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Molecular Diagnosis of Prader-Willi Syndrome: Comparison of Cytogenetic and Molecular Genetic Data Including Parent of Origin Dependent Methylation DNA Patterns

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To the Editor

Recently, Lerer et al. [1994] reported their molecular diagnostic experience with 22 individuals suspected to have Prader-Willi syndrome (PWS) using DNA probe PW71 for locus D15S63 which detects a parent-of-origin-specific methylated site in the PWS critical region at 15q11q13. They determined the deletion or disomy status of chromosome 15 by using (CA)_n dinucleotide repeat polymorphisms of 7 loci of the 15q11q13 chromosome region as well as more distal markers. In 10 PWS individuals, the clinical diagnosis was confirmed by this approach [6 patients showed a paternal deletion (including one patient with an aberrant methylation pattern detected by the PW71 probe but no deletion or disomy was found using loci D15S97, D15S113, GABRB3 and GABRA5 with polymerase chain reaction (PCR) methodology; they proposed that this patient had a paternal microdeletion in the PWS critical region) and 4 patients had maternal uniparental disomy of chromosome 15]. The remaining 12 patients had no recognizable chromosome 15 abnormality using the several molecular diagnostic approaches in their study.

Laboratory evaluation of patients suspected to have either PWS or Angelman syndrome (AS), a different clinical condition but with a similar deletion of the 15q11q13 region, can be helpful particularly in the infant or patient presenting at the time of evaluation without several of the major manifestations of the syndrome. Most patients with these syndromes have recognizable cytogenetic deletions of 15q11q13 (i.e., 60% of PWS patients have a paternal deletion and 70% of AS patients have a maternal deletion) [Butler et al., 1986; Butler, 1990, 1994; Zackowski et al., 1993; Knoll et al., 1993]. Thus, PWS and AS represent the first examples in humans of genetic imprinting or the differential expression of genetic information depending on the parent of origin. With detailed molecular genetic analysis the frequency of deletions in PWS and AS persons approaches 75% [Nicholls, 1994]. Maternal uniparental disomy of chromosome 15 is found in about 25% of PWS patients while less than 5% of AS patients show paternal uniparental disomy of chromosome 15. A small percentage of PWS individuals appear to have biparental inheritance of chromosome 15 but may have genetic imprinting errors or mutations.

Currently the molecular diagnosis of PWS and AS patients includes the analysis of parent-of-origin-dependent methylation sites at 15q11q13 with DNA probe PW71 which recognizes the D15S63 locus as the most commonly used technique. Molecular diagnosis of PWS/AS can be achieved using these assays to identify the parent-of-origin-dependent methylation sites and the detectable DNA fragment regardless of the chromosomal status (i.e., uniparental disomy or deletion of chromosome 15). In addition, molecular analysis of PWS and AS based on the methylation DNA pattern can be achieved without availability of parental DNA.

Herein, I report our experience with parent-of-origin-dependent methylation pattern studies of the D15S63 locus using the DNA probe, PW71B, in 27 patients suspected to have PWS. These patients were studied with high resolution chromosome analysis, fluorescence in situ hybridization (FISH) using 4 probes, quantitative and qualitative Southern hybridization with 7 probes and/or PCR amplification of 17 loci for a combined total of 22 separate loci analysed from the 15q11q13 region.

High resolution chromosome analysis at the 550–850 band level, using Actinomycin D pretreatment and GTG banding methods [Butler et al., 1986] and FISH using four biotin or digoxigenin-labeled DNA probes (D15S10, D15S11, GABRB3 and SNRPN from Oncor, Inc., Gaithersburg, MD, following their protocols) were performed. Based on our studies, 13 of 27 patients referred to confirm or rule out PWS were found to have the 15q11q13 deletion; one patient had a translocation involving chromosomes 15 and 19 and the remaining 13 patients had normal appearing chromosomes. The FISH results confirmed the deletion status in all patients studied with cytogenetic deletion and the nondeletion status in those patients with normal appearing chromosomes.

Genomic DNA was isolated from peripheral blood specimens according to standard procedures. Polymerase chain reaction (PCR) amplification of genomic DNA from the PWS patients and their parents were undertaken with loci D15S11, D15S113, D15S128, D15S122, D15S10, D15S63, GABRB3, D15S97, D15S156, GABRA5, MN-1, D15S543, D15S542, D15S541, D15S165, D15S210, and D15S219 using published primer data and established protocols for identification of microsatellite polymorphisms [Beckmann et al., 1993; Wagstaff et al., 1993; Mutirangura et al., 1993a,b; Weber and May, 1989; Malcolm and Dolon, 1994]. The number of alleles were recorded and sizes compared with published data.

For methylation studies, 5 μ g of genomic DNA from each patient was double digested with Hind III and Hpa II and probed with PW71B as described by Dittrich et al. [1992] and the DNA methylation pattern analyzed. This procedure produces two different size fragments (upper 6.0 kb band from maternal chromosome 15 and a lower 4.4 kb band from the paternal chromosome 15). If only the upper band is present then the typical 15q11q13 deletion or maternal disomy of chromosome 15 would be indicated.

Qualitative and/or quantitative Southern hybridizations were performed according to standard techniques for PW71B and seven additional DNA probes (D15S9, D15S10, D15S11, D15S12, D15S13, D15S18 and SNRPN) from the 15q11q13 region and control

probes from 13q following published protocols [Maniatis et al., 1989; Butler et al., 1993; Tantravahi et al., 1989; Nicholls et al., 1989]. Briefly, quantitative studies of the SNRPN gene were undertaken by using a radiolabeled PCR product generated from primers of exons E and H of the SNRPN gene [Ozcelik et al., 1992] with genomic DNA digested with Bgl II enzyme. This analysis produces a 7.5 kb upper fragment representing the SNRPN pseudogene from chromosome 6 (used as control) and a 5.8 kb lower fragment which represents the SNRPN gene (SNRPN gene is a paternally expressed candidate gene for PWS from the 15q11q13 region). An autoradiograph was evaluated by densitometry to calculate the copy number of the SNRPN gene comparing the control pseudogene upper band with the lower SNRPN gene band intensity from the autoradiograph following the methodology described by Ozcelik et al. [1992]. Table I shows summary clinical, cytogenetic, and molecular genetic data including DNA methylation patterns for our patients referred for PWS.

Our study of suspected PWS patients showed that 13 or 48% had a recognizable cytogenetic deletion of 15q11q13 by high resolution analysis while FISH, Southern hybridization and PCR analysis confirmed the deletion status as well as the presence of the paternal deletion in those studied. Thirteen patients had normal high resolution chromosome findings confirmed by FISH, Southern hybridization and/or PCR studies. Four of these nondeletion patients showed uniparental disomy of chromosome 15 (one with maternal isodisomy and 3 with maternal heterodisomy) by Southern hybridization and PCR analysis of parental and patient genomic DNA showing only a single 6.0 kb fragment with DNA methylation studies; five nondeletion patients showed normal biparental inheritance of chromosome 15 and one patient showed a translocation between chromosomes 15 and 19 with a breakpoint in the 15q11q13 region. FISH data of this PWS patient with the translocation showed two SNRPN hybridization signals (one on the donor chromosome 15 and one on the recipient chromosome 19) [Sun et al., 1995]. No deletion was found using quantitative Southern hybridization with SNRPN, other 15q11q13 loci and PCR amplification of 15q11q13 loci including exons E through H of the SNRPN gene in this translocation patient. DNA was not available on the parents from four patients suspected to have PWS but with normal appearing chromosomes and PCR or Southern hybridization studies of family members could not be performed. However, 2 of these nondeletion patients (PWS 20 and PWS 22) showed DNA methylation patterns consistent with either a deletion or disomy (i.e., only one 6.0 kb fragment observed, but in view of other molecular genetic data, they most likely represent maternal disomy). The 2 remaining nondeletion patients without parental DNA available showed a normal or biparental methylation pattern with 6.0 and 4.4 kb fragments. These latter 2 patients either represent imprinting mutations, very small deletions not detectable with the techniques and loci studied or do not have PWS. All patients with a recognizable 15q11q13 deletion showed the DNA methylation pattern consistent with a paternal deletion (i.e., showed only one 6.0 kb fragment).

In conclusion, our molecular diagnostic experience with a parent-of-origin methylation site at D15S63 was helpful in confirming the diagnosis of PWS in suspected patients. Our data also raise questions about other genetic lesions playing a role in the causation of PWS (e.g., imprinting mutations) or more likely that some suspected patients presenting with manifestations of PWS do not have the diagnosis but have some other cause. Additional

research is needed to gather clinical, cytogenetic and molecular genetic data to further characterize these laboratory diagnostic approaches in PWS.

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TABLE I
Clinical, Cytogenetic, and Molecular Genetic Data of Patients Referred for Prader-Willi Syndrome

Subject number	Sex	Age (yr)	Karyotype	Summary of FISH results ^a	Summary of Southern hybridization and PCR results ^b	Methylation DNA pattern using PW71B
PWS1	M	19	del(15q11q13)	Not available	Paternal deletion	6.0 kb fragment
PWS2	F	16	del(15q11q13)	Deletion	Deletion ^c	6.0 kb fragment
PWS3	F	20	del(15q11q13)	Deletion	Paternal deletion	6.0 kb fragment
PWS4	F	2	del(15q11q13)	Deletion	Paternal deletion	6.0 kb fragment
PWS5	F	12	del(15q11q13)	Not available	Deletion ^c	6.0 kb fragment
PWS6	F	5	del(15q11q13)	Not available	Paternal deletion	6.0 kb fragment
PWS7	M	13	del(15q11q13)	Not available	Paternal deletion	6.0 kb fragment
PWS8	M	18	del(15q11q13)	Deletion	Paternal deletion	6.0 kb fragment
PWS9	F	6	del(15q11q13)	Not available	Paternal deletion	6.0 kb fragment
PWS10	F	3	del(15q11q13)	Deletion	Paternal deletion	6.0 kb fragment
PWS11	M	12	del(15q11q13)	Not available	Paternal deletion	6.0 kb fragment
PWS12	F	23	del(15q11q13)	Not available	Paternal deletion	6.0 kb fragment
PWS13	M	14	del(15q11q13)	Not available	Paternal deletion	6.0 kb fragment
PWS14	F	17	46,XX	Normal	Normal; biparental	4.4/6.0 kb fragments
PWS15	M	7	46,XY	Not available	Normal; mat isodisomy	6.0 kb fragment
PWS16	M	11	46,XY	Not available	Normal; biparental	4.4/6.0 kb fragments
PWS17	F	14	46,XX	Not available	Normal; mat heterodisomy	6.0 kb fragment
PWS18	M	5	46,XY	Not available	Normal ^c	4.4/6.0 kb fragments
PWS19	F	12	46,XX	Not available	Normal ^c	4.4/6.0 kb fragments
PWS20	F	4	46,XX	Not available	Normal ^c	6.0 kb fragment
PWS21	F	0.1	46,XX	Normal	Normal; biparental	4.4/6.0 kb fragments
PWS22	M	0.3	46,XY	Not available	Normal ^c	6.0 kb fragment
PWS23	M	2	46,XY	Normal	Normal; biparental	4.4/6.0 kb fragments
PWS24	M	20	46,XY	Normal	Normal; mat heterodisomy	6.0 kb fragment
PWS25	F	16	46,XX	Normal	Normal; mat heterodisomy	6.0 kb fragment
PWS26	F	3	46,XX	Normal	Normal; biparental	4.4/6.0 kb fragments
PWS27	M	3	46,XY,t(15;19)	Abnormal ^d	Normal; biparental	4.4/6.0 kb fragments

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^aSummary of FISH result using four probes (D15S10, D15S11, GABRB3, SNRPN from Oncor, Inc., Gaithersburg, MD).

^bSummary of qualitative and quantitative Southern hybridization with 7 probes and PCR amplification of 17 loci from 15q11q13 region.

^cParental DNA not available.

^dFluorescence hybridization signals using the SNRPN probe were observed on both the donor chromosome 15 and the recipient chromosome 19 in a t(15;19) patient with PWS phenotype [Sun et al., 1995].