article

Characteristics of two cases with dup(15)(q11.2-q12): one of maternal and one of paternal origin

Rong Mao, MD[1], Syed M. Jalal, PhD[1], Karen Snow, PhD[1], Virginia V. Michels, MD[1], Susan M. Szabo, MD[2], and Dusica Babovic-Vuksanovic, MD[1]

Purpose: The phenotype correlations for interstitial duplications that include the Prader-Willi/Angelman syndrome critical region are not well established. We describe two such duplication cases, one of which was of maternal origin and the other was paternal. **Methods:** High resolution G-banding, fluorescence in situ hybridization (FISH) for SNRP-N and D15S10 were used for cytogenetic analysis. Southern blot analyses based on parent of origin specific DNA methylation at D15S63 (PW71) locus were utilized for detection of methylated and unmethylated fragments. **Results:** The duplication was established by the FISH analysis. The molecular pattern suggested a maternal origin of the duplication in patient 1 and a paternal origin in patient 2. Patient 1 (2 years old) had developmental and speech delays with pervasive developmental disorder or mild autism, strabismus, and normal growth parameters with seizures. Patient 2 (16 years old) had global developmental delay, verbal IQ of 94, depression, obesity, food-seeking behavior, and significant behavioral problems that included self-injurious tendencies. Neither patient had significant dysmorphic features or abnormalities of internal organs. **Conclusion:** The two cases suggest that some patients with 15q11.2q12 duplication may have significant anomalies, and there appear to be phenotypic differences between maternal and paternal transmission of the duplication. ***Genetics in Medicine*, 2000:2(2): 131–135.**

Key Words: *PWS/AS critical region, duplication, clinical features*

Genotype-phenotype correlations have not been consistently demonstrated for chromosome 15 duplications involving q11.2-q13 (reviewed in Table 1). Most proximal duplications without an adverse phenotypic influence appear to represent amplification of pseudogenes.[1] Duplications of 15q12.2-q13.1 have also been well documented as a normal polymorphic variant.[2,3] Neither of these duplications involve the critical regions of Prader-Willi and Angelman syndromes (PWACR). Interstitial duplications that include PWACR often have a dysmorphic phenotype. The duplications transmitted maternally have had autistic type features and variable degrees of developmental delay or mental retardation.[4–8] These nonspecific features may be present in Angelman syndrome and in cases of trisomy or tetrasomy of 15q11-q13 region.[9] The duplication transmitted paternally has been reported to result in no apparent phenotypic dysmorphism.[6,10] However, Mohandas *et al.*[11] reported a case of interstitial duplication that included PWACR in which the duplication was of paternal origin and the patient had developmental delay and communication disorder. It has been suggested that duplications of paternal origin result in PWS-like features and those of maternal origin cause AS-like features.[12] Thus, the genotype-phenotype correlation from duplication of 15q11.2-q13 remains unclear, and additional clinical reports are needed for a better understanding of this issue. We describe the clinical, cytogenetic, and molecular findings in two abnormal patients, where the duplication was maternal in one and paternal in the other.

CASE PRESENTATIONS

Case 1

The patient was the first child of healthy, phenotypically normal, nonconsanguinous parents. Delivery was at term and uncomplicated following an unremarkable pregnancy. Birth weight was 4.01 kg. Early developmental milestones were mildly delayed. He started sitting unsupported at 6–7 months but did not start walking until 16 months. He had surgery for exotropia at 9 months. The first concern about his development was raised when he was 18 months old and not yet talking. He had only limited social contact, without spontaneous engagement in interactive play with other children, poor eye contact, and exhibited hand-flapping behaviors when excited. At age 2, the child received a full developmental assessment that was consistent with mild to moderate autism. His developmental age was estimated to be 12 months, while both receptive and expressive language skills were at a 10 to 11 month-old level. An electroencephalogram was normal. Ophthalmologic examination and audiology were normal. MRI of the head was nor-

From the [1]Mayo Clinic, Rochester, Minnesota; and the [2]LaSalle Clinic, Oshkosh, Wisconsin

Syed M. Jalal, PhD, Cytogenetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic and Mayo Foundation, Rochester, MN 55905.

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Table 1
Reported cases of duplication involving 15q11-q13

No. of cases	Phenotype	Journal	Author
Paternal origin of duplicated PWACR			
1 case	global DD, depression, obesity, and significant behavioral problems	This report	Mao R *et al.*
1 case	phenotypically normal	Am J Hum Genet, 1997	Cook EH *et al.*
1 case	DD and communication disorder	Am J Med Genet, 1999	Mohandas TK *et al.*
Maternal origin of duplicated PWACR			
1 case	developmental and speech delay, autism, and seizures	This report	Mao R *et al.*
4 cases	developmental delay	Am J Hum Genet, 1997	Browne CE *et al.*
1 case (triplication)	multiple minor anomalies, MR, and seizure and autistic behavior	J Med Genet, 1994	Schinzel AA *et al.*
1 case	autism, epilepsy, and ataxia	Dev Med Child Neurol, 1994	Bundey S *et al.*
4 cases	mild facial anomalies, autism, and minor DD	Am J Hum Genet, 1997	Woods CG *et al.*
1 case	autism	Am J Med Genet, 1998	Schroer RJ *et al.*
2 cases	non specific hypotonia, autistic behaviors, and mental retardation	Am J Hum Genet, 1998	Repetto GM *et al.*
2 cases (sibs)	autism, social deficits, and language delay	Am J Hum Genet, 1997	Cook EH *et al.*
Unknown parental origin of duplicated PWACR			
2 cases	one with atypical PWS like features and AS like in the other	Am J Hum Genet, 1993	Mutirangura A *et al.*
Duplications excluding PWACR*			
10 cases	benign	Clin Genet, 1991	Ludowese CJ *et al.*
15 cases from 7 unrelated families	phenotypically silent	Am J Med Genet, 1994	Jalal SM *et al.*

*Table excludes numerous other reports in which presence or absence of PWACR in the duplication is undetermined.

mal. Blood levels of lactate, pyruvate, ammonia, uric acid, amino acids, biotinidase, and lead in blood were all normal. Blood and urine inborn errors screen and urine organic acids were also normal. DNA analysis for fragile X syndrome was normal. Physical examination at 2 years and 3 months showed a nondysmorphic, slightly overweight boy. His weight was 15 kg (90^{th} percentile), height was 90 cm (25^{th}–50^{th} percentile). Head circumference was 49.5 cm (50^{th} percentile). Except for the minimal strabismus, his physical examination, including neurologic exam, was otherwise unremarkable.

Cytogenetic and molecular genetic analyses

G-banded chromosome analysis at *550* band stage or higher did not suggest duplication of PWASCR. FISH analysis involving the PWASCR was conducted to rule out the possibility of mosaicism. Analysis of metaphase FISH for SNRP-N and D15S10 for PWASCR along with two control probes (D15Z1 and PML) was conducted using the Vysis (Downers Grove, IL) probes. Duplication of the PWACR was established by FISH (Fig. 1). Subsequently, the parents of case 1 were tested using FISH for SNRP-N and D15S10, and no duplication was identified in either parent.

DNA extraction from peripheral blood leukocytes and Southern blot hybridization with ^{32}P labeled probes were performed by standard procedures. The isolated genomic DNA (2.5 μg) was digested with *Bgl*II and Cfo I, separated on 0.8% agarose gel, and analyzed by Southern blot with probe PW71B (D15S63). Visualization and densitometry of signals used by a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). PW71B (D15S63) is a 360 bp microdissection clone that maps to the PWACR.[13] Southern blot hybridization of *Bgl*II and methylation sensitive restriction enzyme Cfo I digested DNA with PW71B results in 8.0 kb maternal derived and 6.4 kb paternal derived bands. In patient 1 both 8.0 kb and 6.4 kb bands were present but the 8.0 kb band was twice as intense (Fig. 2).

Case 2

This male patient was born to healthy unrelated parents. Family history showed congestive heart failure and glaucoma on the maternal side, but was otherwise unremarkable. The pregnancy was uncomplicated, and birth was at full term through a normal vaginal delivery. His birth weight was 4 kg. Neonatal period was normal. Early psychomotor development was remarkable for mild global delay. Speech was more severely affected and he was started on speech therapy at age 2. No specific diagnosis was made. Since early childhood the patient expressed various behavioral problems in the form of apathy, defiance, mood lability, difficulties in concentration, and social immaturity. He had a history of uncontrolled appe-

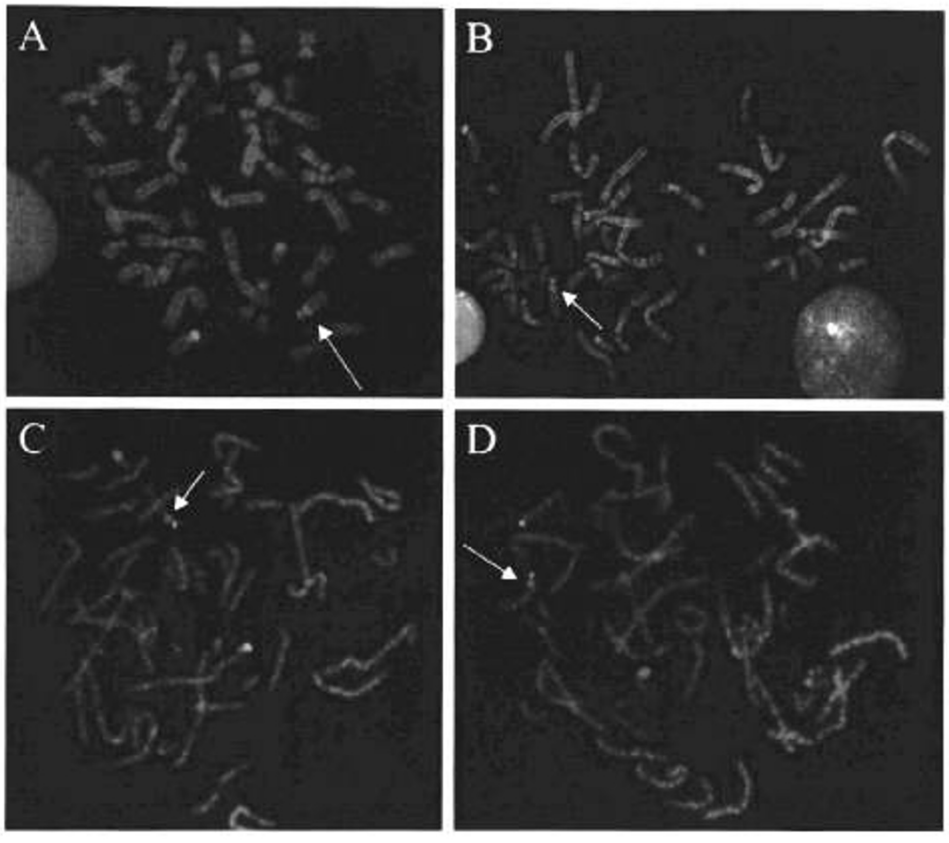

Fig. 1 FISH analysis was performed on metaphases from whole blood cultures. Both SNRP-N and D15S10 probes (red) along with the control probes, D15Zl (green) and PML (red) were used. Results of patient 1 for D15S10 (A) and SNRP-N (B) and of patient 2 for D15S10 (C) and SNRP-N (D) indicate duplication (arrows) of the Prader-Willi/Angelman critical region.

tite and stealing food. The patient also had significant self-injurious tendencies such as pulling out his hair, self-biting, and scratching. His emotional and behavioral problems became aggravated in adolescence, when the patient developed depression and anxiety requiring treatment with antidepressants and he had hospitalization for an acute episode of uncontrollable behavior. At age 12, the patient underwent psychometric testing that showed full scale IQ score of 87, verbal IQ score of 89, and performance IQ score of 89. Physical examination at age 15 showed a nondysmorphic, moderately obese boy with a weight of 57.5 kg (75^{th} percentile) and height of 160 cm (5^{th} percentile). He had numerous scars and excoriations on both forearms and a prominent bald patch on the frontal part of scalp. His examination was otherwise normal.

Cytogenetic and molecular genetic analysis

Twenty metaphases were analyzed and two karyotyped from G-banded cells from stimulated blood and the results were normal 46,XY. The molecular fragile X test was also normal with a CGG repeat of 39. Standard G-banded analysis of two karyotypes and 70 cells established a difference in the length in the region of 15q11.2-q13 between the two homologous chromosomes. At higher resolution a duplication was suspected. The karyotype was 46,XY, ?dup(15)(q11.2q13). A duplication of the PWS/AS region was established by using metaphase FISH (Fig. 1). Vysis probe set for PWS/AS critical region (SNRP-N and S15S10) along with two control probes (D15Z1 and PML) were utilized. Southern blot analysis was used, as described in case 1, to detect alterations in the DNA methylation pattern of the PWS/AS critical region utilizing the probe PW71B (locus D15S63) with a *Bgl*II/Cfo I digest. Southern blot analysis demonstrated the presence of both 8.0 kb and 6.4 kb bands. However, the paternally derived 6.4 kb band appeared twice as intense (Fig. 2). The parents were not available for FISH or molecular analysis.

DISCUSSION

Interstitial duplications of 15q11.2-q13 are often difficult to establish by banded chromosome analysis. The pericentromeric region can have polymorphic variation due to amplification of the pseudogenes.[1] A second established region of polymorphic variation is the 15q12.2-q13.1.[2,3] Both of these variants can lead to a length difference in this region. It is, therefore, important to confirm any suspected duplication of the involvement of PWACR by FISH or molecular methods. When duplications are established by FISH or molecular techniques, the genotype-phenotype correlations are not always clear (Table 1). It is, therefore, of importance to continue to document such cases. Only molecular analysis can provide in-

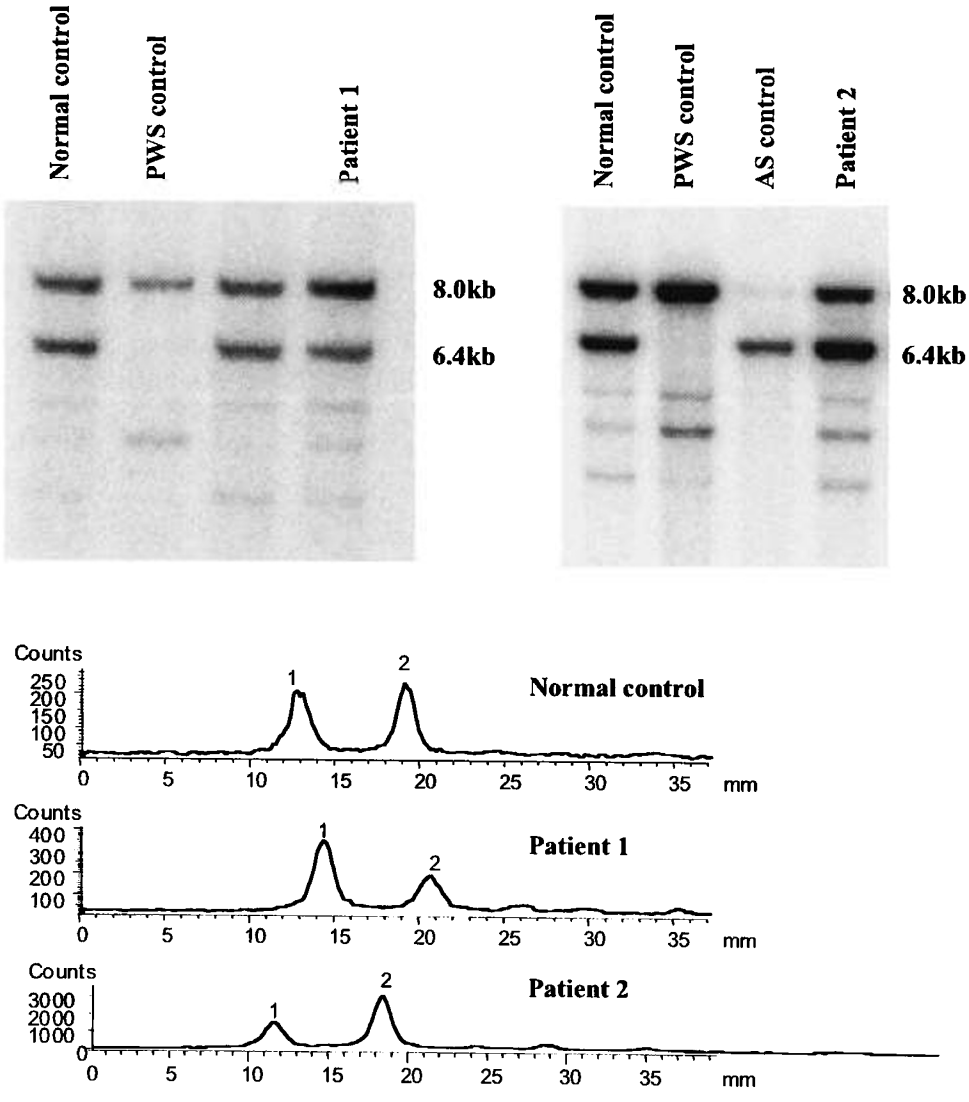

Fig. 2 Southern blot analysis used restriction enzyme digestion with *BglII* and *CfoI* and hybridization with the probe PW71B. Densitometric profiles are shown with peaks 1 and 2 corresponding to the 8.0 kb and 6.4 kb bands, respectively. Area ratios (peak 1/peak 2) were 0.98 for the normal control, 1.88 for patient 1, and 0.47 for patient 2. The unlabeled lane in the left panel shows results for an unrelated patient.

formation about the mode of parental origin of the duplication. We present two abnormal cases of such a duplication in which one case was maternally derived and the other was paternally derived. Our cases are compared with only those reporting interstitial duplications of PWACR that were confirmed by FISH or molecular methods.

Interstitial duplication involving the PWACR have been associated with autistic behavior in which the parental origin was unknown[14] or of maternal origin.[8] The association of autism spectrum disorders and maternally derived duplication has been confirmed by several other investigators.[4,5,7,10] Maternally transmitted duplications have also been associated with developmental delay and ataxia as usually present in Angelman syndrome.[12,15] In a review of these interstitial duplications Browne et al.[6] present 4 of 20 affected cases with a duplication involving the PWACR that were maternal in origin. Two of the affected mothers also carried maternally inherited duplications, whereas the duplication in an unaffected mother was transmitted paternally. It therefore seems that maternally derived duplication of PWACR is associated with some of the nonspecific anomalies that may overlap with Angelman syndrome. Our patient that had a maternal inheritance of PWACR had significant clinical problems including developmental delay, speech delay, and pervasive developmental disorders.

Paternally inherited PWACR duplications have been reported in a normal individual.[10] Mohandas *et al.*[11] report a patient with nonspecific developmental delay and partial agenesis of rostral corpus callosum who had a paternally derived PWACR duplication by FISH and molecular techniques. It has been suggested also that paternal duplication causes PWS features.[12] Our patient with a paternally derived duplication had significant clinical abnormalities including global developmental delay, depression, obesity,

food-seeking behavior, and self-injurious tendencies. However, he did not have the facial dysmorphism, hypopigmentation, and small hands or feet characteristic of PWS.

The estimated proportion of 15q11.2-q13 deletion based Prader-Willi and Angelman syndromes is approximately 1 in 10,000 live births. Using FISH and Southern blot testing for cases referred with a possible diagnosis of Prader-Willi or Angelman syndrome, we estimate that the ratio of duplications to deletions is approximately 1:100. Of course, the relative frequency of duplications among clinically atypical cases, not referred for testing, may be higher. It seems that this region of chromosome 15 is rich in duplicons that are orthologous in 16 nonhuman primates.[16,17] Existence of such repeated sequences provides a mechanism for unequal recombination events that can generate both interstitial deletions as well as duplications. Robinson *et al.*[18] propose multiple breakpoints and either intrachromosomal or unequal exchanges between homologous chromosomes for the origin of these deletions or duplications. This is consistent with pairing of homologous chromosomes or intrachromosomal looping involving duplicons.

The maternally transmitted duplications have a range of dysmorphic features that include autism spectrum disorders and traits such as ataxia overlapping with Angelman syndrome. However, paternally transmitted duplications range from normal phenotype to significant anomalies. The inconsistencies in phenotype of patients with duplication PWACR could be due to differences in the extent of the duplication segment so that different contiguous genes and/or different imprinted genes are involved. The role of imprinting loci and gene dosage is speculative, although the range of clinical abnormalities observed and the difference in maternal versus paternal transmission of the duplication must be in part related to this phenomenon. Further molecular studies in such cases will help to understand the mechanism(s) of duplication in the PWACR and the reason for phenotypic variability associated with the duplications.

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