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Prader-Willi syndrome identified by methylation specific multiplex ligation dependent probe amplification (MS-MLPA)

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ABSTRACT

Prader-Willi syndrome (PWS) is a multisystemic complex genetic disorder caused by lack of expression of genes on the paternally inherited chromosome 15q11.2-q13 region. There are three major genetic subtypes in PWS: paternal 15q11-q13 deletion (70% of cases), maternal uniparental disomy 15 (25-30%) and imprinting defect (1-3%).The clinicians confront the challenge of discern more clearly between the classic PWS and the various PWS-like syndrome (PWLS). It is necessary to study these issues at the molecular level to explain these genetic similarities and to provide appropriate genetic counseling and treatment. We present a case of a 6 years old male patient with severe hypotonia, feeding difficulties in neonatal period, developmental delay in neuromotor acquisition, hyperphagia and obesity (BMI: +4.66 SD).The genetic analysis methylation specific multiplex ligation dependent probe amplification (MS-MLPA) revealed an aberrant methylation of CpG island. It is important to mention that a precise diagnosis of PWS and an early multidisciplinary approach are essential for efficient long-term management, for preventing complications and improve quality of life in this patients.

Keywords: Prader-Willi syndrome, imprinting center, obesity

Abbreviations
PWS – Prader-Willi syndrome
PWLS – PWS-like syndrome
BMI – body mass index
DS – standard deviation
MS-MLPA – methylation specific multiplex ligation dependent probe amplification
SNRPN – small nuclear ribonucleoprotein-associated protein N
IGF1 – insulin growth factor 1
TSH – thyroid stimulating hormone
FT4 – free thyroxine
NDN gene – the encoding gene of necdin
DNA – deoxyribonucleic acid
IC – imprinting center

INTRODUCTION

Prader-Willi syndrome (PWS) is a disorder characterized by neurogenetic, neurometabolic and neurobehavioral alterations. It has an estimated prevalence worldwide in the range of 1 in 10,000 to 30,000 individuals (1). PWS is a genetic condition due to paternal loss of imprinted genes on chromosome 15 and characterized by a range of mental and physical findings including obesity that can be life-threatening (2). Approximately 70% of individuals with PWS are caused by a non-inherited deletion in the paternally derived chromosome 15q11-q13 region; about 25% result from maternal disomy 15 and in less than 3% of cases are individuals genomic imprinting defects (2-4).

Affected infants constantly have significant hypotonia, feeding difficulties, and failure to thrive, followed in early childhood by excessive appetite with gradual development of obesity, short stature, intellectual disabilities and behavioral problems (5).

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In PWS a precocious multidisciplinary approach is fundamental to prevent complications, to improve quality of life and prolong life expectancy.

CASE PRESENTATION

In our department we had the opportunity to observe the case of a child from a non-consanguineous couple, a 6 years old male patient, from an abnormal pregnancy-oligoamnios. He presented diminished fetal activity and he was born at 42 weeks by cesarean section, with a birth weight of 2,100 g (-2.18 SD), a birth height of 47 cm (-1.63 SD) and APGAR score 6/1 minute. During the neonatal period he presented severe hypotonia, feeding difficulties (until 3 weeks of age the child was fed

D [nt]	Gene-Exon	Chr.band	hg18 loc.	Height	Area	Ratio H	Stdev
154	TUBGCP5-8	15q11.2	15-020.398303	8222	49296	*0.92*	0.03
434	NIPA1-4	15q11.2	15-020.612289	396	6929	**0.05**	0
172	MKRN3-1	15q11.2	15-021.362818	8488	54621	*0.96*	0.03
409	MAGEL2-1	15q11.2	15-021.440355	7605	57910	*1*	0.03
445	NDN-1	15q11.2	15-021.482381	7083	52730	*0.99*	0.03
419	***NDN-1 [HHA1]***	15q11.2	15-021.483412	648	5436	**0.08**	0
287	SNRPN-u1b	15q11.2	15-022.619902	8058	51562	*1.01*	0.03
238	SNRPN-u1b*	15q11.2	15-022.626072	12545	78880	*0.99*	0.03
278	SNRPN-Intr.u2	15q11.2	15-022.690980	8852	57458	*0.99*	0.03
270	SNRPN-Intr.u2	15q11.2	15-022.703328	10268	64664	*0.98*	0.03
256	SNRPN-u5	15q11.2	15-022.716714	10000	61727	*0.92*	0.03
391	SNRPN-u5	15q11.2	15-022.717321	8033	57382	*0.94*	0.03
250	***SNRPN-CpG isl***	15q11.2	15-022.751105	819	4598	**0.06**	0
178	***SNRPN-CpG isl***	15q11.2	15-022.751214	**0**	0	**0**	0
190	***SNRPN-CpG isl***	15q11.2	15-022.751480	1057	6430	**0.13**	0
142	***SNRPN-CpG isl***	15q11.2	15-022.751773	1143	9727	**0.15**	0
294	SNRPN-3	15q11.2	15-022.764248	8954	57251	*1.04*	0.03
400	SNRPN-7	15q11.2	15-022.772555	8958	65303	*0.93*	0.03
214	SNRPN-HB2-85-	15q11.2	15-022.848250	17955	109877	*1.04*	0.03
472	SNRPN-HB2-85-	15q11.2	15-022.872658	8189	68867	*0.94*	0.03
328	***SNRPN-HB2-85-***	15q11.2	15-022.888662	1321	8642	**0.11**	0
355	UBE3A-13	15q11.2	15-023.136395	8545	57903	*0.94*	0.03
301	UBE3A-8	15q11.2	15-023.156677	10698	70858	*1.02*	0.03
160	UBE3A-7	15q11.2	15-023.167740	10364	65202	*0.9*	0.03
197	UBE3A-6	15q11.2	15-023.171919	11132	64995	*1.03*	0.03
373	UBE3A-5	15q11.2	15-023.201674	8878	62548	*1*	0.03
184	***UBE3A-1 [HHA1]***	15q11.2	15-023.235184	**0**	0	**0**	0
366	ATP10A-5	15q12	15-023.522207	10569	71690	*1.01*	0.03
226	ATP10A-1	15q12	15-023.659906	9988	60469	*1*	0.03
220	GABRB3-12	15q12	15-024.344242	8575	56340	*1.03*	0.03
382	GABRB3-10	15q12	15-024.363881	429	3511	**0.04**	0
202	***APBA2-14 [HHA***	15q13.1	15-027.196749	404	2421	**0.04**	0
463	***MLH1-1 [HHA1]***	03p22.2	03-037.009621	**0**	0	**0**	0
346	***BLM-1 [HHA1]***	15q26.1	15-089.061432	**0**	0	**0**	0
427	Reference C/M*	01p21.3	01-097.754015	8213	65517	*1.01*	0.03
244	Reference C/M*	05p15.2	05-013.819132	12259	74491	*0.96*	0.03
264	Reference C/M*	07q22.1	07-099.111656	9537	61511	*1.01*	0.03
136	Reference C/M*	10q26.3	10-133.865537	10862	69602	*1.01*	0.03
454	Reference C/M*	11p15.1	11-020.632867	7197	57249	*1.03*	0.03
130	Reference C/M*	11q13.3	11-069.956854	10054	60929	*0.95*	0.03
319	Reference C/M*	11q24.2	11-125.001668	9730	64907	*0.99*	0.03
166	Reference C/M*	11q24.2	11-125.002858	13590	85431	*1*	0.03
208	Reference C/M*	12q13.11	12-046.657475	12938	78148	*0.93*	0.03
337	Reference C/M*	13q14.3	13-049.873373	8638	58908	*0.95*	0.03
148	Reference C/M*	17q12	17-032.463021	12462	73258	*0.92*	0.03
232	Reference C/M*	17q21.31	17-038.421624	11523	70440	*1.02*	0.03
480	Reference C/M*	17q23.2	17-057.116062	8434	74524	*1.05*	0.03
309	Reference C/M*	22q12.2	22-028.400833	11243	72672	*1*	0.03
Median value all probe values:				**8865**	**61220**	**0.99**	**0.03**

FIGURE 1. *Sample report of the data obtained by capillary electrophoresis from a DNA sample analysed with SALSA MS-MLPA probemix ME028 Prader Willi/Angelman probemix. With red are indicated the SNRP-CpG island which are hypomethylated*

by nasogastric tube)and developmental delay in neuromotor acquisition. Later the patient presented hyperphagia with important weight gain and behavioral problems – temper tantrumps and stubbornness. Karyotype from fresh blood was 46,XY. Afterwards a molecular genetic analysis was performed (methylation specific multiplex ligation dependent probe amplification MS-MLPA by using SALSA MS-MLPA ME028 kit) revealing an aberrant methylation of CpG island, which belongs to SNRPN promoter (Fig. 1).

Thereafter, he is admitted to our clinic; physical examination reveals dysmorphic features (narrowed forehead, almond shaped eyes, thinner upper lip), delayed motor and cognitive development, decreased stature (height: -1.05 SD), excessive weight gain (BMI: +4.66 SD) and under development of the genitalia.

Associated comorbidities: congenital microcephaly, cytomegalovirus infection of the central nervous system, severe psychomotor and cognitive retardation, surgically corrected cryptorchidism.

The laboratory investigation revealed IGF1: 62.89 ng/ml, TSH: 1.376 μUI/ml, FT4: 0.94 ng/dl and elevated liver enzymes. The abdominal ultrasound revealed: unilateral hydronephrosis, hepatic steatosis and hepatomegaly.The severe obstructive sleep apnea was diagnosed; the polysomnography revealing apneea-hypopnea score (AHI) > 40. The adenoidectomy for adenoid vegetation was performed and the post-procedure AHI was 9.4. After adenoidectomy growth hormone therapy (rhGH) was initiated. rhGH doses were progressively increased, and after 6 months of treatment an improvement is noted in height: -0.46 SD, BMI: +3.82 SD and height velocity: 9 cm/year.

DISCUSSIONS

It is known that PWS has common clinical aspects with other diseases, which makes it difficult to precisely diagnose. Therefore, the clinicians confront the challenge of distinguish more clearly between the classic PWS and the various PWS-like syndrome (PWLS). It is necessary to study these issues at the molecular level to explain these genetic similarities and to provide appropriate genetic counseling and treatment. PWLS share elements of the PWS phenotype and the gene functions disrupted in PWLS are likely to be involved in genetic pathways that are significant for the expresion of PWS phenotype (6).

Considering these aspects, it is important to choose the correct genetic test to identify patients with classic PWS because Rocha et al. (7) reveals in a meta-analysis other mutation associated with PWS-like phenotype, such us: deletion of 6q, paracentric inversion, fragile X syndrome.

DNA methylation is considered a solid tool to determine paternal-only, maternal-only, or biparental (normal) inheritance, but can not differentiate between maternal uniparental disomy 15 or imprinting defects (5). Compared to conventional DNA methylation, a new semi-quantitative method, namely "methylation-specific multiplex-ligation probe amplification" (MS-MLPA) is recommended for methylation detection in imprinting disorders (ex. Beckwith Wiedemann syndrome, Silver Russel syndrome) and uniparental disomy. MS-MLPA will determine the methylation status by using 5 methylation specific probes (4 for *SNRPN* gene and one for NDN gene) but also detect the copy number changes (deletions on chromosome 15q11), therefore being indicated to establish the diagnosis of PWS (8.9).

For this reason, MS-MLPA analysis might be considered as the first testing when suspecting PWS as a possible diagnosis (10).

Following the MS-MLPA test, our patient was diagnosed with PWS due to the aberrant methylation of the CpG islands belonging to the SNRPN promoter, and thus 15q deletions were ruled out. Unfortunately, because the technique can not distinguish between imprinting defects and chromosome 15 maternal uniparental disomy, additional tests, like DNA polymorphism analysis may be performed in order to indicate the exact cause of the disease, but nevertheless the PWS diagnosis is already established.

Understanding the particular genetic etiology in patients with PWS is essential for the correct genetic counseling of affected families. Genetic defect which produces PWS is correlated with evaluation of recurrent risk (11).

Imprinting defects are a rare but significant cause of PWS. Patients with an imprinting defect have apparently normal chromosome 15 of parental and maternal origin, but present aberrant DNA methylation of SNRPN promoter. When counseling patients with a detected imprinting defect, a recurrence risk is up to 50%, because the mutation is likely dominant and occurred in the paternal grandmother's germ line (12); those without an IC deletion would be expected to have a lower risk.

Maternal uniparental disomy of chromosome 15 is the second cause of PWS and present also aberant methylation frequently de novo. This etiology has a recurrence < 1% (2).

CONCLUSIONS

It is important to mention that a precise diagnosis of PWS and an early multidisciplinary approach are essential for efficient long-term management, to prevent complications and improve the quality of life.The genetic basis of this rare disorder differ but MS-MLPA is a simple, rapid and useful method for identification of PWS cases with aberrant methylation status.

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