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CLINICAL-NEUROLOGIC, CYTOGENETIC AND MOLECULAR ASPECTS OF THE PRADER-WILLI AND ANGELMAN SYNDROMES

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ABSTRACT - The Prader-Willi syndrome (PWS) and the Angelman syndrome (AS) are human neurogenetic disorders involving the imprinting mechanism, at the 15q11-13 chromosome region. The predominant genetic defects in PW are 15q11-13 deletions of paternal origin and maternal chromosome 15 uniparental disomy. In contrast, maternal deletions and paternal chromosome 15 uniparental disomy are associated with a different neurogenetic disorder, the AS. In both disorders, these mutations are associated with parent-of-origin specific methylation at several 15q11-13 loci. We studied 5 patients suspect of PWS and 4 patients suspect of AS who were referred to the Medical Genetics Unit at the University Hospital of Medical School from Ribeirão Preto. Our objective was to establish the correct clinical and etiological diagnosis in these cases. We used conventional cytogenetics, methylation analysis with the probe KB17 (CpG island of the SNRPN gene) by Southern blotting after digestion with the Xba I and Not I restriction enzymes. We studied in patients and their parents the segregation of the $(CA)_n$ repeats polymorphisms by PCR, using the primers 196 and IR4-3R. All the patients had normal conventional cytogenetical analysis. We confirmed 3 cases of PWS: one by *de novo* deletion, one by maternal chromosome 15 uniparental disomy and one case with no defined cause determined by the used primers. We confirmed 2 cases of AS, caused by *de novo* deletion at the 15q11-13 region, and one case with normal molecular analysis but with strong clinical characteristics.

KEY WORDS: medical genetics, mental retardation, Prader-Willi syndrome, Angelman syndrome, molecular genetics, PCR (polymerase chan reaction), Southern blot.

Aspectos clínico-neurológicos, citogenéticos e moleculares das síndromes de Prader-Willi e Angelman

RESUMO - A síndrome de Prader-Willi (SPW) e a síndrome de Angelman (SA) são doenças neurogenéticas consideradas como exemplos do fenômeno de imprinting em seres humanos, estando relacionadas com alterações envolvendo a região cromossômica 15q11-13. As alterações genéticas predominantes na SPW são deleções na região 15q11-13 de origem paterna e dissomia uniparental materna. Na SA encontra-se deleções na região 15q11-13 materna e dissomia uniparental paterna. Estudamos 5 pacientes com suspeita clínica de SPW e 4 pacientes com suspeita clínica de SA atendidos no Setor de Genética Médica do Hospital Universitário da FMRP-USP, com o objetivo de estabelecer o diagnóstico clínico e etiológico de certeza nessa amostra. Para isso utilizamos citogenética convencional, estudo de metilação por Southern blotting utilizando a sonda KB17 (ilha CpG do gene SNRPN) após digestão com as enzimas de restrição Xba I e Not I e análise de polimorfismos de repetição de CA por PCR, usando os primers 196 e IR4-3R. Dos 9 pacientes avaliados, todos tiveram avaliação citogenética convencional normal. Foram confirmados a nível molecular, 1 caso de SPW por deleção nova, 1 caso de SPW por dissomia uniparental materna e 1 caso de SPW em que a causa genética não pode ser esclarecida pela análise de polimorfismo com os primers usados. Foram confirmados a nível molecular 2 casos de SA, ambos por deleção nova na região 15q11-13, e 1 caso de SA cuja clínica é extremamente sugestiva mas no qual não foi evidenciada alteração em qualquer dos exames moleculares utilizados.

PALAVRAS-CHAVE: genética médica; deficiência mental; síndrome de Prader-Willi; síndrome de Angelman; genética molecular; PCR (polymerase chain reaction); Southern blot.

The Prader-Willi and Angelman syndromes are neurogenetic diseases related to changes involving chromosome region 15q11-13 and are considered to be examples of the imprinting phenomenon in human beings, i.e., of the differential expression of a gene depending on parental origin.

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Prader-Willi syndrome (PWS) was first described in 1956[18], but continues difficult to establish the diagnosis solely on a clinical basis due to the fact that many of its characteristics are not specific or change with age. Subdiagnosis is common in childhood and overdiagnosis is common among adolescents and obese mentally deficient adults. The estimated incidence of PWS is 1/10,000 liveborns and has been reported by some Congenital Defect Services as to being among the 5 most common syndromes[8]. It is typically sporadic but 1 to 3% of the cases are familial[21]. Clinically PWS is characterized by central congenital hypotonia, hyperphagia and obesity starting after the first year of life, delayed neuromotor development and later by mental deficiency, hypogenitalism, hypogonadotrophic hypogonadism and some dysmorphisms.

Angelman syndrome (AS) was described by Angelman in 1965[1] in 3 unrelated children with a clinical picture of "flattened head, spasmodic movements, tongue thrusting and paroxysms of laughing conferring the aspect of puppets on them". The incidence of this syndrome is estimated at 1/20,000 births[6]. AS is clinically characterized by central congenital hypotonia, severe mental deficiency, microcephaly with marked occipital flattening, profound speech delay, jerky voluntary movement, a happy disposition with paroxysms of laughther, tongue thrusting, and a characteristic facial appearance, which includes a proeminent jaw wide mouth and midfacial hypoplasia. Seizures are very common and the syndrome is associated with an unusual electroencephalogram (EEG) . The EEG findings are caracteristic and the following three patterns were found separatedly, in association or in sequence in the patients: i) high-amplitude (about 200μV), generalized 4-6Hz, occupying the majority of the record; ii) very high-amplitude (200-500μV) 2-3Hz activity in prolonged runs, more proeminent anteriorly; these were mixed with spikes and sharp waves, thus forming spike-wave complexes; iii) spikes and sharp waves mixed with high-amplitude 3-4 Hz activity, seen posteriorly; these were sometimes asymetrical, typically triggered by eye closure[2]. This syndrome is also difficult to diagnose simply on a clinical basis, especially during the first years of life. Most AS cases are sporadic[23].

Among the genetic mechanisms leading to the onset of PWS, 73% are deletions involving the paternal 15q11-13 region, 25% are maternal uniparental disomy, 2% are mutations in the imprinting center, and 0.1% are translocations[14]. In AS, 73% of the cases involve deletions in the maternal 15q11-13 region, 2% are uniparental paternal disomy, 20% present biparental inheritance, and 5% are mutations in the imprinting center[5]. Laboratory methods for the diagnosis of both syndromes include fluorescent in situ hybridization (FISH), determination of the allele-specific methylation pattern at various loci in the 15q11-13 region, including locus D15S63 studied with the PW71 probe, and the small nuclear ribonucleoprotein peptide N gene (SNRPN)[11,22] and analysis of polymorphism segregation with restriction fragments length polymorphisms (RFLP) or microsatellites (CA repetitions)[16,19]. Although these syndromes have been exhaustively studied, the genes responsible for them have not yet been defined. For PWS there is a strong suspicion of the involvement of gene SNRPN which undergoes imprinting, expressing solely in the paternal copy, and which is located in the critical region of PWS[12]. As to AS, the critical region is estimated at least in 200 kb, but no consensus exists about the gene involved[3].

The objective of the present report was to describe the clinical, cytogenetic and molecular study of 9 patients suspected to have these syndromes (PWS in 5 and AS in 4).

MATERIALS AND METHODS

Patients

We studied 9 patients seen at the Medical Genetics Unit of the University Hospital FMRP-USP with suspected PWS (5 cases) and AS (4 cases). The molecular studies were also extended to the parents.

Clinical Methods

All patients were evaluated clinically by the authors using a protocol based on Holm et al.[13] for PWS and on Williams et al.[24] for AS.

The patients with suspected PWS were divided into 4 subgroups according to the scores obtained by the criteria of Holm et al.[13], excluding alterations in the 15q11-13 chromosome region as a diagnostic criterion, as

proposed by Erdel et al.[9]: *Typical PWS*, patients older than 3 years and with a score of 7 or more and patients younger than 3 years with a score of 5 or more; *Suspected PWS*, patients older than 3 years with a score of 5 to 6.5 or patients too young to permit clinical confirmation of the diagnosis; *Possible PWS*, patients with a score of 2 to 4.5; *non-PWS*, patients with a score of less than 2.

The patients with suspected AS were also divided into subgroups according to the criteria proposed by Erdel et al.[9], adapted from Williams et al.[24], i.e., absence of structural brain defects, exclusion of metabolic disorders, severe mental deficiency with absence of speech, puppet-like movements, paroxysmis of laughter, characteristic EEG abnormalities, and craniofacial dysmorphisms. The classification was based on the following criteria: *Typical AS*, presence of at least 4 of the signs and symptoms listed above; *Suspected AS*, presence of 3 of the signs and symptoms; *Possible AS*, less than 3 signs and symptoms.

Cytogenetic Methods

We studied the karyotype of each suspected patients in peripheral blood samples in order to determine the presence of chromosome rearrangements. The cytogenetic study was carried out using time lymphocyte cultures by the modified technique of Moorhead et al.[15]. Chromosome metaphases were stained by GTG banding according to the technique of Scheres[20].

Molecular Methods

The molecular studies consisted of analysis of the methylation pattern of region 15q11-13 using the KB17 probe[7], which detects the methylation state of the CpG island of gene SNRPN, and the methylation-sensitive restriction enzymes XbAI and NotI. This analysis is used for the diagnosis of PWS and AS at the molecular level. After confirming the presence of PWS or AS, we performed analysis of segregation of the (CA)n repeat polymorphisms by polymorphisms chain reaction (PCR) using primers 196 (D15S113)[10] and IR43R (D15S11)[17] in order to establish the genetic cause of these syndromes.

RESULTS

Clinical Results

Of the 5 patients with suspected PWS, 2 were classified as typical PWS and 3 as suspected PWS, and of the 4 patients with suspected AS, 3 were classified as typical AS and 1 as suspected AS. The clinical findings are listed in Tables 1 and 2.

Cytogenetic results

Cytogenetic analysis of the 9 suspected cases was normal, with no chromosome abnormality detected.

Molecular results

A. Study of methylation in the 15q11-13 region by Southern Blot: in PWS, this technique is fully discriminative, i.e., if the suspected patient has only one band (in this case, the one of maternal origin) he is confirmed as a case of this syndrome, and if two bands are present (a paternal one and a maternal one), the diagnosis of PWS is ruled out. In AS, if the patient has only one band (in this case, the one of paternal origin) he is confirmed as a case of this syndrome, and if two bands are present the diagnosis cannot be ruled out since approximately 20% of the cases of this syndrome have biparental inheritance, and if the clinical picture is really compatible, they represent cases of point mutation (Fig 1).

The following results were obtained: *PWS*: Case PWS-1 (suspected PWS) = 2 bands, excluding the hypothesis of PWS; Case PWS-2 (typical PWS) = 1 band (maternal), confirmed as a PWS case; Case PWS-3 (suspected PWS) = 1 band (maternal), confirmed as a PWS case; Case PWS-4 (typical PWS) = 1 band (maternal), confirmed as a PWS case; Case PWS-5 (suspected PWS) = 2 bands, ruling out the hypothesis of PWS. *AS*: Case AS-1 (typical AS) = 1 band (paternal) confirming a case of AS; Case AS-2 (suspected AS) = 2 bands - with biparental inheritance; a definitive diagnosis depends on clinical criteria; Case AS-3 (typical AS) = 1 band (paternal) confirming the diagnosis of AS; Case AS-4 (typical AS) = 2 bands - with biparental inheritance; the diagnosis depends on clinical criteria.

B. Analysis of segregation of (Ca)n repeat polymorphisms by PCR: as mentioned earlier, this technique is used in confirmed cases of the two syndromes to study the familial pattern of trans-

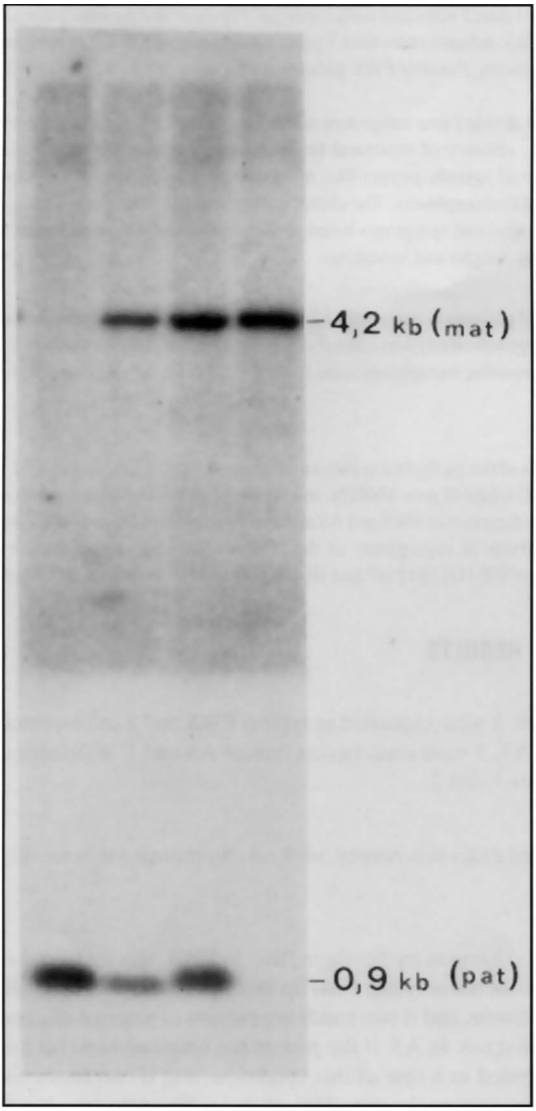

Fig 1. 15q11-13 region methylation study results by Southern Blotting (restriction enzimes: Not I and Xba I; probe: KB17). There are 2 bands: 4,2 Kb (maternal origin), and 0,9Kb (paternal origin). Normal individuals, and in the biparental AS cases, have the two bands; PWS individuals have just the maternal 4,2Kb band; most of the AS patients have only the 0,9Kb paternal band.

mission of alleles from different sites in the 15q11-13 region in order to establish the etiology of each case, i.e., whether the patient is a case of deletion, of uniparental disomy, or of point mutation.

Results obtained: *PWS*: Case SPW-2 - This case resulted from maternal uniparental disomy since the affected subject and the mother present the same haplotype. The father presents an allele identical to that of the mother and the son, but has another allele that differs form theirs; for a better interpretation of this case it should be kept in mind that a deletion of the paternal band was observed in the affected subject by methylation analysis (Fig 2A); Case PWS-3 - The exam was inconclusive in terms of etiology since the family was not informative in the primers studied; it is necessary to study this family with other primers of the 15q11-13 region; Case PWS-4 - This results from a paternal deletion since both the mother and the father are homozygous for different alleles and the affected subject presents only one maternal allele (Fig 2B). *AS*: Case AS-1 - This results from a maternal deletion since the affected subject presents one of the paternal alleles and the mother has the other allele identical to that of the father and another allele that differs from the paternal one and from that presented by the son (Fig 2C); Case AS-2 - This is one of the cases that presented biparental inheritance in the methylation analysis, a result that was confirmed in the present analysis since the affected son presents an allele identical to that of the father and that of the mother; the affected subject is homozygous for this allele, while the father and mother are heterozygous and present a different allele, exactly the one not presented by the son (Fig. 2D); Case AS-3 - This is another case resulting from a maternal deletion since the affected son only presents one of the paternal alleles (the father is a heterozygote); the mother is homozygous for another allele which differs from those of the father and of the affected son (Fig. 2E); Case AS-4 - This is another case that presented biparental inheritance in the methylation analysis since he presents one of the two maternal alleles (the mother is a heterozygote) and one of the paternal alleles (thus, one of the alleles present in the affected son is maternal and the other is paternal; the father is a heterozygote

Table 1. Clinical, cytogenetic and molecular evaluation of patients suspected to have the Prader-Willi syndrome.

	Patients				
Identification	PWS-1	PWS-2	PWS-3	PWS-4	PWS-5
Sex	M	F	M	M	F
Age	9 years and 6 months	2 years and 10 months	4 years and 6 months	13 years	14 years and 1 month
Major diagnostic criteria					
1. Central hypotonia (neonatal and/or in childhood), with weak suction and improving with age	-	+	+	+	+
2. Difficulty in swallowing and low weight gain during the 1st year of life	-	+	-	+	-
3. Excessive or rapid weight gain after the 1st year of life and before 6 years/central obesity	+	+	+	+	+
4. Craniofacial dysmorphisms*	+	+	+	-	-
5. Genital hypoplasia and/or delay or absence of gonadal development after 16 years	+	-	-	+	-
6. Generalized neuromotor delay up to 6 years (mental deficiency or learning deficit)	+	+	+	+	+
7. Hyperphagia	-	-	+	+	-
Minor diagnostic criteria					
1. Reduced fetal movements and/or lethargy in infancy and/or weak crying	-	+	+	+	+
2. Behavioral disorders**	+	-	-	-	-
3. Sleep disorders/sleep apnea	-	-	-	+	-
4. Short stature for the family	-	-	-	-	+
5. Hypopigmentation of skin and hair	-	-	-	-	-
6. Small hands and/or feet	-	+	+	-	+
7. Narrow hands with rectification of the nail border	-	-	-	-	-
8. Ocular alterations (exotropia/myopia)	+	-	-	+	-
9. Thick and viscous saliva accumulating in the corners of the mouth	-	-	-	+	-
10. Problems in speech articulation	+	-	+	+	-
11. Redundant skin	-	-	-	-	-
Score /	5,5	6,0	6,5	8,5	4,5
Clinical classification	Suspected PWS	Typical PWS	Suspected PWS	Typical PWS	Suspected PWS
Laboratory investigation					
Cytogenetics	46, XX	46.XX	46.XX	46,XY	46,XY
Methylation pattern	biparental	maternal uniparental	maternal uniparental	maternal uniparental	biparental
Analysis of the segregation of (CA)n repeats polymorphisms	Not done	Deletion of the paternal allele	Uninformative	Maternal Uniparental Disomy	Not done
Final conclusion	PWS excluded	PWS due to a deletion mechanism	PWS due to a undetermined Marternal Disomy	PWS due to a Uniparental	PWS excluded

**Craniofacial dysmorphisms: considered to be present when the patient presents 3 or more of the following characteristics: dolichocephaly, narrow face or bifrontal diameter, upward slanted palpebral fissures, thin upper lip and downward slanted labial commissure.*

***Behavioral disorders: considered to be present when the patient presents at least 5 of the following behaviors: angry temperament, anger attacks, obsessive-compulsive, possessive, manipulatory, rigid, arguing, contrary, persevering, subreptitious, and lying behavior.*

Table 2. Clinical, cytogenetic and molecular evaluation of suspected cases of Angelman syndrome.

	Patients			
Identification	AS-1	AS-2	AS-3	AS-4
Age	4 years and 5 months	10 years and 6 months	4 years and 7 months	12 years and 9 months
Sex	M	M	M	M
Development and laboratory findings				
Normal prenatal and neonatal history	+	+	+	+
DNPMD	+	+	+	+
No neuromotor involution	+	+	+	+
Normal metabolic investigation	+	+	+	+
Structurally normal brain	- (1)	- (2)	+	+
Consistent findings				
Severe mental deficiency	+	+	+	+
Speech deficiency	+	-	+	+
Ataxic movements	+	+	+	+
Typical behavior*	+	+	+	+
Frequent findings				
Microcephaly	+	+	+	+
Convulsions	+	-	+	+
Abnormal EEG	+	-	-	+
Associated findings				
Flattened occiput	+	+	+	+
Occipital sulcus	-	-	-	-
Protruding tongue	+	-	+	+
Deglutition disorder	-	-	+	+
Prognathism	-	-	+	+
Wide mouth with spaced teeth	+	+	+	+
Frequent salivation	+	-	+	+
Strabismus	+	+	+	+
Hypopigmented skin	-	-	+	+
Increased lower limb reflexes	+	+	+	+
Arm flection while walking	+	+	+	+
Attraction to water	+	+	+	+
Sleep disorders	+	-	-	-
Clinical classification	Typical AS	Suspected AS	Typical AS	Typical AS
Cytogenetics	46,XY	46,XY	46,XY	46,XY
Analysis of the methylation pattern	Paternal Uniparental	Biparental	Paternal Uniparental	Biparental
Analysis of segregation of (CA)n repeats	Deletion of the maternal allele	Biparental	Deletion of the maternal allele	Biparental
Final Conclusion	AS due to deletion	AS ruled out by clinical criteria	AS due to deletion	AS by clinical criteria only (biparental inheritance)

*DNPMD: delayed neuropsychomotor development; EEG, electroencephalogram; *Typical behavior: any combination of frequent laughing/smiling, apparently happy behavior, easily excitable personality, frequent hand flapping, hypermotor behavior. 1, brain atrophy; 2, Dandy-Walker malformation with vermis hypoplasia and cerebellar atrophy.*

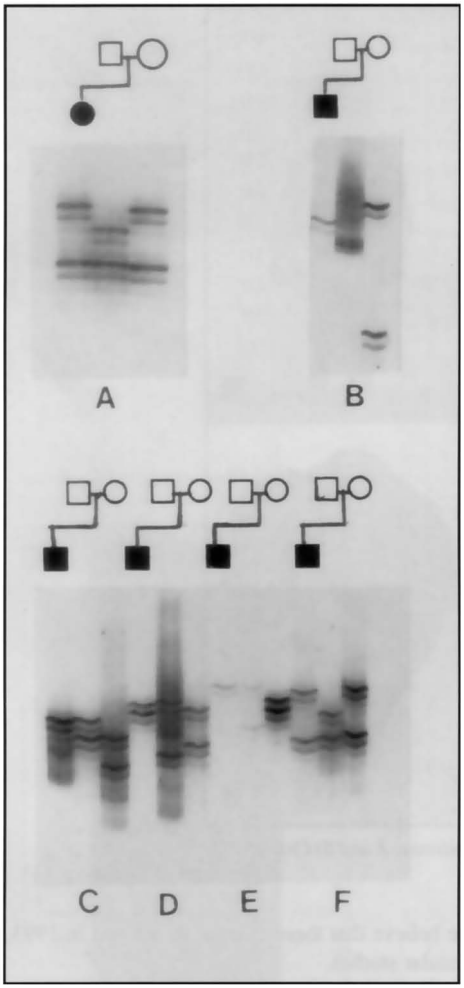

Fig 2. (CA)n repeats analysis with Polymerase Chain Reaction, using 196 primer: A) PWS patient showing Maternal Uniparental Disomy - the proband and his mother present the same haplotype; for a better interpretation, it shoudl be in mind that just the maternal band was observed in the proband by methylation analysis. B) PWS patient showing paternal deletion since the proband presents only one maternal allele and no paternal alleles. C) AS patient showing maternal deletion since the proband presents one paternal allele and no maternal alleles. D) AS patient showing biparental inheritance. E) AS patient showing maternal deletion since the proband presents one paternal allele and no maternal alleles. F) AS patient showing biparental inheritance.

presenting one of the alleles presented by the mother and also by the son, and a different allele) (Fig 2F).

C. Correlations between genotype and phenotype in the patients studied:

The clinical, phenotypic and genotypic correlations are presented in Tables 1 and 2.

In PWS: the cases with a confirmed diagnosis are shown in Figure 3. For the non-confirmed cases, the scores obtained were: Case PWS-1 = 5.5 points; Case PWS-5 = 4.5 points. Case PWS-3, classified as suspected PWS was confirmed as PWS in the methylation study. *In AS*: the cases with a confirmed diagnosis are shown in Figure 4. The cases not confirmed by molecular examination were: Case AS-2, classified as suspected AS, and case AS-4, classified as typical AS. By comparing the clinical findings with the results of molecular investigation we excluded the hypothesis of AS for case AS-2, whereas case AS-4, clinically one of the most typical in the sample (Table 2) is probably a case of AS of biparental inheritance.

DISCUSSION

The methodology used in the present study seems to be adequate for the investigation of suspected PWS and AS cases. In the PWS cases, the study of the methylation pattern with the KB17 probe permits the confirmation or exclusion of virtually 100% of cases, although the investigation of the genetic etiology may occasionally require the use of other polymorphisms detected by PCR for elucidation (as was the case for patients PWS-3). Among the five PWS cases studied, the two classified as typical PWS (PWS-2 and PWS-4) had their diagnosis confirmed by the molecular exams. The three other cases, PWS-1, PWS-3 and PWS-5, were classified as suspected PWS and the molecular exams ruled out the diagnosis of PWS in 2 of them (PWS-1 and PWS-5). PWS-3 was the youngest patient (4 years and 6 months) and had the highest score among suspected PWS cases (6.5 as opposed to 5.5 for PWS-1 and 4.5 for PWS-2), with confirmation of the PWS diagnosis in the methylation study. It is possible that, with age, the children will present other alterations such as behavioral disorders that meet the clinical criterion of Holm et al.[13]. We agree with Erdel et al.[9] about the fact that clinical screening rigidly based on the criteria of Holm et al.[13] may eventually

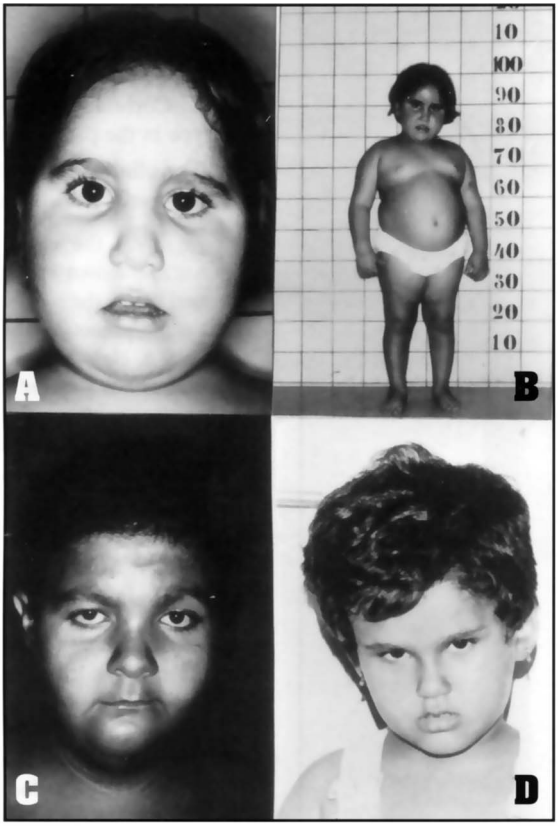

Fig 3. Patients with Prader-Willi syndrome: A and B) Case 16217; C) Case 16226; C) Case 16223.

exclude children with PWS from laboratory tests. We believe that these criteria, developed in 1993, deserve revision in the light of new clinical and molecular studies.

With respect to genetic counseling, cases PWS-2 and PWS-4 presented a deletion of paternal origin on chromosome 15 and case PWS-4 presented uniparental maternal disomy considered to have originated from a correction of trisomy 15 during embryonic life[4]. Case PWS-3, also with a confirmed diagnosis but without a defined etiology, should be studied with other markers for better counseling, although familial PWS cases are extremely rare.

Cases PWS-1 and PWS-5, clinically considered to be suspected PWS and whose PWS diagnosis was excluded by the molecular exams, are currently being reevaluated in the search for a correct diagnosis of their clinical picture.

As to the AS cases, the literature[3,5] shows that most of them (70 to 80%) are due to new deletions on chromosome 15 inherited from the mother, in agreement with our findings. The molecular exams confirmed 2 cases classified as typical AS, i.e., case AS-1 and case AS-3, with 6/7 and 5/7 of the signs and symptoms considered for classification, respectively. With respect to the genetic counseling of these two cases, we concluded that there is no risk of recurrence for the family of the affected subjects, since these are new deletions.

Case AS-4, classified as typical AS, presented biparental inheritance in the analysis of methylation and in the (CA)n repeats polymorphisms used. The literature shows that 2% of AS

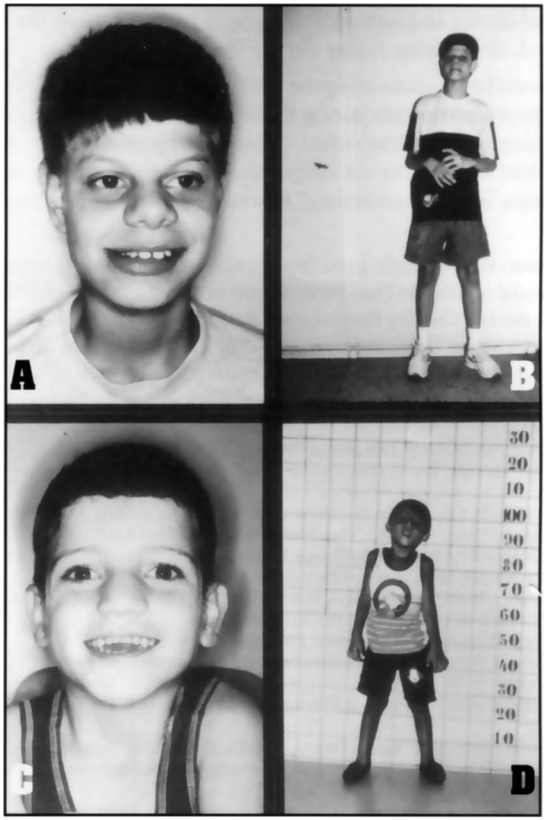

Fig 4. Patients with Angelman syndrome: A and B) Case 16222; C) Case 16214; D) Case 16208.

cases are of biparental inheritance[5] and it is in this group that the familial cases of this syndrome are concentrated, with a 50% recurrence risk. For this reason, we believe that clinical evaluation is extremely important for the confirmation of the diagnosis in these cases. Case AS-4 presented 7/7 signs and symptoms used for patient classification in this study, including the typical electroencephalographic tracing of AS, and was therefore the most typical patient in the sample, leading us to consider him a case of AS of biparental inheritance. This patient presented 23/25 of the diagnostic findings for AS, with all the consistent findings, all the frequent findings and, among the combined findings, the most common ones (Table 2). With respect to genetic counseling, we concluded that there would be a 50% risk of recurrence for the mother in new pregnancies.

Case AS-2, classified as suspected AS, fulfilling only 3/7 of the criteria used for classification, presented molecular results showing biparental inheritance and when we reevaluated him clinically we excluded the possibility that he had AS. Clinical reevaluation of this patient showed that he presented only 14/25 of the diagnostic findings for AS (Table 2) and only 3/7 of the signs used for clinical classification. Among the so-called consistent clinical findings, he did not present speech deficiency, which is a characteristic detected in 100% of AS patients, who are aphasic or only speak a total of 6 words[6,24]. As to the consistent findings, this patient did not present convulsive seizures or EEG alterations, which are encountered in AS patients at a frequency of 86% and 92%, respectively. As to the associated findings, he did not present several signs that are detected in AS at relatively high frequencies (Table 2)[6,24]. A CT scan of the skull indicated as part of the continued investigation revealed the presence of the Dandy-Walker malformation,

with hypoplasia of the vermis and cerebellar atrophy, a fact explaining the ataxia and the delayed development observed, definitively ruling out the diagnosis of AS.

The study of methylation status using the KB17 or PW71 probe has been previously proposed for the evaluation of hypotonic infants during the first year of life[22]. We believe that, in view of the difficulty in establishing an early exclusively clinical diagnosis both in PWS and AS, this test could be very important to confirm the diagnosis, thus permitting the necessary measures for improving the prognosis in both syndromes, as well as genetic counseling and the indication of prenatal diagnosis.

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