A new powerful PCR-based assay for the molecular diagnosis of myotonic dystrophy type I

S. RONDEAU, S. PAISLEY, M. SIMON, A. MUNNICH, J. STEFFANN, J-P. BONNEFONT

Medical Genetic Unit, Necker-Enfants Malades Hospital, Paris-Descartes University, Paris, France.

Introduction

Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease caused by an expansion of CTG triplets (> 50) in the 3'UTR region of *DMPK* gene. In affected alleles, triplets expansions range from 50 to 4000 CTG repeats. Currently, the diagnostic approach in our laboratory consists in carrying out a *DMPK* fluorescent PCR (amplification up to 110 CTG) followed by Southern blot (SB) for apparently homozygous patients. This process can be time-consuming and expensive.
We have assessed the sensitivity and specificity of the new prototype reagents AmplideX® PCR/CE *DMPK* Kit.

Materials and Methods

DNA were isolated from blood, chorionic villi (CVS) and amniotic fluid (AF) from 7 healthy donors and 42 DM1-patients. DM1-patients had CTG repeat size ranging from 58 to 1800.
DNA were amplified using prototype AmplideX® PCR/CE *DMPK* kit reagents (Asuragen) with two gene-specific (GS) primers and one repeat primer located in the repeats (Figure 1). FAM-labeled amplicons were migrated on 3500xL Genetic Analyzer Capillary Electrophoresis (CE) instruments (ThermoFisher) (PCR/CE). 10 samples from DM1-patients (88 to 1500 CTG repeats) were also amplified by the two gene-specific primers only and sized by agarose gel electrophoresis (PCR/AGE) using Reliant Mini 12-well precast 1% SeaKem Gold agarose gels (Lonza), Bionic Buffer (Sigma) and Quick-Load 2-Log DNA ladder (NEB) (Figure 3).

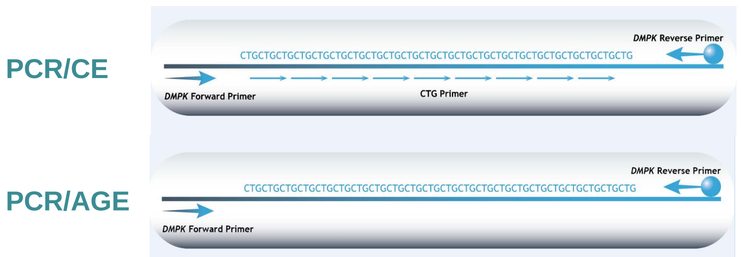

Figure 1 : **Principle of the Asuragen's AmplideX® *DMPK* technology**. The assay consists of two gene-specific primers (GS) (FAM-labeled reverse primer), and one repeat primer that can hybridize and prime anywhere in the repeat region. The three primers are used for CE migration (PCR/CE) and only GS primers for sizing on agarose gel (PCR/AGE).

Patient (P)	Tissues	*DMPK* fluorescent PCR	Southern Blot	AmplideX® PCR/CE	Concordant
1	blood	5	-	5 / 5	YES
2	CVS	5	-	5 / 5	YES
3	blood	5/6	-	5 / 6	YES
4	blood	11/12	-	11 / 12	YES
5	blood	11/13	-	11 / 13	YES
6	blood	14/18	-	14 / 18	YES
7	blood	5/23	-	5 / 22	YES
8	AF	22/57	-	22 / 57	YES
9	blood	5/58	-	5 / 58	YES
10	blood	7/72	-	7 / 73	YES
11	blood	14/78	-	14 / 78	YES
12	blood	12/78	-	12 / 79	YES
13	blood	22/72	-	22 / 73	YES
14	blood	12/88	-	12 / 90	YES
15	blood	5	135	5 / 75-105 / 135 / >200	YES
16	blood	5/144	-	5 / 142	YES
17	blood	12	100-230	12 / >200	YES
18	blood	13	110-230	13 / >200	YES
19	blood	5	130-320	5 / 148 / 189	YES
20	blood	5	170	5 / 130-160 / >200	YES
21	blood	13	170-230	13 / >200	YES
22	blood	11	170-300	11 / >200	YES
23	blood	23	200-500	23 / >200	YES
24	blood	16	220-350	16 / >200	YES
25	blood	12	230-500	12 / >200	YES
26	blood	12	230-500	12 / >200	YES
27	blood	11	230-570	11 / >200	YES
28	blood	12	220-625	12 / >200	YES
29	blood	12	240-700	12 / 66 / >200	YES
30	CVS	5	270-420	5 / >200	YES
31	blood	11	300-600	11 / >200	YES
32	blood	5	350-650	5 / >200	YES
33	blood	5	350-700	5 / >200	YES
34	blood	13	400-600	13 / >200	YES
35	blood	11	400-800	11 / >200	YES
36	CVS	5	500	5 / >200	YES
37	blood	12	500-850	12 / >200	YES
38	CVS	13	800	13 / >200	YES
39	blood	5	730-980	5 / >200	YES
40	blood	5	475-1080	5 / >200	YES
41	blood	13	560-1000	13 / >200	YES
42	blood	5	500-1100	5 / >200	YES
43	blood	11	570-1110	11 / >200	YES
44	blood	5	1000-1300	5 / >200	YES
45	CVS	11	1334	11 / >200	YES
46	CVS	11	1400	11 / >200	YES
47	AF	23	1500	23 / >200	YES
48	blood	11	1500	11 / >200	YES
49	blood	13	1100-1800	13 / >200	YES

Table 1 : Comparison between *DMPK* fluorescent PCR and AmplideX® PCR/CE results

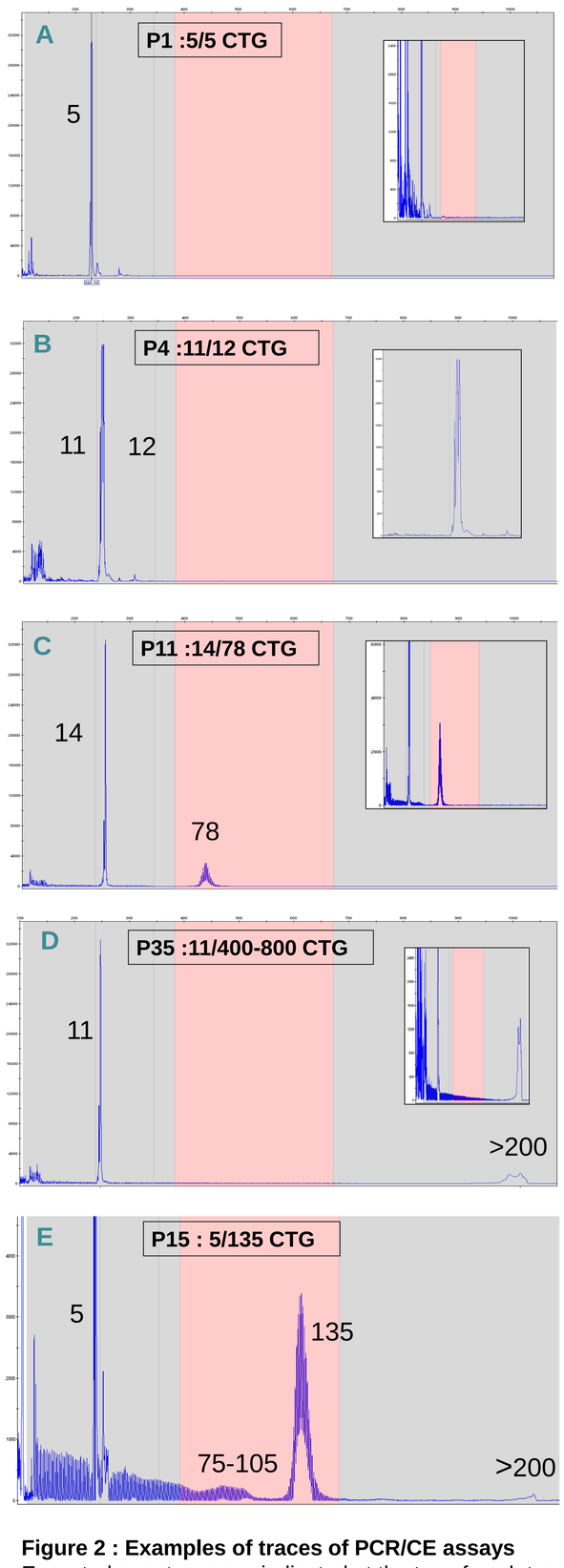

Figure 2 : Examples of traces of PCR/CE assays
Expected genotypes are indicated at the top of each trace

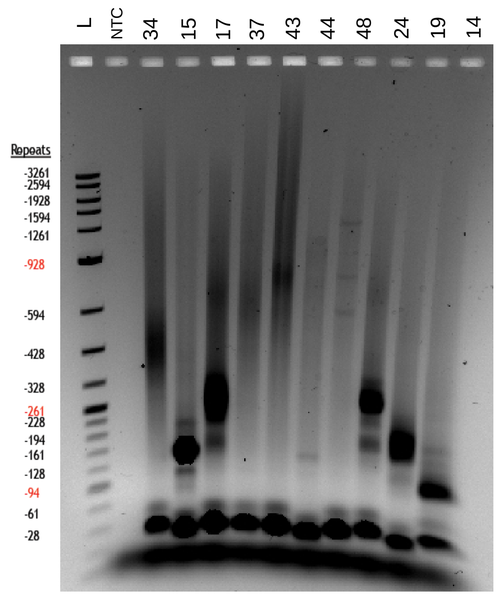

Figure 3 : Migration of PCR/AGE : 10 patients carrier of expansion ranging from 88 to 1500 repeats.
L : ladder, NTC : Negative Template Control

Patient	*DMPK* fluorescent PCR	Southern Blot	AmplideX® PCR/CE	AmplideX® PCR/AGE	Concordant
34	13	400-600	13 / >200	350 - 580	YES
15	5	135	5 / 75-105 / 135 / >200	90 / 120 / 130-180 / 210	YES
17	12	100-230	12 / >200	150-190 / 200-350	YES
37	12	500-850	12 / >200	570-850	YES
43	11	570-1110	11 / >200	650-900	YES
44	5	1000-1300	5 / >200	150	NO
48	11	1500	11 / >200	570 / 800 / 1300	YES
24	16	220-350	16 / >200	150-180 / 260-350	YES
19	5	130-320	5 / 148 / 189	100 / 140-190	YES
14	12/88	-	12 / 90	70-100 / 150	YES

Table 2 : Comparison between SB and AmplideX® PCR/AGE results

Results

- **The AmplideX® PCR/CE assay gave expected results for the 49 samples (100% sensitivity and specificity) (Table 1).**

Normal allele size was precisely determined, all the results were concordant with the *DMPK* fluorescent PCR.
Healthy homozygous patients were distinguished from DM1-patients thanks to the negative repeat profile (Figure 2A). Alleles separated by one triplet were easily differentiated (Figure 2B).

Expansions under 200 repeats were well characterized with a positive repeat profile (Figure 2C).

Large expansions over 200 repeats (ranging from 200 to 1800 repeats) were detected and appeared at the end of the profile (Figure 2D).
The repeat profile was positive for all the samples except for 2 CVS that had a negative repeat profile but for which expansions were amplified by the GS primers.

Previously unidentified mosaicism was revealed in 6 patients (Figure 2E).

- **The AmplideX® PCR/AGE assay gave concordant results except for one patient (Table 2).**

Expansions, ranging from 88 to 1500 repeats, gave various migration profiles with diverse types of mosaicism.
In one sample (patient 44), the expanded allele with 1300 CTG, revealed by Southern blot analysis, was not detected.

Mosaicism was also revealed in 3 patients (14, 15, 48).

Conclusion

The AmplideX® PCR/CE *DMPK* assay is therefore able to efficiently detect expanded alleles, to size alleles up to 200 repeats with a 1 triplet-resolution and to differentiate homozygous genotypes from others.

The AmplideX® PCR/AGE *DMPK* assay is able to size large expanded alleles up to at least 1800 repeats. Its interpretation requires some experience.

AmplideX® PCR *DMPK* technology appears to be simpler and faster than conventional techniques for the molecular DM1 diagnosis.