

ARTICLE

Unique and atypical deletions in Prader–Willi syndrome reveal distinct phenotypes

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Prader–Willi syndrome (PWS) is a multisystem, contiguous gene disorder caused by an absence of paternally expressed genes within the 15q11.2–q13 region via one of the three main genetic mechanisms: deletion of the paternally inherited 15q11.2–q13 region, maternal uniparental disomy and imprinting defect. The deletion class is typically subdivided into Type 1 and Type 2 based on their proximal breakpoints (BP1–BP3 and BP2–BP3, respectively). Despite PWS being a well-characterized genetic disorder the role of the specific genes contributing to various aspects of the phenotype are not well understood. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is a recently developed technique that detects copy number changes and aberrant DNA methylation. In this study, we initially applied MS-MLPA to elucidate the deletion subtypes of 88 subjects. In our cohort, 32 had a Type 1 and 49 had a Type 2 deletion. The remaining seven subjects had unique or atypical deletions that were either smaller ($n=5$) or larger ($n=2$) than typically described and were further characterized by array-based comparative genome hybridization. In two subjects both the PWS region (15q11.2) and the newly described 15q13.3 microdeletion syndrome region were deleted. The subjects with a unique or an atypical deletion revealed distinct phenotypic features. In conclusion, unique or atypical deletions were found in ~8% of the deletion subjects with PWS in our cohort. These novel deletions provide further insight into the potential role of several of the genes within the 15q11.2 and the 15q13.3 regions.

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INTRODUCTION

Prader–Willi syndrome (PWS; OMIM #176270) is an imprinted neurobehavioral condition affecting many organ systems. Major manifestations include early childhood obesity, hyperphagia, hypotonia with poor suck and poor weight gain in infancy, early childhood obesity, mild to moderate mental retardation, hypogonadism, growth hormone insufficiency causing short stature, characteristic facial appearance, characteristic neurobehavior and frequently psychiatric disturbance including abnormal restricted repetitive behavior (eg, skin picking, obsession, compulsion, sameness behavior, etc.).^{1,2}

PWS is caused by an absence of a functionally active paternal contribution in the chromosome 15q11.2–q13 region, in contrast to Angelman syndrome (AS) that is due to an absence of a maternal contribution in the same chromosomal region. PWS occurs via three distinct genetic mechanisms: deletion of paternally expressed genes in 15q11.2 (65–75%), maternal uniparental disomy (UPD; 20–30%) and imprinting defect (ID; 1–3%).^{1,3,4}

Deletions in PWS and AS are subdivided into two main subgroups (Type 1 and 2) and the breakpoints (BPs) are flanked by low-copy repeats (LCR). The common BP regions are proximally at BP1 or BP2, and distally at BP3. Both Type 1 and 2 deletions are almost always *de novo* events. For the purpose of this study, we used the designation of Type 1 deletion to encompass the region between BP1 and BP3 (~6 Mb

in size), and Type 2 deletion to encompass the region from BP2 to BP3 (~5.3 Mb in size).^{5–7} However, there are rare PWS and AS patients with an atypical distal BP at BP4 or BP5.^{8,9} Additionally, in a few patients, the 15q11.2–q13 region may be deleted as a result of an unbalanced translocation, which will yield unique BPs within proximal 15q.^{10,11}

Despite PWS being a well-characterized genetic disorder, specific genetic factors contributing to each of the distinct clinical features of PWS are not clearly understood.^{1,4} Increasing evidence suggest that the loss of the paternally expressed snoRNA gene cluster, *SNORD116*, has a significant role in many of the features of the PWS phenotype.¹² It is unclear whether the four, non-imprinted genes (*NIPA1*, *NIPA2*, *CYFIP1* and *GCP5*) localized to the interval between BP1 and BP2¹³ contribute towards the PWS phenotype. Non-PWS individuals with just a small deletion between BP1 and BP2 have a variable phenotype. For instance, some individuals apparently have developmental delay and/or autistic features, but some of the parents have the same deletion without apparent effect.¹⁴

Several studies have investigated phenotypic characteristics between PWS individuals with Type 1 vs Type 2 deletions, but there has been a lack of consensus among the different studies. For example, Butler *et al*¹⁵ reported individuals with Type 1 deletion ($n=12$) showed worse adaptive behavior, more severe compulsive behavior and more impairments in reading, math skills and visual perception than those with

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Type 2 deletion ($n=14$). In contrast, Dykens and Roof¹⁶ (Type 1, $n=26$; Type 2, $n=29$) and Milner *et al*¹⁷ (Type 1, $n=14$; Type 2, $n=32$) did not find significant phenotypic differences between the two main deletion subtypes. One problem is that none of these studies had a large sample size in each of the two deletion subtypes (size varied from 12 to 32), or used the same measures for the phenotypic assessments. Furthermore, not all of the studies utilized methods that would fine-map the distal BP. Thus, if some of the patients had distal BPs more proximal or distal than BP3, this would affect the genes involved in the deletion and potentially the phenotype.

As the genotype–phenotype relationships become clearer, it will be clinically important to readily subtype the deletion class. Current standard of clinical care in the determination of the deletion class of PWS involves use of fluorescent *in situ* hybridization (FISH) typically using only the *SNRPN* probe in the 15q11.2–q13 region. If the FISH test is positive for a deletion, and DNA methylation analysis reveals an absence of the paternally inherited 15q11.2 region, then few clinical geneticists at this time will delve any further to determine the size of deletion.

Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is a relatively new technique to detect changes of copy number and DNA methylation status, and has been used successfully in PWS and AS diagnosis.^{18,19} Compared with the traditional diagnostic methods, such as chromosomal and FISH analysis, MS-MLPA provides more detailed information on the size of the deletion as well as distinguishing PWS from AS.

The aims of the current study were twofold. One aim was to evaluate the utility of the MS-MLPA technique to delineate the size of the deletion in our cohort of 88 PWS subjects with a deletion. Another aim was to perform genotype–phenotype correlations on the seven subjects who had either a unique deletion (ie, having one or two novel BPs) or an atypical deletion (not a Type 1 or 2 deletion, but having a distal BP in another LCR/duplison like BP5).

MATERIALS AND METHODS

Sample

We obtained DNA samples from 88 PWS individuals with a deletion whose subtypes were unknown (see Supplementary Table S1 for details). In addition, we have examined an additional 145 DNA samples to compare the DNA methylation patterns across the diagnostic categories. These include 16 PWS subjects with maternal UPD, 5 PWS subjects with an ID, 8 AS subjects with a deletion, 1 AS subject with paternal UPD, 2 AS subjects with an ID, 4 family members with a known maternal duplication of the 15q11.2–q13,²⁰ 1 subject with maternal isodicentric duplication of chromosome 15 (47,XX,+inv dup(15)(pter-q11::q11-pter)), 105 subjects with a history of early-onset morbid obesity (EMO) and 4 phenotypically normal control subjects. The research protocol and informed consents were approved by the University of Florida Institutional Review Board.

MS-MLPA

A commercial MS-MLPA kit (ME028, MRC-Holland, Amsterdam, The Netherlands) for PWS/AS was utilized. Two versions were created for ME028 (versions A1 and B1, see Supplementary Table S2 for details). Depending on the version, this kit contains 25–32 probes specific for sequence along the length of the 15q11.2–q13 region to detect copy number changes in genes and other important areas. We used the A1 kit for all our deletion subjects. Some of our subjects were also run on the B1 kit. The manufacturer's protocol was followed for the DNA preparation, ligation, enzyme digestion and multiplex PCR reaction. After the final PCR step, the Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and Peak Scanner Software (version 1.0; Applied Biosystems) were used for capillary electrophoresis and fragment analysis. We utilized the MLPA interpretation protocol developed by Drs Karin Buiting and Bernhard Horsthemke (personal communication) for determination of copy number changes, and manufacturer's protocol to determine the DNA methylation changes. All MS-MLPA results

were reviewed independently by two investigators (SJK and DJD). Whenever the results were inconclusive, the MS-MLPA reaction was repeated.

The array-based comparative genome hybridization

MS-MLPA results from a subset of 35 PWS subjects were verified by an enhanced version of a chromosome 15 specific genomic microarray. These 35 subjects include the 7 subjects with unique or atypical deletions identified by MS-MLPA, and 28 subjects who were study participants recruited as part of our Rare Disease Clinical Research Network center grant. The array-based comparative genome hybridization (aCGH) included a total of over 38 000 oligos across 15q, with a higher density of oligos spanning ~10 Mb of the 15q11.2–q14 interval at the Baylor College of Medicine (Agilent Technologies Inc., Santa Clara, CA, USA).⁷

Clinical evaluation

The clinical evaluations for the subjects with a unique or an atypical deletions were done during clinic visits and/or during clinical research encounters.

RESULTS

Copy number analyses

Among 88 PWS subjects with deletion, we identified 32 subjects (36.4%) with Type 1 deletion and 49 subjects (55.7%) with Type 2 deletion (Supplementary Table S1). The remaining seven PWS subjects (8.0%) had unique or atypical deletions other than Type 1 or 2 (Figure 1). Among eight AS subjects with deletion, five subjects (62.5%) had Type 1 deletion and the remaining three subjects (37.5%) had Type 2 deletion. We also observed microdeletions within *SNRPN* in one of five PWS subjects with an ID and two of two AS subjects with an ID. We incidentally identified two microdeletions among 105 EMO subjects (one with a deletion of the 15q26 region and the other with a deletion between BP1 and BP2). The four subjects with an interstitial duplication of the 15q11.2–q13 region of maternal origin, as well as the subject with maternal isodicentric duplication of proximal 15q showed increased dosage of the respective chromosomal intervals consistent with their molecular diagnoses.

DNA methylation analyses

DNA methylation analyses utilizing the *NDN* and *SNRPN* probes confirmed characteristic DNA methylation patterns for PWS, AS and normal control subjects (Table 1).

Agreement rate between the MS-MLPA and aCGH

The agreement rate between MS-MLPA and aCGH was 100%. However, the aCGH did provide a more precise location of the BPs.

Case studies

The clinical and research records of the seven subjects with a unique or an atypical deletion confirmed by both MS-MLPA and aCGH were reviewed in more detail (Figure 1 and Table 2). A complete description of each case is in the Supplementary Data. An abbreviated description is given below for each case. The deletions and translocation described in these seven cases were all *de novo* in origin.

Case 1 PW235P. MS-MLPA showed a deletion of *MKRN3* to intron 2 of *ATP10A*, sparing exons 1 and 2. aCGH showed a unique deletion of ~2.46 Mb. The features not typical of PWS in this subject include the macrocephaly,²¹ large hands and feet, tall stature for PWS (all before the start of growth hormone), large birth weight, normal skin pigmentation and lower-than-average pain tolerance (Table 2).

Case 2 HBTB-113. The subject came to our attention after his brain was donated to the Human Brain Tissue Bank (HBTB) program at the

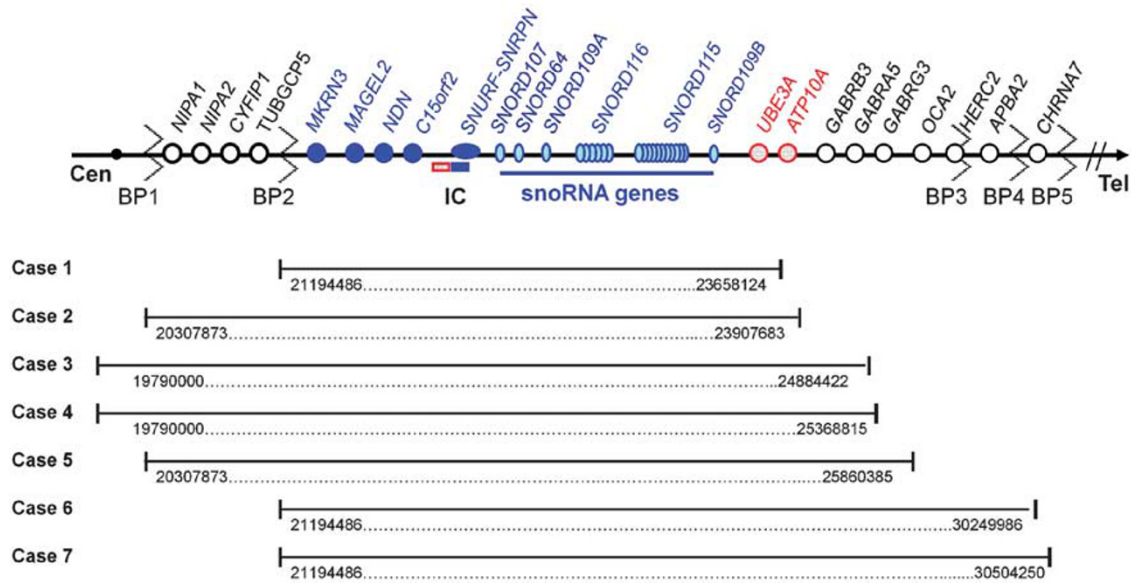


Figure 1 Seven unique or atypical PWS deletions. The position of genes and genetic markers (circles) in the chromosomal 15q11.2-q14 region are shown. In the PWS region (shown in blue), there are six paternal-only expressed unique copy genes (*MKRN3*, *MAGEL2*, *NECDIN*, *C15orf2* and *SNURF-SNRPN* and a family of six snoRNA gene clusters). Only *UBE3A* and *ATP10A* (shown in red), related to AS, have maternal-only expression in mouse and humans, and this imprinted expression is limited to certain tissue-specific regions (ie, mostly regions in the brain). The bipartite imprinting center (IC) lies proximal to *SNURF-SNRPN* and within the 3 Mb PWS/AS imprinted region. The cluster of GABA receptor genes (*GABRB3*, *GABRA5* and *GABRG3*), *OCA2* (Type 2 albinism) and *HERC2* are not imprinted and have biparental expression (shown in open black circle). The jagged vertical lines denote the common PWS deletion BPs, which lie within the segmental duplications associated with BP1–BP5. Type 1 deletions extend from BP1 to BP3 and Type 2 deletions extend from BP2 to BP3. The seven unique or atypical deletions (ie, neither Type 1 or 2) identified from this study are shown in solid lines with base pair positions of BPs confirmed by aCGH. These base pair positions are derived from the UCSC genome browser March 2006 (hg18) freeze (<http://www.genome.ucsc.edu/>). There is a lack of agreement in the literature regarding the order of the genes between BP1 and BP2. Note that there are more copies of the *SNORD115* snoRNA genes than are shown, and map distances are not drawn exactly to scale.

Table 1 DNA methylation analyses from five methylation-sensitive probes^a containing a *HhaI* site in subset of our samples

<i>HhaI</i> site	<i>NDN</i> exon 1	<i>SNRPN</i> U1B	<i>SNRPN</i> exon 1	<i>SNRPN</i> intron 1	<i>SNRPN</i> intron 1
Position ^b	21483420	22619807	22751214	22751483	22751773
PWS (deletion; <i>n</i> =74)	0.79 ± 0.17	0.77 ± 0.10	0.96 ± 0.11	0.99 ± 0.13	0.93 ± 0.15
PWS (UPD; <i>n</i> =14)	0.79 ± 0.13	0.80 ± 0.11	0.93 ± 0.08	0.99 ± 0.09	0.91 ± 0.09
PWS (ID; <i>n</i> =4)	0.81 ± 0.13	0.82 ± 0.09	0.97 ± 0.11	1.04 ± 0.12	0.93 ± 0.03
AS (deletion; <i>n</i> =8)	0.04 ± 0.03	0.03 ± 0.02	0.06 ± 0.05	0.03 ± 0.02	0.02 ± 0.02
AS (UPD; <i>n</i> =1)	0.00	0.00	0.02	0.00	0.00
AS (ID; <i>n</i> =2)	0.07 ± 0.02	0.03 ± 0.01	0.05 ± 0.04	0.03 ± 0.04	0.02 ± 0.03
15q Mat Dup (<i>n</i> =4)	0.51 ± 0.05	0.57 ± 0.07	0.74 ± 0.08	0.72 ± 0.08	0.69 ± 0.08
Mat Isodicentric Dup (<i>n</i> =1)	0.64	0.63	0.81	0.85	0.76
EMO (<i>n</i> =90)	0.39 ± 0.05	0.42 ± 0.05	0.54 ± 0.06	0.52 ± 0.05	0.49 ± 0.05
Control (<i>n</i> =4)	0.38 ± 0.08	0.49 ± 0.07	0.55 ± 0.07	0.58 ± 0.08	0.54 ± 0.06

Abbreviations: AS, Angelman syndrome; control, phenotypically normal individuals; EMO, subjects with a history of early-onset morbid obesity and no known abnormality in the 15q11.2-q13 region; ID, imprinting defect; PWS, Prader-Willi syndrome; 15q Mat Dup, maternally inherited interstitial duplication (three copies of 15q11.2 region); UPD, uniparental disomy; Mat Isodicentric Dup, maternally inherited duplication (four copies of 15q11.2 region).

^aME028 version A1 was used.

^bThe first base pair position derived from the UCSC (The University of California Santa Cruz) genome browser March 2006 (hg18) freeze (<http://www.genome.ucsc.edu/>).

The numbers represent the mean proportion of methylated DNA found in a particular group. The expectation in normal subjects is that this number would be close to 0.5, as the maternal copy would be methylated and the paternal copy would be unmethylated. For PWS, the expectation is 1.0, as they do not have paternal unmethylated copy. For AS, it would be close to 0.0, as they lack a methylated maternal allele. Table 1 shows that there is some small variation, but that the theoretical and the actual numbers match closely especially for the three *SNRPN* probes in either exon 1 or intron 1.

University of Florida. Therefore, we obtained minimal records on him including his autopsy and his hospital records before his death. MS-MLPA found a unique deletion between BP1 and a site telomeric to *ATP10A* sparing the *GABRB3* and more distal genes. These results were confirmed by aCGH, which showed a deletion size of ~3.60 Mb (Table 2).

Case 3 *PWI73P*. A clinical karyotype analysis revealed an unbalanced karyotype interpreted as 45,XY,der(6)t(6;15)(p25;q12),-15. FISH analysis confirmed a 15q11.2 deletion with the *SNRPN* probe. MS-MLPA revealed a deletion of *NIPA1* to *GABRB3* while sparing *OCA2* (there were no MS-MLPA probes for *GABRA5* and *GABRG3*). The research aCGH demonstrated a unique deletion extending from the pericen-

Table 2 Case studies of seven subjects with a unique or atypical deletion

Case #	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
	PW235P	HBTB_113	PW173P	PW246P	PW133P	PW231P	PW259P
<i>Molecular genetics</i>							
Age of PWS diagnosis	11 years	Unknown	22 years	'Infancy'	12 years	3 months	6 weeks
Base pair positions of breakpoints	21,194,486 to 23,658,124	20,307,873 to 23,907,683	19,790,000 to 24,884,422	19,790,000 to 25,368,815	20,307,873 to 25,860,385 (2nd deletion 31.2Mb-31.5Mb) ^a	21,194,486 to 30,249,986	21,194,486 to 30,504,250
Deleted genes	MRKN3 to ATP10A	NIP1 to ATP10A	Centromere to GABRG3	Centromere to GABRG3	NIP1 to OCA2	MRKN3 to CHRNA7	MRKN3 to DKFZp434L187
<i>Demographics</i>							
Gender	Male	Male	Male	Female	Female	Female	Male
Race/ethnicity	White	White	White	White	White	Hispanic	Black
Age of subject for clinical features (years)	21	Died at 42	39	12	30	7.2	4.9
<i>Pregnancy/neonate</i>							
Reduced fetal movement	Yes	Unknown	Unknown	Unknown	Yes	Yes	Yes
Gestational age (weeks)	43	Unknown	Full term	Full term	42	40	40
Birth weight (kg)	3.73	Unknown	3.18	2.1	2.4	3.18	2.36
Hypotonia	Yes	Unknown	Yes	Yes	Yes	Yes (mild)	Yes (severe)
Feeding difficulty as a neonate	Yes	Unknown	Yes	Yes	Yes	Yes (mild)	Yes (severe)
Assisted feeding	Yes (NG tube)	Unknown	Yes (eye dropper)	Unknown	Yes (NG tube)	No	Yes (G-tube until 2.5 years)
Age range of assisted feeding	0-3weeks	Unknown	Several months	Unknown	0-4 months	NA	0-2.5 years
Duration of poor feeding	0-9 months	Unknown	Unknown	Unknown	0-4 months	0 to 5 months	0-2.5 years
<i>Developmental milestones</i>							
Sitting (months)	10	Unknown	Unknown	Unknown	11	8	18
Walking (months)	30	Unknown	36	24	30	20	Not walking at 59
First words (months)	30	Unknown	Unknown	Unknown	Unknown	12	36
Intellectual disability	WJ-III at 19 years; GIA=63, TIA=78	Described as 'very mentally retarded'	WAIS-R at age 31 years; FIQ=62, VIQ=63	Yes	IQ 55-60 per parents	WJ-III at 7.2 years; GIA=89, TIA=109	Moderate/severe MR
<i>Growth</i>							
Growth hormone treatment	10-14 years and 19-21 years	NA	NA	15 years to present	30s-40s	19 months to present	13-39 months
Weight (SDS)	+2.65	+1.28	+2.07	+3.75	-0.24	+2.47	+1.88
Height (SDS)	-0.76	-3.72	-3.72	+0.07	-2.04	+0.65	-1.59
BMI (SDS)	+2.65	+2.53	+2.93	+3.08	+0.76	+2.48	+3.51
Head circumference (in percentile)	98th	Unknown	<2nd	<2nd	<2nd	98th	2nd
Small hands and feet before GH treatment (in percentile)	No, hands=85th, feet=85th	Yes	Yes (<5th),	Yes, hands=25th, feet=3-25th	Yes (<3rd)	No, hands=75th, feet >97th	Yes, hands=3rd, feet=< 3rd

Table 2 (Continued)

Case #	Case 1 PW235P	Case 2 HBTB_113	Case 3 PW173P	Case 4 PW246P	Case 5 PW133P	Case 6 PW231P	Case 7 PW259P
<i>Obesity and appetite</i>							
Childhood obesity	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Subject became obese (BMI > 97%, years)	2	Unknown	Unknown	4.5	3	3	3-9
Voracious appetite began (years)	8	Unknown	Unknown	Unknown	3	6	4-9
<i>Other clinical issues</i>							
Facial gestalt suggestive of PWS	Yes at 5 years before GH, no at 21 years	Unknown	Mild	No	Yes	No	No
Dry/sticky saliva	Yes	Unknown	Yes	Yes	Yes	No	Yes
Hypopigmentation	No	Unknown	No	No	Yes	Only in irides	No
Skin picking	Moderate	Unknown	No	Very mild	Mild to moderate	No	No
Scoliosis	Moderate	Unknown	No	No	Mild	Mild	No
Hypogonadism	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Menstruation	NA	NA	NA	No	Rarely	NA	NA
Seizure activity	No	No	No	No	No	Yes	No clinical seizures, but abnormal EEG
Other medical problems	Hypothyroidism	Right ventricular hypertrophy and chronic gastritis	Hypertension, inguinal hernia	Enuresis	NA	Hypothyroidism, premature adrenarache, hip dysplasia	Reflux, hip dysplasia
Psychiatric and behavioral problems	Destructive behavior; rigid thinking	Unknown	Resistance to change	Imaginary friends still at 14 years	No issues now	Nail biting, resistance to change, stubborn, labile	Plays with feces, self-stimulatory behavior
Pain threshold	Lower than average	Unknown	Higher than average	Unknown	Higher than average	Higher than average	Higher than average
Vomiting	Much lower than average	Unknown	Much lower than average	Much lower than average	Never vomited	Much lower than average	NA due to nissen fundoplication

Abbreviations: BMI, body mass index; EEG, electroencephalography; FIQ, full scale IQ; GIA, general intellectual ability; G tube, gastrostomy tube; IQ, intelligence quotient; MR, mental retardation; NA, not available or not applicable; NS tube, naso-gastric tube; PWS, Prader-Willi syndrome; SDS, standard deviation score; TIA, total achievement score; VIQ, verbal IQ; WAIS-R, Wechsler's adult intelligence scale-revised; WJ-III, Woodcock-Johnson tests of cognitive abilities and achievement, third edition.
*Please see detail in Supplementary data.
Center for Disease Control curves (2000) used for weight, height and BMI SDS.
Head circumference percentages were adopted from Nelthaus.²¹

telomeric 15 region to intron 3 of *GABRG3*, sparing exons and genes telomeric to intron 3 including *OCA2*. A clinical aCGH demonstrated no loss of unique 6p material. The size of the deletion was ~5.09 Mb. Atypical features for PWS in this subject include microcephaly, normal pigmentation, lack of skin picking, and a pleasant, mild-mannered personality with lack of argumentative behavior (Table 2).

Case 4 PW246P. A clinical karyotype and FISH analyses revealed 45,XX,der(14),t(14;15)(p11.1;q13).

MS-MLPA revealed a deletion of *NIPA1* to *GABRB3* while sparing *OCA2*. The research aCGH revealed a unique deletion extending from the pericentromeric 15 region to intron 5 of *GABRG3*. The size of the deletion was ~5.58 Mb. A clinical aCGH demonstrated no loss of unique 14q material. Features not typical of PWS include microcephaly, lower-than-average birth weight for PWS,^{22,23} and later onset of obesity. She lacked the typical PWS facial gestalt. In addition, she was reportedly functioning at a higher level than typical for PWS, according to the experienced group home staff.

Case 5 PW133P. MS-MLPA revealed a deletion of the gene interval from BP1 to within the *OCA2* gene. The aCGH revealed a unique distal BP that was within intron 18 of *OCA2*, sparing exons 1–18 of *OCA2*. The features not typical of PWS in this patient were lower-than-average birth weight for PWS.²²

Case 6 PW231P. MS-MLPA revealed a deletion from *MKRN3* to *APBA2*. The aCGH demonstrated a deletion of ~9.06 Mb extending from (and including) *MKRN3* through *CHRNA7*. Therefore, this subject had an atypical deletion involving BP2 proximally and BP5 distally. The features not typical of PWS include milder hypotonia and feeding difficulties as a neonate, less delay of developmental milestones, macrocephaly (even before the start of growth hormone treatment), frontal bossing, inverted nipples, bilateral transverse palmar creases, large hands and feet, absence of seizures and higher-than-typical IQ for PWS.

Case 7 PW259P. MS-MLPA detected a deletion from *MRKN3* to *APBA2*. The aCGH demonstrated a 9.31 Mb deletion extending from *MKRN3* through *DKFZp434L187*, ~250 kb telomeric of *CHRNA7*. Like case 6, this subject had a BP2–BP5 atypical deletion. The features not typical of PWS include lower-than-typical birth weight, the much more severe developmental delay, the microcephaly, atypical facial features, increased hypotonia and the prolonged failure-to-thrive.

DISCUSSION

A deletion in the paternal 15q11.2–q13 chromosomal region is the most common cause of PWS; however, it has become clear that Type 1 and 2 deletions are not the only type of deletions that occur in individuals with PWS. Among our cohort of 88 deletion subjects with PWS, we found 36.4% with Type 1 deletion, 55.7% with Type 2 deletion and 8% with a unique or an atypical deletion. The higher number of Type 2 *vs* Type 1 is similar to the findings of other studies.^{15–17} The prevalence of unique or atypical deletions was higher than what we expected and has not previously been reported for PWS, but was comparable to the prevalence rate (9.1%) found in AS.⁷

The 15q11.2–q13 region is highly vulnerable to structural rearrangements, such as deletions, duplications, supernumerary marker chromosomes, and translocations due to presence of LCRs in the region.^{24,25} In our subjects with unique or atypical deletion sizes, two subjects (cases 6 and 7) had both proximal and distal BPs involving LCRs (ie, BP2 and BP5). Another three of our subjects (cases 1, 2 and 5) had a proximal BP in an LCR (ie, either BP1 or BP2), but the distal

BP was unique and located proximal to BP3 in an interval between *ATP10A* and *OCA2*. Cases 3 and 4 had an unbalanced translocation with neither BP involving an LCR in the 15q11.2–q13.3 region.

In this study, we also examined genotype–phenotype relationships in our seven subjects with unique or atypical deletion sizes. Three of our subjects (cases 1, 3 and 4) with an intact *OCA2* gene had normal skin pigmentation comparable to other members of their family. This is not surprising given the role of *OCA2* in skin pigmentation.²⁶ Case 2 came to our attention only after his brain was donated to our brain bank, so we cannot comment on his skin pigmentation. The clinic notes we obtained have no mention of his pigmentation. Case 5 had a deletion within intron 18 of *OCA2* and was hypopigmented. Case 6 and 7 had large deletions that included *OCA2*, but hypopigmentation was not noted. In our clinics, we have found that hypopigmentation in PWS deletion cases is much harder to discern in the more deeply pigmented ethnic and racial groups. Case 6 is Hispanic and case 7 is Black, which probably explains why hypopigmentation was not appreciated in these two subjects.

One subject had a much smaller deletion (2.46 Mb) than a typical deletion (Case 1). His deletion spared the GABA_A receptor subunit gene clusters and *OCA2*. Interestingly, he had an above average birth weight, macrocephaly, large hands/feet and tall stature for PWS (before he started growth hormone), lower-than-average pain tolerance, and somewhat delayed onset of the voracious appetite and food-stealing behavior. These atypical features contributed to the delay in his PWS diagnosis. However, the moderate skin picking behavior in this subject was rather puzzling. Given the postulated role of GABA in the mediation and perception of pain,²⁷ we would have anticipated that sparing the GABA_A receptor subunit gene clusters would have lessened this behavior. In addition, he manifested the common behavioral characteristics of individuals with PWS including temper tantrums, easily angered and rigidity. Furthermore, his cognitive IQ was 63, well within the typical range for PWS.²⁸ Unfortunately, we do not have detailed behavioral information on case 2 whose deletion also spared the GABA_A receptor subunit gene clusters. Therefore, based only on this one subject, it would appear that sparing the GABA_A receptor subunit gene clusters does not alter the typical PWS neuro-behavior.

Interestingly our two subjects (cases 6 and 7) with large deletions spanning >9 Mb between BP2 and BP5 had certain similarities and differences in their phenotypic presentation. In both the *CHRNA7* gene was deleted, but case 7 had an additional 250 kb deletion telomeric to *CHRNA7* involving the *DKFZp434L187* transcript. *CHRNA7* encodes the alpha-7 subunit of the neuronal nicotinic acetylcholine receptor.²⁹ Recently several groups have implicated *CHRNA7* as a candidate gene for the 15q13.3 microdeletion syndrome whose clinical manifestations include facial and digital dysmorphology, expressive language deficit, and various neuropsychiatric disorders, such as schizophrenia, autism, epilepsy and mental retardation.^{30–33} The finding that cases 6 and 7 had large deletions encompassing the 15q13.3 region prompted us to request an EEG be done on both of them. Similar to those with the 15q13.3 microdeletion, case 6 had both clinical and EEG-documented seizure activities. Surprisingly, her parents, teachers and health care providers were unaware of her absence seizures until her abnormal seizure related behavior (eg, looking away, lip smacking, etc.) correlated with the EEG wave changes. Case 7 also had an abnormal EEG; however no clinical seizure activity has been noted in this subject. As he is still young, it would be reasonable to assume that he is at increased risk for seizure activities. In addition, both case 6 and 7 lacked the typical facial

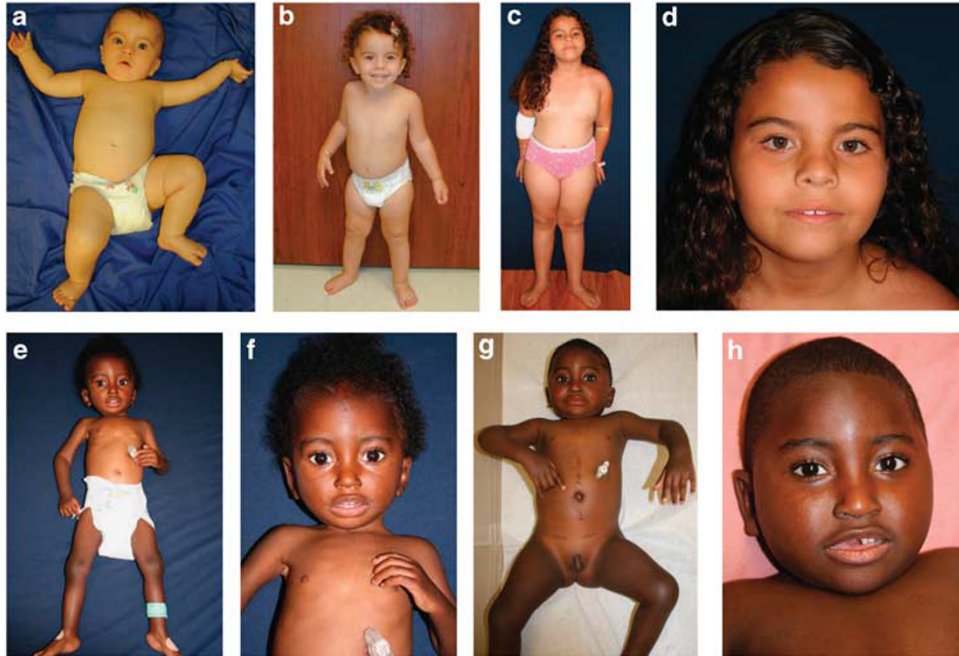


Figure 2 The two PWS subjects with large atypical deletions and lacking the typical PWS facial gestalt. Case 6 at 9 months (a), 2½ years (b) and 7 years (c, d). At 9 months (a) she appears well nourished with a weight for length at the 75th percentile. Features include a depressed and wide nasal bridge, blunted columella, round face and long, smooth philtrum. Case 7 at 13 months (e, f), 24 months (g) and 4 years (h). Note the thin, frail appearance with G-tube in place as an infant with a weight for length at <3rd percentile (e, f). Weight began to increase at 24 months. Facial features include a prominent metopic suture, round face, depressed and wide nasal bridge, blunted columella, and short and smooth philtrum.

features of PWS, but did share some facial features in common with the 15q13.3 microdeletion subjects (Figure 2).

Although sharing some similarities, case 6 and 7 did have drastic differences in their clinical manifestations. Case 6 had a much milder postnatal course, less severe developmental delay, macrocephaly, and higher intellectual and academic functioning. At 7.2 years she had a cognitive IQ score of 89 and an achievement score of 109 both of which are significantly higher than typically found in PWS.²⁸ This is surprising as her deletion encompasses both the PWS region as well as the 15q13.3 microdeletion region. In contrast, case 7 had a much more severe failure-to-thrive phase requiring a feeding tube for 2.5 years, microcephaly, markedly delayed gross and fine motor skills as well as speech development. At 4.9 years he was still not walking independently. Case 7's clinical findings are more in line with what we would have expected from deleting two microdeletion syndrome regions. At this point it is not clear why there is such a dramatic difference between cases 6 and 7 who share a similar-sized deletion of ~9 Mb. There are several possibilities for the differences. First, the genetic variation in the 15q13.3 region on the maternal chromosome may become 'unmasked' by the paternally derived deletion, which may have contributed to the more severe clinical phenotype in case 7. Second, genetic variation elsewhere in the genome may have contributed to the composite clinical features in case 7. A great deal of variability in clinical features has been described for individuals with the 15q13.3 microdeletion including some parents with the deletion who lack the clinical features found in their offspring.³³ Furthermore, the 15q13.3 region, like the 22q11.2 and 16p12.1 regions, may be another example of a 'susceptibility region' that requires a 'second hit' elsewhere in the genome to become fully penetrant as a neurodevelopmental phenotype.^{34,35} Third, the *DKFZp434L187* transcript (deleted in case 7) downstream to *CHRNA7* may require further

evaluation for its potential role in the more severe developmental delay and failure-to-thrive, although this is very unlikely given that this transcript has not been shown to code for any important function to our knowledge.

We identified several strengths and weaknesses of the MS-MLPA technique. MS-MLPA reliably detected the deletion as evidenced by 100% agreement with our laboratory data and aCGH. It also provided more detailed information regarding the size of the deletion than standard FISH due to many (25–32) probes across the 15q11.2–q13 region. Thus, the MS-MLPA technique allows for a reasonable approximation of the BPs and deletion size. Compared with aCGH, the MS-MLPA technique was much more labor/cost-effective, although aCGH provides more precise information regarding the extent of the deletion. In addition, the DNA methylation component of MS-MLPA allows differentiation between PWS and AS deletions, and maternal and paternal 15q11.2 duplications, as well as between uniparental and biparental disomy. Therefore, a compelling argument could be made that MS-MLPA should be the first test used when contemplating AS or PWS as a possible diagnosis, especially since important genotype-phenotype correlations will likely be forthcoming.

In this study, we had an opportunity to evaluate both the A1 and B1 versions of the commercial MS-MLPA kit. The A1 version has two probes for *OCA2*, which are useful to identify unique deletions with distal BPs between *GABRB3* and BP3. If we had not used the A1 version, we would have missed the unique deletion sizes in cases 3, 4 and 5. The B1 version, on the other hand, is designed to provide additional coverage at the bipartite AS and PWS imprinting center and *SNORD116* regions. Taken together, the A1 version appears superior in detecting deletion sizes of AS and PWS, whereas the B1 version is superior in identifying small deletions in the imprinting center and/or the *SNORD116* region.

In conclusion, this study makes several important points. First, deletions should be characterized by accurately determining both their proximal and distal BPs, rather than just their proximal BP. The designation of Type 1 deletion should be strictly reserved for those deletions between BP1 and BP3, and Type 2 deletion for those deletions between BP2 and BP3. This is important for genotype–phenotype counseling in the future, as unique or atypical (ie, not type 1 or 2) deletions due to a novel distal and/or proximal BP could result in a milder or more severe phenotype than either Type 1 or Type 2 deletions. This may also contribute to the lack of current consensus in genotype–phenotype comparisons of Type 1 vs Type 2 deletions, as the distal BPs were not always well delineated in many of these studies. Second, the frequency of deletions that are neither Type 1 nor 2 (8% in our study) is higher than previously recognized in PWS. Third, genotype–phenotype studies on individuals with unique or atypical deletions have the potential to elucidate the role of the various genes in the 15q11.2 region. Fourth, the mechanisms for producing unique deletions need further clarification as at least five of the subjects in this study had at least one BP that did not occur at a 15q11.2–q13 LCR region. Fifth, the differences in clinical manifestations of our two subjects with a deletion extending into the 15q13.3 region further underscore the clinical variability of the 15q13.3 microdeletion syndrome. In our future studies, we plan to obtain more detailed phenotypic information using specific neurobehavioral measures, such as cognitive and behavioral profiles, comorbid psychiatric illnesses, and response to certain medication, to more fully examine genotype–phenotype relationships in these subjects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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