CMD (UCMD) and to a lesser extent in Bethlem myopathy (Lampe and Bushby, 2005). In genetically proven cases of UCMD, hypotonia, hip dislocation, delayed motor milestones, protruded calcaneus and early respiratory failure are usually observed. Some of the less severe UCMD cases may learn to walk but they will lose the ability in their first decade of life (Mercuri *et al.*, 2002; Lampe and Bushby,

2005). In the milder autosomal dominant Bethlem myopathy (BM) joint hyperlaxity is only a minor feature (Lampe and Bushby, 2005). These two conditions were found to be allelic, with mutations in the genes coding for the three subunits of collagen VI: COL6A1/A2 on chromosome 21q22.3 and COL6A3 on chromosome 2q37 (Lampe and Bushby, 2005). The collagen VI protein is a major component of the extracellular matrix (Lampe and Bushby, 2005). Complete loss or reduction of collagen VI due to collagen VI gene mutations has been associated with these disorders (Ishikawa *et al.*, 2004). Over 60 different mutations in all three subunits have been reported in previous studies (Lampe and Bushby, 2005). Recently, cases of CMD with joint hyperlaxity

with preservation of collagen VI in muscle and absence of COL6A1, A2 or A3 mutations have been described (Mercuri *et al.*, 2002; Ishikawa *et al.*, 2004; Baker *et al.*, 2005; Mercuri and Longman, 2005). These observations have led some to suggest that other CMD with hyperlaxity, distinct from UCMD or BM, have yet to be described and their mutated genes identified (Mercuri *et al.*, 2002; Ishikawa *et al.*, 2004; Baker *et al.*, 2005; Mercuri and Longman, 2005). This paper describes a group of French–Canadian cases suffering from a novel CMD with joint hyperlaxity with a milder phenotype than UCMD. This relatively homogeneous cohort further allowed the mapping of the mutated gene to chromosome 3p23–21.3.

Subjects and methods

Clinical evaluation and DNA isolation

We have identified 14 affected individuals from 11 different Quebec kindreds (Fig. 1) presenting a phenotype resembling UCMD. All probands and family members underwent a detailed neurological examination by experienced neurologists. This project was approved by the institutional Ethics Committee of the Centre de recherche du CHUM. Informed consent was obtained from all patients and participating living family members. Genomic DNA was extracted from peripheral blood lymphocytes using a standard method.

Collagen VI gene exclusion by immunohistochemistry, linkage and sequencing

Muscle biopsies were performed using standard techniques on three adult patients (Patients 6, 7 and 11). Standard histochemistry and immunohistochemistry was performed (Carpenter and Karpati, 2001), including immunostaining with the 5C6 anti-collagen VI antibody (Hybridoma Bank, University of Iowa, Iowa) (Freitas *et al.*, 2005). The human muscle for the immunolocalization of collagen VI was purified in acetone and then washed

in phosphate-buffered saline (PBS). The tissues were incubated with the 5C6 anti-collagen VI diluted 1 : 20 for an hour and then with an anti-mouse diluted 1 : 100 as secondary antibody for an hour. Thirty minutes incubation with a Cy3 reagent followed to detect bound antibody. Primary myoblasts were grown from deltoid biopsy samples of Patients 6 (family F), 7 (family G) and 11 (family I) in SKBM complete medium (Cambrex, East Rutherford, NJ, USA) with 10% foetal bovine serum in 5% CO₂ at 37°C. Cultured muscle myoblasts from patients and controls were grown in 24-well chambers on glass slides and were treated with 0.25 mM L-ascorbic acid for 5 days. Samples were fixed with cold paraformaldehyde 4% at room temperature for 10 min. The cell membranes were permeabilized with cold methanol and then blocked for 30 min in PBS–BSA (bovine serum albumin) 3%. The samples were incubated with anti-collagen VI monoclonal antibodies (MAB3303, MAB1944) (Chemicon, Temecula, CA, USA) diluted 1 : 100 in PBS containing 3% BSA for 1 h at room temperature. Then the samples were incubated with an anti-mouse antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) diluted 1 : 200 in PBS 0.05% Tween for 45 min. All the samples were incubated 10 min with a Hoechst solution (Molecular Probes, OR, USA) for nuclear labelling. The antibody VIA4-1 against the glycosylated form of alpha-dystroglycan (Upstate, Charlottesville, VA) was used for the immunostaining on cryostat sections of muscles from three cases (Patients 6, 7 and 11).

The linkage exclusion of the three collagen VI genes was performed using primer sequences of polymorphic markers obtained from deCODE and Marshfield genetic maps [UCSC (<http://genome.ucsc.edu>, May 2004 assembly)]. Polymerase chain reactions (PCRs) were performed using 40 ng genomic DNA in 10- l PCR reactions containing 1  PCR reaction buffer, 3 nM MgCl₂, 10  M primer mix and 0.4 U Taq DNA polymerase (Invitrogen, Burlington, ON, Canada). Amplification conditions were obtained from the genome database (www.gdb.org). PCR product sizing was performed by adding 4  l of STOP loading buffer to each sample, followed by a denaturing step of 7 min at 95°C and the loading of 2  l onto a 64-lane 6% acrylamide gel containing 6 M urea. Data acquisition and analyses were achieved on the LiCOR 4100 automated DNA sequencer using BasemagIR v.4.0 software (Li-COR, ON, Canada). Two-point linkage analysis was performed using FASTLINK v. 2.0.

Screening of reported mutations in the three collagen VI genes and single nucleotide polymorphism (SNP) genotyping was performed by genomic sequencing of exons. Sequencing of COL6A3 was performed as follows: total RNA was isolated from myoblasts using Trizol (Invitrogen, Carlsbad, CA, USA). The samples were treated with M-MLV reverse transcriptase (Invitrogen) to obtain five overlapping cDNAs. The specific primers used for the RT–PCR were 5' CAGGGGCTTCATT-TTCCGCACAG3', 5' CGCCGGGACGACCACCTCAT3', 5' CTGGT-CCCCTGCTCTCCCTCAAAG3', 5' CCGCCACTGGGGGTCTAAC3' and 5' GGTCCCAACGGTGCACATAGATTA3'. The resulting cDNAs were used as a template for PCR amplification of the entire coding region of COL6A3 mRNA transcripts with Amplitaq polymerase (Applied Biosystems, Foster City, CA, USA). RT–PCR products were sequenced on both strands at the Genome Quebec Innovation Center, McGill University. Polymorphisms were confirmed on genomic DNA of the entire cohort by amplification and direct sequencing of the specific regions. Fragments were amplified using the same amplification mix as for genotyping. PCR primers were designed using PrimerSelect 4.03 (DNASTAR) and synthesized by Invitrogen (Burlington, ON, Canada). Sequences were aligned using SeqMan 4.03

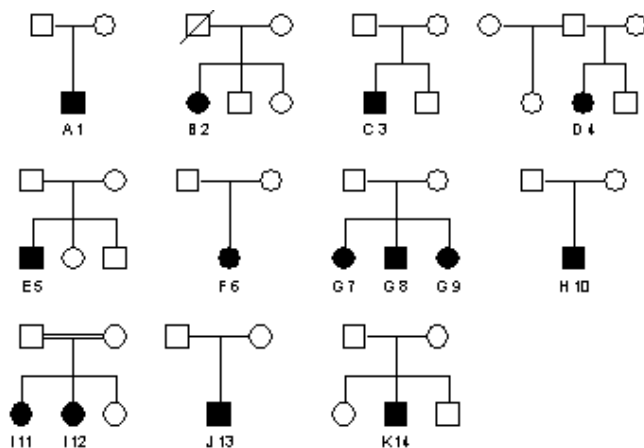


Fig. 1 Pedigrees of the CMDH cohort. We identified 14 patients (7 males and 7 females) belonging to 11 families. Parents of Patients 11 and 12 (family I) are third-degree cousins.

(DNASStar, Wisconsin, USA) and analysed using Chromas 1.62 (Technelysium Pty Ltd, Australia).

Genome scan and linkage analysis

A genome-wide scan with 500 markers was conducted at deCODE Genetics (Reykjavik, Iceland) on 10 participants from families G and I for the identification of a new locus. Fine mapping was performed by typing microsatellite markers from the region (<http://genome.ucsc.edu>, May 2004 assembly). PCR reaction was performed as described for collagen VI genes. Multipoint linkage analysis was performed using GENEHUNTER v.2.1. Marker order and genetic distances were based on the deCODE genetic map and UCSC physical map (<http://genome.ucsc.edu>, May 2004 assembly). For the linkage analyses allele frequencies were assumed to be equal. The congenital muscular dystrophy with hyperlaxity (CMDH) phenotype was analysed as an autosomal recessive trait with 100% penetrance and with an estimated disease gene frequency of 0.001. No phenocopies were incorporated into the analysis.

Results

The French–Canadian CMDH cluster

A total of 14 patients belonging to 11 families with a phenotype resembling a milder form of UCMD were recruited (Fig. 1). On the basis of the review of the 36 French–Canadian cases of CMD followed at the Neuromuscular Clinic of the Marie-Enfant Rehabilitation Center of the Sainte-Justine Hospital in Montreal during the past 20 years, this new CMDH phenotype would account for 45% of all cases. Therefore, CMDH would be the most frequent CMD in French–Canadians, which strongly suggests the existence of a founder effect for CMDH mutations in this population. All recruited families come from the southwestern part of the Province of Quebec, further supporting the presence of a regional founder effect. Segregation in pedigrees strongly suggests an autosomal recessive mode of inheritance (Fig. 1). None of the parents have a muscular dystrophy, though many have distal joint laxity. Furthermore, the child of Case 11 (Family I) is not affected. Only the parents of family I are consanguineous (i.e. third-degree cousins).

The initial recruitment was performed thinking that they were cases of UCMD. Therefore, the UCMD GENRE clinical features were assessed in all cases (Dubowitz, 1997; Freitas *et al.*, 2005). All were hypotonic with contractures at birth. They demonstrated a generalized slowly progressive muscle weakness accompanied by distal joint laxity and proximal contractures (Table 1). Joint laxity is mainly observed at the fingers (93%), wrists (43%) and toes (43%). In our cohort, we also observed proximal laxity of the elbows in some patients (43%). The contractures are mostly present at the ankle (71%), knee (21%) and shoulder (21%) (Fig. 2A–C). Rigidity of the spine was not observed, while important cervical spine hypermobility is frequently observed. A long myopathic face is rarely observed. Intelligence is normal or only mildly impaired. Creatine phosphokinase (CPK) levels are normal to mildly elevated (range: 17–959 U/l). Pulmonary vital capacity in our cohort was usually diminished on average by 50% (range: 21–100%), but seemed to be stable through decades. This probably explains the prolonged life expectancy. Unlike in UCMD and many other CMDs described to date (Jimenez-Mallebrera *et al.*, 2005), respiratory failure in CMDH appears not to be a problem despite the usual abnormal pulmonary function. All three adult cases tested had normal ECG and cardiac ultrasounds (Patients 2, 3 and 6). All cases learned to walk between 14 months and 3 years of age (Table 1). Three of our patients became wheelchair-bound: Patient 2 (Family B) at the age of 28, Patient 6 (Family F) at the age of 10 and Patient 11 (Family I) at the age of 32. For the latter it followed a car accident and a prolonged period in an intensive care bed during which she developed severe proximal contractures of the hips and lumbosacral spine. Scoliosis, though a frequent feature (64%), is not found in all cases and varies from mild to severe (Fig. 2D). Of the 11 cases older than 18, only 2 (14%) were operated for their scoliosis. Other distinctive features from UCMD are the absence of high arched palate, torticollis or protruded calcaneus. Together the shared clinical features of these cases define a new type of CMD with joint hyperlaxity distinct from other recessive CMDs and milder than molecularly confirmed cases of UCMD.

Table 1 Clinical data on the 14 CMDH patients

Case number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Family	A	B	C	D	E	F	G	G	G	H	I	I	J	K
Sexe	male	female	male	female	male	female	female	male	female	male	female	female	male	male
Age at diagnosis	2	4	6	3	6	1	5	5	5	3	4	2	0	7
Age in 2005	16	40	28	29	18	22	24	17	19	10	39	24	19	35
Distal laxity	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Contractures	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CNS involvement	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Scoliosis	++	++	++	++	–	+	+	–	+	+	+	–	+	–
Ability to walk	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lost ability to walk (age)	–	28	–	–	–	10	–	–	–	–	32	–	–	–
Abnormal pulm. f.	+	+	+	+	+	+	+	+	+	+	+	+	+	–
% VC at age	30	59	50	93	69	21	53	77	66	100	63	86	38	NA

VC = Vital Capacity; 0 = Birth; NA = Not available.



Fig. 2 Clinical features of the CMDH patients. **(A)** Ankle and toe contractures (Patient 6, family F). **(B)** Finger hyperlaxity (Patient 6, family F). **(C)** Wrist hyperlaxity (Patient 6, family F). **(D)** Severe previously operated scoliosis (Patient 4, family D).

Absence of deficiency or mutations of collagen VI

The review of pathological reports of old muscle biopsies performed since the 1970s in four different institutions confirmed the presence of variable muscular abnormalities in all cases. To better characterize our cohort, Cases 6 with the most severe phenotype (family F) and two belonging to the most informative families (Case 7 from family G and 11 from family I) had new deltoid biopsies. No shared structural abnormality or homogeneous histopathology was observed, though all biopsies showed variation in fibre sizes, the presence of central nuclei and increased endomysial connective tissue. In particular, no hyaline bodies were observed (Onengut *et al.*, 2004). In the most affected Case 6, aged 21 at the time of the biopsy, there is fibre size variability and in certain moderate-sized areas fibres are replaced by adipose tissue (Fig. 3A). In numerous muscle fibres, there was a single, large centrally situated nucleus (Fig. 3A, arrows) surrounded, on oxidative enzyme-stained specimens, either by a diformazan-free zone or by conspicuous peculiarly shaped diformazan deposits (Fig. 3B, arrow). The overall myopathological pattern is reminiscent of an atypical centronuclear myopathy. In Case 11, aged 38, fibre size variability was also observed with the presence of markedly atrophic angular or rounded fibres as well as moderately hypertrophied fibres (Fig. 3C and D). Loss of muscle fibres was marked by islands of adipocytes (Fig. 3C). Some muscle fibres contain normal appearing central myonuclei. Several scattered muscle fibres harbour small rimmed vacuoles (Fig. 3D), which on electron microscopy were found to contain whorls of cytomembranes and heterogeneous cell debris (data not shown). There was a marked numerical preponderance of the histochemical type

1 fibres (Fig. 3E). In the least affected Case 7, at age 23 her biopsy only showed scattered atrophic polygonal fibres and rare normal size central myonuclei (data not shown). Immunostaining for the glycosylated form of alpha-dystroglycan was performed for three patients and three controls. No deficiency was observed (data not shown). The differences in histopathology between the three cases seem to correlate with the severity of the clinical presentations and, as we show below, may correspond to differences in genotypes.

Most cases of recessive CMD associated with distal joint hyperlaxity have been found to be caused by recessive mutations and only rarely dominant mutations (Zhang *et al.*, 2002; Pan *et al.*, 2003; Jimenez-Mallebrera *et al.*, 2005), in one of the three genes coding for the subunits of collagen VI (Lampe and Bushby, 2005). These patients have been diagnosed as affected by UCMD. However, cases with overlapping phenotypes have clearly been described as not being caused by mutations in these genes (Ishikawa *et al.*, 2004; Baker *et al.*, 2005; Mercuri *et al.*, 2005). Since the initial presumptive diagnosis of our cases was UCMD, we studied the presence of collagen VI on muscle biopsies, and completed linkage analyses and searched for COLVI mutations. In mutation-proven UCMD cases, a complete or partial deficiency of the collagen VI protein is observed in muscle (Lampe and Bushby, 2005). Presence of collagen VI was observed by immunostaining in all three biopsies (Fig. 3F on Case 11) and in cultures of myoblasts from the same patients (data not shown). None of the mutations previously reported in the genes coding for the three subunits of collagen VI were uncovered by genomic sequencing of all cases. Genotyping analysis of microsatellite markers flanking the COL6A1 and COL6A2 genes located on chromosome 21 was performed. Linkage of our families to these markers was excluded [log of the odds (LOD) score ≤ -2 for markers D21S1255, D21S1893 and one intragenic marker to COL6A2 (data not shown)]. Linkage analysis with markers in close proximity to the COL6A3 gene did not allow such exclusion. We therefore decided to sequence the entire coding region of this gene. The sequencing by RT-PCR of the entire gene from three of our cases did not uncover any mutations (Patients 6, 7 and 11). Together these results suggest that our cases share a distinct milder CMD with joint hyperlaxity phenotype that is caused by mutations in another gene.

Mapping of the CMDH locus

Ten DNA samples from affected and unaffected participants belonging to two unrelated CMDH families (families G and I) were sent to deCODE genetics (Reykjavik, Iceland) for a genome-wide scan (GWS). Genealogical data confirmed that parents in family I were third-degree cousins, thereby increasing the odds that Cases 11 and 12 be homozygous at the disease locus. Genotypes were generated for 500 polymorphic microsatellite markers separated on average by 7 cM. Multipoint autosomal recessive parametric linkage

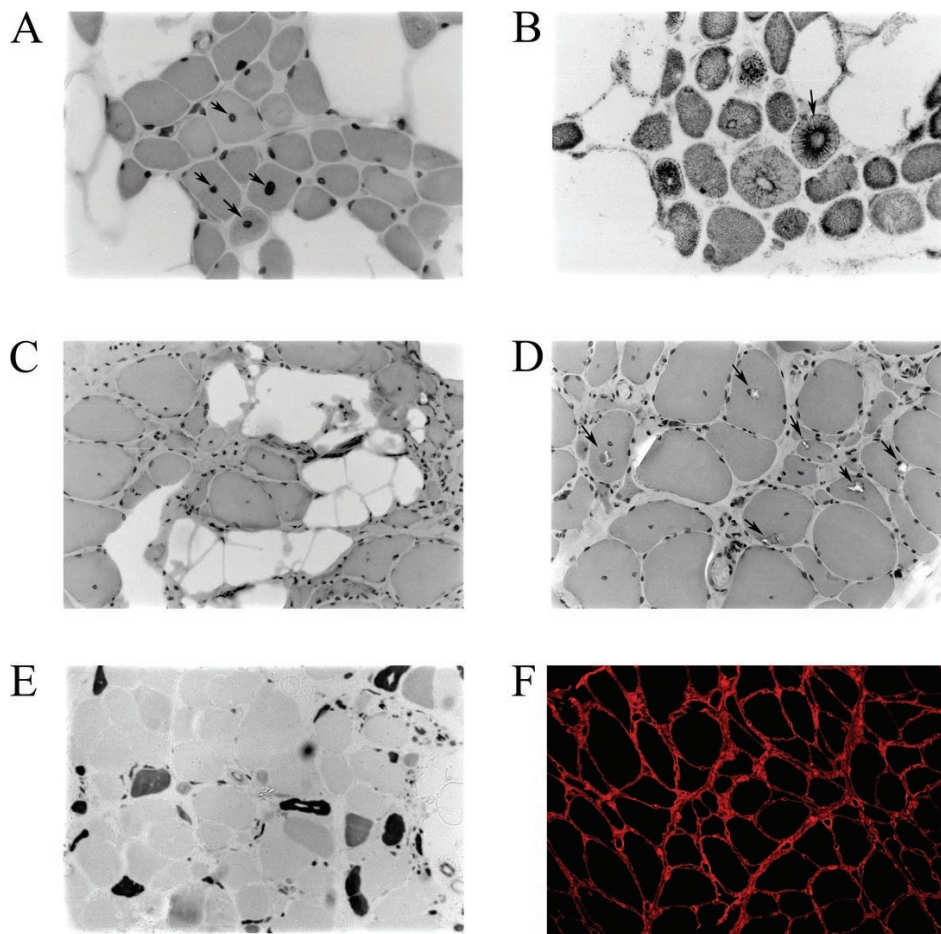


Fig. 3 Pathological features in two adult cases of CMDH: Patient 6 (**A, B**) and Patient 11 (**C, D, E, F**). (**A**) Several muscle fibres have large centrally situated myonuclei (arrows). There is appreciable variability in fibre cross-sectional area. There is regional replacement of fibres by adipose tissue. [haematoxylin and eosin (H & E), 350 \times] (**B**) Several muscle fibres show clear areas corresponding to central nuclei. Central myonuclei are surrounded by diformazan, which sometimes gives a pattern reminiscent of spokewheels (arrow) (NADH tetrazolium reductase, 350 \times). (**C**) Islands of muscle fibres are lost and replaced by adipocytes (H & E). (**D**) Scattered atrophic angular or rounded fibres are mixed with normal-sized and hypertrophied fibres. Several muscle fibres (arrows) have typical rimmed vacuoles. Endomysial connective tissue is increased in some areas. A few muscle fibres have centrally situated myonuclei (H & E, 350 \times). (**E**) The vast majority of muscle fibres are of histochemical type I. The remaining few type II fibres are atrophic (myofibrillar ATPase, preincubation pH 10.2, 350 \times). (**F**) Collagen VI immunostaining demonstrating the presence of the protein in the patient muscle.

was computed using Genehunter v.2.1. Haplotypes were reconstructed in a single section using the MAXPROB method of Genehunter v.2.1. The resulting haplotypes were imported in Cyrillic v.2.0. Multipoint LOD scores >2 was obtained for one locus. A multipoint LOD score of 2.5 was obtained at that locus. The allele homozygosity for six markers between D3S2385 and D3S3521 (32 cM) observed for the two cases of family I further supported this locus. Genotyping of the entire 11 families with 40 polymorphic markers, spanning 34 cM (38 Mb) confirmed linkage of these to a 5-cM (6 Mb) region on chromosome 3p23-21.3 (D3S1768-D3S3522). Maximum multipoint LOD score value of 5.3 was obtained for marker D3S2417 (Fig. 4). As shown in Table 2, allele and haplotype sharing suggest that few CMDH mutation-carrying chromosomes are present in our cohort, with two more common explaining 78% of the chromosomes. The three biopsied cases, though they all carry

one copy of the most common carrier chromosome, differ as to their other chromosome. This suggests that the variability observed in phenotypes and pathology may be explained by different mutations. Only the identification of the CMDH mutations will settle this issue. Our recent experience of the study of rarer French-Canadian regional founder effect diseases suggests that we should expect more than one mutation (Duquette *et al.*, 2005; Roddier *et al.*, 2005). By looking at the more common chromosome, the haplotype analysis suggests five putative historical recombinations at marker D3S3639, making marker D3S3639 the centromeric flanking marker (Table 2). On the telomeric side, we observed for the same common chromosome three putative ancestral recombinations at marker D3S1611. This marker becomes the telomeric flanking marker. This defines a 1.6-cM (1.3 Mb) candidate interval based on the Marshfield genetic map and UCSC (<http://genome.ucsc.edu>) (Table 2).

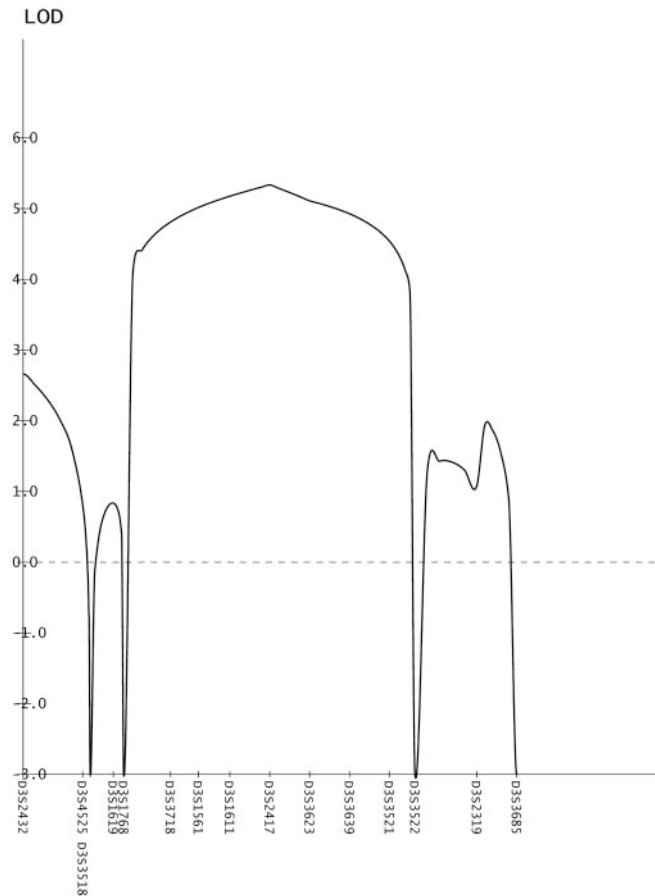


Fig. 4 Linkage analysis. Multipoint LOD scores for chromosome 3 markers defining a 5-cM candidate interval (maximum LOD score of 5.3).

Conclusion

CMDH: a disorder distinct from UCMD

This paper describes a cohort of French–Canadian patients with a novel recessive congenital muscular dystrophy with joint hyperlaxity (CMDH) with clinical overlap with UCMD. We chose not to refer to it as a congenital myopathy because of its clear evolution and the absence of specific morphological findings despite its more favourable prognosis than most CMDS described to date. All cases clearly have a different and milder CMD phenotype than classical UCMD. They share with UCMD the presence of congenital hypotonia, weakness, contractures, distal joint hyperlaxity, scoliosis, normal intelligence and frequent delayed motor milestones (Jimenez-Mallebrera *et al.*, 2005). However, certain clinical features are clearly different from UCMD: their strength is better preserved, they may have concomitant distal and proximal hyperlaxity, they do not have protruding calcani, they all acquire the ability to walk, most continue to walk in adulthood and they do not develop respiratory failure. The apparent stability of their pulmonary function, despite their frequent scoliosis, probably explains in large part their prolonged life expectancy. Case 2, the oldest now aged 40, can still stand from her wheelchair and walk a few steps. The

recessive mode of transmission and the more severe phenotype of the CMDH patients help distinguish it from Bethlem myopathy (Lampe and Bushby, 2005). The absence of rigid spine on examination or need for assisted ventilation tells it apart from rigid spine syndrome (Mercuri *et al.*, 2002). The normal intelligence and absence of central nervous pathology help distinguish it from the numerous other forms of CMD associated with mental retardation such as merosin-deficient or Fukuyama CMD. The absence of skin hyperelasticity or other skin abnormality combined with the predominant muscle weakness excludes the diagnoses of connective tissue disorders such as Ehlers–Danlos or Marfan syndromes (Lampe and Bushby, 2005). The absence of hyaline bodies in muscle and the presence of important contractures and joint hyperlaxity allow its distinction from the hyaline body myopathy that was also mapped to chromosome 3p in a Turkish family (Onengut *et al.*, 2004).

A growing number of cases of CMD with UCMD-like phenotypes, preservation of collagen VI in muscle and absence of collagen VI subunit mutations are being reported (Mercuri *et al.*, 2002; Ishikawa *et al.*, 2004; Baker *et al.*, 2005; Mercuri and Longman, 2005). Most of these cases have more severe phenotypes than the French–Canadian cases described in this paper. They may have protruding calcani (Ishikawa *et al.*, 2004), many never achieve independent ambulation (Mercuri *et al.*, 2002; Ishikawa *et al.*, 2004) and some have severe restricted respiratory function (Ishikawa *et al.*, 2004; Mercuri *et al.*, 2004; Baker *et al.*, 2005), short stature (Ishikawa *et al.*, 2004; Mercuri *et al.*, 2004) or intellectual impairment (Ishikawa *et al.*, 2004; Mercuri *et al.*, 2004). However, some older cases with positive collagen VI staining appear to have a milder form of CMD very reminiscent of CMDH (Mercuri *et al.*, 2002). Though the 5-cM candidate interval defined by the multipoint LOD scores slightly overlaps with the autosomal recessive hyaline body myopathy locus (Onengut *et al.*, 2004), the smaller 1.6-cM haplotype-defined CMDH interval is clearly telomeric. Furthermore, no hyaline bodies were observed in the three recent biopsies. The shared French–Canadian background of our patients further supports the fact that they are affected by a new form of CMD with a founder effect in this population that is well known for the higher prevalence of certain recessive disorders (Laberge *et al.*, 2005). The clinical and pathological differences between these diseases and the mapping of our families to an original chromosome 3p23–21.3 locus further supports the fact that CMDH is a distinct form of the growing number of CMD associated with joint hyperlaxity.

CMDH's 3p23–21.3 candidate region

Though >40 genes are present in the large 5-cM (6 Mb) and 16 in the 1.6-cM CMDH candidate regions, three stand out as excellent candidates: ITGA9, LAMR1 and ACVR2B. ITGA9 and ACVR2B lie in the smaller haplotype-defined CMDH region. All three genes could be involved in normal

Table 2 Haplotype results of CMDH carrier chromosomes in 11 families for 3p23-21 markers

Marker	D3S1619	D3S1768	D3S3718	D3S1561	D3S1611	D3S2417	D3S3623	D3S3639	D3S3521	D3S3522	D3S2319	D3S3685	D3S3687	D3S3624
deCODE*	59,79	60,05	61,22	61,92	62,71	62,73	62,73	66,4	66,4	64,99	66,54	68,77	68,77	68,77
Mb*	34,09	34,6	36,14	36,46	37,04	37,41	37,42	38,37	38,84	40,76	42,36	42,44	44,58	44,58
Family	Patient													
A	1	3	4	7	3	5	2	3	2	3	3	5	2	0
A	1	2	2	7	3	5	2	5	3	3	3	12	3	0
A	2	5	1	2	3	5	1	3	3	3	1	6	2	6
B	2	4	2	2	3	5	1	5	0	3	1	8	2	3
B	2	3	2	7	3	5	1	5	0	3	1	8	2	3
C	3	4	5	5	2	5	3	5	4	3	3	8	2	5
C	3	5	2	7	5	2	3	1	1	4	1	10	3	3
D	4	5	2	2	1	5	1	4	4	1	3	7	1	5
D	4	5	2	4	4	5	1	5	1	2	3	10	2	5
D	4	5	2	4	3	5	1	4	3	2	3	7	2	3
E	5	4	2	7	3	5	2	4	3	2	3	7	2	3
E	5	4	2	4	3	5	2	5	3	3	3	12	2	4
F	6	2	2	2	3	5	1	5	3	2	2	12	3	4
F	6	3	4	7	2	2	3	2	5	3	3	12	2	4
F	6	4	4	7	2	2	3	2	5	3	3	12	2	4
G	7-8-9	5	4	7	2	5	2	4	1	2	3	5	2	4
G	7-8-9	6	3	2	3	5	1	4	5	3	1	6	2	3
H	10	5	2	2	2	6	4	2	3	2	3	10	2	0
H	10	3	4	2	3	1	3	5	4	2	3	1	2	0
I	11-12	4	4	2	2	5	1	4	5	2	3	9	2	4
I	11-12	4	4	2	3	5	1	4	5	2	3	12	3	3
J	15	3	3	7	3	5	2	5	6	0	3	5	2	3
J	15	3	2	7	3	3	2	5	1	0	3	12	2	3
K	16	3	4	4	2	5	1	4	0	2	3	9	2	2
K	16	4	4	7	3	5	2	5	0	3	3	6	2	2

*genome.ucsc.edu/

extracellular matrix and basal lamina integrity. Integrins are known to mediate cell–cell and cell–matrix adhesion. ITGA9 is highly expressed in lung and muscle and forms heterodimers with integrin beta 1 (ITGB1) (Palmer *et al.*, 1993). ITGB1 bound to integrin alpha 7 (ITGA7) known to be mutated in a congenital myopathy (Mayer *et al.*, 1997). Unfortunately, the clinical description of the original ITGA7 cases is limited and none of the cases seemed to have joint hyperlaxity. Mutations in ITGA9 could interfere with normal basal lamina function and lead to abnormal anchorage to the interstitium as was observed in Japanese Ullrich-like cases with no collagen VI mutations (Ishikawa *et al.*, 2004). Another candidate is the laminin receptor 1 (LAMR1), a member of the large extracellular matrix glycoproteins. The heterodimeric structure is similar to other extracellular matrix receptors like fibronectin and vitronectin (Gehlsen *et al.*, 1988). The activin A IIB receptor (ACVR2B), a dimeric growth and differentiation factor that belongs to the transforming growth factor beta (TGF ) superfamily, is also a promising candidate. The expression of TGF  is known to be dysregulated in Marfan syndrome cases, and the TGF R2 gene is mutated in the Marfan syndrome type II (Mizuguchi *et al.*, 2004; Dietz *et al.*, 2005). Joint hyperlaxity is observed in these diseases, making ACVR2B an interesting candidate gene for CMDH. Further narrowing of the candidate interval by the recruitment of other families will help the uncovering of the CMDH gene. In this report, we describe the clinical features and mapping of a large French–Canadian cohort affected by a novel autosomal recessive CMDH. Mutation in the CMDH gene may also be found in some of the growing number of cases with an Ullrich-like phenotype that do not carry mutations in the collagen VI genes. CMDH expands the growing spectrum of CMDs associated with joint hyperlaxity.

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