METHYLATION OF THE CPG SITES IN THE MYOTONIC DYSTROPHY LOCUS DOES NOT CORRELATE WITH CTG EXPANSION SIZE OR WITH THE CONGENITAL FORM OF THE DISEASE

Claudia Spits, Sara Seneca, Pierre Hilven, Inge Liebaers, Karen Sermon

► To cite this version:

Claudia Spits, Sara Seneca, Pierre Hilven, Inge Liebaers, Karen Sermon. METHYLATION OF THE CPG SITES IN THE MYOTONIC DYSTROPHY LOCUS DOES NOT CORRELATE WITH CTG EXPANSION SIZE OR WITH THE CONGENITAL FORM OF THE DISEASE. *Journal of Medical Genetics*, 2010, 47 (10), pp.700. 10.1136/jmg.2009.074211 . hal-00557380

HAL Id: hal-00557380

<https://hal.science/hal-00557380>

Submitted on 19 Jan 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

METHYLATION OF THE CPG SITES IN THE MYOTONIC
DYSTROPHY LOCUS DOES NOT CORRELATE WITH CTG
EXPANSION SIZE OR WITH THE CONGENITAL FORM OF THE
DISEASE.

Claudia Spits¹, Sara Seneca², Pierre Hilven¹, Inge Liebaers^{1,2}, Karen Sermon¹

¹ Department of Embryology and Genetics, Vrije Universiteit Brussel (VUB),
Laarbeeklaan 101, 1090, Brussels, Belgium

² Centre for Medical Genetics, Universitair Ziekenhuis Brussel (UZ Brussel),
Laarbeeklaan 101, 1090, Brussels, Belgium

Corresponding author: Claudia Spits, Department of Embryology and Genetics, Vrije
Universiteit Brussel (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium. Tel 32 2 477 46
34, Fax 32 2 477 66 60, e-mail claudia.spits@uzbrussel.be

ABSTRACT

We have studied the methylation status of the sequence 152 nucleotides upstream of
the CTG repeat of the DM1 locus in patients' peripheral blood. We used the methylation-
sensitive endonucleases SacII, HpaII and HhaI, followed by PCR. This allowed to
correlate the methylation status of each CTG allele with its size. Contrary to previous
findings (Steinbach P et al., 1988, Am.J.Hum.Genet. 62:278-285), only the SacII site is
often but not always differentially methylated amongst expanded CTG alleles.
Importantly, this methylation was not restricted to congenital DM1, nor to large
expansions, as it was also present in DM1 patients with a classical phenotype and various
expansion sizes. On the other hand, we did not find any methylated alleles on the HhaI

27 and HpaII sites, as was reported by Steinbach et al, which is in line with the results of
28 Shaw and collaborators (1993, J.Med.Genet. 30:189-192). The size range of the repeat
29 expansions with methylation was from as small as 300 to as large as 2800 repeats.
30

31 REPORT

32 Myotonic dystrophy type 1 (DM1, [MIM 160900]) is a dominantly inherited
33 multisystemic disorder, with an incidence of approximately 1 in 8000 individuals. DM1
34 has a complex phenotype, with symptoms including progressive skeletal muscle wasting,
35 impaired muscle relaxation (myotonia), cardiac conduction defects resulting in
36 arrhythmias, early-onset iridescent cataracts, insulin resistance and hyperinsulinemia
37 (Harper, 2001). These symptoms are caused by several mechanisms, which include the
38 aberrant processing of the *DMPK* mRNA, leading to decreased levels of DMPK (Krahe et
39 al., 1995), toxic gain of function of the mRNA (Mankodi et al., 2000), decreased
40 expression of the neighbouring *SIX5* gene (Klesert et al., 1997; Thornton et al., 1997)
41 and disrupted alternative splicing of numerous other genes (Ranum and Cooper, 2006).

42 DM1 is caused by a CTG expansion in the 3' untranslated region of the *DMPK* gene
43 located on chromosome 19q13.3. Expanded CTG tracts show both somatic and germinal
44 instability; unaffected individuals have from 5 to 40 CTG repeats, whereas individuals
45 with the disease carry more than 100 repeats. Disease severity and age of onset
46 correlate with repeat size, and expansions of >1500 repeats often result in a severe
47 congenital form of DM1.

48 CpG methylation has been suggested to play a role in the stability of repetitive
49 sequences in general (Gourdon et al., 1997, Nichol and Pearson, 2002), and more
50 particularly in the behaviour of the CTG repeat in the DM1 locus (Steinbach et al., 1998,
51 Brock et al., 1999, Gorbunova et al., 2004) as well as in the pathogenesis of the disease
52 (Shaw et al., 1993, Steinbach et al., 1998, Filippova et al., 2001).

53 The relationship between the semiology of the disease and the methylation of the CpG
54 sites in the DM1 locus was first investigated by Shaw and collaborators (1993), who

could not find any clear correlation between the methylation and the age of onset or the severity of the disease. On the other hand, Steinbach and coworkers found that several CpG sites close to the CTG repeat were fully methylated in blood samples containing expansions larger than 1000 repeats. They also reported that, since these expansions appeared as single well-defined signals on Southern blot, the methylation of these CpG sites would correlate with triplet repeat stability. This idea was later supported by *in vitro* and *in silico* work (Brock et al., 1999, Gorbunova et al., 2004, Gourdon et al., 1997, Nichol and Pearson, 2002). The work of Filippova built further on the idea that methylation would be related to the pathogenesis of congenital DM1 (CMD). The authors discovered that there are two binding sites for the zinc-finger protein CTCF flanking the CTG repeat, and that the hypermethylation of these sequences, which coincide with the region described by Steinbach and collaborators (1998), would be incompatible with the binding of the CTCF and its proper function mediating the inhibition of promoter-enhancer interactions by insulator elements (Bell et al., 1999). Conversely, Libby and collaborators (2008) found that CpG methylation of CTCF binding sites may rather lead to repeat instability, instead of stability as previously suggested.

In this work we studied 8 of the 18 CpG sites in 152 nucleotides upstream of the CTG repeat of the DM1 locus (see figure 1). These sites are part of the CpG sites investigated by Steinbach and collaborators and did not include the sites affecting the CTCF binding (Filippova et al., 2001). We did not investigate the distal SacII site, located over 1 Kb upstream of the CTG repeat, which Steinbach and collaborators reported as constitutively methylated, nor the HpaII and HhaI sites located in this same region. We analysed our samples in duplicate by methylation sensitive endonuclease digestion, followed by PCR. The PCRs were designed so that they included both the restriction site and the CTG repeat and were performed on small quantities of DNA. Consequently, smears could be resolved into individual bands, and sizing was much more accurate than by using Southern blot on total genomic DNA. Furthermore, it enabled us to correlate the methylation status with the exact length of each allele, which would not have been possible with Southern blot on total genomic DNA or bisulphite sequencing. It is

84 important to notice that in the case of the HpaII restriction enzyme, the studied region
85 contained 6 sites. This means that we could not distinguish the methylation state of each
86 independent site, but could only asses whether at least one of the sites was
87 unmethylated.

88 The aim of this study was to establish the threshold for the expansion size necessary for
89 the hypermethylation of the CpG sites close to the CTG repeat and this within one DNA
90 sample. We also aimed to investigate the possibility that hypermethylation is patient- or
91 allele specific.

92 DNA samples from peripheral blood from 22 DM1 patients were digested with the
93 methylation-sensitive restriction enzymes SacII, HhaI and HpaII (Biolabs, Westburg,
94 Leusden, The Netherlands). Both digested and undigested samples were analysed by
95 PCR. In the case of the SacII digested samples, we used the primer set indicated in
96 figure 1 as SacII primer and reverse primer. The DNA input was of 200 pg. For the HhaI
97 and HpaII samples, we used the HhaI-HpaII and the reverse primers. Initially, the DNA
98 input was of 200 pg, but after the first results, DNA input was increased to 2 ng to
99 ensure the detection of possible uncleaved alleles present at a very low quantity. The
100 PCR protocol was further performed as previously described (De Temmerman et al.,
101 2008). The expansions were visualized by denaturing Southern blot using a GAC probe,
102 and the size calculated by comparison to two molecular weight markers (markers VI and
103 VII, Roche diagnostics, Vilvoorde, Belgium).

104 Table 1 shows the results for the 22 DM1 patients included in this study. The table
105 outlines the results of the PCR before and after endonuclease cleavage by SacII, along
106 with the disease status and inheritance of the mutation in all those patients for which
107 these data were known. The cohort of patients includes cases of congenital DM1, infantile
108 and classic DM1, both paternally and maternally inherited. The age at sampling ranges
109 from birth to 43 years, and expansions were between 180 to 2800 repeats. Figure 2
110 shows an example of the Southern blot results after SacII restriction enzyme cleavage
111 and PCR. The results for HhaII and HpaII cleavage, and the complete data for the SacII
112 digestion can be found in the supplementary figures 1, 2 and 3.

After HhaI endonuclease cleavage, none of the DNA samples showed amplification, whereas the undigested samples worked as a positive control for the PCR. This meant that all alleles were unmethylated, both the wild type alleles as well as all of the expansions. We verified that the lack of amplification in the digested samples was not due to DNA degradation by amplifying the material with a PCR for 4 short tandem repeats located on chromosome 19q13.41, 19q13.33, 19p13.3 and 19p13.2 (tandem repeats located using the database www.microsatellites.org). The primer sequences and PCR conditions are available upon request. The results showed that the DNA was intact in all samples, and proved that the restriction by HhaI had been specific for its target. These results suggest that, contrary to the findings of Steinbach and collaborators, not only is this CpG site constitutively unmethylated, but also that it is not necessarily methylated in expansions larger than 1000 repeats or in congenital DM1 patients.

Identical results were obtained after cleavage with HpaII. Both the wild type and the expanded alleles were unmethylated (for at least one of the six HpaII sites in the sequence) in all the samples studied. These results are in concordance with the findings of Shaw and collaborators (1993) and contradict those of Steinbach and co-workers (1998).

From our results it seems that of the CpG sites we studied the SacII site immediately upstream of the repeat is the only one in the region that can be differentially methylated between wild type alleles, which are never methylated, and expanded alleles, which may be methylated. Our data are difficult to harmonize with the previously published study (Steinbach et al., 1998). We did not find a correlation between the methylation status of this site on one hand and the allele size or on the other hand with the presence of a smear on the Southern blot –indicative of triplet repeat instability- , as was suggested by Steinbach et al. Alleles as small as 400 repeats could be methylated (patient 20), and alleles of 1600 repeats unmethylated (patient 8), indicating that there is no correlation between the allele size and the methylation status. We found several patients showing intermediate methylation patterns (some alleles were methylated while others were not), and even patients without any methylation at this site. A patient could present a smear

in which all alleles appeared to be methylated (i.e. patient 7), indicating that the methylation status apparently does not correlate with the stability of the repeat. However, it is clear that the presence of CTG length mosaicism either with or without methylation does not necessarily make one the cause or effect of the other. The time during which one occurred may or may not have coincided with the other. Furthermore, the link between methylation of this site and congenital DM1 does not seem to be as strict as previously assumed. It is true that both congenital DM1 cases analysed here showed methylation of the SacII site in all the expanded CTG alleles (patients 4 and 6), but non-congenital DM1 patients also presented it (patients 5 and 7). Furthermore, we did not find the correlation previously described (Steinbach *et al.*, 1998) between the methylation of the SacII site and of the HhaI and HpaII sites, since we never found methylation in the latter two, not even in the congenital DM1 patients. The fact that we did not find a methylation pattern specific to the CMD patients contradicts the hypothesis that differential methylation may be responsible for the distinct features of CMD by modulating the binding of CTCF in the DM1 locus (Flippova *et al.*, 2001). It is, however, important to bear in mind that we did not study the methylation of all the CpG sites present in the CTCF binding sites, and that methylation may still play an important role in the pathogenesis of DM1.

These discordances do not seem attributable to incomplete restriction or technical differences. In the works of Shaw *et al.* (1993) and Steinbach *et al.* (1998), as in ours, incomplete restriction would have been detected by the incomplete cleavage of the wild type allele. To ensure that the results for HpaII and HhaI were not due to the degradation of the samples after restriction, we confirmed the presence of intact DNA by PCR for microsatellite markers. The fact that in our work we analysed PCR products by Southern blot, whereas Shaw *et al.* (1993) and Steinbach *et al.* (1998) performed direct Southern blotting of the restricted DNA only adds to the resolution of the expanded alleles. Furthermore, it is possible that our method is more sensitive in picking up a few unrestricted alleles. Nevertheless, for the HhaI and HpaII digests, we never found any uncleaved allele.

In the work of Shaw et al (1993) it is suggested that the patient's age might play a role on the methylation levels in CMD. The authors suggest that, since their CMD patients were rather of mature age, the methylation could have been lost. It is important to bear in mind that these authors were investigating a possible relationship between imprinting of the DM1 locus and CMD, and hypothesized that this imprinting would only be of importance *in utero*, and would be subsequently lost after birth. In Steinbach's paper, the oldest CMD patient is 24 years, and shows full methylation of the expanded allele for all the SacII, HhaI and HpaII sites. Shaw et al. (1993) did not mention the exact age of the patients, but if they were older than 24 years, their hypothesis would harmonize the difference in results between Shaw and Steinbach's papers, but not with ours, where the two CMD patients were very young, and showed no methylation for the HhaI and HpaII sites. An age effect on the classical DM1 patients can be ruled out, as both Steinbach's work as ours has patients of all ages (Steinbach's range was 19-62, ours 4-45 years). Furthermore, there does not seem to be a parent of origin effect.

In conclusion, we have studied the methylation status of the proximal sequence upstream of the CTG repeat of the DM1 locus. From our results, we establish that, contrary to previous findings, only one of the studied sites is differentially methylated in wild type and expanded CTG alleles, and that this methylation does not strictly correlate with the length of the allele, nor with the disease status of the patient. Further research on this topic is necessary to elucidate the reasons for the discrepancy with some of the previously published results.

AKNOWLEDGEMENTS

The authors wish to thank their colleagues at the Centre for Medical Genetics for the fruitful collaboration, and Prof. C.E. Pearson for his constructive comments on the manuscript. This work has been supported by grants from the Fund for Scientific Research Flanders (Fonds voor Wetenschappelijk Onderzoek (FWO) Vlaanderen). CS is a postdoctoral fellow at the FWO Vlaanderen.

License for publication

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in Journal of Medical Genetics and any other BMJPGJ products and sublicences such use and exploit all subsidiary rights, as set out in our licence (<http://group.bmj.com/products/journals/instructions-for-authors/licence-forms>).

Competing interests

None declared

REFERENCES

- Bell, A.C., West, A.G., and Felsenfeld, G. (1999) The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. *Cell*. 98,387-96.
- Brock, G. J., Anderson, N. H., and Monckton, D. G. (1999). Cis-acting modifiers of expanded CAG/CTG triplet repeat expandability: associations with flanking GC content and proximity to CpG islands. *Hum. Mol. Genet.* 8, 1061-1067.
- De Temmerman, N., Seneca, S., Van Steirteghem, A., Haentjens, P., Van der Elst, J., Liebaers, I., and Sermon, K.D. (2008). CTG repeat instability in a human embryonic stem cell line carrying the myotonic dystrophy type 1 mutation. *Mol. Hum. Reprod.* 14, 405-12.
- Filippova, G. N., Thienes, C. P., Penn, B. H., Cho, D. H., Hu, Y. J., Moore, J. M., Klesert, T. R., Lobanenko, V. V., and Tapscott, S. J. (2001). CTCF-binding sites flank CTG/CAG repeats and form a methylation-sensitive insulator at the DM1 locus. *Nat. Genet.* 28, 335-343.
- Gorbunova, V., Seluanov, A., Mittelman, D., and Wilson, J. H. (2004). Genome-wide demethylation destabilizes CTG.CAG trinucleotide repeats in mammalian cells. *Hum. Mol. Genet.* 13, 2979-2989.
- Gourdon, G., Dessen, P., Lia, A. S., Junien, C., and Hofmann-Radvanyi, H. (1997). Intriguing association between disease associated unstable trinucleotide repeat and CpG island. *Ann. Genet.* 40, 73-77.
- Harper, P.S. (2001). *Myotonic Dystrophy*. 3rd edn. WB Saunders, London, UK.
- Klesert, T.R., Otten, A.D., Bird, T.D., and Tapscott, S.J. (1997). Trinucleotide repeat expansion at the myotonic dystrophy locus reduces expression of DMAHP. *Nat. Genet.* 16,402-6.

Krahe, R., Ashizawa, T., Abbruzzese, C., Roeder, E., Carango, P., Giacanelli, M., Funanage, V. L., and Siciliano, M. J. (1995). Effect of myotonic dystrophy trinucleotide repeat expansion on DMPK transcription and processing. *Genomics* 28, 1-14.

Libby, R.T., Hagerman, K.A., Pineda, V.V., Lau R, Cho, D.H., Baccam, S.L., Axford, M.M., Cleary, J.D., Moore, J.M., Sopher, B.L. et al. (2008). CTCF cis-regulates trinucleotide repeat instability in an epigenetic manner: a novel basis for mutational hot spot determination. *PLoS Genet.* 4:e1000257.

Mankodi, A., Logigian, E., Callahan, L., McClain, C., White, R., Henderson, D., Krym, M., and Thornton, C. A. (2000). Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. *Science* 289, 1769-1773.

Nichol, K. and Pearson, C. E. (2002). CpG methylation modifies the genetic stability of cloned repeat sequences. *Genome Res.* 12, 1246-1256.

Ranum, L. P. and Cooper, T. A. (2006). RNA-Mediated Neuromuscular Disorders. *Annu. Rev. Neurosci.*

Shaw, D. J., Chaudhary, S., Rundle, S. A., Crow, S., Brook, J. D., Harper, P. S., and Harley, H. G. (1993). A study of DNA methylation in myotonic dystrophy. *J. Med. Genet.* 30, 189-192.

Steinbach, P., Glaser, D., Vogel, W., Wolf, M., and Schwemmle, S. (1998). The DMPK gene of severely affected myotonic dystrophy patients is hypermethylated proximal to the largely expanded CTG repeat. *Am. J. Hum. Genet.* 62, 278-285.

Thornton, C.A., Wymer, J.P., Simmons, Z., McClain, C., and Moxley, R.T. 3rd (1997). Expansion of the myotonic dystrophy CTG repeat reduces expression of the flanking DMAHP gene. *Nat. Genet.* 16,407-9.

FIGURE LEGENDS

Figure 1. Schematic overview of the sequence analyzed in this study and its relationship to the genomic regions studied by Shaw et al (1993) and Steinbach et al (1998). The figures are modified versions of the original figures found in the respective publications. A: Genomic region studied by Shaw et al.(1993). Three fragments were created using EcoRI and EcoRV, and then restricted by HpaII. The results showed that at least some of the HpaII sites contained unmethylated cytosines, independently of the form of the disease or expansion size. B: Genomic region studied by Steinbach et al. (1998). The samples were restricted using combinations of SacI and HindIII, and SacII, HpaII or HhaI. The results showed that the upstream SacII site was constitutively methylated, and the downstream SacII site only methylated on very largely expanded alleles of patients with congenital DM1. The HpaII and HhaI sites were also all

280 methyated in these patients. C: Sequence studied in this paper. Genomic DNA was
281 restricted using SacII, HpaII or HhaI and amplified by PCR using the primers indicated on
282 the sequence as arrows. The highlighted sequence corresponds to the CTCF binding site
283 1 (Fillippova et al., 2001). The results showed no correlation between the methylation of
284 the SacII site and the disease status of the patient or the expansion size. No methylation
285 of the HhaI site was detected, and the results for HpaII suggested that there was at least
286 always one unmethylated site present in the sequence.

287 **Figure 2.** Example of the Southern blot results after SacII restriction enzyme cleavage
288 and PCR. Lanes 1, 2, 3, 4 and 5 show restricted samples, lanes 1', 2', 3', 4' and 5' are
289 the same DNA samples, but unrestricted. These numbers correspond also to the patient
290 identification numbers found in table 1. The lane marked with MWM contains the
291 molecular weight marker VII (Roche Diagnostics, Vilvoorde, Belgium). The lane numbers
292 correspond to the patient numbers. Unrestricted samples still contain the wild type allele
293 (wt), whereas in the restricted samples, the wt is completely cleaved. This can be used
294 as an internal restriction control.

295 **Supplementary figures 1, 2 and 3.** Raw data of the Southern blot analysis of the
296 PCRs after restriction enzyme digestion of the patient's DNA samples. Figure 1 shows the
297 results for the SacII cleavage, figure 2 for the HpaII cleavage, and figure 3 for the HhaI
298 cleavage. All Southern blots contain the PCR products of restricted and unrestricted DNA
299 samples, for comparison and as PCR control. The lanes are labeled with the patient's
300 identification numbers. When the number is followed by a 'd', it is a digested sample. All
301 blots show one or two molecular weight markers. On the left side, molecular weight
302 marker VI is shown, on the right, marker VII of Roche Diagnostics (Vilvoorde, Belgium).
303 Some samples have been analyzed in duplicate on the same blot, and in some cases a
304 replicate can be found on another blot. In the case of the HpaII and HhaI cleavage, the
305 blots show smears in the control lanes because the PCRs were performed with a higher
306 input to ensure the detection of possible uncleaved alleles present in a low quantity.
307 Nevertheless, none of the digested samples showed any amplification, revealing that
308 they all contained unmethylated sites. The signal at the lower end of the blot corresponds

309 to the wild type allele. Its full cleavage serves as an internal control for the digestion, as
310 it is assumed to be always unmethylated. In some lanes, heteroduplexes can be seen as
311 a signal between the wild type and the expanded allele.
312

Table 1. Details of the studied patients and results after SacII cleavage

Patient	Disease status and origin of mutation	Age at sampling	Expansion size (repeats)	Description of the expansion	Alleles after SacII digestion	Description of the alleles after SacII digestion
1	DM, paternal	33	900-2800	Numerous discrete bands	900-2800	Numerous discrete bands; full methylation
2	Unknown	30	400-1200	Smear	Fully cleaved	Fully unmethylated
3	DM, paternal	33	200-400	Two groups of very close bands	Fully cleaved	Fully unmethylated
4	CMD, probably maternal	3 months	1400	One band	1400	One band; full methylation
5	DM, paternal	41	300-900	Two groups of very close bands	300-900	Two groups of very close bands; full methylation
6	CMD, maternal	Newborn	700-1300	Smear with predominant band at 1300	700-1300	Smear with predominant band at 1300; full methylation
7	Unknown	4	700-1300	Smear with predominant band at 1300	700-1300	Smear with predominant band at 1300; full methylation
8	DM, maternal	20	700-1600	Smear with predominant allele at 1600	860	One band; partial methylation
9	Unknown	Unknown	200-500	Smear	Fully cleaved	Fully unmethylated
10	DM, paternal	33	700-1600	Smear with predominant allele at 1600	700-1160	Bands of 700, 990, 840 and 1160; partial methylation
11	DM, maternal	30	600-1200	Smear	680-1170	Bands of 680 and 1170; partial methylation
12	DM, paternal	24	200-480	Smear	Fully cleaved	Fully unmethylated
13	DM, probably maternal	33	200-480	Smear	Fully cleaved	Fully unmethylated
14	DM, maternal	27	680-900	Smear	680-860	Bands of 680 and 860; partial methylation
15	DM, paternal	45	500-1300	Smear	840-990	Bands of 840 and 990; partial methylation
16	DM, paternal	38	500-1300	Smear	Fully cleaved	Fully unmethylated
17	Unknown	43	500-1650	Smear	1300	One band; partial methylation
18	Unknown	30	220-370	Smear	Fully cleaved	Fully unmethylated
19	DM, maternal	29	250-1400	Smear	850-1000	Bands of 850 and 1000; partial methylation
20	DM	Unknown	180-2200	Smear	400-1700	discrete bands of 400, 900 and 1700; partial methylation
21	Unknown	Unknown	300-2600	Smear	600-2600	discrete bands of 600, 950, 1200, 1500, 1600 and 2600; partial methylation
22	Infantile form, maternal inheritance	14	1000-2600	Smear	1300	One band; partial methylation

DM: disease onset is not congenital, CMD: disease onset is congenital.

Full methylation indicates that all alleles appeared to carry a methylated SacII site.

1 1' 2 2' 3 3' 4 4' 5 5' MWM

1 1' 2 2' 3 3' 4 4' 5 5' MWM

Supplementary figure 1: SacII digested DNA samples.

Supplementary figure 2: HpaII digested DNA samples.

Supplementary figure 3: HhaI digested DNA samples.