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Molecular and Clinical Studies of Polish Patients with Prader-Willi Syndrome

A. Szpecht-Potocka, E. Obersztyn, M. Karwacki, E. Bocian, J. Bal, T. Mazurczak

Department of Genetics, National Research Institute of Mother and Child, Warsaw, Poland

Abstract. A group of 30 patients clinically described as having the Prader-Willi Syndrome (PWS) were studied using microsatellites from 15q11-13 and methylation analysis with probe PW71B (D15S63). The patients were categorized according to clinical symptoms. 80% of all patients were informative using molecular and cytogenetic methods. Among 8 patients with an atypical PWS phenotype, 2 showed uniparental disomy, and 2 had a mosaic deletion for 15q. The last 4 atypical and 2 typical patients had neither molecular defects confirmed by microsatellite analysis nor a parent-of-origin-specific methylation pattern for PWS. Our results confirm that methylation pattern analysis provides an additional and alternative microsatellite analysis to diagnose PWS.

Key words: Prader-Willi syndrome, Microsatellite analysis, Methylation pattern

INTRODUCTION

Prader-Willi syndrome (PWS) is the clearest example of genomic imprinting in humans. This mental retardation disorder is characterized by a complex of neurodevelopmental, dysmorphic and behavioral symptoms: diminished fetal movement, infantile hypotonia and feeding difficulties, increased appetite and onset of obesity in early childhood, retarded motor and mental development, characteristic features of the face, short stature, small hands and feet and hypogenitalism. A clinical diagnosis of PWS may be difficult because of variability in phenotype expression and diversity of clinical manifestation with age. PWS is associated with abnormalities of chromosome 15q11-13. Approximately 60% of patients have a de novo interstitial chromosome 15q deletion. Among clinically typical patients, 40% have a normal karyotype in HRT chromosomal analysis. Molecular studies show that (a) most of PWS patients (75%) carry a paternally derived deletion of the Prader-Willi chromosome region (PWCR); (b) maternal disomy is

detected in 25% of PWS patients, and (c) imprinting mutations are very rare [1]. The loss of functional paternally derived gene(s), which is (are) responsible for clinical manifestation, is observed in these three molecular classes of PWS patients.

PATIENTS AND METHODS

Clinical, cytogenetic and molecular studies were performed in 30 patients with a clinical diagnosis of PWS. The analyzed group of PWS patients consisted of 22 males and 8 females ranging in age from 5 months to 25 years. 9 patients were younger than 3 years old. The PWS patients were categorized according to clinical symptoms using consensus diagnostic criteria described by Holm et al. [2]. Every patient was scored for 8 major and 11 minor diagnostic traits. PWS patients were classified as clinically typical when they fitted 8 points for children over 3 years and 5 points for 3 years of age and younger. Patients who did not fulfil this criterion were qualified as atypical.

Four dinucleotide repeat markers from loci D15S11, D15S10, D15S113 and GABRB3 in the PWCR were used in microsatellite polymorphism analysis [3, 4]. Parent-of-origin-specific DNA methylation in 15q11-13 was tested by genomic probe PW71B (D15S63) [5].

RESULTS AND DISCUSSION

The results of the clinical, molecular and cytogenetics analysis are summarized in table 1. Using molecular and cytogenetic methods, we were able to confirm the clinical

Table 1 - Results of molecular study in clinical and cytogenetic categories of PWS patients

Clinical category	typical (n = 22)		atypical (n = 8)	
Cytogenetics	normal	deletion	normal	deletion in mosaicism
Number of patients	11	11	6	2
Molecular classification				
Deletion	0	8	0	0
Heterodisomy	1	0	0	0
Heterodisomy excluded	8	0	2	0
Microsatellites not informative	2	3	4	2
Confirmation by methylation analysis	2	1	0	0
Mean clinical criteria score[1]	9.8 (5-11.5)	9.9 (7.5-12)	4.16 (3.5-5.5)	4.5

[1]Range given in parentheses.

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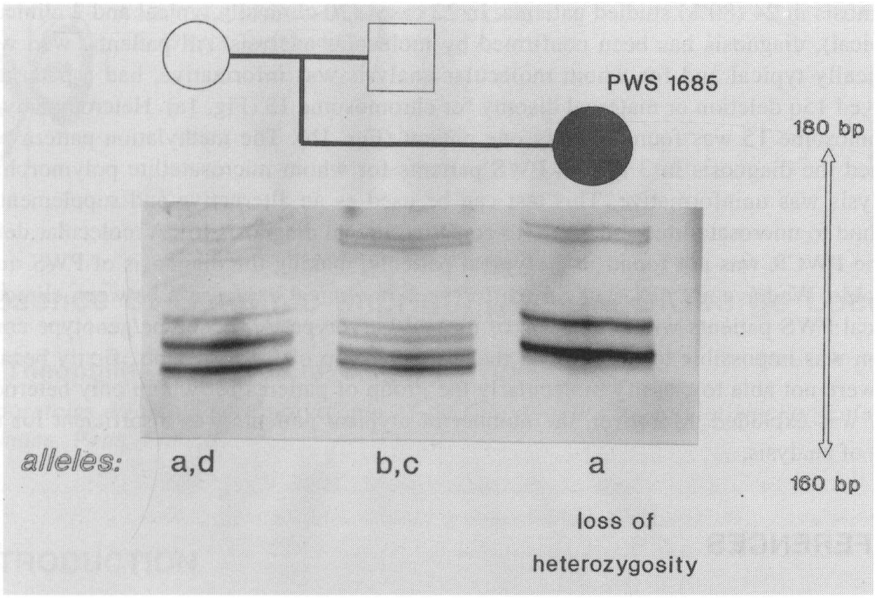

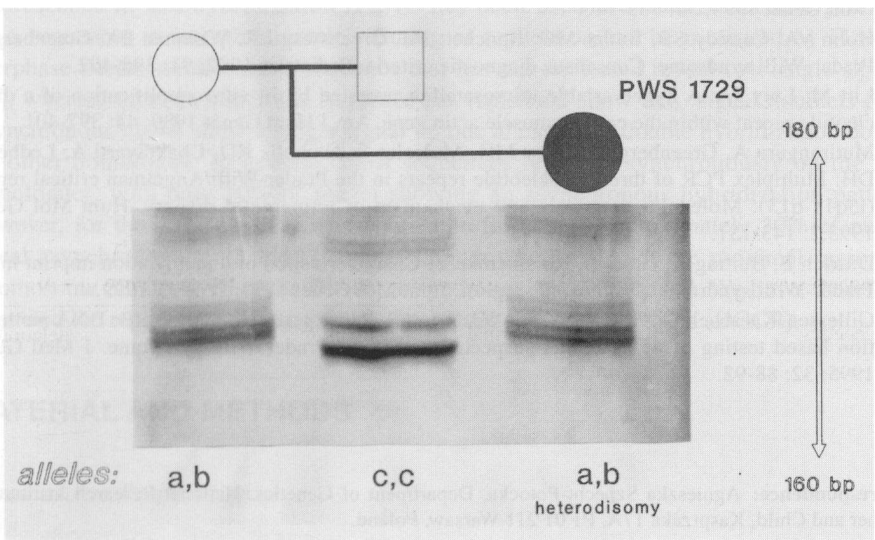

Fig. 1 - Microsatellite polymorphism analysis at the locus GABRB3. Undernaturated DNA samples were run on the native polyacrylamide minigels and silver-stained for detection. Each allele is accompanied by a "conformational" band of reduced mobility, which is often slightly fuzzy in appearance. Presence of conformational bands is probably due to the repeat sequence within the PCR product taking up an altered conformation. The distance behind the proper allele is unique to each repeat but remains constant for alleles of different size. *a*) Loss of heterozygosity in the patient PWS 1685. *b*) Heterodisomy detected in the patient PWS 1729.

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diagnosis in 24 (80%) studied patients. In 22 cases (20 clinically typical and 2 clinically atypical), diagnosis has been confirmed by molecular analysis. All patients, who were clinically typical and for whom molecular analysis was informative, had a paternally derived 15q deletion or maternal disomy for chromosome 15 (Fig. 1a). Heterodisomy for chromosome 15 was found in only one patient (Fig. 1b). The methylation pattern confirmed the diagnosis in 3 typical PWS patients for whom microsatellite polymorphism analysis was uninformative. This test can be used as an alternative and supplementary method to microsatellites and RFLP to confirm clinical diagnosis [6]. A molecular defect in the PWCR was not found in 4 atypical patients, making the diagnosis of PWS questionable. We have not observed any differences in clinical expression between clinically typical PWS patients with a deletion or normal karyotype. A phenotype/genotype correlation was impossible to establish in the studied group of PWS patients, firstly because we were not able to classify molecularly the group of patients for whom only heterodisomy was excluded. Moreover, the number of atypical patients was insufficient for this type of analysis.

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Correspondence: Agnieszka Szpecht-Potocka, Department of Genetics, National Research Institute of Mother and Child, Kasprzaka 17A, P1 01 211 Warsaw, Poland.