Short Report

Prader–Willi syndrome resulting from an unbalanced translocation: characterization by array comparative genomic hybridization

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Prader–Willi syndrome (PWS) is caused by lack of expression of paternally inherited genes on chromosome 15q11→15q13. Most cases result from microdeletions in proximal chromosome 15q. The remainder results from maternal uniparental disomy of chromosome 15, imprinting center defects, and rarely from balanced or unbalanced chromosome rearrangements involving chromosome 15. We report a patient with multiple congenital anomalies, including craniofacial dysmorphology, microcephaly, bilateral cryptorchidism, and developmental delay. Cytogenetic analysis showed a *de novo* 45,XY,der(5)t(5;15)(p15.2;q13), -15 karyotype. In effect, the proband had monosomies of 5p15.2→pter and 15pter→15q13. Methylation polymerase chain reaction analysis of the promoter region of the *SNRPN* gene showed only the maternal allele, consistent with the PWS phenotype. The proband's expanded phenotype was similar to other patients who have PWS as a result of unbalanced translocations and likely reflects the contribution of the associated monosomy. Array comparative genomic hybridization (array CGH) confirmed deletions of both distal 5p and proximal 15q and provided more accurate information as to the size of the deletions and the molecular breakpoints. This case illustrates the utility of array CGH in characterizing complex constitutional structural chromosome abnormalities at the molecular level.

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Prader–Willi syndrome (PWS) is a well-defined multiple congenital anomaly disorder whose distinctive phenotype is caused by the lack of normally expressed paternal genes at chromosome 15q11→q13 (1). PWS was first described in 1956, and major criteria include hypotonia in infancy with associated feeding difficulties and failure to thrive (1, 2). This is followed by rapid weight gain, resulting in significant obesity if uncontrolled. Other major criteria include hypogonadism and developmental delay. Characteristic facial features include bitemporal narrowing, almond-shaped palpebral fissures, strabismus, narrow nasal bridge, and down-turned corners of the mouth with a thin upper lip. Musculoskeletal findings may include small hands and feet, scoliosis, and kyphosis. Individuals with PWS may have characteristic behavioral issues, including tantrums, manipulative behavior, and obsessive-compulsive tendencies. The incidence of PWS is reported to be 1/10,000–1/15,000 individuals (3).

Approximately 75% of patients with PWS have a microdeletion in the long arm of the paternally inherited chromosome 15, and most of the remainder have uniparental disomy (UPD) for maternal chromosome 15 (4, 5). Both UPD, in

which an individual inherits two copies of the maternal chromosome and no copies of the paternal chromosome, and a deletion in the paternal chromosome lead to the exclusive expression of maternally inherited genes in the PWS region. In approximately 1% of patients who have neither a deletion nor UPD, an imprinting mutation may be found, which results in only maternal expression of genes in the PWS region (6). The vast majority of deletions are interstitial, but in approximately 3.5–5% of patients, a structural rearrangement is involved (7, 8).

In this study, we report a patient with PWS and several additional dysmorphic features. Using conventional cytogenetics, fluorescence *in situ* hybridization (FISH), and array-based comparative genomic hybridization (array CGH), we demonstrate that this patient has a 5;15 translocation, leading to not only a deletion in the usual PWS region of 15q11→13 but also a distal deletion on chromosome 5p.

Case report

Clinical description

The proband was the first child born to healthy, non-consanguineous parents. The mother was 28 years old and the father was 27 years old. Family history was unremarkable. The prenatal course was uneventful until approximately 7 months, when the mother developed polyhydramnios. Prenatal ultrasonography was normal. The infant was delivered by cesarean section at 42 weeks due to failure to progress. He was severely hypotonic at birth and required intubation for respiratory failure, which subsequently resolved. The infant's growth parameters were as follows: birth weight was 3.5 kg (50th percentile), length was 51 cm (50th percentile), and head circumference was 36 cm (75th percentile). As described in the legend to Fig. 1, at birth the proband had dysmorphic features. He had scrotal hypoplasia with bilateral cryptorchidism and generalized hypotonia with markedly decreased deep tendon reflexes. An initial evaluation included a brain magnetic resonance imaging that demonstrated bilateral periventricular cysts, diffuse mild prominence of extra-axial spaces, and mildly abnormal bifrontal cortical gyral patterns. Upon bronchoscopy, the patient was found to have laryngeal dyskinesis. Abdominal ultrasonography showed undescended testes. Ophthalmologic, cardiac, auditory, electroencephalographic, and skeletal survey evaluations were normal.

The patient's surgical history was significant for gastroesophageal reflux treated with a Nissen fundoplication, the placement of a gastrostomy

Birth — 15 months

27 months — 38 months

Fig. 1. Proband at indicated ages. Dysmorphic features included a high, posteriorly slanted forehead, hypoplasia of the supraorbital ridges, epicanthal folds bilaterally and a short nose with a bulbous tip. Ears were mildly low-set with thickened, overturned superior helices and prominent antihelices. The mouth had down-turned corners with a thin, tented upper lip, and there was retromicrognathia. Hands and feet were of normal size.

tube at 5 months of age and bilateral orchiopexy at 8 months. He gradually began to feed by mouth and currently does not require gastrostomy feedings. His developmental progress has been delayed: he rolled over at 12 months, pulled to stand at 24 months, walked at 3 years, and spoke approximately 10 words by 3 years of age.

Consistent with the typical pattern seen in PWS, by 22 months, the patient's weight had increased to the 75th–90th percentile. His height, meanwhile, decreased to the 5th percentile. At the age of 3 years and 9 months, his weight was >95th percentile and length was at the 25th percentile. His head circumference was <10th percentile, demonstrating the development of a relative microcephaly. Other notable physical findings included a small phallus and hypoplastic scrotum.

Cytogenetic and fluorescence *in situ* hybridization analyses

Cytogenetic analysis and GTG-banding were performed using standard techniques on metaphases from peripheral blood lymphocytes. This analy-

sis showed an abnormal male karyotype containing an unbalanced translocation resulting in loss of one chromosome 15 and a derivative 5 chromosome: 45,XY,der(5)t(5;15)(p15.2;q13),-15 (Fig. 2). In effect, the proband had monosomies of 5p15.2→pter and 15pter→15q13. Parental karyotypes were normal.

Dual color FISH was performed with SpectrumGreen^TM-labeled 5pter (84c11) and SpectrumOrange^TM-labeled 5qter (D5S2907) probes (Vysis, Downer's Grove, IL), according to the manufacturer's instructions. The 5qter probe was present on both the normal and der(5) chromosomes. The 5pter probe was deleted from the der(5) chromosome (Fig. 3a). FISH with a probe for the cri-du-chat syndrome critical region (Oncor, Gaithersburg, MD) at 5p15.2 showed a signal on the der(5), localizing the 5p breakpoint distal to this region (Fig. 3b). FISH with a SNRPN probe (Vysis) and a D15S10 probe (Oncor) showed that these loci were deleted from the der(5) chromosome (data not shown).

Methylation polymerase chain reaction analysis

Genomic DNA was extracted from peripheral blood lymphocytes using a Puregene® DNA isolation kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's instructions. Methylation analysis for Prader–Willi and Angelman syndromes was performed by methylation polymerase chain reaction (mPCR) analysis from the SNRPN locus. Bisulfite modification of genomic DNA was performed with the CpGenome® DNA modification kit (Oncor) according to the manufacturer's instructions. mPCR reactions with maternal and paternal oligonucleotide primers for the CpG island of the SNRPN gene were performed as described (9).
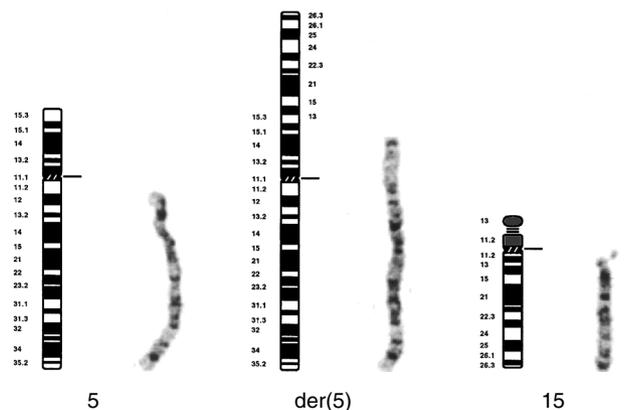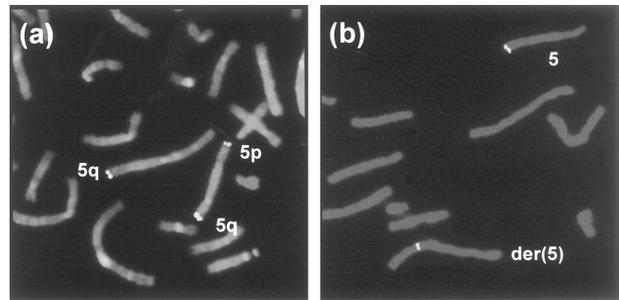

Fig. 3. Fluorescence in situ hybridization using commercially available 5pter (84c11), 5qter (D5S2907) and cri-du-chat critical region (5p15.2) probes. (a) The 5qter probe can be seen on both the normal and der(5) chromosomes. The 5pter probe is seen only on the normal chromosome 5. (b) The cri-du-chat syndrome critical region probe showed signal on the normal chromosome 5 and on the der(5), localizing the 5p breakpoint distal to this region.

mPCR analysis of the proband showed only the 174-bp maternal allele (Fig. 4), consistent with the imprinting pattern seen in PWS.

Array comparative genomic hybridization analysis

In order to analyze the chromosome deletions at a higher resolution, array CGH analysis was performed using a microarray consisting of 2464 bacterial artificial chromosomes (BAC), PAC, and P1 clones printed in triplicate (HumArray2.0) as previously described (10, 11). In brief, DNA was isolated from peripheral blood lymphocytes using a QIAamp DNA Blood Midi kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. The patient's DNA and normal male reference DNA were labeled by random priming with Cy3 or Cy5 labeled nucleotides and hybridized for 2 days to the array. Sixteen bit 1024 × 1024 pixel
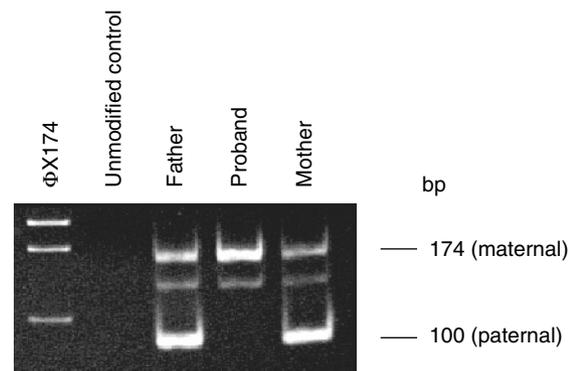

Fig. 2. Partial karyotype and ideogram of the normal chromosomes 5, chromosome 15 and the der(5) chromosome from the proband.

Fig. 4. Methylation-PCR of the CPG island in the SNRPN gene showed the presence of only the maternal allele in the proband. Lane 1 – molecular weight marker X174 (Pharmacia, Piscataway, NJ); lane 2 – control DNA without bisulfite modification; lanes 3–5 – paternal, proband and maternal DNAs after bisulfite modification.

DAPI, Cy3, and Cy5 images were collected using a custom CCD camera system (12), and the data were analyzed using UCSF SPOT (13) to automatically segment the array spots and to calculate the $\log_2$ ratios of the total Cy3 and Cy5 intensities for each spot after background subtraction. A second custom program, SPROC, was used to calculate averaged ratios of the triplicate spots for each clone, standard deviations of the triplicates, and plotting position for each clone on the array on the July 2003 freeze of the draft human genome sequence (http://www.genome.ucsc.edu). SPROC also implements a filtering procedure to reject data based on a number of criteria, including low reference/DAPI signal intensity, and low correlation of the Cy3 and Cy5 intensities with a spot. The data files were edited to remove ratios on clones for which only one of the triplicates remained after SPROC analysis and/or the standard deviation of the $\log_2$ ratios of the triplicates was >0.2 (10).

Array CGH analysis of the proband demonstrated two aberrations in the genome. A deletion of the short arm of one copy of chromosome 5 was seen, as indicated by nine BACs (Fig. 5a) with the average $\log_2$ ratio $= -0.91 \pm 0.09$, close to the expected $\log_2$ ratio $= -1$ for a single copy deletion in a diploid genome (linear ratio $1:2=0.5$). This was concordant with the standard cytogenetic analysis shown in Fig. 2, as these BACs have also been cytogenetically mapped to this location (14). The estimated size of the deletion was between 6.3 and 7.4 Mb as determined by array CGH analysis. The molecular breakpoint determined by array CGH was within 5p15.31 based on the UCSC draft human genome sequence. Furthermore, it was possible to localize the breakpoint to a region less than 1 Mb defined by the map position of the deleted BAC and the flanking BAC. In addition to the deletion on chromosome 5p, a deletion of the long arm of one copy of chromosome 15 was present, as indicated by five BACs (Fig. 5b) with the average $\log_2$ ratio $= -0.82 \pm 0.09$. This deletion was concordant with the standard cytogenetic analysis shown in Fig. 2, encompassing the proximal portion of 15q. Array CGH analysis estimated the size of the deletion between 8 and 13 Mb and defined the molecular breakpoint to a 4.5 Mb region proximal to 15q13.3. One copy of the Vysis *SNRPN* 176 A BAC clone was deleted by array CGH analysis ($\log_2$ ratio $= -0.78 \pm 0.03$). This deletion was consistent with FISH analysis. No other copy number alterations were detected. The average $\log_2$ ratio of all other genomic clones not included in these deletions was 0 ± 0.13.
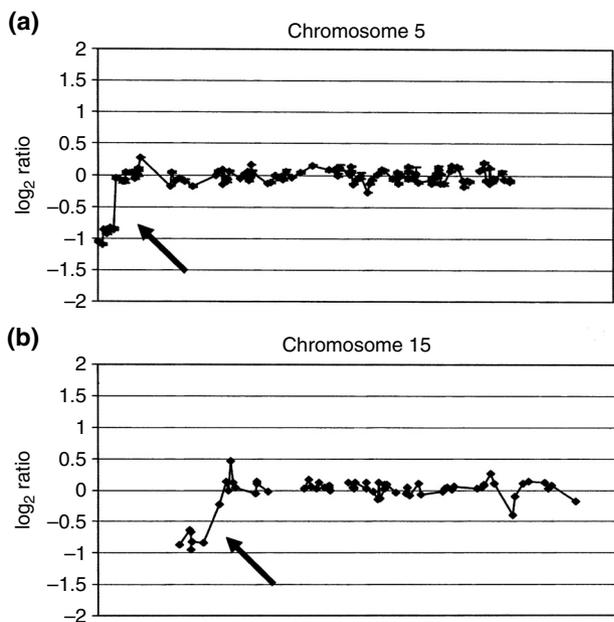
(a)

(b)

Fig. 5. Array comparative genomic hybridization analysis. (a) A deletion of the short arm of one copy of chromosome 5 was present, as indicated by nine bacterial artificial chromosomes (BACs) with average $\log_2$ ratio $= -0.91 \pm 0.09$. The estimated size of the deletion is between 6.3 and 7.4 Mb. (b) A deletion of the long arm of one copy of chromosome 15 was present, as indicated by five BACs with average $\log_2$ ratio $= -0.82 \pm 0.09$. The estimated size of the deletion is between 8 and 13 Mb.

Discussion

PWS, as the result of an unbalanced translocation, is often associated with an expanded phenotype compared to that seen with the more common interstitial microdeletion (15). For example, in this case, the patient had atypical craniofacial features, required gastrostomy feedings, and developed postnatal microcephaly. The typical PWS microdeletions are thought to be restricted to the region between 15q11 and 15q13 because of the presence of homologous recombination sites (16). The additional findings often seen in structural rearrangements can be explained by a deletion that is larger than that typically seen in PWS, or monosomy or trisomy of the other chromosome affected by the translocation. Thus, the augmented features in our patient were likely caused by deletion of genes on distal chromosome 5p.

The majority of *de novo* unbalanced translocations involving loss of proximal chromosome 15q result in PWS due to loss of the paternal allele, which is consistent with the lack of expression of paternally inherited genes as the etiology of PWS. One report has demonstrated that in most PWS translocations, proximal chromosome 15q

is transposed to the telomeric sequences of the recipient chromosome (17). However, in the proband, array CGH and FISH analyses demonstrated an approximate 7 Mb deletion of distal 5p suggesting a mechanism other than affinity to telomeric sequences. Proximal 15q is rich in repeat sequences and duplicons that are involved in the microdeletion seen in PWS, as well as in the formation of chromosome 15 supernumerary markers (18, 19). Such low-copy genomic repeats are increasingly implicated in chromosomal rearrangements (20).

While no clear genotype–phenotype correlations have been demonstrated in PWS caused by unbalanced translocations, two recent reports illustrate the tendency toward expanded phenotypes in these cases (21, 22). To our knowledge, only one instance of an unbalanced translocation (5;15) has been reported where the authors describe a PWS-like phenotype (23). This patient had severe mental retardation, a seizure disorder and hypotonia, small hands and feet, hypoplastic genitals, and truncal obesity. With the exception of brachycephaly, the facial characteristics were not similar to those seen in our patient.

It is likely that the 5p deletion resulting from the complex rearrangement in our patient contributed to his phenotype. Deletions of 5p are involved in cri-du-chat syndrome, although assignment of various aspects of the phenotype to specific regions of deletion is not yet definitive. Potential critical regions have been identified in 5p15.2 and 5p15.3, but more distal regions may also be involved (24–26). Consistent with these reports, our patient had the distal deletion boundary in 5p15.31 as defined by array CGH. He had features frequently seen in cri-du-chat syndrome, including microcephaly, micrognathia, epicanthal folds, and low-set ears.

Array CGH analysis was performed to further define the breakpoints at the molecular level and to determine the size of the chromosomal aberrations more precisely in the proband. Array CGH is a technology that measures copy number change across the entire genome and maps these changes onto the genome sequence (10, 12). The array used in this study consisted of genomic clones covering the genome with an average resolution of 1.4 Mb, which is significantly higher than standard GTG-banding. Molecular analysis by array CGH accurately confirmed the deletions demonstrated by conventional cytogenetics and refined the breakpoints at the molecular level. Because the density of genomic clones on our array is relatively higher on chromosome 5 as compared to chromosome 15, the breakpoint on 5p was localized within 5p15.31 to a region span-

ning approximately 1 Mb, whereas the molecular breakpoint on 15q was localized proximal to 15q13.3 to a region spanning approximately 4.5 Mb. By accurately defining breakpoints, the size of the deletions was more precisely determined, with the deletion on 5p approximating 7 Mb and the deletion on 15q approximating 10 Mb.

We have analyzed on our microarray several additional patients with *SNRPN* deletions who were identified by standard molecular cytogenetic studies (data not shown). In those cases studied by array CGH, the same genomic clones on 15q were deleted as in the case presented here. Because of imprinting in this region, mPCR analysis must be undertaken to determine the parental origin of the deletion.

In summary, we present a patient with PWS caused by an unbalanced translocation (5;15) resulting in monosomies of distal 5p and proximal 15q. The results of this study provide further evidence of the expanded phenotype in PWS caused by chromosomal translocations, presumably because of larger monosomic regions on 15q and/or the effect on the other translocation partner, which in our case was chromosome 5. In addition to defining the complex translocation by GTG-banding, FISH, and mPCR, we used array CGH to map the deletions relative to the genome sequence. The high resolution of array CGH allows breakpoints to be localized at the molecular level, providing accurate sizing of chromosomal aberrations and providing finer mapping of candidate genes which may be implicated in specific malformations. Analysis by array CGH has the potential to assist in refining genotype–phenotype correlations in complex chromosomal rearrangements.

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