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High Resolution Chromosome Analysis and Fluorescence In Situ Hybridization in Patients Referred for Prader-Willi or Angelman Syndrome

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To the Editor:

Recently, Delach et al. [1994] reported their experience in evaluating fluorescence in situ hybridization (FISH) studies and high resolution chromosome analysis in 14 patients referred to confirm or rule out Prader-Willi syndrome (PWS) and 5 patients referred to confirm or rule out Angelman syndrome (AS). In 4 patients (3 from the PWS category and one from the AS group) the chromosome analysis suggested a deletion but FISH failed to confirm the finding. They concluded that patients reported with a cytogenetic deletion of 15q11q13 and clinical findings inconsistent with PWS or AS should be re-evaluated by molecular genetic techniques.

Laboratory testing is helpful in the evaluation of patients suspected to have either PWS or AS because most of these patients 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, 1994b; Zackowski et al., 1993]. Maternal uniparental disomy of chromosome 15, identified by molecular genetic techniques, is found in about 20–25% of PWS patients while the remaining PWS patients have submicroscopic deletions or biparental inheritance of chromosome 15 [Mascari et al., 1992]. Paternal uniparental disomy of chromosome 15 is seen in 2–3% of AS patients while the remaining AS patients have normal appearing chromosomes [Zackowski et al., 1993]. About 20% of families have more than one affected relative but the condition rarely recurs in PWS families. Thus, PWS and AS represent the first examples in humans of genetic imprinting or the differential expression of genetic information depending on the parental origin [Nicholls et al., 1989]. Herein, I report our experience with FISH and high resolution chromosome analysis in patients referred to confirm or rule out PWS or AS.

High resolution chromosome analysis at the 500–850 band level, using actinomycin D pretreatment and GTG banding methods [Butler et al., 1986], and FISH studies were performed with 5 probes (D15S11, D15S10, GABRB3, SNRPN, and D15Z1) available as biotin or digoxigenin labeled DNA kits (Oncor, Gaithersburg, MD) following the protocols

provided by the manufacturer. All high resolution chromosomes were analyzed but attention was focused on chromosome 15. The chromosomes were scored as either normal or deleted. In our laboratory the determination of a deletion of the 15q11 or 15q12 bands was made on the basis of absence of these bands on the proximal long and/or a shorter distance between the centromere and 15q14 band in the deleted chromosome.

Based on high resolution chromosome analysis, 11 of the 21 patients referred to confirm or rule out PWS were reported with the 15q11q13 deletion while 7 had normally appearing chromosomes, 2 had questionable 15q11q13 deletions, and one patient had a t(15q;15q). Similarly, 4 of the 5 patients referred to confirm or rule out AS were reported with the 15q11q13 deletion and one patient had a de novo 15q11q13 duplication (see Table I). In 2 subjects, the high resolution chromosome analysis was performed in another laboratory.

Initially, the D15S11 FISH probe (Oncor Region A) was used for the evaluation of PWS patients and GABRB3 (Oncor Region B) for AS patients. Subsequently, SNRPN (a paternally expressed candidate gene for PWS [Ozcelik et al., 1992]) and D15S10 FISH probes (which are thought to be more sensitive for the detection of the deletion) in PWS and AS, respectively, were used. A minimum of 20 metaphases was analyzed for the presence or absence of hybridization signals (an internal control PML probe was cohybridized generating a signal at 15q22 and commercially available in the labeled DNA kit with the proximal 15q probes [D15S11, SNRPN, D15S10, or GABRB3]). A proximal 15q deletion was determined if a hybridization signal was seen in the 15q11q13 region in only one of the chromosome 15s while the control signal was present at 15q22 in both 15s. A chromosome 15 was interpreted as normal if hybridization signals were seen in both the distal long arm (i.e., control PML probe signal) and proximal long arm (i.e., candidate probe [e.g., SNRPN] signal) in most (e.g., 95%) metaphases. In addition, a chromosome 15 alpha satellite probe (D15Z1), which localizes to the pericentromeric area of chromosome 15 particularly the short arm, was hybridized for the detection of centromeric polymorphisms and to rule out translocations involving the short arm of chromosome 15 with other acrocentric chromosomes [Butler and Greenstein, 1991].

Four patients referred to rule out PWS showed either a discrepancy between the high resolution chromosome findings and the FISH data or the FISH data appeared more informative than the high resolution results (e.g., 2 patients from the rule out PWS group [PWS 2 and PWS 3] were reported with normal chromosome 15s but FISH data showed a deletion with 4 of 4 15q11q13 probes for PWS 2 but only one probe [SNRPN] of 4 15q11q13 probes for PWS 3; one patient from the rule out PWS group [PWS 7] had a reported deletion of 15q11q13 but FISH data with 4 of 4 15q11q13 probes showed normal chromosome 15s; and one patient from the rule out PWS group [PWS 8] reported with a questionable 15q11q13 deletion had normal chromosome 15s using the SNRPN probe). One patient in the confirmed PWS group was reported with a questionable 15q11q13 deletion and the FISH data with 2 of 2 15q11q13 probes showed a deletion. There were no high resolution cytogenetic and FISH discrepancies in the evaluation of the rule out AS or confirmed AS patients (see Table I).

In summary, our FISH studies using proximal 15q probes did support the high resolution chromosome findings in most rule out or confirm PWS or AS patients; however, there were discrepancies similar to those reported by Delach et al. [1994]. Approximately 15% of our patients (2 with reported normal chromosomes but FISH showed a deletion [one patient with only SNRPN deleted] and 2 with a reported deletion or questionable deletion of 15q11q13 but no deletion by using FISH) showed a discrepancy between the high resolution chromosome interpretation and the FISH results. Our study further supports that FISH is useful in the laboratory evaluation of patients referred for PWS or AS, particularly in view of overrepresentation of short arm variants or pericentromeric heteromorphisms reported in the chromosome 15 with the deletion in these patients [Butler, 1994a]. Additional studies are required to better characterize the status of the high resolution chromosome results in PWS or AS patients as well as the sensitivity and reliability of FISH in the laboratory evaluation of these patients.

REFERENCES

- Butler MG (1990): Prader-Willi syndrome: Current understanding of cause and diagnosis. *Am J Med Genet* 35:319–332. [PubMed: 2309779]
- Butler MG (1994a): Are specific short arm variants or heteromorphisms over-represented in the chromosome 15 deletion in Angelman or Prader-Willi syndrome patients? *Am J Med Genet* 50:42–44. [PubMed: 8160752]
- Butler MG (1994b): Prader-Willi and Angelman syndromes: Examples of genetic imprinting in man. In Seth PK, Seth S (eds): “Human Genetics” New Delhi, India: Omega Scientific Publishers, pp 185–200.
- Butler MG, Greenstein MA (1991): Molecular cytogenetics of Prader-Willi and Angelman syndromes. *Lancet* 338:1276.
- Butler MG, Meaney FJ, Palmer CG (1986): Clinical and cytogenetic survey of 39 individuals with Prader-Labhart-Willi syndrome. *Am J Med Genet* 23:793–809. [PubMed: 3953677]
- Delach JA, Rosengren SS, Kaplan L, Greenstein RM, Cassidy SB, Benn PA (1994): Comparison of high resolution chromosome banding and fluorescence in situ hybridization (FISH) for the laboratory evaluation of Prader-Willi syndrome and Angelman syndrome. *Am J Med Genet* 52:85–91. [PubMed: 7977469]
- Mascari MJ, Ladda RL, Gottlieb W, Butler MG, Rogan PK, Armour J, Jeffreys A, Waller D, Nicholls RD (1992): Molecular diagnosis in Prader-Willi syndrome identifies a common occurrence of maternal uniparental disomy. *N Engl J Med* 326:1599–1607. [PubMed: 1584261]
- Nicholls RD, Knoll JHM, Butler MG, Karam S, Lalande M (1989): Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. *Nature* 342:281–285. [PubMed: 2812027]
- Ozcelik T, Leff S, Robinson W, Donlon T, Lelande M, Sanjines E, Schinzel A, Francke U (1992): Small nuclear ribonucleoprotein polypeptide N (SNRPN). An expressed gene in the Prader-Willi syndromes critical region. *Nature Genet* 2:265–269. [PubMed: 1303277]
- Zackowski JL, Nicholls RD, Gray BA, Bent-Williams A, Gottlieb W, Harris PJ, Waters MF, Driscoll DJ, Zori RT, Williams CA (1993): Cytogenetic and molecular analysis in Angelman syndrome. *Am J Med Genet* 46:7–11. [PubMed: 8098583]

TABLE I.

Clinical, Cytogenetic, and FISH Data on Patients Referred for PWS or AS

Subject	Age (year)	Sex	Referred group	High resolution chromosome results	D15Z1	D15S11	SNRPN	D15S10	GABRB3
PWS 1 ^a	3	M	R/O PWS	Normal	ND ^b	—	—	—	—
PWS 2	3	F	R/O PWS	Normal ^c	—	D ^d	D	D	D
PWS 3	5	F	R/O PWS	Normal	ND	N ^e	D	N	N
PWS 4 ^a	6	M	R/O PWS	Normal	—	N	—	—	N
PWS 5	6	F	R/O PWS	t(15q;15q)	—	—	N	N	—
PWS 6	13	F	R/O PWS	Normal	ND	—	N	N	—
PWS 7	23	F	R/O PWS	del(15q) ^c	—	N	N	N	N
PWS 8	24	F	R/O PWS	?del(15q)	—	—	N	—	—
PWS 9	1	F	PWS	del(15q)	—	D	—	—	D
PWS 10	2	F	PWS	del(15q)	DD ^f	—	D	—	—
PWS 11	3	M	PWS	del(15q)	DD	—	—	—	—
PWS 12	6	F	PWS	del(15q)	ND	—	—	—	—
PWS 13 ^a	8	M	PWS	Normal	—	N	—	—	N
PWS 14	9	M	PWS	del(15q)	ND	D	—	—	D
PWS 15	16	F	PWS	del(15q)	—	D	—	D	D
PWS 16	16	M	PWS	del(15q)	DD	—	—	—	—
PWS 17 ^a	17	F	PWS	Normal	—	—	N	—	—
PWS 18	18	M	PWS	del(15q)	—	D	—	—	D
PWS 19	20	F	PWS	del(15q)	DD	D	—	—	D
PWS 20	20	M	PWS	del(15q)	DD	D	—	D	D
PWS 21	38	F	PWS	?del(15q)	DD	D	—	—	D
AS 1	3	F	R/O AS	dup(15q)	—	dup	N	N	dup
AS 2	2	M	AS	del(15q)	DD	—	—	—	—
AS 3	4	F	AS	del(15q)	DD	—	—	—	—
AS 4	5	F	AS	del(15q)	—	D	—	—	D
AS 5	12	M	AS	del(15q)	DD	D	—	—	D

^gBiparental inheritance found using polymerase chain reaction microsatellite probes [e.g., GABRB3, D15S11, D15S113, D15S10, etc.; Mascari et al., 1992; Nicholls et al., 1989; Zackowski et al., 1993].

^hNo detectable difference in size of D15Z1 hybridization signal.

ⁱChromosome results from an outside laboratory.

^jHybridization signal found on only one of the chromosome 15s (deletion).

^eTwo hybridization signals, one on each of the chromosome 15s (nondeletion).

^fTwo D15Z1 hybridization signals seen but the signals were of different size.