Integrated approach to the Prader-Willi Syndrome

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INTRODUCTION

Prader-Willi Syndrome (PWS) is caused in 99% of cases by an alteration of the pattern of imprinting of the chromosome 15 q11-q13 region. The main cause of this syndrome is the deletion in the region 15q11-13 that accounts for approximately 70% of the cases. The second cause is the presence of maternal uniparental disomy that accounts for 25% of the cases. In rare cases the pathology is determined by defects of the center controlling imprinting. The early diagnosis and the corrected determination of the genetic cause are fundamental for an optimal therapeutic approach to the PWS. In particular, for a correct genotype-phenotype correlation, is emerged the importance of the characterization of the break points (Figure 1) in case of deletions.

Figure 1: Schematic representation of q11-q13 region of chromosome 15. The different deletion breakpoints (BP) and the locus of imprinting control (IC) are shown.

DIAGNOSIS

The diagnosis of the PWS as early as possible is the key for a correct treatment of the condition and may drammaticly improve the quality of life of the patients. The basis is a correct clinical diagnosis that may be based on age specific signs and symptoms like hypotonia, failure to thrive in the first years of life, low stature, small hand and feet, caracteristic facies, later in life by the hyperfagia and the neuropsichiatric signs (Table 2). The molecular diagnosis confirms the clinical suspect and can be performed by different techniques (Table 1). Among the tests available the most cost effective is definitely methylation specific PCR. The downside of this technique is that it detects the PWS but not the underlyning cause. FISH diagnosis detects only the deletions but not the other causes of PWS. Besides this FISH requires working with cells that rises the costs and is laborious to do for foreign patients. STR studies offer a means to detect the presence of the sole UPD having also the parents DNA. A newer method is methylation specific MLPA that is able not only to confirm the diagnosis of PWS but also to detect deletions on the molecular level.

TEST	DETECTABLE MUTATIONS	SENSIBILITY
MS-PCR	Methylation anomalies	99%
FISH/qPCR	Deletion of the PW critical region (PWCR)	70%-75%
UPD analysis	PWCR UPD	25%-29%
Sequencing	Defects of imprinting locus	<1%
MS-MLPA	Methylation anomalies, deletion	99%

Table 1: Molecular tests available for PWS. MS-PCR identifies the presence of methylation anomalies but not the cause; MS-MLPA detects the different causes of methylation anomalies and allows the discrimination between deletions with related breakpoints

Birth to two years:
- Hypotonia with poor suck in the neonatal period

Two to six years:
- Hypotonia with history of poor suck
- Global developmental delay

Six to 12 years:
- History of hypotonia with poor suck (hypotonia often persists)
- Global developmental delay
- Excessive eating with central obesity if uncontrolled

13 years to adulthood:
- Cognitive impairment, usually mild mental retardation
- Excessive eating with central obesity if uncontrolled
- Hypothalamic hypogonadism and/or typical behaviour problems

Table 2: Gunay-Aygun diagnostic criteria for PWS; Gunay-Aygun in 2001 suggested the presence of the following age specific findings to be sufficient to justify a molecular test

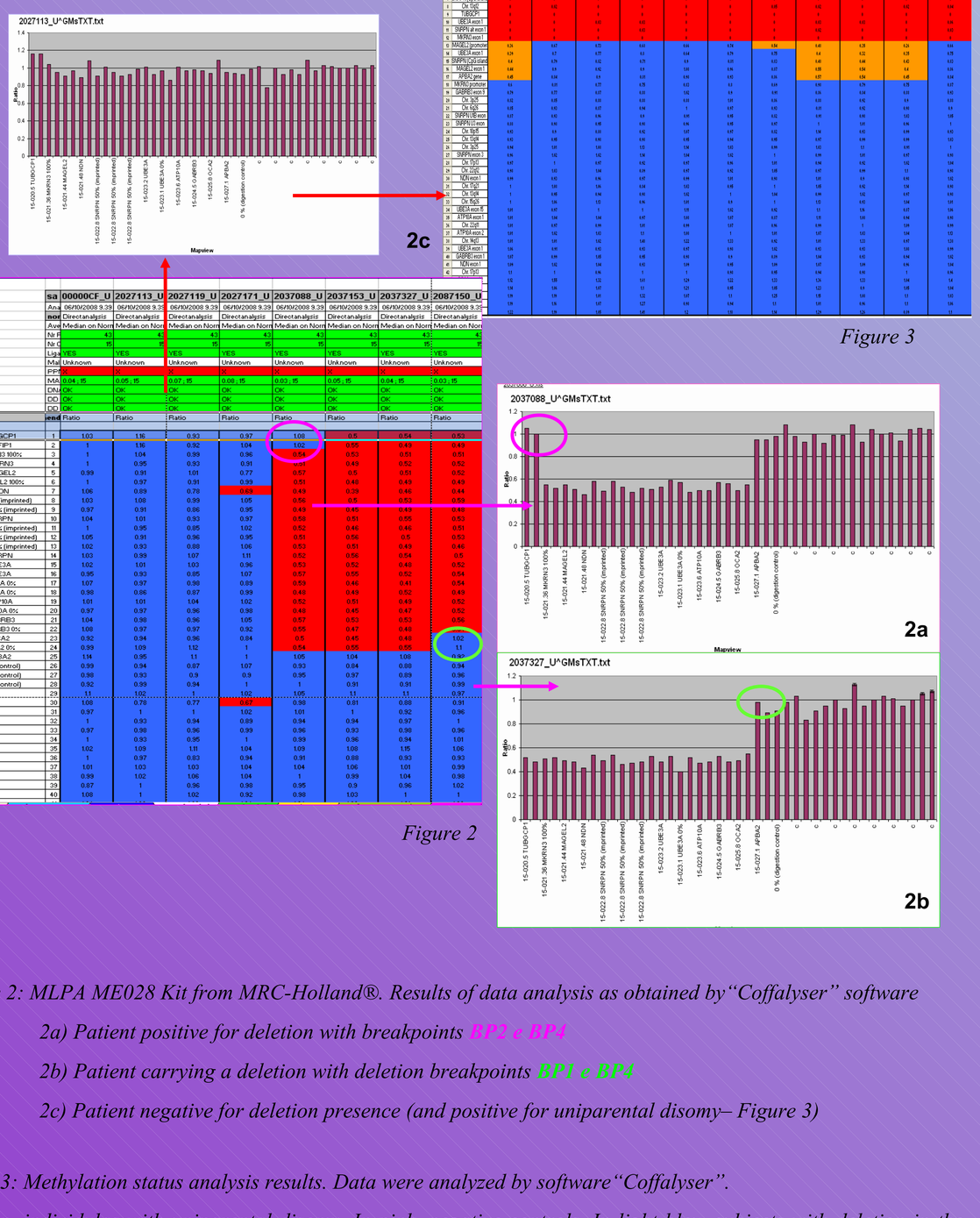

Figure 2

Figure 3

Figure 2: MLPA ME028 Kit from MRC-Holland®. Results of data analysis as obtained by"Coffalyser" software

*2a) Patient positive for deletion with breakpoints **BP2 e BP4***

*2b) Patient carrying a deletion with deletion breakpoints **BP1 e BP4***

2c) Patient negative for deletion presence (and positive for uniparental disomy– Figure 3)

Figure 3: Methylation status analysis results. Data were analyzed by software"Coffalyser".

In green, individulas with uniparental disomy. In pink, negative controls. In light bleu, subjects with deletion in the region.

MS-MLPA TECHNIQUE

MS-MLPA. MRC Holland MS-MLPA ME028 kit (Figure 2 and 3) allows the simultaneous determination of copy number variation and DNA methylation status in a semiquantitative fashion. It consists of a probe mix which contains 43 probes, 25 of which are specific to the PWS/AS critical region genes. Mix contains also 18 probes not related to PWS/AS region as control for copy number variation. Among the probes specific for the PWS/AS region, 15 probes are methylation sensitive (including 3 control probes for methylation mapping on other chromosomes) and contained a *HhaI* restriction site.
MS-MLPA dosage analysis allows to detect gross deletions, molecular lesions most frequent in patients with PWS and AS. MS-MLPA is the diagnostic method of election for PWS diagnosis considering that it allows the investigation of methylation status of several loci, then reducing the risk of false positives or of false negatives due to SNPs; furthermore if a probe fails there are other 4 probes to assess the methylation status. MS-MLPA is abele to detect IC deletion in case of Prader Willi patients wiyh imprinting defects.

REHABILITATION AND TREATMENT

Current guidelines suggest a pluridisciplinar approach that takes into account the different needs of the subject during different phases of life:

1.In the first phase, from 0 to 3 years, the objectives are: reducing hypotonia, sufficient nutrition, developement of normal motricity both by quality and quantity.

• These objectives require an equipe formed by a: neuropsichomotricist, psichomotricist, logopedist, dietist

1.In the second phase, from 3 years (since the developement of hyperphagia) the objectives are: weight control and control of the daily calory intake, prevention and management of "secondary" pathologies, developing and maintaining autonomies

• These objectives require an equipe formed by a: phisioterapist, dietist, logopedist, educator, endocrinologist, ortopedic, dentist, oftalmologist, dermatologist, pneumologist, neuropsychiater

PWS patients require access to surgical treatments, pharmacological treatments (growth hormone, sexual hormones, psychiatric drugs, insulin), psychological support .

B.I.R.D. Rehabilitation cycles

The main target of the rehabilitation cycles is giving PWS families some professional assistance while the PWS patients continue to live in their families.
The Italian culture gives great importance to the family and PWS patients are often taken care of at home with one of the parents dedicating to their needs. During infancy, PWS patients are followed on the medical and therapeutic as well as the educational, psychological, social aspects but in the adulthood with the exception of the medical part the patients receive much less attention that is mostly concentrated in occupational therapy in day centers.

Our answer to the many aspects that need to be addressed in the adult PWS patients are the PWS specific rehabilitation cycles. The cycles are organized every 2 months and are of three to four weeks. A number of adult PWS patients between 6 and 10 bot male and female is admitted to each cycle. The main goals are: treatment of obesity, occupational therapy with a moderate diet, multispecialistic approach, relief to the family.
During the PWS cycles the PWS guests are seen by a team of specialists that determine their health status, their social skills and their autonomies. The basis of the weight management are in a moderate diet of 1500 kcal/day distributed over five meals and a lot of aerobic activities. The association of the two proved to be extremely effective since a non restrictive diet proves to be less stressing and focusing on physical activity helps in reducing the generalized edema that is often present in PWS patients.
The PWS diet is based on a Mediterranean diet with as little oil as possible and abundance of raw vegetables.
The physical activities are varied and include several different specialists in order to avoid repetitive tasks that could be found frustrating. The activities include: neuropsychomotricity, physiotherapy, music therapy, social activities, cultural trips, pet therapy and many more.

The results of the rehabilitation cycles point to a constant weight loss in all the PWS patients that attend at least three cycles per year with a good weight control by atending to two cycles per year. In general the families report an increse in social skills and autonomies after participating to a cycle. The stimulative environment and the presence of only PWS patients incentivates the partecipants to do their best.

CONCLUSIONS

The Prader – Willi syndrome requires a multidisciplinar approach, on one side is the diagnosis of the disease, clinical and molecular, on the other side is "fighting" with the disease. Being it with hormonal therapies, or diets or education of all the persons that interact with a patient.

PWS demands a strict medical monitoring due to the many known complications and life threatening situations but primarly requires a strict control on their environment, in particular access to food sources.

Our institute focuses its attention in particular to the diagnosis, education of the families and professionals, treatment cycles for PWS adults.

We provide aid in the clinical diagnosis and offer molecular diagnoses that range from a 24 hour floppy infants screening in the first days of life, to MLPA tests, to free of charge methylation tests.

We furthermore offer counselling to families and others regarding the disease and its characteristics.

We also offer therapeutic cycles for adult PWS patients, where we concentrate in a three-four week period a custom diet with aerobic activities and medical observations.

Our goal is simply to be a partner for the families where they can have their questions answered and that can aid them in all phases of life.