Simultaneous detection of copy number changes and CpG methylation of genes in the Chromosome 15q11 imprinted region with a novel method; Methylation-specific MLPA (MS-MLPA)

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Introduction

Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS) are autosomal dominant disorders and the vast majority of the cases are caused by copy number changes in the chromosome 15q11 imprinting center (IC) or by uniparental disomy (UPD). The recently developed MLPA method has increased the possibilities for multiplex detection of copy number changes in a routine laboratory. Here we describe a novel robust method: the Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) which can detect changes in both CpG methylation as well as copy number of up to 40 chromosomal sequences in one simple reaction. In MS-MLPA, ligation of MLPA probe oligonucleotides is combined with digestion of the sample DNA-probe hybrid complexes with methylation-sensitive endonucleases. We successfully used MS-MLPA to detect copy number and methylation status of the genes in the chromosome 15q11 IC in 12 DNA samples of patients with Prader-Willi Syndrome (PWS) or Angelman Syndrome (AS). Digestion of the sample DNA-probe complex, rather than double stranded sample DNA, allows the use of poor quality DNA derived from paraffin embedded formalin treated tissues.

Methods

MS-MLPA assay

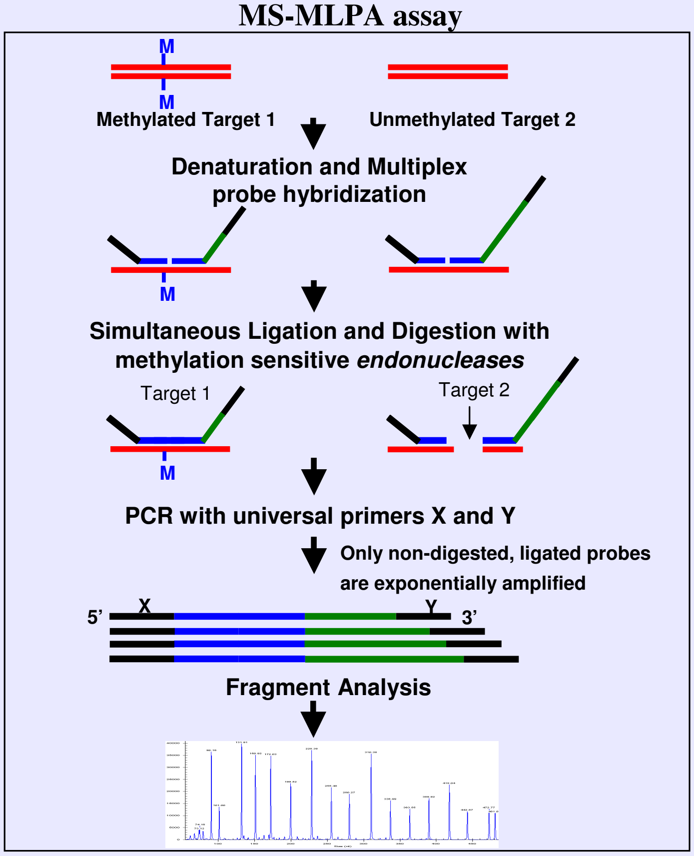

Figure 1. In MS-MLPA, ligation of the MS-MLPA probe oligonucleotides is combined with digestion of a probe-sample DNA hybrid complex with methylation-sensitive endonucleases such as *Hha*I or *Hpa*II. MS-MLPA probes are designed similarly to ordinary MLPA probes except that the sequences detected by these probes contain a recognition sequence for *Hha*I or *Hpa*II. Upon digestion with one of these enzymes, probes of which the recognition sequence is unmethylated will not generate a signal. In contrast, a probe signal will be detected if the site is methylated. Each assay contains a number of probes without any *Hha*I or *Hpa*II site that serve as controls.

Results

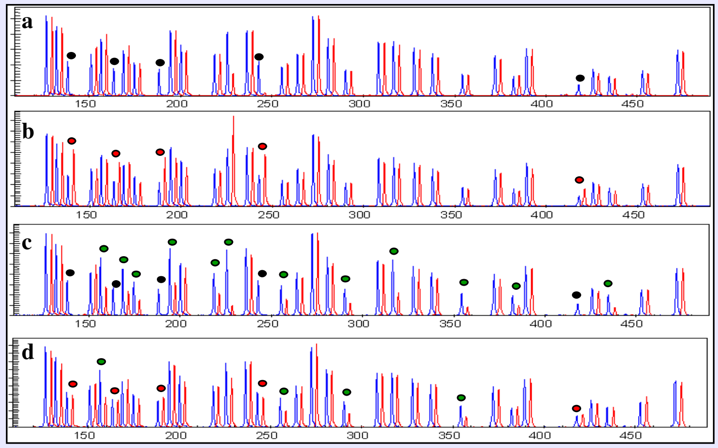

Figure 2. Detection of the methylation status of the chromosome 15q11 imprinting center by MS-MLPA. DNA from patients diagnosed with either PWS or AS and control DNA were subjected to MS-MLPA using the P028 probe mix. Blue signals represent control DNA digested with *Hha*I. Red signals represent the digested patient samples. The gaps indicate places for MS-MLPA probes located in unmethylated CpG islands both within and outside the Chr. 15q11 IC, serving as digestion control probes. Red dots represent loss of methylation (AS) compared to the control sample. Black dots represent gain of methylation (PWS). Green dots represent deletions. **a)** CE pattern generated from AS patient 88 with paternal UPD. **b)** CE pattern generated from PWS patient 93 with maternal UPD. **c)** CE pattern generated from AS patient 91 with a maternally inherited deletion of the Chr. 15q11 IC. **d)** CE pattern generated from PWS patient 95 with a paternally inherited deletion encompassing the *SNRPN* and *UBE3A* genes.

Patient sample no

Gene	87	88	89	90	91	92	93	94	95	96	97	98
TUBGCP1-D02-154-M	1.200	1.050	0.560	1.000	1.070	0.510	1.170	1.160	1.040	1.150	0.980	1.000
CYFIP1-D01-136-M	1.040	1.000	0.540	0.950	0.970	0.460	1.110	1.130	1.010	1.060	1.000	0.940
MKRN3-D02-172-M	1.190	1.080	0.540	0.560	0.500	0.570	1.130	1.230	1.110	0.620	0.520	0.560
MAGEL2-D02-178-M	1.060	1.070	0.530	0.520	0.440	0.510	1.060	1.060	0.990	0.530	0.510	0.530
MAGEL2-D01-193-M	1.070	0.990	0.590	0.580	0.420	0.480	1.110	1.080	1.180	0.640	0.520	0.490
NDN-D02-319-M	1.040	0.990	0.560	0.520	0.520	0.540	0.990	1.020	1.000	0.530	0.560	0.550
NDN-D01-418-M	1.000	1.020	0.480	0.550	0.430	0.540	0.900	0.880	0.980	0.500	0.540	0.470
SNRPN-M09-142-M	1.050	1.100	0.600	0.500	0.540	0.530	1.110	1.100	0.620	0.610	0.530	0.520
SNRPN-D06-166-M	1.00	1.04	0.64	0.47	0.54	0.53	1.13	1.11	0.58	0.60	0.53	0.48
SNRPN-M08-190-M	1.05	1.06	0.62	0.54	0.61	0.53	1.07	1.13	0.58	0.60	0.51	0.53
SNRPN-D05-229-M	1.110	1.030	0.630	0.550	0.550	0.510	1.120	1.110	0.590	0.570	0.530	0.510
SNRPN-D04-247-M	1.100	1.110	0.630	0.560	0.530	0.570	1.050	1.070	0.580	0.580	0.510	0.520
SNRPN-D02-256-M	1.02	0.97	0.50	0.50	0.61	0.49	0.99	1.09	0.48	0.57	0.56	0.55
SNRPN-D01-292-M	0.950	1.040	0.540	0.490	0.440	0.510	1.050	1.150	0.490	0.500	0.530	0.510
UBE3A-D01-160-M	1.040	1.030	0.640	0.490	0.550	0.580	1.140	1.120	0.540	0.570	0.540	0.520
UBE3A-M01-184-M	1.01	1.13	0.56	0.49	0.51	0.58	1.10	1.16	0.49	0.58	0.58	0.58
UBE3A-D02-355-M	1.030	1.130	0.570	0.590	0.550	0.590	1.060	1.010	0.510	0.540	0.510	0.530
UBE3A-M02-400-M	1.120	0.940	0.510	0.590	0.530	0.510	0.890	0.830	0.620	0.570	0.490	0.490
ATP10A-D01-364-M	1.050	1.060	0.520	0.520	0.540	0.530	1.020	1.000	1.150	0.480	0.560	0.540
ATP10A-D02-382-M	0.92	0.92	0.43	0.44	0.52	0.54	0.94	0.91	0.92	0.47	0.45	0.47
GABRB3-D01-220-M	1.040	0.990	0.600	0.540	0.550	0.510	1.130	1.040	1.070	0.540	0.490	0.530
GABRB3-D02-409-M	0.940	0.930	0.460	0.540	0.530	0.540	0.860	0.920	1.050	0.540	0.560	0.520
OCA2-D01-436-M	1.140	1.030	0.450	0.610	0.520	0.640	1.030	0.930	1.180	0.580	0.540	0.520
OCA2-D02-445-M	0.950	0.890	0.500	0.500	0.520	0.500	0.900	0.860	0.990	0.520	0.480	0.440
APBA2-D01-202-M	0.970	0.930	1.030	0.950	0.920	0.940	1.050	1.090	1.030	1.140	0.960	1.000
BLM-M01-346-M	0.990	0.990	1.000	1.030	0.960	1.030	0.960	1.080	1.030	0.910	1.050	1.020
IGF1R-D01-454-M	1.030	0.970	1.000	1.130	1.000	1.030	0.910	0.970	1.110	1.040	1.050	0.990
Control Chr 17p13	1.04	1.07	0.93	1.06	1.15	1.20	0.97	1.11	0.89	0.94	1.09	1.13
Control Chr 17q21	1.00	0.91	0.84	1.00	0.97	1.01	1.00	1.00	0.86	0.98	0.99	1.00
Control Chr 22q11	0.870	0.960	0.920	0.980	1.060	0.990	0.950	0.980	0.930	0.930	1.020	0.920
Control Chr 22q12	1.050	0.990	1.060	1.010	1.020	1.000	1.010	1.090	1.040	1.000	1.000	0.970
Control Chr 5q31	1.05	1.12	1.18	1.20	1.01	1.20	1.05	1.09	1.22	1.10	1.21	1.12
Control Chr 2p16	0.970	1.000	0.940	0.950	1.000	1.000	1.000	0.980	0.920	0.990	0.990	0.960
Control Chr 3p25	0.980	1.090	1.010	1.160	1.120	1.150	0.870	0.970	1.110	0.930	1.160	1.110
Control Chr 3p22	1.06	1.01	1.11	0.97	0.99	0.96	1.12	1.14	1.04	1.11	0.97	0.98
Control Chr 6q26	0.84	1.00	1.05	0.98	0.96	0.99	1.00	0.92	1.01	1.06	0.94	1.01
Control Chr 10p15	1.030	1.030	0.640	0.990	0.880	0.970	1.040	1.000	0.990	1.060	0.980	0.940
Control Chr 13q12	1.030	0.960	1.150	1.000	0.870	0.950	1.090	0.980	1.000	1.110	0.960	1.020
Control Chr 13q14 a	0.970	1.020	1.060	0.990	1.000	0.960	0.990	0.950	0.990	0.980	0.970	0.970
Control Chr 13q14 b	0.980	1.030	0.960	0.970	1.010	1.040	1.040	1.090	0.930	1.000	1.060	1.060
Control Chr 14q13	1.010	1.170	1.030	1.090	1.130	1.120	1.040	1.100	1.030	1.050	1.100	1.110
Methylation status	0%	0%	0%	0%	0%	0%	100%	100%	100%	100%	100%	100%
Syndrome	AS	AS	AS	AS	AS	AS	PWS	PWS	PWS	PWS	PWS	PWS
Cause	PAT UPD	PAT UPD	MAT DEL	MAT DEL	MAT DEL	MAT DEL	MAT UPD	MAT UPD	PAT DEL	PAT DEL	PAT DEL	PAT DEL

Table 1. Summary of the deletions detected using MS-MLPA with the P028 probe mix. Relative copy numbers were obtained by comparing the sample peak ratio with the average peak ratio from four control DNA samples. Deletions are highlighted in gray.

Conclusions

MS-MLPA is a simple and easy procedure that allows the analysis of:

- **a large number of samples simultaneously.**
- **the methylation status and the copy number of many genes using only a small amount of DNA (20ng)**
- **DNA derived from paraffin-embedded tissue samples**

For more information on MLPA, contact MRC Holland bv, Hudsonstraat 68, 1057 SN Amsterdam, The Netherlands.

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